

UK National Screening Committee

cfDNA testing in the fetal anomaly screening programme

19 November 2015

Aim

1. To ask the UK National Screening Committee (UK NSC) to make a recommendation based upon the evidence presented in this document on the introduction of cfDNA as a contingent test within the UK Fetal Anomaly Screening Programme (FASP).

This document provides background on the items addressing this proposed modification to the Fetal Anomaly Screening programme.

Current programme policy and area impacted by the proposed change

2. Currently fetal anomaly screening is offered to all pregnant women in the first or second trimester. Women can opt to be screened for Down's syndrome, Edwards' syndrome and Patau's syndrome (T21, 18 and 13 respectively). The proposed change to the programme is for cfDNA testing to be offered to women following a risk score of 1/150 or greater for T21 or T18/13.

Programme Modification Proposal

3. Specifically, the consultation asked for responses on the recommendation that:

A cfDNA test be offered after any of the following combined test outcomes:

- The combined test risk score for trisomy 21 (T21) is greater than or equal to 1 in 150
- The combined test risk score for trisomy 18 (T18) and trisomy 13 (T13) is greater than or equal to 1 in 150

Women are advised that a cfDNA test is not diagnostic and that an invasive diagnostic test is required to receive a definitive diagnosis.

4. Key reasons supporting this proposal:

- The RAPID study and UK NSC review both noted the number of women with unaffected pregnancies that are offered an invasive test is markedly reduced when cfDNA testing is introduced. The UK NSC review suggested that when the risk threshold is reduced and women are offered cfDNA tests with a risk lower than 1 in 150 this benefit is less clear. Although more cases are found when women with a lower risk are offered cfDNA tests, the UK NSC did not consider that this benefit outweighs the effect on the numbers of invasive tests offered.

- The UK NSC review found that this approach would not reduce the number of babies found with trisomies.
- The availability of cfDNA testing is limited in the UK. It is thought that the introduction of cfDNA testing in this way will not exceed the current capacity for testing in the UK.
- Both the systematic review and RAPID study concluded that this implementation strategy will have a minimal effect on the expenditure on the screening programme, compared to alternatives. The review has highlighted a number of uncertainties in implementing cfDNA testing in the screening programme. It is therefore pragmatic to introduce the test in this way and learn from the implementation.
- Finally, retaining the current 1 in 150 risk threshold will mean that changes to the current screening programme pathway will be minimised by the introduction of cfDNA testing. This is particularly important, as there are a number of issues with cfDNA testing that are not clear (e.g. test failure, impact on uptake). By offering the test at this threshold, the test is available to those at the highest risk without disrupting the screening programme and there is opportunity to explore these uncertainties.

Consultation

5. A three month consultation was hosted on the UK NSC website which closed on the 30th October 2015. The consultation was sent directly 38 organisations. Annex A

Responses were received from the 30 stakeholders. Annex A

Consultation responses

6. The responses received were varied. Many of the responses were positive, supporting the recommendation of implementation of Non Invasive Prenatal Testing (NIPT) as a contingent test following a combined test risk score of 1 in 150 or higher. However, a number of responses were not supportive of the recommendations. To those that were not supportive of the recommendations, a programme with termination as a possible outcome is unacceptable, and these respondents were of the view that the number of unaffected fetuses saved by the proposal would be outweighed by an increase in the number of affected fetuses terminated.
7. The current demand for the test in the health system was highlighted by a number of respondents, in terms of the number of women currently accessing the test privately and the positive response that has been received where it has been implemented already. The Royal College of Midwives (RCM) and British Pregnancy Advisory Service (BPAS) responses both highlighted the anxiety felt by women in choosing an invasive test with a potential risk of miscarriage.
8. A number of respondents raised factors relating to cfDNA as a **primary screen**. The greater cost to the NHS of implementing cfDNA as primary screen was acknowledged. It was raised that implementation of cfDNA following combined test would not alter the number of false negatives, and implementing the pathway with cfDNA as the primary screen would pick up a

much greater percentage of cases of trisomy. The lower Positive Predictive Value (PPV) of cfDNA testing in a low risk population was acknowledged.

9. A number of responses made reference to the **proposed cut-off**. Many responses agreed with the approach of using a cut-off of 1/150. A number of respondents also raised the point that the test accuracy at the proposed cut off is uncertain. One respondent suggested a cut off of 1/800, with a different approach to recall (see Annex C for further comment)
10. A large number of respondents raised points related to the **pathway** on implementation. A number of respondents expressed concerns that the consultation only looks at NIPT following the combined test, and raised that it should also be offered to women following the Quadruple test as the primary screen. A number of respondents raised points relating to the screening pathway if ultrasound abnormalities were detected or particularly raised nuchal translucency measurements (See Annex E for pathway information).
11. A number of respondents raised concerns regarding the **pathway** if following a high risk result in the combined test, women were required to have NIPT prior to invasive prenatal diagnosis (IPD). It was suggested that women should be offered the choice in order to be able to make a decision taking into account their risk and gestation. It was raised that women value being able to make an informed choice as early in a pregnancy as possible. Concerns were raised over failure rates potentially causing further delays in the pathway. The BPAS highlights that for the majority of women with a positive diagnosis who choose not to continue their pregnancy this will mean it may be harder to obtain a choice of method of termination. A number of respondents raised that cfDNA will lengthen the pathway overall and cause additional anxiety. Response from the RAPID team highlights choices made by women. (See Annex E and F for pathway options and further modelling and literature review undertaken by Warwick University).
12. It was highlighted that in conducting Chorionic Villus Sampling (CVS) following cfDNA there will be a higher risk of confined placental mosaicism being the cause, particularly for T13/18. Therefore issues around which invasive test should be used following cfDNA testing were raised.
13. There were a number of queries relating to the **economic model**, particularly in relation to some of the costs used. Points were also raised regarding the true rate of procedural loss following invasive testing, which would impact on the estimated reduction in test-related miscarriage, and comments were also made regarding the test accuracy used in the model. Comments were also made regarding study quality and risk of bias in the studies in the **systematic review**. (See appendix C for response to these comments from the systematic reviewers)
14. A number of responses related to the **specifications for the laboratory**; for example a number of respondents made reference to different failure rates from their experience, and around turn-around time. Other comments relate to varying predictive values, and fetal fraction, and ensuring sufficient throughput for cost-effectiveness. The Association for

Clinical Genetic Scientists suggest that there is currently re-configuration of laboratories that needs to be addressed by the screening programme. They note that the profession would not support a single provider for the service as part of a screening programme.

