Analysis of Cell-Free DNA in Maternal Blood in Screening for Aneuploidies: Meta-Analysis

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Key Words
Cell-free fetal DNA · Non-invasive prenatal testing · Trisomy 21 · Trisomy 18 · Trisomy 13 · Turner syndrome · Fetal aneuploidy

Abstract
Objective: To review clinical validation or implementation studies of maternal blood cell-free (cf) DNA analysis in screening for aneuploidies and to explore the potential use of this method in clinical practice. Methods: Searches of PubMed and MEDLINE were performed to identify all peer-reviewed articles on cfDNA testing in screening for aneuploidies between 2011, when the first such study was published, and 20 December 2013. Results: Weighted pooled detection rates (DR) and false-positive rates (FPR) in singleton pregnancies were 99.0% (95% CI 98.2–99.6) and 0.08% (95% CI 0.03–0.14), respectively, for trisomy 21; 96.8% (95% CI 94.5–98.4) and 0.15% (95% CI 0.08–0.25) for trisomy 18; 92.1% (95% CI 85.9–96.7) and 0.20% (95% CI 0.04–0.46) for trisomy 13; 88.6% (95% CI 83.0–93.1) and 0.12% (95% CI 0.05–0.24) for monosomy X, and 93.8% (95% CI 85.9–98.7) and 0.12% (95% CI 0.02–0.28) for sex chromosome aneuploidies other than monosomy X. For twin pregnancies, the DR was 94.4% (95% 74.2–99.0) and the FPR was 0% (95% CI 0.00–1.84) for trisomy 21. Conclusion: An analysis of cfDNA in maternal blood provides effective screening for trisomies.
creased maternal age, increased fetal NT and decreased serum PAPP-A, screening using the algorithm for trisomy 21 can detect about 90% of trisomy 21 cases and 70–75% of cases of trisomies 18 and 13 at an FPR of 4–5% [5, 6]. However, with the use of specific algorithms for each trisomy, which incorporate not only their similarities but also their differences in biomarker pattern, including high serum-free β-hCG in trisomy 21 and low levels in trisomies 18 and 13 and high fetal heart rate in trisomy 13, it is possible to increase the DR of trisomies 18 and 13 to about 95% at the same overall FPR of about 4–5% [5, 6].

In addition to trisomies 21, 18 and 13, invasive testing in the screen-positive group from the combined test detects many other clinically significant aneuploidies [7]. However, the biomarker profile for many of the rare aneuploidies and microdeletion/duplication syndromes is not clearly defined and it is uncertain whether their incidence in the screen-positive group for trisomy 21 is higher than in the screen-negative group. The only exceptions are monosomy X, presenting with very high fetal NT, and triploidy, presenting with either very high serum-free β-hCG and high NT or very low serum-free β-hCG and PAPP-A [2, 8–10].

In some countries, such as the UK, there is a national program of screening for trisomy 21 based on the combined test and the offer of invasive testing at a risk cut-off which aims to maintain the FPR at 3% or less [11]. In many countries there are no national guidelines on screening and individual practitioners offer a variety of first- and/or second-trimester methods often driven by market forces and the rules of supply and demand. In some parts of Europe the rate of invasive testing is in excess of 20% [12].

Screening for Aneuploidies by Analysis of Cell-Free DNA in Maternal Blood

Several studies in the last 3 years have reported the clinical validation and/or implementation of analysing cell-free (cf) DNA in maternal blood in screening for trisomies 21, 18 and 13 and in a few cases screening for sex chromosome aneuploidies and triploidy [13–45].

The studies used one of three methods for analysis of cfDNA in maternal blood: massively parallel sequencing (MPSS) [46, 47], chromosome-selective sequence analysis (CSS) [26] and single nucleotide polymorphism-based analysis (SNP) [27, 48]. Other methods of examining fetoplacental nucleic acids in maternal blood have been investigated, but these have not yet been implemented in clinical practice [49–56].

Objectives

This study reviews the findings of the clinical validation or implementation studies of maternal blood cfDNA testing in screening for aneuploidies and explores the potential use of this method in clinical practice.

Systematic Review and Meta-Analysis

Literature Search and Study Selection

Searches of MEDLINE, EMBASE and The Cochrane Library were performed without language restriction to identify all peer-reviewed articles published on clinical validation or implementation of maternal cfDNA testing in screening for aneuploidies. The search period was limited to between January 2011, when the first such study was published [13], and 20 December 2013. The list of relevant citations was generated from these databases using the following: maternal blood cfDNA, noninvasive prenatal diagnosis or non-invasive prenatal diagnosis.

The abstracts of citations were examined by two reviewers (M.M.G. and R.A.) to identify all potentially relevant articles which were then examined in full text. Reference lists of relevant original and review articles were searched for additional reports. Agreement about potential relevance was reached by consensus and by consultation with the third reviewer (K.H.N.). The inclusion criteria were studies reporting on clinical validation or implementation of maternal cfDNA testing in screening for aneuploidies, in which the laboratory scientists carrying out the tests were not aware of the fetal karyotype or pregnancy outcome.

Data Extraction and Construction of Contingency Tables

Data regarding sample size, gestational age at analysis, method used for cfDNA testing and DR and FPR for non-mosaic trisomies 21, 18, 13 and sex chromosome aneuploidies were obtained from each study included in the systematic review and documented in contingency tables. In the calculation of FPR we included all euploid and aneuploid cases other than the aneuploidy under investigation. In the table where there was a zero in any cell, Hal-dane correction was used, which added 0.5 to each count in the table to allow for estimation of variance and pooled effects. The DR and FPR for each study and weighted pooled data are provided in tables 1–5 and illustrated in figures 1–4.
Table 1. Studies reporting on the application of cfDNA analysis of maternal blood in screening for trisomy 21

