Original Paper

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

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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.

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Introduction

Current Practice in Screening for Aneuploidies

Prenatal diagnosis of fetal aneuploidies necessitates invasive testing, which is essentially carried out by chorionic villus sampling at 10–15 weeks' gestation or amniocentesis at or after 16 weeks. However, invasive testing is expensive and can cause miscarriage in up to 1% of pregnancies; it is therefore reserved for cases identified by screening as being at high risk for aneuploidies [1].

In the last 40 years prenatal screening for aneuploidies has focused on trisomy 21. The method of screening has evolved from maternal age in the 1970s, with a detection rate (DR) for trisomy 21 of 30% and a false-positive rate (FPR) of 5%, to a combination of maternal age and second-trimester serum biochemistry in the 1980s and 1990s, with a DR of 60–70% and an FPR of 5%, to a combination of maternal age, fetal nuchal translucency (NT) thickness and serum-free β-hCG and PAPP-A in the first trimester in the last 20 years, with a DR of 90% and an FPR of 5% [2].

A beneficial consequence of screening for trisomy 21 is the early diagnosis of trisomies 18 and 13, which are the second and third most common chromosomal abnormalities, with a relative prevalence to trisomy 21 at 11–13 weeks' gestation of 1:3 and 1:7, respectively [3, 4]. Since all three trisomies are similar in being associated with in-

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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 shotgun 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 nonmosaic 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, Haldane 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

Author	Method	Gestational age (range), weeks	Trisomy 21		Non-trisomy 21	
			total, n	detection, n	total, n	false positive, n
Chiu et al. [14], 2011	MPSS	13 (–)	86	86 (100.0, 95.8–100.0)	146	3 (2.05, 0.43–5.89)
Ehrich et al. [15], 2011	MPSS	16 (8–36)	39	39 (100.0, 91.0–100.0)	410	1 (0.24, 0.01–1.35)
Palomaki et al. [16], 2011	MPSS	15 (8–21)	212	209 (98.6, 95.9–99.7)	1,471	3 (0.20, 0.04–0.60)
Sehnert et al. [17], 2011	MPSS	15 (10–28)	13	13 (100.0, 75.3–100.0)	34	0 (0.00, 0.00–10.28)
Ashoor et al. [18], 2012	CSS	12 (11–13)	50	50 (100.0, 92.9–100.0)	347	0 (0.00, 0.00–1.06)
Bianchi et al. [19], 2012	MPSS	15 (10–23)	89	89 (100.0, 95.9–100.0)	404	0 (0.00, 0.00–0.91)
Jiang et al. [21], 2012	MPSS	– (10–34)	16	16 (100.0, 79.4–100.0)	887	0 (0.00, 0.00–0.42)
Lau et al. [22], 2012	MPSS	12 (11–28)	11	11 (100.0, 71.5–100.0)	97	0 (0.00, 0.00–3.73)
Nicolaides et al. [23], 2012	CSS	12 (11–13)	8	8 (100.0, 63.1–100.0)	1,941	0 (0.00, 0.00–0.19)
Norton et al. [24], 2012	CSS	16 (10–38)	81	81 (100.0, 95.6–100.0)	2,888	1 (0.04, 8.7e^{-04}–0.19)
Sparks et al. [26], 2012	CSS	18 (11–36)	36	36 (100.0, 90.3–100.0)	131	0 (0.00, 0.00–2.78)
Zimmerman et al. [27], 2012	SNP	17 (9–36)	11	11 (100.0, 71.5–100.0)	134	0 (0.00, 0.00–2.72)
Guex et al. [30], 2013	MPSS	12 (11–13)	30	30 (100.0, 88.4–100.0)	146	0 (0.00, 0.00–2.50)
Liang et al. [32], 2013	MPSS	21 (11–39)	37	37 (100.0, 90.5–100.0)	367	0 (0.00, 0.00–1.00)
Nicolaides et al. [34], 2013	SNP	13 (11–13)	25	25 (100.0, 86.3–100.0)	204	0 (0.00, 0.00–1.79)
Song et al. [37], 2013	MPSS	16 (11–21)	8	8 (100.0, 63.1–100.0)	1,733	0 (0.00, 0.00–0.21)
Stumm et al. [38], 2013	MPSS	15 (11–32)	39	38 (97.4, 86.5–99.9)	430	0 (0.00, 0.00–0.85)
Verweij et al. [39], 2013	CSS	14 (10–28)	18	17 (94.4, 72.7–99.9)	502	0 (0.00, 0.00–0.73)
Pooled analysis, %						
Fixed-effects model				99.0 (98.2–99.6)		0.07 (0.03–0.12)
Random-effects model				99.0 (98.2–99.6)		0.08 (0.03–0.14)
Cochrane's Q				7.747 (0.9717)		19.337 (0.3095)
I^2 statistic, %				0 (0–43.7)		12.1 (0.0–50.3)
Egger bias				–0.02745 (0.8957)		0.2299 (0.1831)

Detection and false-positive values include percentages and 95% CI in parentheses. Pooled analysis and I^2 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

Author	Method	Gestational age (range), weeks	Trisomy 18		Non-trisomy 18	
			total, n	detection, n	total, n	false positive, n
Chen et al. [13], 2011	MPSS	–	37	34 (91.9, 78.1–98.3)	252	5 (1.98, 0.65–4.57)
Sehnert et al. [17], 2011	MPSS	15 (10–28)	8	8 (100.0, 63.1–100.0)	39	0 (0.00, 0.00–9.03)
Ashoor et al. [18], 2012	CSS	12 (11–13)	50	49 (98.0, 89.4–99.9)	347	0 (0.00, 0.00–1.06)
Bianchi et al. [19], 2012	MPSS	15 (10–23)	36	35 (97.2, 85.5–99.9)	460	0 (0.00, 0.00–0.80)
Jiang et al. [21], 2012	MPSS	– (10–34)	12	12 (100.0, 73.5–100.0)	891	1 (0.11, $2.8e^{-03}$–0.62)
Lau et al. [22], 2012	MPSS	12 (11–28)	10	10 (100.0, 69.2–100.0)	98	0 (0.00, 0.00–3.69)
Nicolaides et al. [23], 2012	CSS	12 (11–13)	2	2 (100.0, 15.8–100.0)	1,947	2 (0.10, 0.01–0.37)
Norton et al. [24], 2012	CSS	16 (10–38)	38	37 (97.4, 86.2–99.9)	2,888	2 (0.07, $8.4e^{-03}$–0.25)
Palomaki et al. [25], 2012	MPSS	14 (9–22)	59	59 (100.0, 93.9–100.0)	1,912	5 (0.26, 0.09–0.61)
Sparks et al. [26], 2012	CSS	18 (11–36)	8	8 (100.0, 63.1–100.0)	159	0 (0.00, 0.00–2.29)
Zimmerman et al. [27], 2012	SNP	17 (9–36)	3	3 (100.0, 29.2–100.0)	142	0 (0.00, 0.00–2.56)
Guex et al. [30], 2013	MPSS	12 (11–13)	20	19 (95.0, 75.1–99.9)	156	0 (0.00, 0.00–2.34)
Liang et al. [32], 2013	MPSS	21 (11–39)	13	13 (100.0, 75.3–100.0)	391	0 (0.00, 0.00–0.94)
Nicolaides et al. [34], 2013	SNP	13 (11–13)	3	3 (100.0, 29.2–100.0)	226	0 (0.00, 0.00–1.62)
Song et al. [37], 2013	MPSS	16 (11–21)	2	2 (100.0, 15.8–100.0)	1,738	1 (0.06, $1.5e^{-03}$–0.32)
Pooled analysis, %						
Fixed-effects model				96.8 (94.5–98.4)		0.15 (0.08 – 0.22)
Random-effects model				96.8 (94.5–98.4)		0.15 (0.08 – 0.25)
Cochrane's Q				7.2471 (0.9248)		16.7753 (0.2684)
I^2 statistic, %				0 (0 – 46.4)		21.2 (0.0 – 54.9)
Egger bias				–0.1668 (0.4172)		0.1589 (0.5504)