15. Respondents highlight the need for appropriate counselling and **support for women** when going through the screening pathway and making decisions. Further comments on the importance of information for women highlight that this should include information regarding failure rates, detection rates in trisomy 13 and 18, twins, and provide false positive values/ positive predictive values. It was also highlighted that information for women should be balanced and include information relating to life prospects of people with Down Syndrome, the impact on families, support available.
16. A number of responses related to the **potential impact of implementation**. It was raised that on implementation it is likely that there will be reduced numbers of invasive tests carried out, and that is important that skills in this area are maintained. Comments were also made querying the start-up costs and funding flows on implementation.
17. Responses highlighted requirements for **data collection** to inform knowledge if implemented, suggesting an early review 6 months following implementation, reviewing the 1/150 cut off, patient experience, financial impact (particularly if the cost of the test changes), assessing choice between IPD/NIPT, gathering information regarding procedural related miscarriage following IPD, failure rates , and predictive values.
18. Concerns regarding the **ethics** of the recommendations were raised. Particularly, concerns were raised that the recommendation is in conflict with the UN Convention of the Rights of People with Disabilities (CRPD) and the Report of the UNESCO International Bioethics Committee, and disability rights in general. Concerns were raised that the proposal runs counter to the principle articulated in these statements and would discriminate against a disabled unborn child, and increase stigma and stereotyping of people with disabilities. Concerns were raised that cfDNA is seen only as an approach to lead to termination of affected babies, and not to help prepare parents to take care of a disabled child and plan best medical care and support.
19. Concerns were raised by a number of respondents regarding the ethical issues relating to collecting and storage of DNA samples, and other tests that samples could be used for. Particularly, concerns were raised regarding the potential for cfDNA testing to be used to enable sex-selective abortion. (See appendix G for further information regarding ethical issues)
20. Concerns were raised that NIPT may be seen as diagnostic, and that this may lead to reproductive choices in the absence of appropriate diagnostic procedures.

Recommendation

21. The committee is asked to recommend that;

Further evaluative work is undertaken, with input from scientific and ethical expertise, to better understand

- a. what would need to be answered before full implementation, and approach to addressing these issues
- b. the practical impact to the screening programme if implemented after the following test outcomes:
 - i. The screening test risk score for trisomy 21 (T21) is greater than or equal to 1 in 150
 - ii. The combined test risk score for trisomy 18 (T18) and trisomy 13 (T13) is greater than or equal to 1 in 150

That the evaluative work be based on a pathway to test the idea that women could have the option of cfDNA testing or invasive testing following a high risk result, (see appendix D for pathway options and rationale for option a)

And that the evaluative work should be informed by work on ethics and NIPT currently being initiated by the Nuffield Council



List of organisations contacted:

1. Antenatal Results and Choices
2. Ariosa
3. BLISS
4. British Heart Foundation
5. British Maternal & Fetal Medicine Society
6. The British Medical Ultrasound Society
7. British Pregnancy Advice Service
8. CDH UK
9. Child Growth Foundation
10. Children's Heart Federation
11. CLAPA
12. Contact a Family
13. CRUSE
14. DIPex
15. Down Syndrome Education International
16. Down Syndrome Research Foundation UK
17. Down's Heart Group
18. Down's Syndrome Association
19. Down's syndrome Medical Interest Group
20. Downs Syndrome Scotland
21. Elfrida Society
22. Genetic Alliance UK
23. Little Hearts Matter
24. Marie Stopes International
25. MENCAP
26. Miscarriage Association
27. PHG Foundation
28. Restricted Growth Foundation
29. Royal College of General Practitioners
30. Royal College of Midwives
31. Royal College of Obstetricians and Gynaecologists
32. Samantha Walsh (NHSE)
33. SANDS
34. SHINE Charity
35. Society and College of Radiographers
36. Tiny Tickers
37. Together for Short Lives
38. Wolfson Institute of Preventive Medicine

List of stakeholder whom commented on the consultation

1. Guy's and St Thomas NHS Foundation Trust
2. a Screening and Immunisation Coordinator
3. the Wolfson Institute for Preventative Medicine
4. Genetic Alliance UK
5. Royal College of Obstetricians and Gynaecologists
6. Royal College of Pathologists
7. University Hospitals of Leicester NHS Trust
8. Fetal Medicine Unit, University of Leicester, East Surrey Hospital
9. British Maternal and Fetal Medicine Society
10. Guy's Genetics Centre
11. The Association for Clinical Genetic Science
12. Saving Down Syndrome
13. The Down Syndrome Research Foundation UK
14. Down Syndrome Scotland
15. RAPID Research Team
16. Roche Diagnostics Limited
17. Royal College of Midwives
18. Society and College of Radiographers
19. University Hospital Southampton NHS Trust
20. Right to Life
21. Stop Gendercide
22. Evangelical Alliance
23. Christian Concern
24. E A Mcreadie
25. LIFE Charity
26. Society for the Protection of Unborn Children
27. Society for the Protection of Unborn Children Scotland
28. Hayley Goleniowska, the Chrisian Medical Fellowship
29. Daniel Marsden, Practice Development Nurse for people with learning disabilities
30. British Pregnancy Advisory Service

Compiled consultation responses

(See separate document)

Response from Systematic Reviewers, Warwick University

University of Warwick Comments on Consultation Responses

Sian Taylor-Phillips 3/11/2015

Detailed below are the consultation feedback responses that relate to the Warwick review and economic model (in italics) with our comments. Comments are grouped by subject for ease of interpretation. We have provided a response where comments relate to the methods or assumptions of our report, but have not provided a response when views regarding implementation, or interpretation of findings are given.