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>Gestational age (range), weeks</th>
<th>Trisomy 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total, n</td>
<td>Detection, n</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Non-trisomy 21</td>
<td></td>
<td>Total, n</td>
<td>False positive, n</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chiu et al. [14], 2011</td>
<td>MPSS</td>
<td>13 (–)</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>86 (100.0, 95.8–100.0)</td>
<td>146</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 (2.05, 0.43–5.89)</td>
</tr>
<tr>
<td>Ehrlich et al. [15], 2011</td>
<td>MPSS</td>
<td>16 (8–36)</td>
<td>39</td>
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<tr>
<td></td>
<td></td>
<td>39 (100.0, 91.0–100.0)</td>
<td>410</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>1 (0.24, 0.01–1.35)</td>
</tr>
<tr>
<td>Palomaki et al. [16], 2011</td>
<td>MPSS</td>
<td>15 (8–21)</td>
<td>212</td>
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<tr>
<td></td>
<td></td>
<td>209 (98.6, 95.9–99.7)</td>
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<td>MPSS</td>
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<td>13 (100.0, 75.3–100.0)</td>
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<td>0 (0.00, 0.00–10.28)</td>
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<tr>
<td>Ashoor et al. [18], 2012</td>
<td>CSS</td>
<td>12 (11–13)</td>
<td>50</td>
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<tr>
<td></td>
<td></td>
<td>50 (100.0, 92.9–100.0)</td>
<td>347</td>
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<tr>
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<td>0 (0.00, 0.00–1.06)</td>
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<td>Bianchi et al. [19], 2012</td>
<td>MPSS</td>
<td>15 (10–23)</td>
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<td>0 (0.00, 0.00–0.91)</td>
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<td>Jiang et al. [21], 2012</td>
<td>MPSS</td>
<td>– (10–34)</td>
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<tr>
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<td>16 (100.0, 79.4–100.0)</td>
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<td>0 (0.00, 0.00–0.42)</td>
</tr>
<tr>
<td>Lau et al. [22], 2012</td>
<td>MPSS</td>
<td>12 (11–28)</td>
<td>11</td>
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<tr>
<td></td>
<td></td>
<td>11 (100.0, 71.5–100.0)</td>
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<td>0 (0.00, 0.00–0.373)</td>
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<td>12 (11–13)</td>
<td>8</td>
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<td></td>
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<td>8 (100.0, 63.1–100.0)</td>
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<td>0 (0.00, 0.00–0.19)</td>
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<td>Norton et al. [24], 2012</td>
<td>CSS</td>
<td>14 (10–38)</td>
<td>81</td>
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<td>81 (100.0, 95.6–100.0)</td>
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<td>1 (0.04, 8.7e–04–0.19)</td>
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<td>Sparks et al. [26], 2012</td>
<td>CSS</td>
<td>18 (11–36)</td>
<td>36</td>
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<td>36 (100.0, 90.3–100.0)</td>
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<tr>
<td>Zimmerman et al. [27], 2012</td>
<td>SNP</td>
<td>17 (9–36)</td>
<td>11</td>
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<td></td>
<td>11 (100.0, 71.5–100.0)</td>
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<td>0 (0.00, 0.00–2.72)</td>
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<td>Guex et al. [30], 2013</td>
<td>MPSS</td>
<td>12 (11–13)</td>
<td>30</td>
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<td>30 (100.0, 88.4–100.0)</td>
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<td>0 (0.00, 0.00–2.50)</td>
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<td>Liang et al. [32], 2013</td>
<td>MPSS</td>
<td>21 (11–39)</td>
<td>37</td>
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<td></td>
<td>37 (100.0, 90.5–100.0)</td>
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</tr>
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<td>Nicolaides et al. [34], 2013</td>
<td>SNP</td>
<td>13 (11–13)</td>
<td>25</td>
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<tr>
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<td></td>
<td>25 (100.0, 86.3–100.0)</td>
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<td>0 (0.00, 0.00–1.79)</td>
</tr>
<tr>
<td>Song et al. [37], 2013</td>
<td>MPSS</td>
<td>16 (11–21)</td>
<td>8</td>
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<tr>
<td></td>
<td></td>
<td>8 (100.0, 63.1–100.0)</td>
<td>1,733</td>
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<td>0 (0.00, 0.00–0.21)</td>
</tr>
<tr>
<td>Stumm et al. [38], 2013</td>
<td>MPSS</td>
<td>15 (11–32)</td>
<td>39</td>
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<tr>
<td></td>
<td></td>
<td>38 (97.4, 86.5–99.9)</td>
<td>430</td>
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<td>0 (0.00, 0.00–0.85)</td>
</tr>
<tr>
<td>Verweij et al. [39], 2013</td>
<td>CSS</td>
<td>14 (10–28)</td>
<td>18</td>
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<td></td>
<td>17 (94.4, 72.7–99.9)</td>
<td>502</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>0 (0.00, 0.00–0.73)</td>
</tr>
</tbody>
</table>

Pooled analysis, %

<table>
<thead>
<tr>
<th></th>
<th>Fixed-effects model</th>
<th>Random-effects model</th>
<th>Cochrane’s Q</th>
<th>I² statistic, %</th>
<th>Egger bias</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>99.0 (98.2–99.6)</td>
<td>99.0 (98.2–99.6)</td>
<td>7.747 (0.9717)</td>
<td>0 (0–43.7)</td>
<td>–0.02745 (0.8957)</td>
</tr>
<tr>
<td></td>
<td>0.07 (0.03–0.12)</td>
<td>0.08 (0.03–0.14)</td>
<td>19.337 (0.3095)</td>
<td>12.1 (0.0–50.3)</td>
<td>0.2299 (0.1831)</td>
</tr>
</tbody>
</table>
| Detection and false-positive values include percentages and 95% CI in parentheses. Pooled analysis and I² statistic include 95% CI in parentheses. Cochrane’s Q and Egger bias include p values in parentheses.

Quality Assessment

Quality and integrity of this review and meta-analysis were validated with PRISMA (preferred reporting items for systematic reviews and meta-analyses), which focuses on randomized trials, but it can also be used as a basis for reporting systematic reviews of other types of research [57].

The methodological quality of the selected studies, in terms of risk of bias and applicability, was evaluated by three assessors (M.M.G., R.A. and K.H.N.) using the quality assessment tool for diagnostic accuracy studies (QUADAS-2) [58].

Meta-Analysis of Data from All Studies

A meta-analysis of extracted data was carried out in two steps: firstly, summary statistics with 95% confidence intervals (CIs) were derived for each study and secondly, individual study statistics were combined to obtain a pooled summary estimate which was calculated as a weighted average of the individual study estimates. The pooled summary statistics were estimated using both the fixed-effects (inverse variance) and the random-effects model (DerSimonian-Laird). The fixed-effects model weighs each study by the inverse of its variance and only considers variability in results within studies and not between studies. The random-effects model allows for between-study variability in results by weighting studies using a combination of their own variance and the between-study variance. Random-effects models are generally preferable as they provide a conservative estimate of pooled statistics with wider CIs [59].

