Consultation for cell-free DNA testing in the first trimester in the Fetal Anomaly Screening Programme

1. Introduction

Cell-free DNA (cfDNA) in a pregnant woman’s blood can be used as a screening test for trisomies. The cfDNA can be used to detect Down’s syndrome (trisomy 21), Edwards’ syndrome (trisomy 18) or Patau’s syndrome (trisomy 13).

During pregnancy a small amount of the baby’s DNA can be found in the mother’s blood, but the majority of the cfDNA in the blood comes from the mother herself. If the baby has trisomy 21 there will be slightly more cfDNA from chromosome 21 than expected in the mother’s blood. Similarly, if the baby has trisomy 18 or trisomy 13 there will be slightly more cfDNA from chromosome 18 or 13 respectively. Thus analysis of this cfDNA can be used as a screening test for these trisomies.

The fetal anomaly screening programme (FASP) in England currently offers all pregnant women a screening test to discover the chance that their baby has a trisomy. Women are offered blood and ultrasound tests, which combined together, give an indication of the chance that her baby has a trisomy. Women can opt to be screened for Down’s syndrome, Edwards’ syndrome and Patau’s syndrome.

Women who choose to have screening and who are found to have a risk of 1 in 150 or more of having a baby with a trisomy are offered an invasive test (amniocentesis or chorionic villus sampling) to provide a diagnosis. Invasive testing carries a 0.5-1 in 100 risk of causing of miscarriage. By setting the risk level in combined testing at 1 in 150, most women offered invasive testing will find their baby does not have a trisomy. Therefore there is a risk that women with an unaffected baby may miscarry following an invasive procedure.

By offering cfDNA testing to women who are identified as having a risk of 1 in 150 or more, women have the opportunity of a safe test that will provide a much better estimate of the chance that her baby has a trisomy. This means that many women will be able to avoid an invasive test.

2. UKNSC recommendation

The UK national screening committee (UKNSC) commissioned a full review of the published scientific and cost evidence (systematic review) relating to cfDNA testing following combined testing. This was presented to the UKNSC in June 2015, along with the results from a pilot study evaluating cfDNA testing for trisomy 21 undertaken in University College London Hospital, Queen’s Hospital Romford, Salisbury District Hospital, and St George’s Hospital (the RAPID study - Reliable Accurate Prenatal non-Invasive Diagnosis).

Based on the evidence from the systematic review and the pilot study, the UKNSC wishes to consult on cfDNA testing in the following circumstances.

1) 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

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

The UKNSC was presented with a number of different ways in which cfDNA testing could be implemented. This included testing at various levels of risk following a combined test result and using cfDNA testing as a first line (primary) screen.

The UKNSC agreed to consult on offering cfDNA testing to women with a 1 in 150 or greater risk of trisomy, based on the following:

• The RAPID study and UKNSC 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 UKNSC 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 UKNSC did not consider that this benefit outweighs the effect on the numbers of invasive tests offered.
• The UKNSC 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 (for further details see the additional questions at the end of the document).

The UKNSC is also consulting on not offering cfDNA testing to all women (primary screen) based on the following:

• The systematic review estimated that offering cfDNA testing as the primary screening test would find approximately 289 more babies with trisomies with 5,711 fewer invasive tests, than the current combined test screening programme in one year. However, the UKNSC were concerned that this represented a large opportunity cost and that these resources might be better used by the NHS.
3. **Summary of the evidence**

**Test accuracy**

The UKNSC systematic review aimed to identify the sensitivity and specificity of cfDNA testing in a variety of risk groups. Although the findings in different risk groups were limited, the review was able to report a pooled sensitivity and specificity from 41 studies.

| Table 1. UKNSC review pooled sensitivity and specificity of cfDNA (taken from 41 included studies) |
|----------------------------------|---|---|---|
| **Sensitivity** | **T21** | **T18** | **T13** |
| Sensitivity | 99% (99-100%) | 97% (96-98%) | 97% (86-100%) |
| Specificity | 99.9% (99.9-100%) | 99.9% (99.9-100%) | 99.9% (99.9-100%) |

When these test accuracy values are applied to a high risk population the positive predictive values are 91%, 84% and 87% for T21, T18 and T13 respectively. This means that if 100 women were given a positive result (ie that their baby had Down’s syndrome) 91 would actually have Down's syndrome.