Detection and false-positive values include percentages and 95% CI in parentheses. Pooled analysis and I^2 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

Author	Method	Gestational age, weeks	Trisomy 13		Non-trisomy 13	
			total, n	detection, n	total, n	false positive, n
Chen et al. [13], 2011	MPSS	–	25	25 (100.0, 86.3–100.0)	264	3 (1.14, 0.24–3.28)
Bianchi et al. [19], 2012	MPSS	15 (10–23)	14	11 (78.6, 49.2–95.3)	485	0 (0.00, 0.00–0.76)
Jiang et al. [21], 2012	MPSS	– (10–34)	2	2 (100.0, 15.8–100.0)	901	0 (0.00, 0.00–0.41)
Lau et al. [22], 2012	MPSS	12 (11–28)	2	2 (100.0, 15.8–100.0)	106	0 (0.00, 0.00–3.42)
Palomaki et al. [25], 2012	MPSS	14 (9–22)	12	11 (91.7, 61.5–99.8)	1,959	16 (0.82, 0.47–1.32)
Zimmerman et al. [27], 2012	SNP	17 (9–36)	2	2 (100.0, 15.8–100.0)	143	0 (0.00, 0.00–2.55)
Ashoor et al. [28], 2013	CSS	13 (11–26)	10	8 (80.0, 44.4–97.5)	1,949	1 (0.05, $1.3e^{-03}$–0.29)
Guex et al. [30], 2013	MPSS	12 (11–13)	13	13 (100.0, 75.3–100.0)	163	0 (0.00, 0.00–2.24)
Liang et al. [32], 2013	MPSS	21 (11–39)	3	3 (100.0, 29.2–100.0)	401	1 (0.25, $6.3e^{-03}$–1.38)
Nicolaides et al. [34], 2013	SNP	13 (11–13)	1	1 (100.0, 2.5–100.0)	228	0 (0.00, 0.00–1.61)
Song et al. [37], 2013	MPSS	16 (11–21)	1	1 (100.0, 2.5–100.0)	1,740	0 (0.00, 0.00–0.21)
Pooled analysis, %						
Fixed-effects model				92.1 (86.0–96.7)		0.19 (0.11–0.29)
Random-effects model				92.1 (85.9–96.7)		0.20 (0.04–0.46)
Cochrane's Q				10.0423 (0.4368)		36.7126 (<0.0001)
I^2 statistic, %				10.3 (0–51.4)		72.8 (42.8–83.7)
Egger bias				–0.4082 (0.2714)		0.7184 (0.2004)

Detection and false-positive values include percentages and 95% CI in parentheses. Pooled analysis and I^2 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

Author	Method	Gestational age (range), weeks	Monosomy X		Non-monosomy X	
			total, n	detection, n	total, n	false positive, n
Sehnert et al. [17], 2011	MPSS	15 (10–28)	2	2 (100.0, 15.8–100.0)	45	0 (0.00, 0.00–7.87)
Bianchi et al. [19], 2012	MPSS	15 (10–23)	20	15 (75.0, 50.9–91.3)	462	1 (0.22, $5.5e^{-03}$–1.20)
Jiang et al. [21], 2012	MPSS	– (10–34)	3	3 (100.0, 29.2–100.0)	899	1 (0.11, $2.8e^{-03}$–0.62)
Lau et al. [22], 2012	MPSS	12 (11–28)	8	8 (100.0, 63.1–100.0)	100	0 (0.00, 0.00–3.62)
Zimmerman et al. [27], 2012	SNP	17 (9–36)	1	1 (100.0, 2.5–100.0)	144	0 (0.00, 0.00–2.53)
Guex et al. [30], 2013	MPSS	12 (11–13)	15	15 (100.0, 78.2–100.0)	161	0 (0.00, 0.00–2.27)
Liang et al. [32], 2013	MPSS	21 (11–39)	5	5 (100.0, 47.8–100.0)	399	1 (0.25, $6.3e^{-03}$–1.39)
Mazloom et al. [33], 2013	MPSS	– (10–20)	21	17 (81.0, 58.1–94.6)	390	1 (0.26, $6.5e^{-03}$–1.42)
Nicolaides et al. [34], 2013	SNP	13 (11–13)	2	2 (100.0, 15.8–100.0)	227	0 (0.00, 0.00–1.61)
Nicolaides et al. [40], 2013	CSS	12 (11–13)	47	43 (91.5, 79.6–97.6)	116	0 (0.00, 0.00–3.13)
Samango-Sprouse et al. [36], 2013	SNP	13 (9–36)	12	11 (91.7, 61.5–99.8)	175	0 (0.00, 0.00–2.09)
Song et al. [37], 2013	MPSS	16 (11–21)	3	2 (66.7, 9.4–99.2)	1,737	0 (0.00, 0.00–0.21)
Pooled analysis, %						
Fixed-effects model				88.6 (83.0–93.1)		0.12 (0.05–0.24)
Random-effects model				88.6 (83.0–93.1)		0.12 (0.05–0.24)
Cochrane's Q				10.9584 (0.4468)		7.1744 (0.7848)
I^2 statistic, %				0 (0–49.8)		0 (0–49.8)
Egger bias				–0.1712 (0.7599)		0.2315 (0.1161)

Detection and false-positive values include percentages and 95% CI in parentheses. Pooled analysis and I^2 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