Assessment of Heterogeneity between Studies

The heterogeneity between studies was estimated using Cochrane’s Q, which is calculated as the weighted sum of squared differences between individual study effects and the pooled effect across studies, with the weights being those used in the pooling method. Heterogeneity
Table 2. Studies reporting on the application of cfDNA analysis of maternal blood in screening for trisomy 18

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>Gestational age (range), weeks</th>
<th>Trisomy 18 total, n detection, n</th>
<th>Non-trisomy 18 total, n false positive, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen et al. [13], 2011</td>
<td>MPSS</td>
<td>–</td>
<td>37 34 (91.9, 78.1–98.3)</td>
<td>252 5 (1.98, 0.65–4.57)</td>
</tr>
<tr>
<td>Sehnert et al. [17], 2011</td>
<td>MPSS</td>
<td>15 (10–28)</td>
<td>8 8 (100.0, 63.1–100.0)</td>
<td>39 0 (0.00, 0.00–9.03)</td>
</tr>
<tr>
<td>Ashoor et al. [18], 2012</td>
<td>CSS</td>
<td>12 (11–13)</td>
<td>50 49 (98.0, 89.4–99.9)</td>
<td>347 0 (0.00, 0.00–1.06)</td>
</tr>
<tr>
<td>Bianchi et al. [19], 2012</td>
<td>MPSS</td>
<td>15 (10–23)</td>
<td>36 35 (97.2, 85.5–99.9)</td>
<td>460 0 (0.00, 0.00–0.80)</td>
</tr>
<tr>
<td>Jiang et al. [21], 2012</td>
<td>MPSS</td>
<td>– (10–34)</td>
<td>12 10 (100.0, 73.5–100.0)</td>
<td>891 1 (0.11, 2.8e–03–0.62)</td>
</tr>
<tr>
<td>Lau et al. [22], 2012</td>
<td>MPSS</td>
<td>12 (11–28)</td>
<td>10 10 (100.0, 69.2–100.0)</td>
<td>98 0 (0.00, 0.00–3.69)</td>
</tr>
<tr>
<td>Nicolaides et al. [23], 2012</td>
<td>CSS</td>
<td>12 (11–13)</td>
<td>2 2 (100.0, 15.8–100.0)</td>
<td>1,947 2 (0.10, 0.01–0.37)</td>
</tr>
<tr>
<td>Norton et al. [24], 2012</td>
<td>CSS</td>
<td>16 (10–38)</td>
<td>38 37 (97.4, 86.2–99.9)</td>
<td>2,888 2 (0.07, 8.4e–03–0.25)</td>
</tr>
<tr>
<td>Palomaki et al. [25], 2012</td>
<td>MPSS</td>
<td>14 (9–22)</td>
<td>59 59 (100.0, 93.9–100.0)</td>
<td>1,912 5 (0.26, 0.09–0.61)</td>
</tr>
<tr>
<td>Sparks et al. [26], 2012</td>
<td>CSS</td>
<td>18 (11–36)</td>
<td>8 8 (100.0, 63.1–100.0)</td>
<td>159 0 (0.00, 0.00–2.29)</td>
</tr>
<tr>
<td>Zimmerman et al. [27], 2012</td>
<td>SNP</td>
<td>17 (9–36)</td>
<td>3 3 (100.0, 29.2–100.0)</td>
<td>142 0 (0.00, 0.00–2.56)</td>
</tr>
<tr>
<td>Guex et al. [30], 2013</td>
<td>MPSS</td>
<td>12 (11–13)</td>
<td>20 19 (95.0, 75.1–99.9)</td>
<td>156 0 (0.00, 0.00–2.34)</td>
</tr>
<tr>
<td>Liang et al. [32], 2013</td>
<td>MPSS</td>
<td>21 (11–39)</td>
<td>13 13 (100.0, 75.3–100.0)</td>
<td>391 0 (0.00, 0.00–0.94)</td>
</tr>
<tr>
<td>Nicolaides et al. [34], 2013</td>
<td>SNP</td>
<td>13 (11–13)</td>
<td>3 3 (100.0, 29.2–100.0)</td>
<td>226 0 (0.00, 0.00–1.62)</td>
</tr>
<tr>
<td>Song et al. [37], 2012</td>
<td>MPSS</td>
<td>16 (11–21)</td>
<td>2 2 (100.0, 15.8–100.0)</td>
<td>1,738 1 (0.06, 1.5e–03–0.32)</td>
</tr>
</tbody>
</table>

Pooled analysis, %
- Fixed-effects model: 96.8 (94.5–98.4)
- Random-effects model: 96.8 (94.5–98.4)
- Cochrane’s Q: 7.2471 (0.9248)
- I² statistic, %: 0 (0 – 46.4)
- Egger bias: –0.1668 (0.4172)

Detection and false-positive values include percentages and 95% CI in parentheses. Pooled analysis and I² statistic include 95% CI in parentheses. Cochrane’s Q and Egger bias include p values in parentheses.

Table 3. Studies reporting on the application of cfDNA analysis of maternal blood in screening for trisomy 13

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>Gestational age, weeks</th>
<th>Trisomy 13 total, n detection, n</th>
<th>Non-trisomy 13 total, n false positive, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen et al. [13], 2011</td>
<td>MPSS</td>
<td>–</td>
<td>25 25 (100.0, 86.3–100.0)</td>
<td>264 3 (1.14, 0.24–3.28)</td>
</tr>
<tr>
<td>Bianchi et al. [19], 2012</td>
<td>MPSS</td>
<td>15 (10–23)</td>
<td>14 11 (78.6, 49.2–95.3)</td>
<td>485 0 (0.00, 0.00–0.76)</td>
</tr>
<tr>
<td>Jiang et al. [21], 2012</td>
<td>MPSS</td>
<td>– (10–34)</td>
<td>2 2 (100.0, 15.8–100.0)</td>
<td>901 0 (0.00, 0.00–0.41)</td>
</tr>
<tr>
<td>Lau et al. [22], 2012</td>
<td>MPSS</td>
<td>12 (11–28)</td>
<td>2 2 (100.0, 15.8–100.0)</td>
<td>106 0 (0.00, 0.00–3.42)</td>
</tr>
<tr>
<td>Palomaki et al. [25], 2012</td>
<td>MPSS</td>
<td>14 (9–22)</td>
<td>12 11 (91.7, 61.5–99.8)</td>
<td>1,959 16 (0.82, 0.47–1.32)</td>
</tr>
<tr>
<td>Zimmerman et al. [27], 2012</td>
<td>SNP</td>
<td>17 (9–36)</td>
<td>2 2 (100.0, 15.8–100.0)</td>
<td>143 0 (0.00, 0.00–2.55)</td>
</tr>
<tr>
<td>Ashoor et al. [28], 2013</td>
<td>CSS</td>
<td>13 (11–26)</td>
<td>10 8 (80.0, 44.4–97.5)</td>
<td>1,949 1 (0.05, 1.3e−03–0.29)</td>
</tr>
<tr>
<td>Guex et al. [30], 2013</td>
<td>MPSS</td>
<td>12 (11–13)</td>
<td>13 13 (100.0, 75.3–100.0)</td>
<td>163 0 (0.00, 0.00–2.24)</td>
</tr>
<tr>
<td>Liang et al. [32], 2013</td>
<td>MPSS</td>
<td>21 (11–39)</td>
<td>3 3 (100.0, 29.2–100.0)</td>
<td>401 1 (0.25, 6.3e−03–1.38)</td>
</tr>
<tr>
<td>Nicolaides et al. [34], 2013</td>
<td>SNP</td>
<td>13 (11–13)</td>
<td>1 1 (100.0, 2.5–100.0)</td>
<td>228 0 (0.00, 0.00–1.61)</td>
</tr>
<tr>
<td>Song et al. [37], 2012</td>
<td>MPSS</td>
<td>16 (11–21)</td>
<td>1 1 (100.0, 2.5–100.0)</td>
<td>1,740 0 (0.00, 0.00–0.21)</td>
</tr>
</tbody>
</table>