Modelling Lifetime Costs of Caring for Child with Downs Syndrome

RCOG. BMFMS: It has been queried why the lifetime costs of caring for a child with down's syndrome were not included in the economic analysis. I wondered if you had a rationale or thoughts around this decision?

Warwick Response: We agreed in collaboration with the NSC very early on that this type of modelling would be unethical. Calculating the cost of caring for a child with Down syndrome, then using this as part of a model evaluating testing with a view to abort that child is not ethical. Furthermore if we were to model the costs of caring for that child, it would make sense to model the QALY gains by the child living also. This would add further ethical issues including how to value the quality of life of a person with Down syndrome. For this and related reasons the analysis did not include any modelling of women's choices following diagnosis.

Roche Diagnostics: We believe that the assessment of the financial impact of having fewer invasive procedures should not be limited to the cost of the procedure itself but also take into account the cost associated with procedure related complications such as premature rupture of membranes and miscarriage. Other countries have also included cost directly related to consequences arising from a false negative screening test³.

Warwick Response: Similarly to above we agreed to model up to the point of test accuracy, but consistently include none of the downstream consequences of test accuracy due to the ethical complexities that arise.

Estimates in the Economic Model

Cost of cfDNA

Royal College of Pathologists: The estimated cost of £232 for cfDNA seems low – RAPID quote £250 and other UK websites quote £400 to £700. A US study quotes £518 (Song et al 2013). Another responses suggests that the cost of cfDNA testing will drop and the analysis should incorporate this

Guys Genetic's Centre: Estimated cost of NIPT at £232. Although it is acknowledged in the consultation that there is uncertainty regarding this price, in our opinion the price is remarkably low and some essential peripheral costs do not appear to have been included. These include costs for phlebotomy, counselling, sample transfer. RAPID quote £250 plus an extra £30 for phlebotomy/counselling/feedback/repeat tests. In addition does the price include any patent/licence fee, currently £50/report to Illumina/Sequenome. Will this be paid centrally? Start-up costs for the NIPT will be significant and training of health professionals must be included.

Saving Down Syndrome: Question 5 Economic Evaluation A model was constructed. The £232 figure for cfDNA is notional. There was no representative figures for the additional costs such as training, counselling, and staff which would also increase any budgets.

Warwick Response: In consultation with the UK NSC we agreed that the published quotes by Lyn Chitty's group would be the most appropriate for the UK context, recognising the inherent difficulty in predicting the future cost of a rapidly evolving technology. Therefore we used the estimate from Lyn Chitty's paper. This is unsurprisingly very similar to the £250 Lyn quoted in her economic analysis related to the RAPID trial. Because of the uncertainty in the estimate we did a sensitivity analysis (produced different versions of the economic model) with costs ranging from £100 to £500

Cost of Invasive Testing

Guys Genetic's Centre: Cost of invasive procedures and testing at £650. This is higher than the Guy's costings which come in at £465 (invasive procedure £240, QF-PCR £75, sample prep £150). Although these costs will vary nationally, we wonder if the estimated price has included aCGH/karyotyping. The cases being considered for NIPT would have a nuchal of less than 3.5mm and therefore no aCGH/karyotyping would be required in line with national guidelines which have been implemented in a number of regions. The cost of arrayCGH/karyotyping should not therefore be included in the cost of invasive procedures and testing

Warwick Response: This is a misunderstanding. Our estimates for the cost of Amniocentesis was £383.31 and CVS was £318.90 using the NHS FASP decision planning tool inflated using the Hospital & community health services pay & prices index reported in PSSRU Unit Costs of Health and Social Care 2014. We did a sensitivity analysis to determine the effects of using the higher estimates of £515 for both amniocentesis and CVS. I don't understand what the reference to £650 relates to, this was not used in the Warwick model.

Miscarriage rate following Invasive Testing

Royal College of Gynaecologists: The miscarriage risk following invasive testing has also been queried, suggesting the figures used were derived from an older study and a more recent study (Adolekar 2015) suggests figures of 0.11% for CVS and 0.22% for amnio.

Guys Genetic's Centre:

Test-related miscarriage of 0.6 to 0.7%. Much of the justification for NIPT comes from the reported risk of invasive procedures and this risk is therefore fundamental to the whole premise of the NIPT.

The systematic review discusses this on p77 and details the published variability for this figure. The figure chosen for the economic model to calculate loss is 0.6 to 0.7%. However, the UK data support the lower figure of 0.1% for AF and 0.2% for CVS. The review calculates that with this risk, introduction of cfDNA testing would avoid 10 miscarriages per year.

Warwick Response: We included the Akolekar study as a sensitivity analysis in the model. There is not consensus amongst professionals about the actual rate, so we used the most accepted rate as our main analysis, (O'Leary et al.) and did sensitivity analysis to determine the effects of using the higher estimates of Tabor et al. (2009) and lower estimates of Akolekar et al. (2015).