Author	Method	Gestational age (range), weeks	47,XXX; 47,XXY; 47,XYY		Non-SCA	
			total, n	detection, n	total, n	false positive, n
Bianchi et al. [19], 2012	MPSS	15 (10–23)	9	8 (88.9, 51.8–99.7)	453	0 (0.00, 0.00–0.81)
Jiang et al. [21], 2012	MPSS	– (10–34)	3	3 (100.0, 29.2–100.0)	896	0 (0.00, 0.00–0.41)
Lau et al. [22], 2012	MPSS	12 (11–28)	1	1 (100.0, 2.5–100.0)	99	0 (0.00, 0.00–3.66)
Zimmerman et al. [27], 2012	SNP	17 (9–36)	3	3 (100.0, 29.2–100.0)	141	0 (0.00, 0.00–2.58)
Guex et al. [30], 2013	MPSS	12 (11–13)	5	5 (100.0, 47.8–100.0)	156	0 (0.00, 0.00–2.34)
Liang et al. [32], 2013	MPSS	21 (11–39)	3	3 (100.0, 29.2–100.0)	396	1 (0.25, 0.01–1.40)
Mazloom et al. [33], 2013	MPSS	– (10–20)	8	8 (100.0, 63.1–100.0)	382	0 (0.00, 0.00–0.96)
Nicolaides et al. [40], 2013	CSS	12 (11–13)	9	9 (100.0, 66.4–100.0)	107	1 (0.93, 0.02–5.10)
Samango-Sprouse et al. [36], 2013	SNP	13 (9–36)	3	3 (100.0, 29.2–100.0)	172	0 (0.00, 0.00–2.12)
Pooled analysis, %						
Fixed-effects model				93.8 (85.9–98.7)		0.12 (0.02–0.28)
Random-effects model				93.8 (85.9–98.7)		0.12 (0.02–0.28)
Cochrane's Q				1.5726 (0.9914)		5.6194 (0.6898)
I^2 statistic, %				0 (0–54.4)		0 (0–54.4)
Egger bias				0.0253 (0.9408)		0.2576 (0.1714)

Detection and false-positive values include percentages and 95% CI in parentheses. Pooled analysis and I^2 statistic include 95% CI in parentheses. Cochrane's Q and Egger bias include p values in parentheses. SCA = Sex chromosome abnormality.

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Author	DR	Weights, %		FPR	Weights, %
Chiu et al. [14], 2011	100.0 (95.8–100)	10.52		2.06 (0.43–5.89)	1.52
Ehrich et al. [15], 2011	100.0 (91.0–100)	4.84		0.24 (0.01–1.35)	4.02
Palomaki et al. [16], 2011	98.6 (95.9–99.7)	25.76		0.20 (0.04–0.60)	11.87
Sehnert et al. [17], 2011	100.0 (75.0–100)	1.69		0.00 (0.00–10.28)	0.37
Ashoor et al. [18], 2012	100.0 (92.9–100)	6.17		0.00 (0.00–1.06)	3.45
Bianchi et al. [19], 2012	100.0 (95.9–100)	10.88		0.00 (0.00–0.91)	3.97
Jiang et al. [21], 2012	100.0 (79.4–100)	2.06		0.00 (0.00–0.42)	7.93
Lau et al. [22], 2012	100.0 (71.5–100)	1.45		0.00 (0.00–3.73)	1.02
Nicolaides et al. [23], 2012	100.0 (63.1–100)	1.09		0.00 (0.00–0.19)	14.54
Norton et al. [24], 2012	100.0 (95.6–100)	9.92		0.04 ($8.8e^{-06}$–0.19)	18.89
Sparks et al. [26], 2012	100.0 (90.3–100)	4.47		0.00 (0.00–2.78)	1.37
Zimmerman et al. [27], 2012	100.0 (71.5–100)	1.45		0.00 (0.00–2.72)	1.40
Guex et al. [30], 2013	100.0 (88.4–100)	3.75		0.00 (0.00–2.50)	1.52
Liang et al. [32], 2013	100.0 (90.5–100)	4.60		0.00 (0.00–1.00)	3.63
Nicolaides et al. [34], 2013	100.0 (86.3–100)	3.14		0.00 (0.00–1.79)	2.09
Song et al. [37], 2013	100.0 (63.1–100)	1.09		0.00 (0.00–0.21)	13.41
Stumm et al. [38], 2013	97.4 (86.5–99.9)	4.84		0.00 (0.00–0.85)	4.20
Verweij et al. [39], 2013	94.4 (72.7–99.9)	2.30		0.00 (0.00–0.73)	4.83
Pooled analysis	99.0 (98.2–99.6)	100.00		0.08 (0.03–0.14)	100.00

DR with 95% CI (%) — 50 60 70 80 90 100
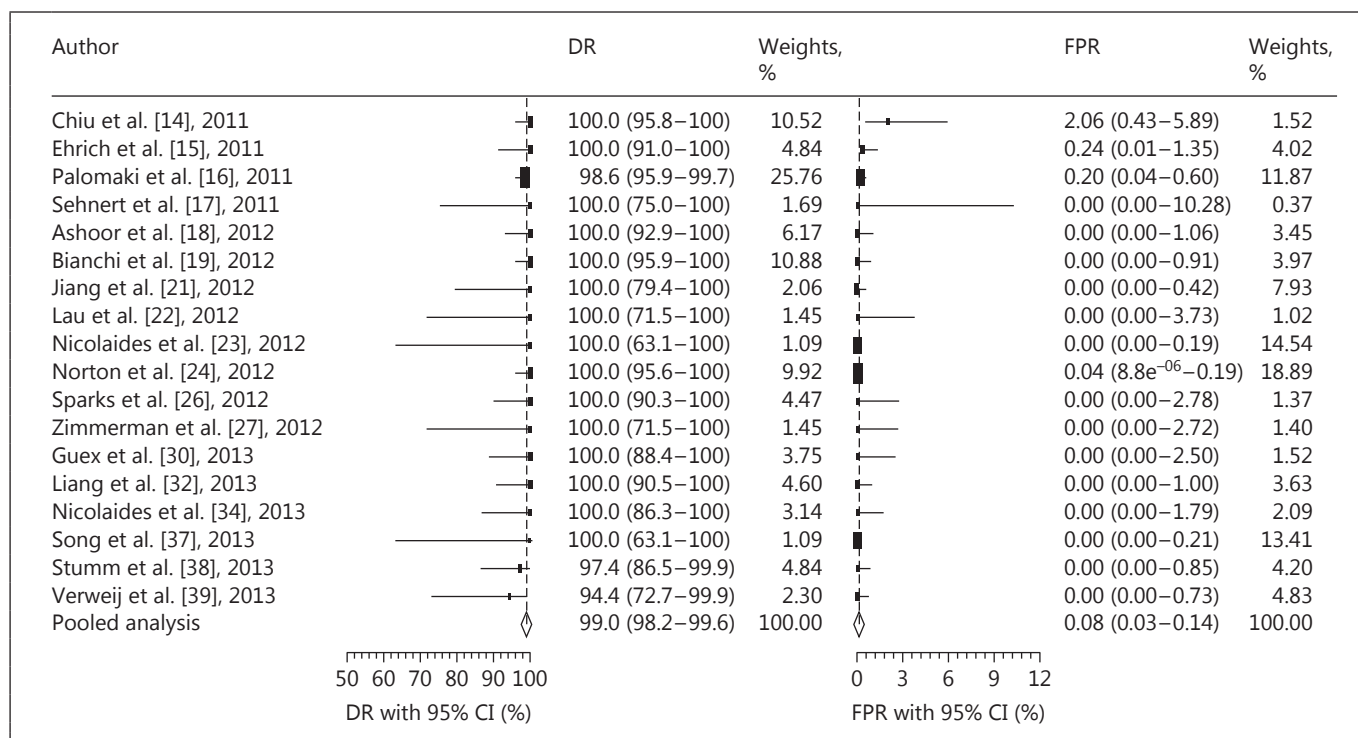
FPR with 95% CI (%) — 0 3 6 9 12