Pooled analysis, %
- Fixed-effects model: 92.1 (86.0–96.7)
- Random-effects model: 92.1 (85.9–96.7)
- Cochrane’s Q: 10.0423 (0.4368)
- I² statistic, %: 10.3 (0–51.4)
- Egger bias: –0.4082 (0.2714)

Detection and false-positive values include percentages and 95% CI in parentheses. Pooled analysis and I² statistic include 95% CI in parentheses. Cochrane’s Q and Egger bias include p values in parentheses.
### Table 4. Studies reporting on the application of cfDNA analysis of maternal blood in screening for monosomy X

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>Gestational age (range), weeks</th>
<th>Monosomy X total, n detection, n</th>
<th>Non-monosomy X total, n false positive, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sehnert et al. [17], 2011</td>
<td>MPSS</td>
<td>15 (10–28)</td>
<td>2 (100.0, 15.8–100.0)</td>
<td>45 (0.00, 0.00–7.87)</td>
</tr>
<tr>
<td>Bianchi et al. [19], 2012</td>
<td>MPSS</td>
<td>15 (10–23)</td>
<td>20 (75.0, 50.9–91.3)</td>
<td>462 (0.22, 5.5e–03–1.20)</td>
</tr>
<tr>
<td>Jiang et al. [21], 2012</td>
<td>MPSS</td>
<td>– (10–34)</td>
<td>3 (100.0, 29.2–100.0)</td>
<td>899 (1.11, 2.8e–03–0.62)</td>
</tr>
<tr>
<td>Lau et al. [22], 2012</td>
<td>MPSS</td>
<td>12 (11–28)</td>
<td>8 (100.0, 63.1–100.0)</td>
<td>100 (0.00, 0.00–3.62)</td>
</tr>
<tr>
<td>Zimmerman et al. [27], 2012</td>
<td>SNP</td>
<td>17 (9–36)</td>
<td>1 (100.0, 2.5–100.0)</td>
<td>144 (0.00, 0.00–2.53)</td>
</tr>
<tr>
<td>Guex et al. [30], 2013</td>
<td>MPSS</td>
<td>12 (11–13)</td>
<td>15 (100.0, 78.2–100.0)</td>
<td>161 (0.00, 0.00–2.27)</td>
</tr>
<tr>
<td>Liang et al. [32], 2013</td>
<td>MPSS</td>
<td>12 (11–13)</td>
<td>5 (100.0, 47.8–100.0)</td>
<td>399 (1.25, 6.3e–03–1.39)</td>
</tr>
<tr>
<td>Zimmerman et al. [33], 2013</td>
<td>SNP</td>
<td>– (10–20)</td>
<td>21 (81.0, 58.1–94.6)</td>
<td>390 (0.26, 6.5e–03–1.42)</td>
</tr>
<tr>
<td>Nicolaides et al. [34], 2013</td>
<td>CSS</td>
<td>12 (11–13)</td>
<td>47 (91.5, 79.6–97.6)</td>
<td>116 (0.00, 0.00–3.13)</td>
</tr>
<tr>
<td>Lau et al. [36], 2013</td>
<td>SNP</td>
<td>13 (9–36)</td>
<td>12 (100.0, 61.5–99.8)</td>
<td>175 (0.00, 0.00–2.09)</td>
</tr>
<tr>
<td>Song et al. [37], 2013</td>
<td>MPSS</td>
<td>16 (11–21)</td>
<td>3 (66.7, 9.4–99.2)</td>
<td>1,737 (0.00, 0.00–0.21)</td>
</tr>
</tbody>
</table>

Pooled analysis, %

- Fixed-effects model: 88.6 (83.0–93.1)
- Random-effects model: 88.6 (83.0–93.1)

Cochrane’s Q: 10.9584 (0.4468), I² statistic, %: 0 (0–49.8)

Egger bias: –0.1712 (0.7599)

Detection and false-positive values include percentages and 95% CI in parentheses. Pooled analysis and I² statistic include 95% CI in parentheses. Cochrane’s Q and Egger bias include p values in parentheses.

### Table 5. Studies reporting on the application of cfDNA analysis of maternal blood in screening for sex chromosome abnormalities other than monosomy X

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>Gestational age (range), weeks</th>
<th>47,XXX; 47,XXY; 47,XYY total, n detection, n</th>
<th>Non-SCA total, n false positive, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bianchi et al. [19], 2012</td>
<td>MPSS</td>
<td>15 (10–23)</td>
<td>9 (88.9, 51.8–99.7)</td>
<td>453 (0.00, 0.00–0.81)</td>
</tr>
<tr>
<td>Jiang et al. [21], 2012</td>
<td>MPSS</td>
<td>– (10–34)</td>
<td>3 (100.0, 29.2–100.0)</td>
<td>896 (0.00, 0.00–0.41)</td>
</tr>
<tr>
<td>Lau et al. [22], 2012</td>
<td>MPSS</td>
<td>12 (11–28)</td>
<td>1 (100.0, 2.5–100.0)</td>
<td>99 (0.00, 0.00–3.66)</td>
</tr>
<tr>
<td>Zimmerman et al. [27], 2012</td>
<td>SNP</td>
<td>17 (9–36)</td>
<td>3 (100.0, 29.2–100.0)</td>
<td>141 (0.00, 0.00–2.58)</td>
</tr>
<tr>
<td>Guex et al. [30], 2013</td>
<td>MPSS</td>
<td>12 (11–13)</td>
<td>5 (100.0, 47.8–100.0)</td>
<td>156 (0.00, 0.00–2.34)</td>
</tr>
<tr>
<td>Liang et al. [32], 2013</td>
<td>MPSS</td>
<td>21 (11–39)</td>
<td>3 (100.0, 29.2–100.0)</td>
<td>396 (1.25, 6.3e–03–1.40)</td>
</tr>
<tr>
<td>Mazloom et al. [33], 2013</td>
<td>SNP</td>
<td>– (10–20)</td>
<td>8 (100.0, 63.1–100.0)</td>
<td>382 (0.00, 0.00–0.96)</td>
</tr>
<tr>
<td>Nicolaides et al. [40], 2013</td>
<td>CSS</td>
<td>12 (11–13)</td>
<td>9 (100.0, 66.4–100.0)</td>
<td>107 (0.93, 0.02–5.10)</td>
</tr>
<tr>
<td>Samango-Sprouse et al. [36], 2013</td>
<td>SNP</td>
<td>13 (9–36)</td>
<td>3 (100.0, 29.2–100.0)</td>
<td>172 (0.00, 0.00–2.12)</td>
</tr>
</tbody>
</table>