Right to Life: The UKNSC claims from its expert review that implementation of cfDNA testing in the FASP could reduce the number of miscarriages that occur due to IDP procedures. This is questionable given that the RAPID study projects around half the reduction in miscarriages that the systematic review does. This discrepancy seriously undermines the confidence that can be put on the UKNSC's cited data.

Warwick Response: As above there is uncertainty around the estimates of miscarriage rate from invasive testing, and therefore uncertainty around the number of miscarriages averted by a reduction of invasive testing in affected fetuses. This is described in the model sensitivity analyses.

Uptake of NIPT

Saving Down Syndrome: We also note that the review used a survey which is termed as 'antenatal clinic survey data', this is misleading. The survey results were derived from Antenatal clinic survey data together with users of the ARC website and MUMSnet website, this brings into question, again, the figures produced by the ensuing model.

The antenatal clinic survey data referred to was Lewis et al (2014) used to estimate what choices women would make if offered NIPT i.e. the uptake. We discuss in the report the difficulty in making such an estimation in advance of implementation, and in recognition of this difficulty provide a sensitivity analysis using estimates of Gil et al. of actual uptake of NIPT when offered as part of a UK study.

Results of Economic Models

Roche Diagnostics: As part of this process we believe that the assumptions behind the present health-economic assessments should be critically reviewed. For example, in the final report to the committee a cut-off of 1 in 1000 seemed to result in more invasive procedures and related terminations than a primary NIPT test strategy (Table 8). However, almost 50% of invasive procedures were assumed to be chosen by women without the NIPT result, which may be an over-estimate.

Warwick Response: the reason for the higher estimate for invasive procedures when implementing combined test at 1/1000 then offer of cfDNA, in comparison to cfDNA as the primary screen is that

setting the threshold for high risk at the combined test at 1/1000 will classify a large number of women as 'high risk'. These 'high risk' women will all be offered cfDNA or the choice of straight to invasive testing, which produces a large number of invasive tests. In comparison using cfDNA as the primary screen would have a higher threshold, so fewer women would be referred to invasive testing. We are happy with these model assumption, but note in the report that there is uncertainty surrounding several of the model assumptions, most of which could not be resolved without actually implementing cfDNA. We address these uncertainties will a full set of sensitivity analyses.

Alternative Pathways

BMFMS: To fully investigate cfDNA as primary screen – would want to see analysis including NT scan (no biomchemistry) for all, with IPT offered to those with a risk >1 in 10, NT>3.5 or anomalies, and all other women offered cfDNA testing (quad offered to those with failed cfDNA).

Warwick Response: This is a genuinely new suggestion that was not discussed in our scoping discussions with the UK NSC, so our report does not analyse the clinical or cost effectiveness of this implementation. Evaluating this option would require understanding of the test accuracy of NT which we did not investigate, and I am not familiar with the research literature on this. In summary this is not similar to the remit or question we addressed.

Related to this we discussed with the NSC the option of a combination of NIPT with parts of the combined test. We report that there is no evidence about test accuracy for any version of that, but one study where Dave Wright did some modelling work around the potential of this option.

Different Manufacturer's tests

Roche Diagnostics: We would like to point out that while sensitivity may be comparable among most cfDNA tests (>99%), the False-positive Rate (FPR) can differ significantly; i.e. <0.1 – 1.46%.^{4,5,6} The FPR of NIPT has significant impact on clinical management and consequently on cost-effectiveness models. A higher FPR will limit the reduction of invasive procedure rates after NIPT, increasing procedure related cost as well as cost due to procedure related complications. Thus, choosing a cfDNA test with a low FPR appears key to meet the expectations put forward by the current cost calculations.

4. Norton M et al., *Am J Obstet Gynecol.* 2012 Aug;207(2):137.e1-8.

5. Palomaki G et al., *Genet Med.* 2011 Nov;13(11):913-20.

6. Bianchi D et al., *Obstet Gynecol.* 2012 May;119(5):890-901.

Warwick Response: This refers to implementation, these issues and references are included in our report.

Roche Diagnostics: Robust clinical validation is key to validate the performance of NIPT. Some cfDNA tests lack validation in blinded, prospective studies published in peer-reviewed journals and rely on retrospective registry studies without outcome information on all patients.⁷ Actual clinical

performance (i.e. sensitivity/specificity), which provides the basis for a robust health economic assessment, is therefore hard to establish. We therefore believe it is critical to review the individual clinical dataset of any cfDNA test in consideration for implementation in the NHS, rather than solely relying on theoretical health-economic models produced with pooled sensitivity and specificity data.

7. Jani J, et al., *Ultrasound Obstet Gynecol.* 2015 Aug 24.

Warwick Response: This refers to implementation. In our review we give thorough assessment of study quality using QUADAS 2, and discuss issues around incomplete follow up in some studies.

Roche Diagnostics: Issue: The impact on cost efficiency, turn around times for results and test accuracy of the various cfDNA tests should be considered when implementing NIPT in the NHS. NIPT can be performed using different technical approaches. Most cfDNA tests are based on massively parallel shotgun sequencing (MPSS). MPSS is not selective in the chromosomal origin of the sequenced cfDNA. It is therefore necessary to sequence many million DNA fragments, originating from the complete genome, to ensure the analysis of sufficient chromosome 21 fragments to statistically detect significant differences between normal and trisomic fetuses. In national screening programs, where only a limited number of chromosomal conditions are to be evaluated, this is a less time- and cost-efficient approach compared to other NIPT methodologies. Targeted NIPT methods on the other hand, focus on cfDNA from the chromosomes of interest rather than analysing the complete genome where much of the information that is obtained remains unused or may even reveal conditions that were not intended to be screened for in the first place. Targeted NIPT also allows for deeper analysis, and yields more accurate results with a lower cost overall.^{8,9} Furthermore targeted approaches allow for use of methods other than costly Next-Generation Sequencing (NGS) for cfDNA quantification such as Microarray analysis - a robust, reliable method for DNA analysis that has been in use in UK for many years. Additional benefits of targeted technology result in faster turn-around times than with most MPSS systems providing laboratory results within 3 days⁹.