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.
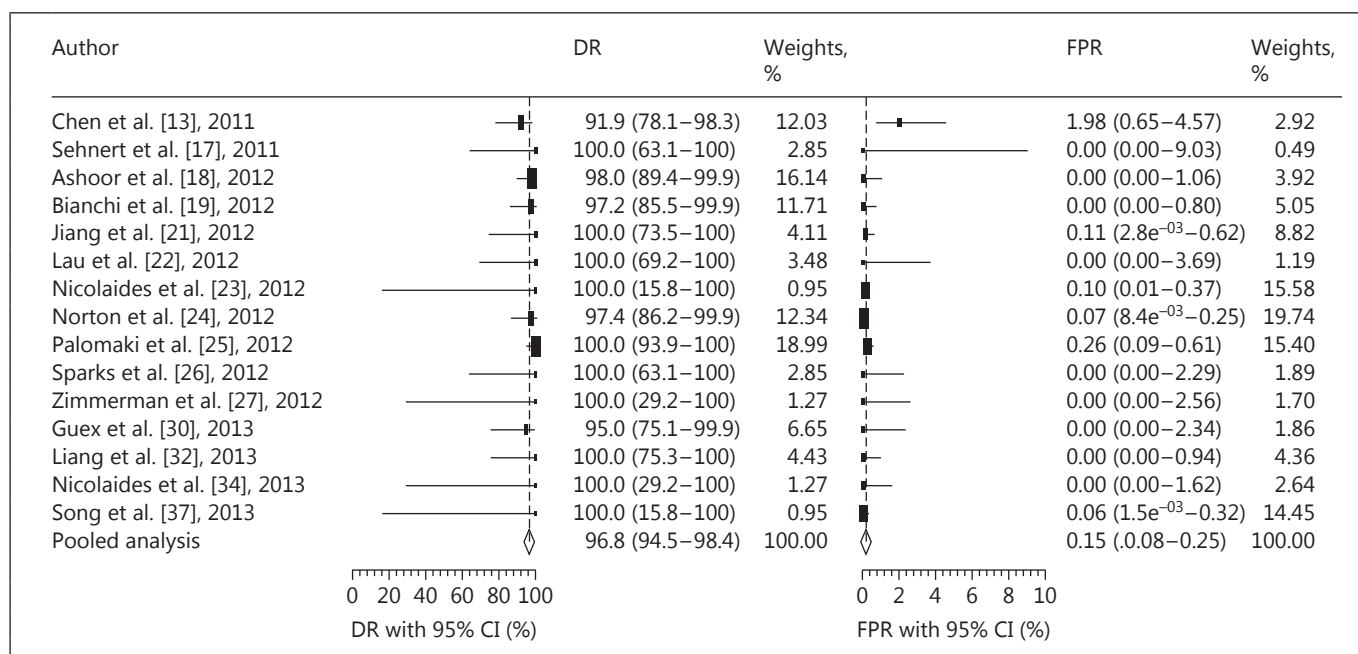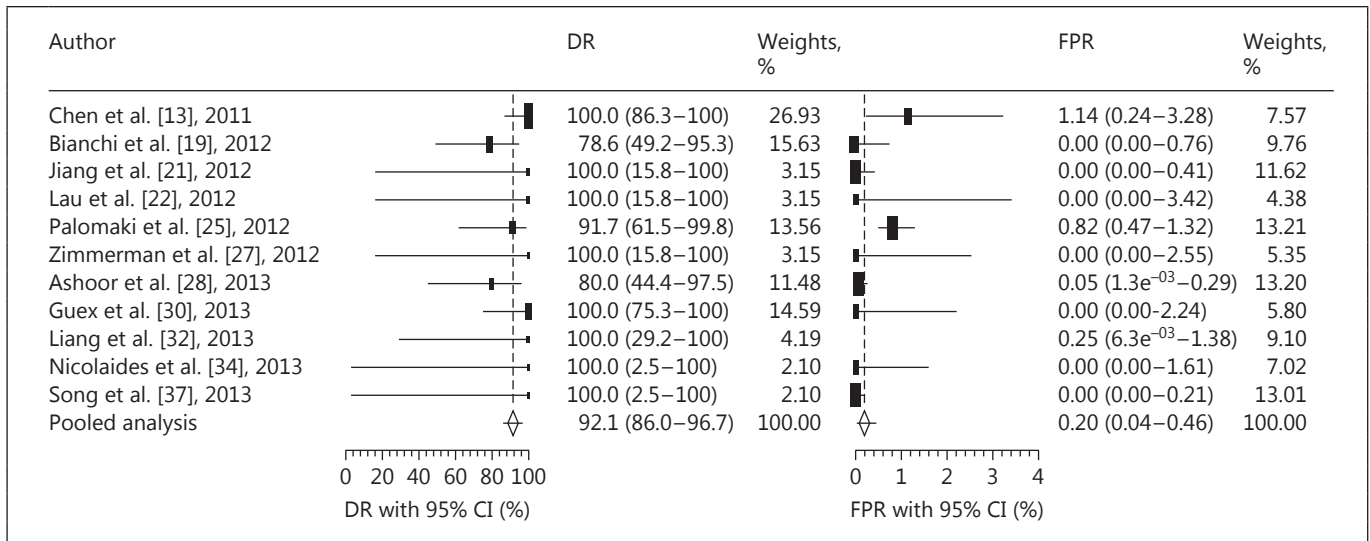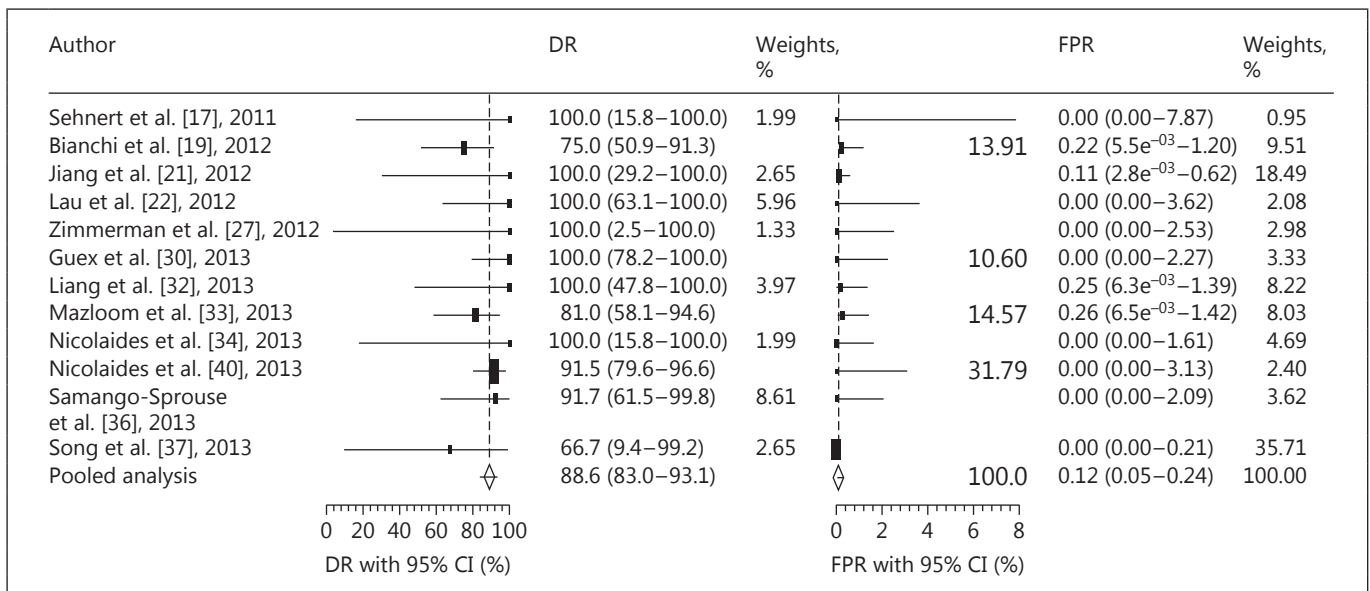
Author	DR	Weights, %		FPR	Weights, %
Chen et al. [13], 2011	91.9 (78.1–98.3)	12.03		1.98 (0.65–4.57)	2.92
Sehnert et al. [17], 2011	100.0 (63.1–100)	2.85		0.00 (0.00–9.03)	0.49
Ashoor et al. [18], 2012	98.0 (89.4–99.9)	16.14		0.00 (0.00–1.06)	3.92
Bianchi et al. [19], 2012	97.2 (85.5–99.9)	11.71		0.00 (0.00–0.80)	5.05
Jiang et al. [21], 2012	100.0 (73.5–100)	4.11		0.11 ($2.8e^{-03}$–0.62)	8.82
Lau et al. [22], 2012	100.0 (69.2–100)	3.48		0.00 (0.00–3.69)	1.19
Nicolaides et al. [23], 2012	100.0 (15.8–100)	0.95		0.10 (0.01–0.37)	15.58
Norton et al. [24], 2012	97.4 (86.2–99.9)	12.34		0.07 ($8.4e^{-03}$–0.25)	19.74
Palomaki et al. [25], 2012	100.0 (93.9–100)	18.99		0.26 (0.09–0.61)	15.40
Sparks et al. [26], 2012	100.0 (63.1–100)	2.85		0.00 (0.00–2.29)	1.89
Zimmerman et al. [27], 2012	100.0 (29.2–100)	1.27		0.00 (0.00–2.56)	1.70
Guex et al. [30], 2013	95.0 (75.1–99.9)	6.65		0.00 (0.00–2.34)	1.86
Liang et al. [32], 2013	100.0 (75.3–100)	4.43		0.00 (0.00–0.94)	4.36
Nicolaides et al. [34], 2013	100.0 (29.2–100)	1.27		0.00 (0.00–1.62)	2.64
Song et al. [37], 2013	100.0 (15.8–100)	0.95		0.06 ($1.5e^{-03}$–0.32)	14.45
Pooled analysis	96.8 (94.5–98.4)	100.00		0.15 (.0.08–0.25)	100.00