Pooled analysis, %

- Fixed-effects model: 93.8 (85.9–98.7)
- Random-effects model: 93.8 (85.9–98.7)

Cochrane’s Q: 1.5726 (0.9914), I² statistic, %: 0 (0–54.4)

Egger bias: 0.0253 (0.9408)

Detection and false-positive values include percentages and 95% CI in parentheses. Pooled analysis and I² statistic include 95% CI in parentheses. Cochrane’s Q and Egger bias include p values in parentheses. SCA = Sex chromosome abnormality.
Fig. 1. Forest plots of DR and FPR with 95% CI and weighted pooled summary statistics using the random-effects model in assessing cfDNA analysis in screening for trisomy 21.

Fig. 2. Forest plots of DR and FPR with 95% CI and weighted pooled summary statistics using the random-effects model in assessing cfDNA analysis in screening for trisomy 18.
was also assessed using the $I^2$ statistic, which describes the percentage of variation across studies which is due to heterogeneity rather than chance. $I^2$ values of up to 40% might be unimportant, 30–60% moderate, 50–90% substantial and 75–100% considerable [60].

**Estimation of Bias**
The publication bias in studies included in the review was assessed graphically using funnel plots and by using Egger’s bias, which assesses the asymmetry of the funnel plot [61].

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**Fig. 3.** Forest plots of DR and FPR with 95% CI and weighted pooled summary statistics using the random-effects model in assessing cfDNA analysis in screening for trisomy 13.

**Fig. 4.** Forest plots of DR and FPR with 95% CI and weighted pooled summary statistics using the random-effects model in assessing cfDNA analysis in screening for monosomy X.
The statistical software package StatsDirect version 2.7.9 (StatsDirect Ltd., Cheshire, UK) was used for data analysis.

Results

Data Sources

The search identified 834 potentially relevant citations but 198 were excluded because they were not peer-reviewed papers but conference abstracts, and 562 papers were excluded based on title or abstract. In the remaining 74 citations the full manuscripts were assessed and 41 were excluded because they were review articles, opinions or studies that reported laboratory techniques without providing data on clinical validation of maternal blood cfDNA analysis.

In total, 33 relevant studies were identified and then divided into two groups. The first group of studies was used for the meta-analysis on the performance of screening by cfDNA testing in screening for aneuploidies [13–40]. These studies reported cfDNA results in relation to fetal karyotype from invasive testing or clinical outcome; 26 studies examined prospectively collected blood or stored plasma samples obtained before invasive diagnostic testing in patients identified by conventional methods of screening as being at high-risk for aneuploidies. Only two studies reported on findings in a general population [23, 37].

The second group of studies also reported on the clinical implementation of cfDNA testing in screening for aneuploidies, but in a high proportion of the included cases there was no pregnancy outcome and they were therefore not used for assessment of the performance of the test [41–45].

Quality of Studies, Pooled Outcome Measures, Heterogeneity and Bias

Heterogeneity between studies is summarized in tables 1–5. Funnel plots for assessment of publication bias are presented in figure 5. The methodological quality of the selected studies using QUADAS-2 is illustrated in figure 6.

No Result Rate from cfDNA Testing

One issue with cfDNA testing as a method of screening for aneuploidies is its failure to provide a result. One of the reasons for not providing results relates to problems with blood collection and transportation of the samples to the laboratory and the second is the inability to extract sufficient DNA, low fetal fraction or assay failure. In prospective multicentre studies in high-risk pregnancies, no result was given from cfDNA testing in 4.6% of 3,228 eligible samples analysed by CSS [24], in 3.8% of 1,916 analysed by MPSS [37] and in 5.4% of 242 samples analysed by the SNP-based method [34].

Six clinical implementation studies provided data on the no result rates [37, 41–45]. One laboratory-based study reported that the no result rate in 6,123 blood samples they received was 2.4% [44]. The reasons included improper labelling of blood bottles, inadequate blood volume, transportation problems, cancelling the test by the patient or physician, presence of interfering substance in the sample or inability to extract sufficient DNA. In a large multicentre study of 11,184 singleton pregnancies results were provided for 99.3% cases, but in about 1% of cases repeat sampling was necessary [41]. In another four smaller studies the no result rate ranged from 0 to 3.8% [37, 42, 43, 45].

Another issue complicating cfDNA testing is that, in some studies, in addition to some cases not receiving a result there are others in which the result is unclassifiable. The rate of unclassifiable results appears to be higher for sex chromosome aneuploidies than for the major trisomies. For example, Bianchi et al. [19] reported an unclassifiable rate of 9.2% (49 of 532) for sex chromosome aneuploidies, compared to 1.3% (7 of 532) for trisomy 21.

The time interval between taking a sample from the patient and receiving results from the laboratory was on average 9 calendar days and results were given within 14 days in 95–98% of cases [41–45].

Performance of Screening for Aneuploidies

Trisomy 21

A total of 18 studies reported on the performance of screening by cfDNA analysis for trisomy 21 in a combined total of 809 trisomy 21 and 12,272 non-trisomy 21 singleton pregnancies (table 1). In individual studies the DR varied between 94.4 and 100% and the FPR between 0 and 2.05%. The pooled weighted DR and FPR were 99.0% (95% CI 98.2–99.6) and 0.08% (95% CI 0.03–0.14), respectively.

Trisomy 18

A total of 15 studies reported on the performance of screening by cfDNA analysis for trisomy 18 in a combined total of 301 trisomy 18 and 11,646 non-trisomy 18 singleton pregnancies (table 2). In individual studies the DR varied between 91.9 and 100% and the FPR between 0 and 1.98%. The pooled weighted DR and FPR were 96.8% (95% CI 94.5–98.4) and 0.15% (95% CI 0.08–0.25), respectively.
Trisomy 13

A total of 11 studies reported on the performance of screening by cfDNA analysis for trisomy 13 in a combined total of 85 trisomy 13 and 8,339 non-trisomy 13 singleton pregnancies (table 3). In individual studies the DR varied between 78.6 and 100% and the FPR between 0 and 1.14%. The pooled weighted DR and FPR were 92.1% (95% CI 85.9–96.7) and 0.20% (95% CI 0.04–0.46), respectively.