8. Sparks AB et al., *Prenat Diagn.* 2012 Jan;³²(1):3-9.

9. Juneau K, et al., *Fetal Diagn Ther.* 2014;³⁶(4):282-6.

Issue: Consideration should be given to including measurement of the proportion of fetal cfDNA in maternal plasma sample as a quality metric when implementing NIPT into the NHS.

For NIPT, ensuring that a sufficient proportion of cell-free DNA in the maternal plasma is "fetal" - in other words, originates from the pregnancy rather than the mother - is widely considered to be an important quality metric. Having insufficient fetal fraction for statistically reliable analysis can potentially lead to a higher likelihood of a false negative result as well as incorrect calls for fetal sex.⁷ As measuring fetal fraction is complicated and associated with an increase in cost, some NIPT providers are not measuring the cell-free fetal DNA (cffDNA) amount. This has been shown to lead to samples with insufficient cffDNA for analysis (e.g. non-pregnant samples) to be given a reassuring NIPT result.^{10,11}

7. Jani J, et al., *Ultrasound Obstet Gynecol.* 2015 Aug 24.

10. Bevilacqua E, et al., *Ultrasound Obstet Gynecol.* 2015 Jul 6.

11. Takoudes T, et al., *Ultrasound Obstet Gynecol.* 2015 Jan;⁴⁵(1):112.

Warwick Response: This refers to implementation. In our report we refer to one study which concludes that multiplexing reduces test accuracy, and discuss as follows: “The level of multiplexing affects the sequencing depth and an optimal level of multiplexing needs to be determined before consideration of cfDNA testing for implementation. While increased levels of multiplexing (lower sequencing depth) can reduce costs⁶⁰ it has also been shown to decrease the accuracy of the MPSS technology.⁵⁷ Therefore, a balance should be found between optimal test performance and costs of testing.⁶⁰ Liao et al. (2014)⁶⁰ therefore pointed out that it is important to determine the fetal fraction in the sample to be analysed and the sequencing depth required for analysis.” (pg 83)

Detailed comments from Saving Down Syndrome:

The Systematic Review often referred to the studies involved as having high risk of bias, including study bias, patient spectrum bias, publication bias and gestational bias. Thus any move to implement mass programmes would likely be very problematic and could not be ethically carried out following this review.

Twin pregnancies, multiple pregnancies (more than twin) – were certainly not discussed in the review, BMI issues, and Trisomy issues – were all singled out as possibly leading to test failure.

Question 1 a) What is the accuracy of cfDNA testing in predicting T21, T18 and T13 in pre-defined high risk (1:150) pregnant women following a test? The review told us that there were no studies reporting relevant performance and concluded that while it was a very good test even using our highest estimates of accuracy it must not be considered a diagnostic test.

Question 1b) How does changing the threshold for defining high risk following a combined test affect the accuracy of cfDNA testing? The reviewers told us that they were unable to present cfDNA testing at different risk cut-offs ranging from very high to low risk or present an optimal risk cut-off to maximise cfDNA testing performance in clinical practise. This was another major issue. There was no ideal study available in order to test accuracy; therefore a synthesis was undertaken in an attempt to answer this.

It states in the review that no firm conclusions could be drawn, regarding this question.

Question 2 What is the most accurate primary prenatal screening tool for T21, T18 and T13 in the first trimester when cfDNA testing and the combined testing are compared in a general population?

The review stated that ‘due to a lack of studies’ comparison between the current combined testing and cfDNA testing was not possible.

Question 3 What diagnostic accuracy is achievable by integrating cfDNA testing into the combined test? The review states that there were no studies which demonstrated test accuracy after implementing this approach and reviewers were unable to determine if the combination test with cfDNA would offer increased accuracy. Only a narrative could be provided for this question.

Question 4 What is the rate of cfDNA testing failure (number of inconclusive and excluded samples/ total number of samples? The studies showing failure were up to 12.7% (of course bias may be a reason for such varying results in our opinion.) There seemed to be some evidence that earlier gestational age and trisomies may lead to failure. We feel that this is a major concern in a system which currently has an extremely high level of throughput for termination, if strict guidelines weren't in place then failed tests may be wrongly perceived as diagnostic. Tables and a narrative summary were provided.

Question 5 Economic Evaluation A model was constructed. The £232 figure for cfDNA is notional. There was no representative figures for the additional costs such as training, counselling, and staff which would also increase any budgets. In addition, no model could be provided for using the combined test with the new cfDNA test. Elevated detection rates may include Trisomy pregnancies which may miscarry (a significant number of Trisomy pregnancies miscarry naturally). There is no cost included for any termination of pregnancies which may have miscarried naturally but may be detected earlier and terminated. Some of the economic models exhibited predicted high costs.

Other Points on the Systematic Review

The Systematic Review and cost consequence assessment of cell-free DNA testing for T21, T18, T13 was a truly comprehensive review of studies on the issue, most impressive. According to the authors the major limitation of the review were lack of data and no evidence of test accuracy when cfDNA and first trimester screening are combined which led to an inability to analyse the impact and model the scenario. None of the articles involved were of 'optimal quality'. Further limitation was the inability to provide a comparison of combined testing v cfDNA, therefore diagnostic performance (Question 2) was limited to narrative review. The main limitation was that it relied on published data; together with studies which may have had a high risk of bias classification due to unclear reporting. On page 16 it stated that a review to evaluate the performance of such tests is needed before implementation into UK clinical practice can be considered.