The systematic review estimated how well cfDNA testing would work if it was used in a high risk population of 10,000 pregnancies where 3.3% of fetuses have Down’s syndrome, 1.5% have Edwards’ syndrome and 0.5% have Patau’s syndrome. It predicted that there would be 324 cases of Down’s syndrome detected, with 9 missed and 31 false positive results, 140 cases of Edwards’ syndrome detected with 11 missed and 26 false positive results, and 47 cases of Edwards’ syndrome detected, with 3 missed and 7 false positive results.

Without the addition of cfDNA testing, women with a risk of 1 in 150 or more have between 5% and 10% chance of having an affected pregnancy (i.e. 5 to 10 in 100 women).

This suggests that cfDNA is a more accurate test and would significantly reduce the number of women who receive a false positive result.

The systematic review was subjected to a number of sensitivity analyses that aimed to explore uncertainties that might have a significant effect on the accuracy of the test. It found that having a multiple pregnancy made the test less accurate. A tool was used to look at the quality of evidence included and it was found that a number of the studies in the review may have reported outcomes that are more favourable than that would be seen in practice.

Finally, the systematic review found that the test was not diagnostic. Although a cfDNA test may be able to provide a very strong indication of trisomy there is still a small risk that the finding of the test may be incorrect. This is particularly important in T18 and T13, where the test performance is not as good. Invasive tests should be retained and offered to women who opt for a definitive diagnosis.

**Economic analysis**

Both the systematic review and RAPID study produced an economic model to test the outcomes and costs of a screening programme in which cfDNA testing is offered to women identified as high risk through combined testing (table 2).

In the systematic review model, at a threshold of 1 in 150 following combined testing, the model predicts that 9,912 cfDNA tests would be carried out and 350 retests would be carried
out. The number of invasive tests would fall from 7,910 to 1,434. The number of test-related miscarriages, the majority of which would be trisomy-free pregnancies, fell from 46 to 3 per year. Using an estimated cost for each cfDNA test of £232, the addition of the test into the pathway should not add a significant extra cost to the programme. However, there is some uncertainty regarding the actual cost of the test, and this was subject to sensitivity analysis in the review. The actual cost of implementation will be influenced by the cost of the test at the time of implementation.

At lower risk thresholds, the number of trisomies detected does not increase greatly and the number of test related miscarriages does not decrease greatly. However, the total cost and cost per trisomy detected does start to increase substantially.

### Table 2 - Predictions for annual FASP performance in England and Wales, for various options of cfDNA cut off

<table>
<thead>
<tr>
<th></th>
<th>Combined test alone</th>
<th>cfDNA testing if combined test result &gt;1/150</th>
<th>cfDNA testing alone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of combined tests performed</strong></td>
<td>448676</td>
<td>448676</td>
<td>0</td>
</tr>
<tr>
<td><strong>Number of initial cfDNA tests performed</strong></td>
<td>0</td>
<td>9912 (9513,10300)</td>
<td>448676</td>
</tr>
<tr>
<td><strong>Number of cfDNA test failures</strong></td>
<td>0</td>
<td>385.3 (300.3,503.1)</td>
<td>13410 (12240,14610)</td>
</tr>
<tr>
<td><strong>Number of cfDNA retests performed</strong></td>
<td>0</td>
<td>349.7 (271,458.3)</td>
<td>12160 (11040,13330)</td>
</tr>
<tr>
<td><strong>Number of amniocenteses performed</strong></td>
<td>3781 (3683,3879)</td>
<td>698.7 (466.2,896)</td>
<td>1051 (677.5,1319)</td>
</tr>
<tr>
<td><strong>Number of CVS performed</strong></td>
<td>4129 (4022,4236)</td>
<td>763 (509.1,978.5)</td>
<td>1148 (739.8,1441)</td>
</tr>
<tr>
<td><strong>T18 cases detected by testing</strong></td>
<td>196.4 (180.0,213.6)</td>
<td>197.2 (117.3,247)</td>
<td>250.3 (144.5,312.2)</td>
</tr>
<tr>
<td><strong>T13 cases detected by testing</strong></td>
<td>73.7 (64.4,83.5)</td>
<td>74.45 (42.71,98.36)</td>
<td>109 (61.33,141)</td>
</tr>
<tr>
<td><strong>T21 cases detected by testing</strong></td>
<td>763.9 (730.4,797.7)</td>
<td>784.1 (466.2,954.7)</td>
<td>961.9 (556.6,1173)</td>
</tr>
<tr>
<td><strong>Total trisomies detected</strong></td>
<td>1031.65 (964.08,1102.51)</td>
<td>1055.75 (626.21,1300.06)</td>
<td>1321.2 (762.43,1626.2)</td>
</tr>
<tr>
<td><strong>Test-related miscarriage of healthy pregnancy</strong></td>
<td>46.05 (29.98,68.85)</td>
<td>2.717 (1.135,5.614)</td>
<td>5.884 (3.116,10.3)</td>
</tr>
<tr>
<td><strong>Total cost (£m)</strong></td>
<td>14.93 (14.86,15)</td>
<td>15.06 (14.87,15.22)</td>
<td>107.7 (107.3,108)</td>
</tr>
<tr>
<td><strong>Cost per trisomy detected through testing (£/trisomy)</strong></td>
<td>14472 (13605,15414)</td>
<td>14265 (11707,23746)</td>
<td>81517 (66412,140734)</td>
</tr>
</tbody>
</table>