DR with 95% CI (%) — 0 20 40 60 80 100

FPR with 95% CI (%) — 0 2 4 6 8 10

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.

Author	DR	Weights, %	FPR	Weights, %
Chen et al. [13], 2011	100.0 (86.3–100)	26.93	1.14 (0.24–3.28)	7.57
Bianchi et al. [19], 2012	78.6 (49.2–95.3)	15.63	0.00 (0.00–0.76)	9.76
Jiang et al. [21], 2012	100.0 (15.8–100)	3.15	0.00 (0.00–0.41)	11.62
Lau et al. [22], 2012	100.0 (15.8–100)	3.15	0.00 (0.00–3.42)	4.38
Palomaki et al. [25], 2012	91.7 (61.5–99.8)	13.56	0.82 (0.47–1.32)	13.21
Zimmerman et al. [27], 2012	100.0 (15.8–100)	3.15	0.00 (0.00–2.55)	5.35
Ashoor et al. [28], 2013	80.0 (44.4–97.5)	11.48	0.05 ($1.3e^{-03}$–0.29)	13.20
Guex et al. [30], 2013	100.0 (75.3–100)	14.59	0.00 (0.00–2.24)	5.80
Liang et al. [32], 2013	100.0 (29.2–100)	4.19	0.25 ($6.3e^{-03}$–1.38)	9.10
Nicolaides et al. [34], 2013	100.0 (2.5–100)	2.10	0.00 (0.00–1.61)	7.02
Song et al. [37], 2013	100.0 (2.5–100)	2.10	0.00 (0.00–0.21)	13.01
Pooled analysis	92.1 (86.0–96.7)	100.00	0.20 (0.04–0.46)	100.00

DR with 95% CI (%)
FPR with 95% CI (%)

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.