Monosomy X

A total of 12 studies reported on the detection of monosomy X by cfDNA analysis for a combined total of 139 singleton pregnancies with fetal monosomy X and 4,855 with no monosomy X (table 4). In individual studies the DR varied between 66.7 and 100% and the FPR between 0 and 0.26%. The pooled weighted DR and FPR were 88.6% (95% CI 83.0–93.1) and 0.12% (95% CI 0.05–0.24), respectively. There is some evidence that it may be
Cell-Free DNA in Screening for Aneuploidies

more difficult to distinguish between affected and unaffected cases of monosomy X than between trisomies 21 and 18. Two studies using MPSS on a combined total of 41 cases of monosomy X classified 32 cases (78.0%) correctly; 2 were classified as non-monosomy X and 7 (17.1%) were unclassified [19, 33].

Sex Chromosome Aneuploidies other than Monosomy X

A total of nine studies reported on the performance of screening by cfDNA analysis for sex chromosome abnormalities other than monosomy X in a combined total of 44 affected and 2,802 non-sex chromosome aneuploidy singleton pregnancies (table 5). Most studies included very few affected cases and in only three the number of such aneuploidies was more than five [19, 33, 40]. The pooled weighted DR and FPR were 93.8% (95% CI 85.9–98.7) and 0.12% (95% CI 0.02–0.28), respectively.

Triploidy

Triploidy presents with two phenotypes, depending on whether the origin of the extra haploid set is paternal (diandric) or maternal (digynic). In the digynic type the placenta is very small and the fetus is severely growth restricted, whereas in the diandric type the placenta is enlarged and partially molar. In the combined test, digynic triploidy is suspected from the very low serum-free β-hCG and PAPP-A (<0.1 multiple of the median), the small fetus and normal fetal NT, whereas in diandric triploidy the fetal NT tends to be high and maternal serum-free β-hCG is about 10 times higher than normal [8].

The SNP method of cfDNA testing is the only one at present that can detect triploidy because it analyses allele distributions and does not require the use of a disomic reference chromosome. A study of samples from 8 cases of triploidy and euploid controls detected 4 cases of diandric triploidy from the presence of multiple paternal haplotypes (indicating fetal trisomies 21, 18 and 13) and raised the suspicion for digynic triploidy because the fetal fraction, corrected for maternal weight, was very low [35].

Studies in Twin Pregnancies

Three studies reported on the performance of screening by cfDNA analysis for trisomies in twin pregnancies (table 6). Two studies used MPSS and one CSS [20, 29, 31]. In a combined total of 18 trisomy 21 and 209 euploid pregnancies the DR was 94.4% (95% CI 74.2–99.0) and the FPR was 0% (95% CI 0.00–1.84). There were also 2 trisomy 13 pregnancies which were correctly classified. One other study examined 5 twin pregnancies, 4 in a training set and 1 in a test set, and correctly categorized the 2 with trisomy 21 fetuses (both in the training set) and the 3 with euploid fetuses [17].
Clinical Implementation Studies without Complete Pregnancy Outcome

There are five studies in singletons and one in twins that examined the clinical implementation of cfDNA analysis in screening for aneuploidies. They reported useful information but because they do not provide data on complete pregnancy outcome they cannot be used for assessment of the performance of screening.

Dan et al. [41] reported cfDNA results in 11,105 singleton pregnancies. The result was screen positive in 190 cases (143 for trisomy 21 and 47 for trisomy 18). In 182 of these 190 cases, invasive testing was carried out and in 180 the prediction from cfDNA testing was confirmed, but in 1 case of trisomy 21 and in 1 of trisomy 18 the karyotype was normal (false positive). In the screen-negative group, the cfDNA result was confirmed by karyotyping or by the birth of phenotypically normal babies in 7,342 of the 10,915 cases (67.3%); in the remaining cases there was no follow-up or the pregnancies resulted in stillbirth or termination without karyotyping.

Futch et al. [44] reported cfDNA results in 5,974 singleton pregnancies. This was screen positive in 284 cases (155 for trisomy 21, 66 for trisomy 18, 19 for trisomy 13, 40 for monosomy X and 4 double aneuploidy), screen negative in 5,517 and unclassifiable in 173 (2.9%). In the screen-negative group information on pregnancy outcome was available for less than half of the cases and in this group there were 5 false-negative results (2 cases of trisomy 21, 2 of trisomy 18 and 1 of monosomy X). In the screen-positive group in 193 (68.0%) of 284 cases there was no follow-up or confirmation of the cfDNA result, in 74 (26.1%) the suspected aneuploidy was confirmed by invasive testing and in 7 (6.9%) the fetal karyotype was normal. The 17 cases with a false-positive result (1 case of trisomy 21, 6 of trisomy 18, 5 of trisomy 13, 3 of monosomy X and 2 of double trisomy) included 2 cases of confined placental mosaicism, 2 with a history of co-twin demise and 1 of maternal malignancy with concordant cytogenetics.

Gil et al. [45] reported cfDNA results in 984 singleton pregnancies. The result was screen positive in 17 cases (11 for trisomy 21, 5 for trisomy 18 and 1 for trisomy 13) and screen negative in 968. The suspected trisomies were confirmed by invasive testing, except in 1 case of trisomy 18 in which the karyotype was normal. More than 95% of the pregnancies with screen-negative results are continuing and it is therefore uncertain if there are any aneuploidies in this group. However, on the basis of the maternal age distribution of the study population, the expected and observed numbers for each of the three trisomies were similar. The study showed that the FPR was 0.1% for the cfDNA test and 3.4% for the combined test.

Lau et al. [42] reported cfDNA results in 567 singleton pregnancies. The result was screen positive in 9 cases (8 for trisomy 21 and 1 for trisomy 18), which were all confirmed by invasive testing. Most pregnancies with screen-negative results are continuing. Fairbrother et al. [43] reported cfDNA results in 284 singleton pregnancies and the risk for each trisomy was less than 1:10,000 in all cases; all pregnancies are continuing.

One prospective study examined 68 twin pregnancies at 10–13 weeks’ gestation [29]. Risks for trisomies were provided for 63 cases (92.6%). The result was screen positive in 3 cases (2 for trisomy 21 and 1 for trisomy 18) and screen negative in 60. In the screen-positive group, invasive testing was carried out and karyotyping confirmed the suspected trisomy in 1 of the twins for all cases. All pregnancies with screen-negative results are continuing.