An economic evaluation was also completed as part of the RAPID study, in which both current screening programme data and data from the study were used to assess the costs of implementing cfDNA testing at different risk thresholds. In this study, the outcome was identification of T21 only (not T18 or T13). This analysis estimated that at a risk threshold of 1 in 150, offering cfDNA would be cost neutral.

**Summary**
The UKNSC report suggests that using a risk of 1 in 150 it is predicted that the number of invasive tests would fall from 7,910 to 1,434, and the number of test-related miscarriages would fall from 46 to 3 per year. At this threshold, the additional costs to the screening programme are minimal and potentially “cost neutral”.

4. More questions

The UKNSC noted that there are a number of areas of uncertainty that should be addressed during implementation to allow development and improvement of a programme and information to women.

Failure rate

The rate of cfDNA test failure was assessed as part of the systematic review. Most studies reported there was a degree of test failure, ranging from 0% to 12.7%.

There is some evidence that the rate of test failure is higher when the test is done earlier in pregnancy, if the woman has a high body mass index, and in pregnancies with babies with trisomies. However this was not consistently the case in studies included in the systematic review.

If cfDNA testing is to be offered, failure rates should be closely monitored to gain a better understanding of rates in the screening programme setting.

Uptake

From the work undertaken to date, there is not universal agreement regarding the impact introducing cfDNA within the screening pathway on the following:

- The number of women choosing to accept screening
- The number of women choosing to have further investigation or invasive testing following a combined test result of 1 in 150 or greater

The systematic review used two cfDNA test uptake rates to explore the choices women make after the combined test. In the table below is an extract from that analysis which shows the difference between the number of test-related miscarriages when fewer women choose to have a cfDNA test before an invasive test.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Women opting for cfDNA (%)</th>
<th>Women opting for invasive tests (%)</th>
<th>Women who have no further tests (%)</th>
<th>Test-related miscarriages of healthy pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analysis 1 – using data from Lewis et al, 2014</td>
<td>90.7</td>
<td>2.9</td>
<td>6.4</td>
<td>2.8</td>
</tr>
<tr>
<td>Analysis 2 – using data from Gil et al, 2015</td>
<td>57</td>
<td>40</td>
<td>3</td>
<td>30.6</td>
</tr>
</tbody>
</table>

It is clear that the uptake rate of cfDNA affects the benefits offered from its implementation. If implemented, the impact on uptake rates should also be monitored to better understand this issue and the likely implications on the number of invasive tests offered.

Test accuracy in T18 and T13
It was noted that the literature for T18 and T13 is less detailed than for T21; partly because they are much rarer conditions and so the true performance of cfDNA is less certain in these trisomies.

The systematic review showed that the accuracy for T18 and T13 is poorer than that for the T21.

The UKNSC is consulting on offering cfDNA for all three trisomies because literature and models suggest that, even at lower test accuracy estimates for T18 and T13, cfDNA testing offers a substantial benefit to women.

The implementation of cfDNA in the UK should pay particular attention to T18 and T13 to ascertain the performance of the test for these trisomies, in particular any way the cfDNA test can be integrated with the combined test to improve the accuracy of the entire screening test.

**Turnaround time**

It is expected that the turn-around time for the cfDNA test should be a maximum of 10 days. The RAPID pilot data and experience has shown that operationally this is achievable within a NHS laboratory. This will be monitored as the pathway is implemented.