Author	DR	Weights, %	FPR	Weights, %
Sehnert et al. [17], 2011	100.0 (15.8–100.0)	1.99	0.00 (0.00–7.87)	0.95
Bianchi et al. [19], 2012	75.0 (50.9–91.3)	13.91	0.22 ($5.5e^{-03}$–1.20)	9.51
Jiang et al. [21], 2012	100.0 (29.2–100.0)	2.65	0.11 ($2.8e^{-03}$–0.62)	18.49
Lau et al. [22], 2012	100.0 (63.1–100.0)	5.96	0.00 (0.00–3.62)	2.08
Zimmerman et al. [27], 2012	100.0 (2.5–100.0)	1.33	0.00 (0.00–2.53)	2.98
Guex et al. [30], 2013	100.0 (78.2–100.0)	10.60	0.00 (0.00–2.27)	3.33
Liang et al. [32], 2013	100.0 (47.8–100.0)	3.97	0.25 ($6.3e^{-03}$–1.39)	8.22
Mazloom et al. [33], 2013	81.0 (58.1–94.6)	14.57	0.26 ($6.5e^{-03}$–1.42)	8.03
Nicolaides et al. [34], 2013	100.0 (15.8–100.0)	1.99	0.00 (0.00–1.61)	4.69
Nicolaides et al. [40], 2013	91.5 (79.6–96.6)	31.79	0.00 (0.00–3.13)	2.40
Samango-Sprouse et al. [36], 2013	91.7 (61.5–99.8)	8.61	0.00 (0.00–2.09)	3.62
Song et al. [37], 2013	66.7 (9.4–99.2)	2.65	0.00 (0.00–0.21)	35.71
Pooled analysis	88.6 (83.0–93.1)	100.0	0.12 (0.05–0.24)	100.00

DR with 95% CI (%)
FPR with 95% CI (%)

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.

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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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 pro-spective 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.
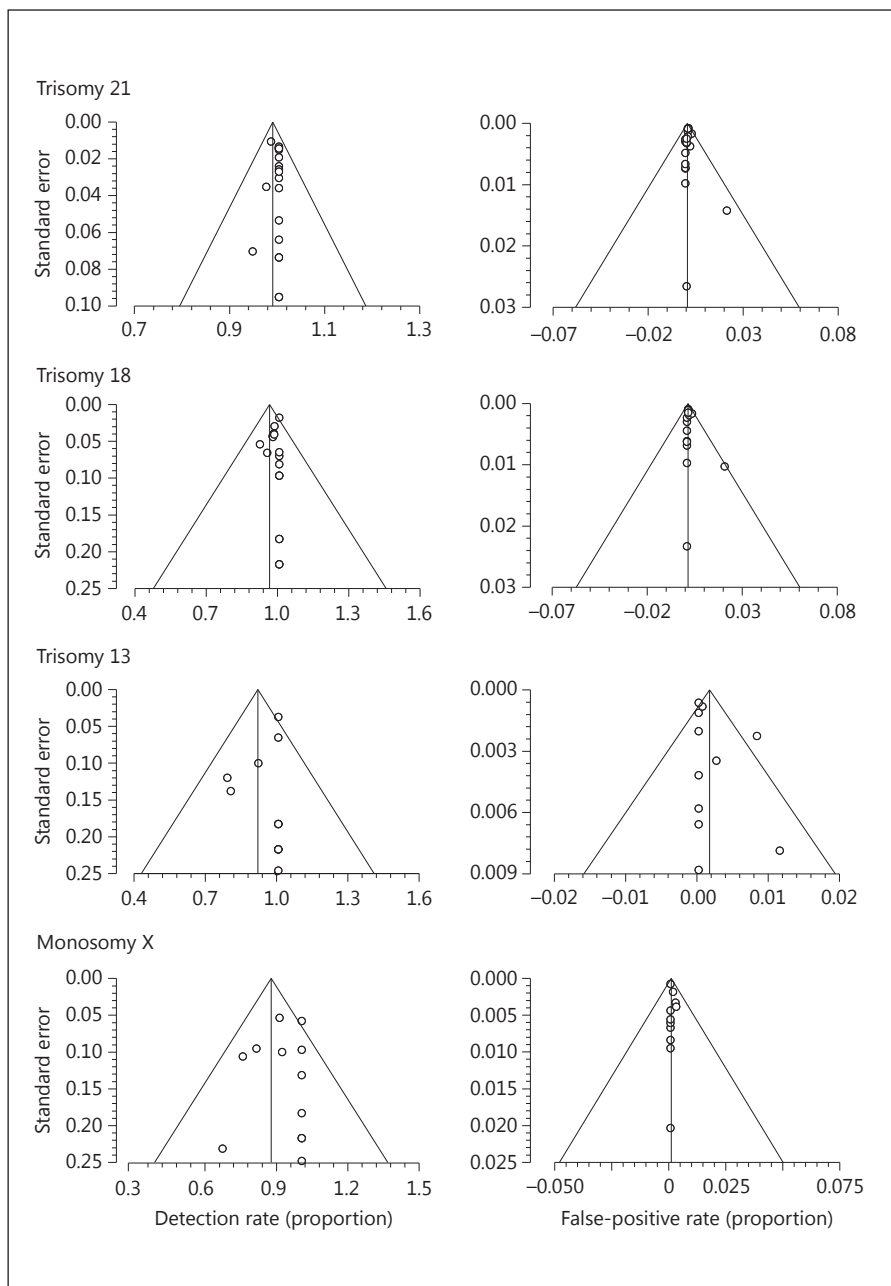
Fig. 5. Funnel plots demonstrating assessment of publication bias in screening for trisomies 21, 18, 13 and monosomy X.

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

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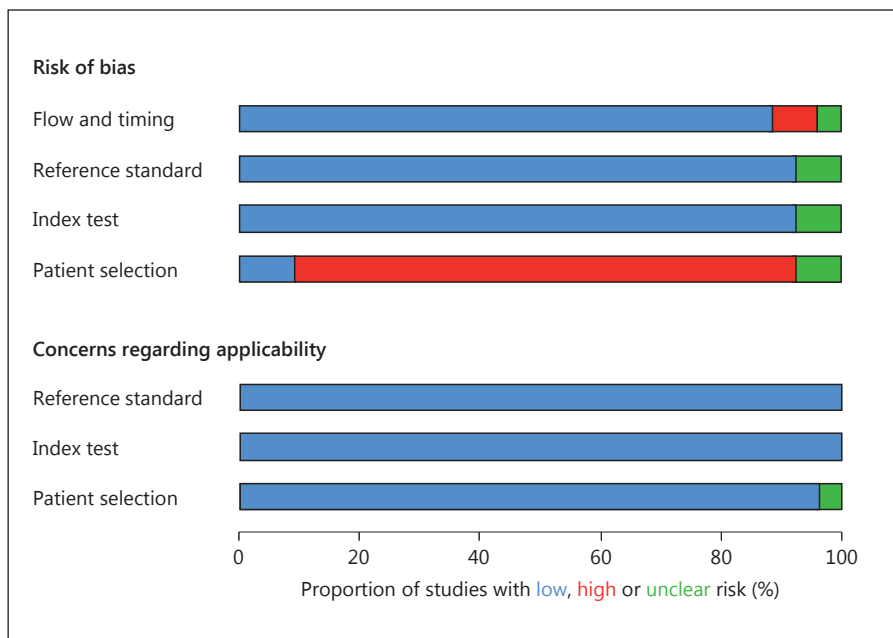
Fig. 6. Summary of the quality of included studies using the QUADAS-2 checklist.