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Table 6. Studies reporting on the application of cfDNA analysis of maternal blood in screening for trisomies in twin pregnancies

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>Gestational age (range), weeks</th>
<th>Karyotype</th>
<th>Trisomic total, n</th>
<th>detection, n</th>
<th>Non-trisomic total, n</th>
<th>false positive, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canick et al. [20], 2012</td>
<td>MPSS</td>
<td>14 (10–18)</td>
<td>Trisomy 21</td>
<td>7</td>
<td>7 (100)</td>
<td>17</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Trisomy 13</td>
<td>1</td>
<td>1 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lau et al. [31], 2013</td>
<td>MPSS</td>
<td>13 (11–20)</td>
<td>Trisomy 21</td>
<td>1</td>
<td>1 (100)</td>
<td>11</td>
<td>0.0</td>
</tr>
<tr>
<td>Gil et al. [29], 2013</td>
<td>CSS</td>
<td>13 (12–13)</td>
<td>Trisomy 21</td>
<td>10</td>
<td>9 (90.0)</td>
<td>181</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Trisomy 13</td>
<td>1</td>
<td>1 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>Trisomy 21</td>
<td>18</td>
<td>17 (94.4)</td>
<td>209</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Trisomy 13</td>
<td>2</td>
<td>2 (100)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Detection and false-positive values include percentages in parentheses.
Discussion

Meta-Analysis of Studies Reporting on Performance of Screening by cfDNA Testing

The studies included in the meta-analysis differed in the method of recruitment of patients, cfDNA analysis, algorithms and reporting of results. In particular, the studies were inconsistent in reporting the failure rate and reasons for failure to provide results. In two studies the apparent performance of screening may have been over-estimated because some of the results were unclassified and these were excluded from their calculations of DR and FPR [19, 33]. It was also sometimes unclear if all the different algorithms used to improve the performance of the test were developed without a previous knowledge of the results and performance of the previous algorithm [14].

A major limitation of pooling data from case series, some of which were very small, is that they are highly prone to several sources of bias; however, it is acceptable that systematic reviews can include case series on new technologies or interventions which are unlikely to be studied in randomized controlled trials [62, 63]. Similarly, although small case series are generally regarded as particularly susceptible to publication bias, an extensive health technology assessment report has shown that there is a consistent lack of association between sample size and outcome and recommended that size limitations should not be used as inclusion criteria [62, 64].

In our review, the risk for most of the common sources of bias was low, because the outcome measure of a specific aneuploidy is well defined and objectively ascertained and the included studies reported on consecutive cases with known outcome. The low risk for bias in our review is supported by the findings of the funnel plots and the markedly low heterogeneity between studies.

Screening for Trisomies 21, 18 and 13

The DR of cfDNA analysis in the identification of pregnancies with fetal trisomies 21, 18 and 13 is superior to all other methods combining maternal age, first- or second-trimester ultrasound findings and first- or second-trimester serum biochemical analysis. Additionally, cfDNA testing is associated with a substantial reduction in the FPR and therefore with the need for invasive testing.

In singleton pregnancies, the combined data from studies involving a large number of affected and unaffected pregnancies indicate that with cfDNA analysis the DR for trisomies 21, 18 and 13 is about 99, 97 and 92%, respectively, at a combined FPR of 0.43%.

Screening for Sex Chromosome Aneuploidies

The combined data from studies examining cfDNA testing for monosomy X and other sex chromosome aneuploidies indicate that the DR is about 89 and 94%, respectively, at a combined FPR of 0.24%. In the MPSS methods the poorer performance of cfDNA analysis in screening for trisomy 13 and monosomy X, compared to trisomies 21 and 18, could, at least in part, be due to the highly variable amplification of chromosomes X and 13 because of a lower guanosine-cytosine content [13, 46]. An additional factor that complicates cfDNA screening for sex chromosome aneuploidies is the maternal and fetal mosaicism for these aneuploidies.

Detection of Other Aneuploidies

Two proof-of-principle studies have reported that with deep sequencing (20–100 million sequence tags per sample) it is possible to identify trisomies other than those affecting chromosomes 21, 18 and 13 and subchromosomal deletions and duplications [30, 67]. The clinical utility of this approach remains to be determined.

Screening for Triploidy

The SNP-based method of cfDNA analysis can identify diandric triploidy and raise the suspicion of digynic triploidy because of an abnormally low fetal fraction [35]. These conditions can also be suspected by the combined test because of the highly atypical levels of serum-free β-hCG and PAPP-A and the ultrasound features of molar placenta or severe asymmetrical fetal growth restriction. However, in some developed countries, including the USA, the combined test is carried out in less than half of the population, and should cfDNA testing be applied as a first-line method of screening, identification of triploidy would be beneficial; diandric triploidy can cause maternal complications, including severe early-onset pre-eclampsia and choriocarcinoma [65, 66].

Screening for Aneuploidies in Twin Pregnancies

In twin pregnancies, there is some evidence that cfDNA testing can be effective in identifying trisomy 21. However, in twins, cfDNA testing is more complex than in singleton pregnancies because the 2 fetuses could be either monozygotic and therefore genetically identical, or dizygotic, in which case only 1 fetus is likely to have aneuploidy when present. There is evidence that in dizygotic twins each fetus can contribute different amounts of cfDNA into the maternal circulation, which could vary by nearly 2-fold [68, 69]. It is therefore possible that in a dizygotic twin pregnancy discordant for aneuploidy, the fe-
tual fraction of the affected fetus is below the threshold of 4% for successful cfDNA testing. This could lead to an erroneous result of low risk for aneuploidy because a high contribution from the disomic co-twin could result in a satisfactory total fetal fraction. To avoid this potential mistake it was proposed that in cfDNA testing in twin pregnancies the lower fetal fraction of the 2 fetuses, rather than the total, should be estimated in the assessment of risk for aneuploidies [70]. However, an inevitable consequence of such policy is that the no result rate in twins is likely to be higher than in singleton pregnancies.

Limitations of cfDNA Testing

The clinical implementation studies of cfDNA testing have shown the following: firstly, in 1–5% of singleton pregnancies no result is given after first sampling, either because of problems with sample collection and transportation to the laboratory, low fetal fraction or assay failure [41–45]; secondly, on repeat sampling, a result is obtained in about 100, 50 and 75% of cases in which on first sampling there was a sample collection and transportation problem, low fetal fraction or assay failure, respectively [45], and thirdly, the average interval between sampling and providing results is about 10 calendar days; for 95–98% of cases a result is available within 14 days, but in 2% of cases a result may not be available in less than 3–4 weeks, especially in those requiring repeat sampling.