We also note that the review used a survey which is termed as 'antenatal clinic survey data', this is misleading. The survey results were derived from Antenatal clinic survey data together with users of the ARC website and MUMsnet website, this brings into question, again, the figures produced by the ensuing model.

Reviewers also said that due to publication bias, test performance may be overstated, and that the failure rate was very variable. They also state that there is limited evidence in the UK and generalising findings to the UK should be carefully considered. Oddly, there was no mention of spontaneous miscarriage for Trisomy 21, which is relevant to the review. We object to the use of the phrase 'normal fetus', there are more sensitive terms to use. On pages 25 and 81 there was also the questionable use of the term 'disease'. We object to the use of the phrase 'healthy pregnancies' on pages 65 and 76, 'unaffected' might be a more sensitive term.

Warwick Response: These are very thoughtful comments, and we are pleased that they considered our review comprehensive. Most of the comments refer to the interpretation of the review, rather than how we carried it out and so we will not comment on interpretation. However we can clarify a couple of points. Firstly the decision to conduct a meta-analysis was not driven by a lack of an 'ideal study', but rather conducting a meta-analysis is considered the best practice method to obtain an overall estimate of test accuracy rather than relying on a single study. The comments regarding estimates in the economic model are covered in the relevant sections above. Use of the term 'normal' rather than 'unaffected' fetus, and use of the term 'healthy' rather than 'unaffected' pregnancies was an error for which we apologise.

Comments from Down Syndrome Research Foundation UK:

The study was unable to provide full answers to the questions due to –

- *Bias – Some studies were sponsored by NIPT interested parties. Some studies were on selected patients, some studies were carried out on later gestations leading to more accuracy of results and publication bias where only favourable studies are published.*
- *Lack of data*
- *Other issues such as changes to the length of testing pathway, false positive results, false negative results, test failures, retest failure, diagnostic test numbers, financial implications, and termination numbers.*

Warwick Response: This refers to interpretation of our report rather than the report itself.

Society for the Protection of Unborn Children:

The research findings are presented with extensive data, but the recommendations summarising the findings are incomplete, and use misleadingly simple statistics. The unreliability of the figures is pointed up on page 55 of the Systematic Review:

“Findings should be interpreted with caution. Assessment using QUADAS-2 identified high risk of bias in included studies, particularly for selection of women and flow. Deeks' funnel plots indicated there was high risk of publication bias in included studies.”

Much of the research data is from studies of commercially marketed test systems, and we recommend that the impartiality of these studies be checked very carefully. The Systematic Review's statistical analysis is highly complex, with some data derived by narrative analysis – an inherently subjective process. Furthermore it is uncertain to assume that results achieved in laboratory trials would be matched in everyday clinical application of the tests.

Warwick Response: There was a high risk of bias in many of the included studies, as described in the QUADAS2 summary. Where there were insufficient quantity of studies for meta-analysis, we have summarised the studies narratively. All included studies were described, so none were

systematically excluded based on our judgement, and the same data extraction sheet and quality assessment methods were applied to all, with comparative data tabulated where possible.

Further comment regarding consultation response 8, Wolfson Institute of Preventative Medicine:

1. The idea of not recalling the woman is appealing, but the idea of taking an extra blood sample from everyone is not appealing. I presume you would also have to consent everyone to both combined and NIPT initially, even though only 10% get NIPT.
2. Would reflex testing in this way necessitate use of a particular type of cfDNA test? I don't understand why the test and the protocol are related. Are they?
3. Does the research group have their own cfDNA test? I can't find any paper that shows the test accuracy of their particular test, just modelling of the different pathways.
4. The paper "Performance of antenatal reflex DNA screening for Down's syndrome"
 - a. Is an economic model essentially comparing primary screen with cfDNA to combined test at threshold 1/800 then cfDNA. Its similar to our model in its gist. The fact that they take both samples at the same time has little effect on the model
 - b. The accuracy of cfDNA is taken from one of the Palomaki papers (included in our review)
 - c. The detection rate of 91% they refer to is the sensitivity of combined test at 1/800 followed by cfDNA, the 0.025% false positive rate they refer to is 1-specificity of combined test at 1/800 followed by cfDNA. Both are results of their economic model based on cfDNA accuracy from Palomaki

Work being undertaken to develop implementation plans

A group has been established to look at some of the issues related to the practicalities of implementing cfDNA in the fetal anomaly screening programme. The activity of this group is addressing a number of the points raised in the consultation responses. The work programme of this group and the areas being addressed are as follows:

Pathway and clinical issues:

- Pathway issues, including QUAD pathway, criteria for referral to fetal medicine following screening tests, pathway following test failures, multiple pregnancies
- Test characteristics and laboratory specifications (to feed in to procurement)
- Implications for clinical and laboratory changes

Data collection and monitoring:

- Standards - consider setting standards for the following:
 - o Coverage (cannot measure offer so cannot measure uptake at present)
 - o Turnaround times – DNA laboratories
 - o Throughput – link to lab specification
 - o Failure rate- related to gestational age- need to be clear about what failure rate we are meaning and there is more than one point where failure can occur.
- Data items and sources
 - o Number of women screened and gestational age
 - T21
 - T18/T13
 - o Number of high risk women choosing further investigation
 - cfDNA with gestational age
 - invasive testing with gestational age
 - no further testing (how do we ensure outcome on all?) Need to make links with NIPE SMART
 - o Outcome of
 - each test
 - each pregnancy
- Consider IT requirements

Patient and public information and training requirements

- Training requirements
- Patient and public information (including PPV communication)
- Communication requirements

Finance and procurement

- Procurement options and processes
- Commissioning and contracting
- Finance
- Implementation plans and impact

Pathways

Two possible pathways have been developed, the recommended pathway is pathway a, for the following reasons;

- a. A focus group held with women highlighted the importance of choice in the pathway and the importance of taking into consideration the individual circumstances of someone within the pathway, particularly regarding gestational age, risk score in primary screen, and the woman's own values and decisions.
- b. A further piece of work conducted by Warwick University completed a literature review highlighting choices that women make regarding NIPT and IPD, and women's views around NIPT. This has shown a range of uptake choices between NIPT and IPD and highlight that this is difficult to predict (appendix F)
- c. A further economic analysis conducted by Warwick University suggests that offering women the choice of NIPT or IPD does not have a large impact on the cost of implementation, at varying levels of uptake of NIPT or IPD following combined test (appendix F).