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 fe-

tus 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].

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

Author	Method	Gestational age (range), weeks	Karyotype	Trisomic		Non-trisomic	
				total, n	detection, n	total, n	false positive, n
Canick et al. [20], 2012	MPSS	14 (10–18)	Trisomy 21	7	7 (100)	17	0 (0.0)
			Trisomy 13	1	1 (100)		
Lau et al. [31], 2013	MPSS	13 (11–20)	Trisomy 21	1	1 (100)	11	0 (0.0)
Gil et al. [29], 2013	CSS	13 (12–13)	Trisomy 21	10	9 (90.0)	181	0 (0.0)
			Trisomy 13	1	1 (100)		
Total			Trisomy 21	18	17 (94.4)	209	0 (0.0)
			Trisomy 13	2	2 (100)		

Detection and false-positive values include percentages in parentheses.

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 17 (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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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 overestimated 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.

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 preeclampsia and choriocarcinoma [65, 66].

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 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-

tal 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 Pregnancies

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
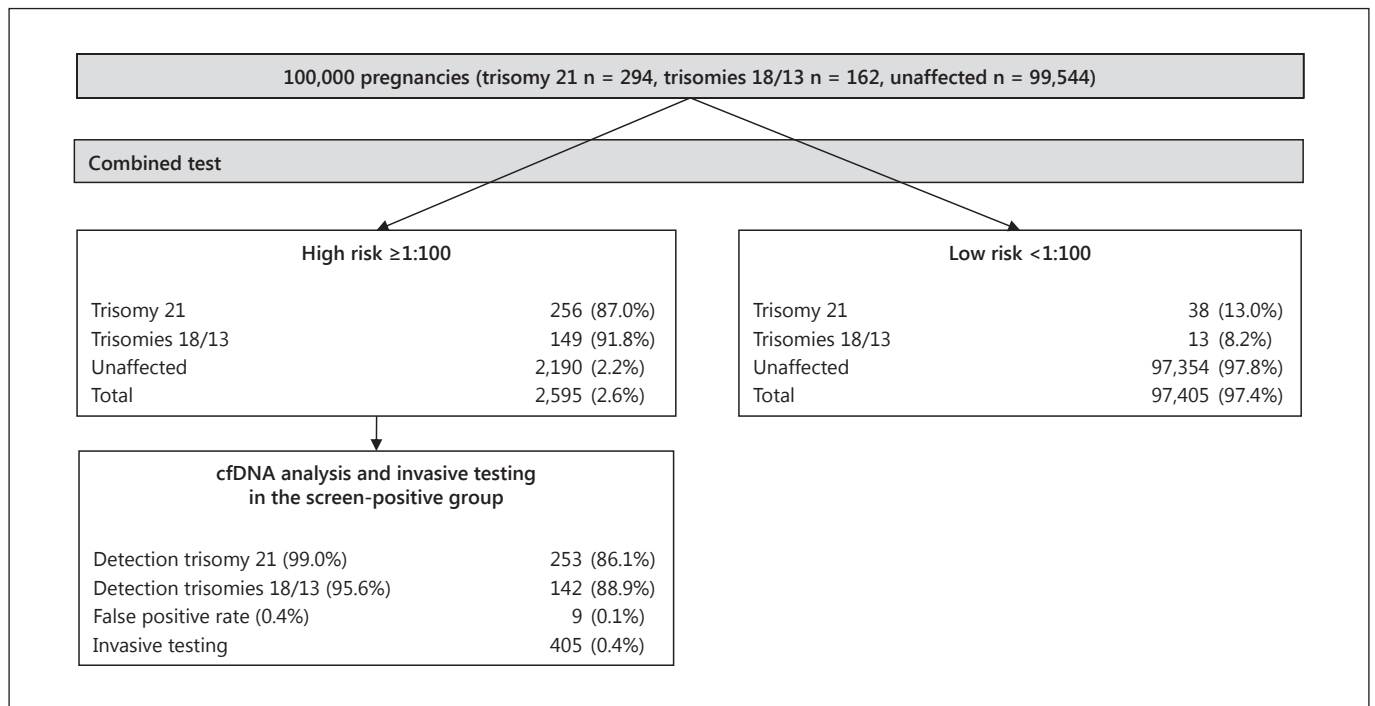
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Nicolaides

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%.