There are three main limitations of cfDNA testing in the implementation of this method of screening for aneuploidies. The first limitation is that the cost of the test is similar to or higher than that of invasive testing and karyotyping and considerably higher than that of the currently available screening methods. Widespread uptake of the test will inevitably lead to a reduction of cost, but the speed and extent of such a reduction is currently uncertain. The second limitation of the test relates to the 1-to 2-week interval between collecting maternal blood and receiving results. Such a delay may reverse the beneficial shift in screening and diagnosis of aneuploidies from the second to the first trimester of pregnancy achieved in the last 20 years. First-trimester screening and diagnosis of aneuploidies lead to early reassurance of the majority of parents that their fetus is unlikely to be trisomic, and for the few with an affected fetus the parents have the option of an earlier and safer termination of pregnancy. The third limitation of the test arises from the 1–5% rate of failure to provide results. An important cause of failure of cfDNA testing is low fetal fraction which is often a consequence of maternal obesity and this problem may be difficult to overcome [16, 71–73]. Another potential disadvantage of cfDNA testing is the loss of useful information, beyond the detection of trisomies, which is derived from current methods of screening for trisomy 21 and invasive testing in the high-risk group [7].

Models for Clinical Implementation of cfDNA Testing in Maternal Blood

There are essentially two options in the clinical implementation of cfDNA testing: firstly, routine screening of the whole population and secondly, contingent screening based on the results of first-line screening by another method, preferably the first-trimester combined test. In the latter option, cfDNA testing could be offered to the high-risk group as an alternative to invasive testing or to the intermediate-risk group as a method of selecting the small subgroup that could benefit from invasive testing.

In a population of 100,000 pregnancies, with the maternal age distribution in England and Wales in 2011 (median age 29 years), the estimated prevalence of trisomy 21 and trisomies 18 or 13 at 12 weeks’ gestation is 294 and 162, respectively [6]. If combined screening is carried out in the whole population and the high-risk group is defined by the risk cut-off of 1:100, this group would include 87.0% of the cases of trisomy 21, 91.8% of those with trisomy 18 or 13 and 2.2% of pregnancies unaffected by these trisomies [6] (fig. 7).

cfDNA Testing as a First-Line Method of Screening for All Pregnanncies

We estimated that if cfDNA testing of maternal blood was offered as a first-line method of screening to all pregnancies about 99% of fetuses with trisomy 21 and 95% with trisomies 13 and 18 could be detected at an overall invasive testing rate of 1% [74].

The best approach to implement primary screening for trisomies 21, 18 and 13 by cfDNA testing is to take the maternal blood at 10 weeks’ gestation [45]. The results of the test would then be available at the time of the scheduled first-trimester ultrasound examination, which is ideally performed at 12 weeks. Such an approach retains the advantages of, firstly, diagnosis of the major trisomies within the first trimester and, secondly, early diagnosis of major fetal defects and assessment of risk for pregnancy complications [75].

If cfDNA testing reports a high risk for trisomies 21, 18 or 13 it would be important to confirm or refute the result with invasive testing. In contrast, if cfDNA testing reports a low risk for trisomy 21 or 18 the parents can be reassured that it is highly unlikely that the fetus has one...
of these aneuploidies. In the case of trisomy 13 the number of cases examined by cfDNA testing is too small for accurate assessment of performance of screening. In the combined data from published studies with a DR of 92.1% and an FPR of 0.20% the positive and negative likelihood ratio of the test is 461 (92.1/0.20) and 0.08 (7.9/99.80), respectively. Consequently, if cfDNA testing shows a positive result for trisomy 13 there is a 461-fold increase in the risk for this trisomy and therefore such patients should be offered the option of invasive diagnostic testing. If the result is negative for trisomy 13 there is a 12.5-fold decrease in the a priori odds. However, if the ultrasound examination at 12 weeks demonstrates holoprosencephaly, exomphalos or megacystis, where the risk for aneuploidies is very high, the 12.5-fold reduction in risk following a negative cfDNA test is unlikely to reassure the parents and they should still be offered the option of invasive testing.

Invasive testing should also be considered when the fetal NT is ≥3.5 mm because in this group there is high risk not only for the common trisomies but also for other rare aneuploidies [7, 76].

In those cases where cfDNA testing does not provide a result the parents would still have the option of first-trimester screening for aneuploidies by a combination of maternal age, fetal NT and serum-free β-hCG and PAPP-A.

cfDNA Testing in the High-Risk Group from the First-Trimester Combined Test

In this model of clinical implementation of cfDNA testing, first-line screening is done by the combined test, and in the high-risk group (more than 1:100) cfDNA testing rather than invasive testing is carried out. If invasive testing was carried out in all cases in the high-risk group, about 87% of fetuses with trisomy 21 and 92% with trisomies 18 or 13 could be detected at an overall invasive testing rate of 2.6% (fig. 7). In a policy of carrying out cfDNA testing in the high-risk group and reserving invasive testing for the screen-positive cases,

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**Fig. 7.** In a population of 100,000 pregnancies, with the maternal age distribution in England and Wales in 2011, the estimated prevalence of trisomy 21 and trisomies 18 or 13 at 12 weeks’ gestation is 294 and 162, respectively [6]. If combined screening is carried out in the whole population and the high-risk group is defined by the risk cut-off of 1:100, this group would include 87.0% of the cases of trisomy 21, 91.8% of those with trisomy 18 or 13 and 2.2% of pregnancies unaffected by these trisomies. On the basis of data from this systematic review, we estimated that if cfDNA testing is carried out in the high-risk group followed by invasive testing in those with a screen-positive result, 86.1% of the fetuses with trisomy 21 and 88.9% of those with trisomy 18 or 13 would be detected at an invasive testing rate of 0.4%.
out followed by invasive testing for those with a screen-positive result. Such a strategy would detect about 98% of the fetuses with trisomies 21, 18 and 13, at an overall invasive testing rate of 0.8% (fig. 8).

The intermediate-risk group requiring cfDNA testing constitutes about 24% of the population. However, this proportion can be reduced to about 17% if first-line screening includes serum PLGF and AFP, in addition to fetal NT, FHR and serum-free β-hCG and PAPP-A [74]. A further reduction in the need for cfDNA testing can be achieved by a first-line method of screening which includes ductus venosus pulsatility index, in addition to fetal NT, FHR and serum-free β-hCG, PAPP-A, PLGF and AFP [74].

This approach of cfDNA testing contingent on the results of first-line screening by ultrasound and biochemical testing retains the major advantages of cfDNA testing.
in increasing DR and decreasing FPR, but at consider-
ably lower cost than offering the test to the whole popu-
lation.

Conclusion

On the basis of clinical validation and/or implementa-
tion studies, the DR of trisomies 21, 18 and 13 by cfDNA
analysis of maternal blood is about 99, 97 and 92%, re-
spectively, at an overall FPR of 0.4%. We propose differ-
ent options for clinical implementation of the test in
screening for major trisomies, whereby cfDNA analysis is
offered as a first-line method of screening or contingent
on the results of first-line screening by the combined test.

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