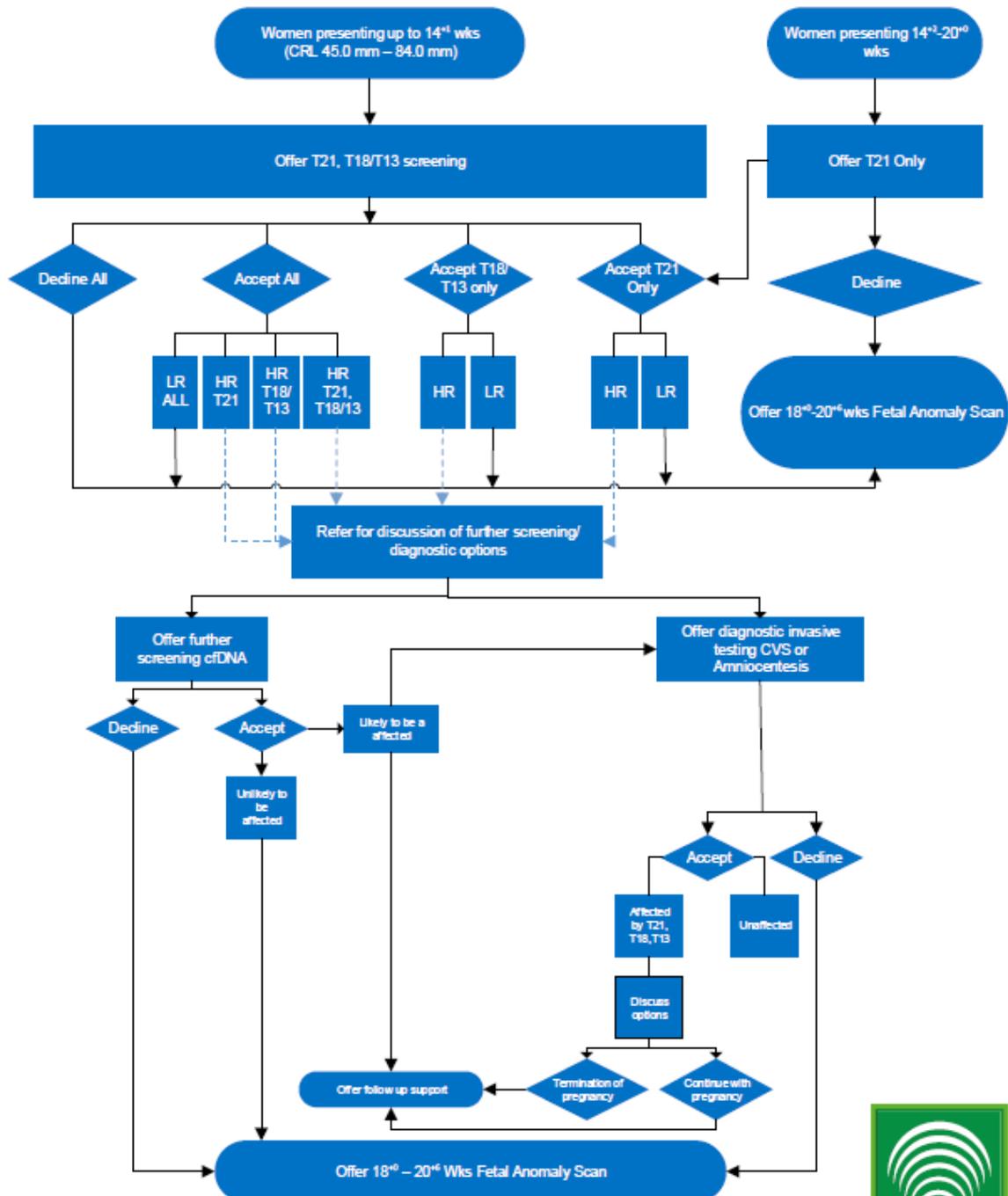
Down's (T21), Edwards' (T18) and Patau's (T13)
For Singleton Pregnancies Only