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 13 and 18 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,
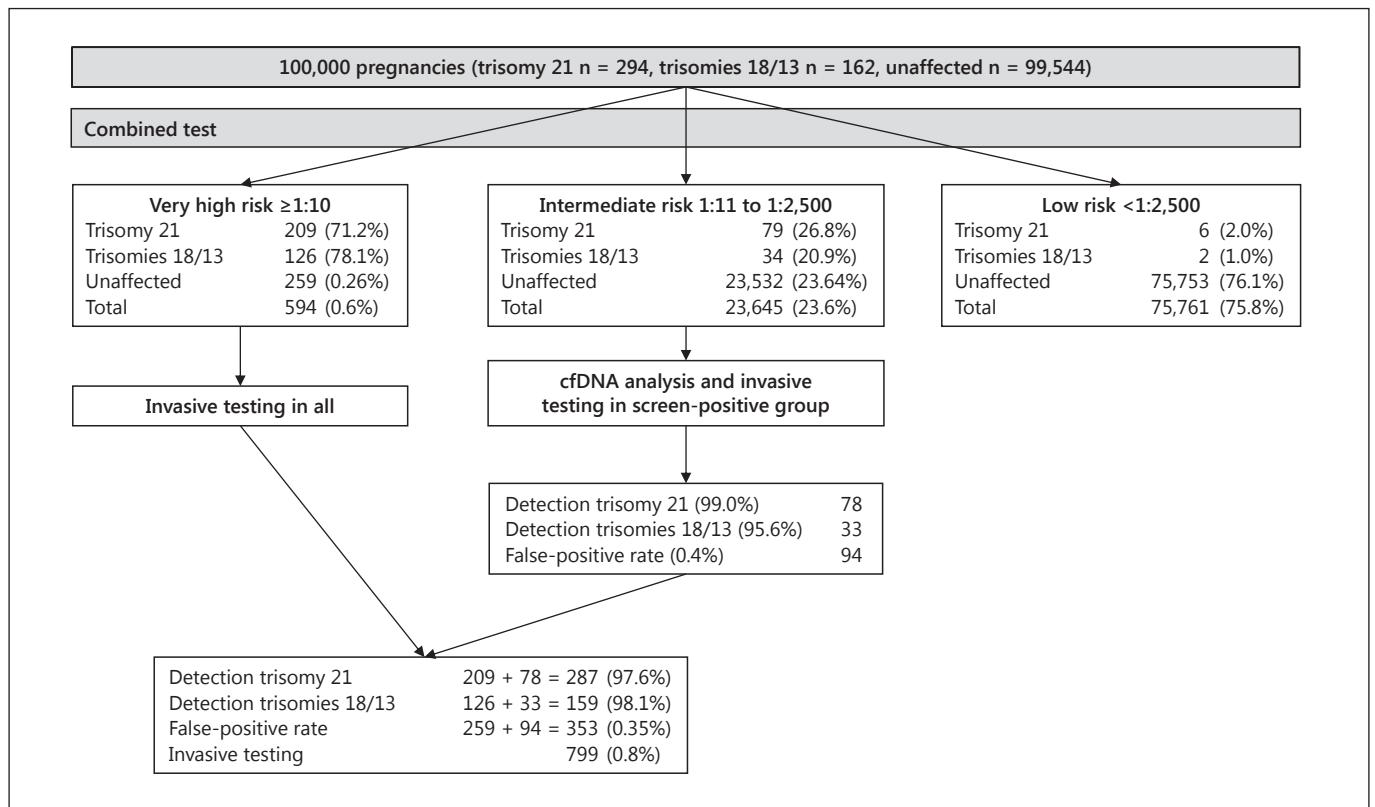
Cell-Free DNA in Screening for
Aneuploidies

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┌─────────────────────────────────────────────────────────────────────────────────────────┐
│  ┌─────────────────────────────────────────────────────────────────────────────────┐    │
│  │   100,000 pregnancies (trisomy 21 n = 294, trisomies 18/13 n = 162, unaffected    │    │
│  │   n = 99,544)                                                                     │    │
│  └─────────────────────────────────────────────────────────────────────────────────┘    │
│  ┌─────────────────────────────────────────────────────────────────────────────────┐    │
│  │  Combined test                                                                    │    │
│  └─────────────────────────────────────────────────────────────────────────────────┘    │
│                                                                                           │
│  ┌───────────────────────────┐  ┌────────────────────────────┐  ┌──────────────────────┐ │
│  │ Very high risk ≥1:10       │  │ Intermediate risk 1:11 to  │  │ Low risk <1:2,500    │ │
│  │ Trisomy 21      209 (71.2%)│  │ 1:2,500                    │  │ Trisomy 21   6 (2.0%)│ │
│  │ Trisomies 18/13 126 (78.1%)│  │ Trisomy 21       79 (26.8%)│  │ Trisomies 18/13      │ │
│  │ Unaffected      259 (0.26%)│  │ Trisomies 18/13  34 (20.9%)│  │              2 (1.0%)│ │
│  │ Total           594 (0.6%) │  │ Unaffected   23,532 (23.64%)│ │ Unaffected           │ │
│  │                            │  │ Total        23,645 (23.6%)│  │          75,753 (76.1%)│ │
│  │                            │  │                            │  │ Total  75,761 (75.8%)│ │
│  └───────────────────────────┘  └────────────────────────────┘  └──────────────────────┘ │
│                                                                                           │
│  ┌───────────────────────────┐  ┌────────────────────────────┐                            │
│  │ Invasive testing in all    │  │ cfDNA analysis and invasive│                            │
│  └───────────────────────────┘  │ testing in screen-positive │                            │
│                                  │ group                      │                            │
│                                  └────────────────────────────┘                            │
│                                  ┌────────────────────────────┐                            │
│                                  │ Detection trisomy 21 (99.0%)  78 │                        │
│                                  │ Detection trisomies 18/13 (95.6%) 33 │                    │
│                                  │ False-positive rate (0.4%)    94 │                        │
│                                  └────────────────────────────┘                            │
│  ┌─────────────────────────────────────────────────────────┐                             │
│  │ Detection trisomy 21       209 + 78 = 287 (97.6%)         │                             │
│  │ Detection trisomies 18/13  126 + 33 = 159 (98.1%)         │                             │
│  │ False-positive rate        259 + 94 = 353 (0.35%)         │                             │
│  │ Invasive testing                     799 (0.8%)           │                             │
│  └─────────────────────────────────────────────────────────┘                             │
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Fig. 8. In a population of 100,000 pregnancies, with the maternal age distribution in England and Wales in 2011, combined screening is used to divide the population into very high-risk (≥1:10), intermediate-risk (1:11–1:2,500) and low-risk (<1:2,500) groups. In the very high-risk group, invasive testing is carried out in all cases. In the intermediate-risk group, cfDNA testing is carried out followed by invasive testing for those with a screen-positive result. Such a strategy could detect about 98% of the fetuses with trisomies 21, 18 and 13, at an overall invasive testing rate of 0.8%.

the overall invasive testing rate would be reduced to 0.4% with detection of about 86% of fetuses with trisomy 21 and 89% with trisomies 13 and 18. Such a strategy would be cost neutral if the cost of cfDNA testing was similar to that of invasive testing, and would reduce the number of invasive testing-related miscarriages. The disadvantage relates not only to the decrease in the detection of trisomies 21, 18 and 13 but also to the ineffectiveness of cfDNA testing, compared to invasive testing, in detecting other aneuploidies.

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

In this model, combined screening is used to divide the population into very high-risk (≥1:10), intermediate-risk (1:11–1:2500) and low-risk (<1:2,500) groups. In the very high-risk group invasive testing is carried out in all cases. In the intermediate-risk group cfDNA testing is carried out followed by invasive testing for those with a screen-positive result. Such a strategy would detect about 98% of 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 to less than 10%, without affecting the overall performance of screening, can potentially 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

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in increasing DR and decreasing FPR, but at considerably lower cost than offering the test to the whole population.

Conclusion

On the basis of clinical validation and/or implementation studies, the DR of trisomies 21, 18 and 13 by cfDNA analysis of maternal blood is about 99, 97 and 92%, respectively, at an overall FPR of 0.4%. We propose different 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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