Screening Programmes

Abbreviations:
LR = Low Risk
HR = High Risk

Fetal Anomaly

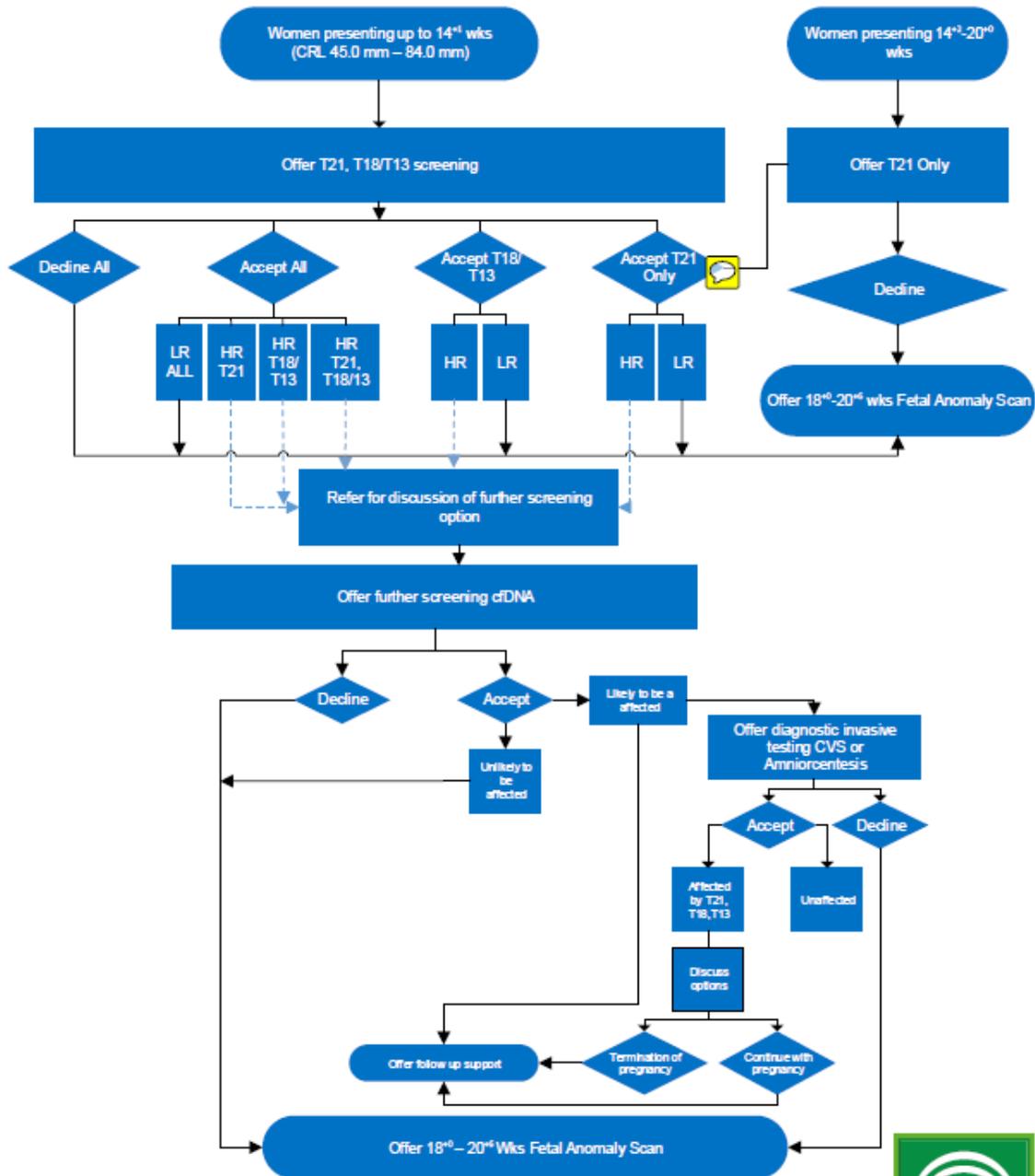


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Screening Programmes

Fetal Anomaly

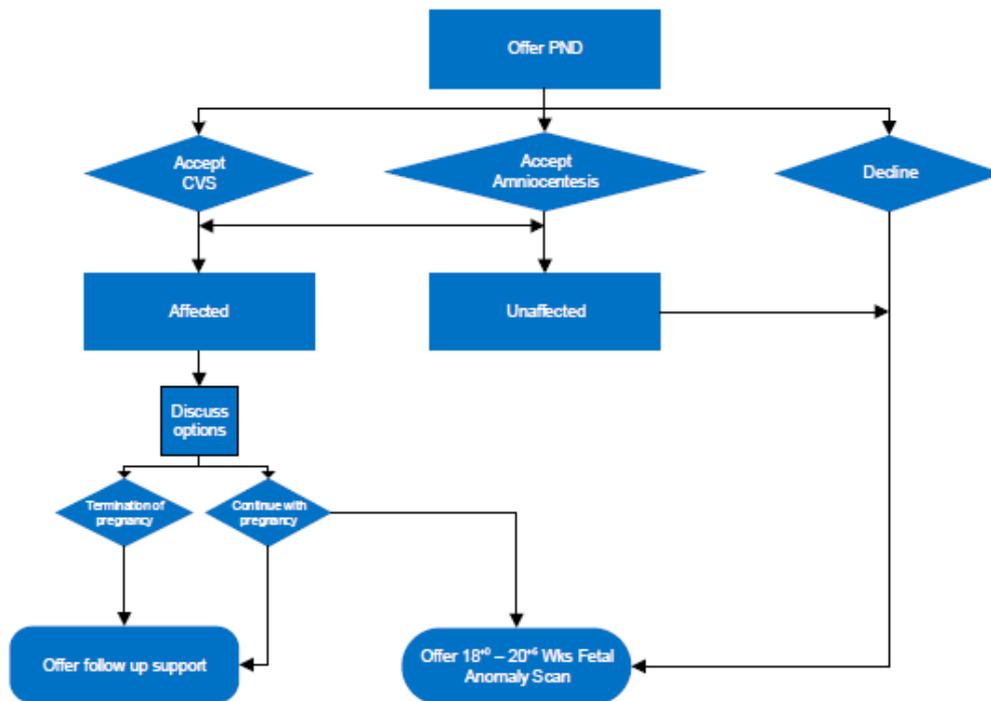


**Diagnostic Testing Pathway Following a Screen Positive Result
For Down's (T21), Edwards' (T18) and Patau's (T13)**



Screening Programmes

Fetal Anomaly



Addition to systematic review and economic model reviewing women's choices between NIPT and IPD

See separate attachment

