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Replacing the Combined Test by Cell-Free DNA Testing in Screening for Trisomies 21, 18 and 13: Impact on the Diagnosis of Other Chromosomal Abnormalities

Argyro Syngelaki[a] Eugene Pergament[b] Tessa Homfray[a] Ranjit Akolekar[c]
Kypros H. Nicolaides[a]

[a]Harris Birthright Research Centre of Fetal Medicine, King's College Hospital, London, UK; [b]Northwestern Reproductive Genetics, Chicago, Ill., USA; [c]Fetal Medicine Unit, Medway Maritime Hospital, Gillingham, UK

Key Words
Cell-free DNA testing · Chorionic villus sampling · Fetal karyotyping · Aneuploidies · First-trimester screening

Abstract
Objective: To estimate the proportion of other chromosomal abnormalities that could be missed if combined testing was replaced by cell-free (cf) DNA testing as the method of screening for trisomies 21, 18 and 13. ***Methods:*** The prevalence of trisomies 21, 18 or 13, sex chromosome aneuploidies, triploidy and other chromosomal abnormalities was examined in pregnancies undergoing first-trimester combined screening and chorionic villus sampling (CVS). ***Results:*** In 1,831 clinically significant chromosomal abnormalities in pregnancies with combined risk for trisomies 21, 18 and 13 ≥1:100, the contribution of trisomies 21, 18 or 13, sex chromosome aneuploidies, triploidy and other chromosomal abnormalities at high risk of adverse outcome was 82.9, 8.2, 3.9 and 5.0%, respectively. Combined screening followed by CVS for risk ≥1:10 and cfDNA testing for risk 1:11–1:2,500 could detect 97% of trisomy 21 and 98% of trisomies 18 and 13. Additionally, 86% of monosomy X, half of 47,XXY, 47,XYY or 47,XXX, half of other chromosomal abnormalities and one third of triploidies, which are currently detected by combined screening and CVS for risk ≥1:100, could be detected. ***Conclusions:*** Screening by cfDNA testing, contingent on results of combined testing, improves detection of trisomies, but misses a few of the other chromosomal abnormalities detected by screening with the combined test.

Introduction

Diagnosis of fetal chromosomal abnormalities relies on invasive testing, by chorionic villus sampling (CVS) or amniocentesis, in pregnancies identified by screening to be at high risk for such abnormalities. In the last 40 years prenatal screening for chromosomal abnormalities has focused on trisomy 21 and has evolved from maternal age in the 1970s with detection rate (DR) of 30% at false-positive rate (FPR) of 5%, to a combination of maternal age, fetal nuchal translucency (NT) thickness, fetal heart rate (FHR) and serum-free β-hCG and PAPP-A in the first trimester in the last 15 years, with DR of 90% and FPR of 5% [1]. The emphasis in introducing new methods of screening has resulted in both an increase in DR but also

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Prof. K.H. Nicolaides
Harris Birthright Research Centre for Fetal Medicine
King's College Hospital
Denmark Hill, London SE5 9RS (UK)
E-Mail kypros@fetalmedicine.com

a decrease in FPR. For example, in the last 40 years there has been a major shift in the age of childbirth so that now in many developed countries more than 20% of pregnant women are 35 years or older, compared to about 5% in the 1970s. Consequently, if maternal age had remained the basis of screening the FPR would have increased to more than 20%.

A beneficial consequence of screening for trisomy 21 by the combined test is the early diagnosis of 70–75% of trisomies 18 and 13, because all three trisomies are similar in being associated with increased maternal age, increased fetal NT and decreased serum PAPP-A. 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 FHR 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 5% [2, 3]. In addition to trisomies 21, 18 and 13, invasive testing in the screen-positive group from the combined test detects many other clinically significant chromosomal abnormalities, including sex chromosome aneuploidies, triploidy, rare trisomies, deletions or duplications and mosaicism. However, the biomarker profile for many of these abnormalities is not clearly defined and it is uncertain whether their prevalence in the screen-positive group for trisomies 21, 18 and 13 is higher than in the screen-negative group; if it is not higher, then the DR of these chromosomal abnormalities would inevitably decrease with the introduction of better methods of screening for trisomies 21, 18 and 13, because the FPR and rate of unnecessary invasive testing would decrease.

Several studies in the last 2 years have reported the clinical validation and implementation of screening for aneuploidies by analysis of cell-free (cf) DNA in maternal blood [4]. Most studies have reported on screening for trisomies 21, 18 and 13 and a few have also reported findings in sex chromosome aneuploidies. Some proof-of-principle studies have examined the potential value of cfDNA testing in the detection of triploidy, trisomies other than those affecting chromosomes 21, 18 and 13 and subchromosomal deletions and duplications [5–7]. 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 99.0, 96.8 and 92.1%, respectively, at FPR of 0.08, 0.15 and 0.20% [4].

In this study of singleton pregnancies undergoing first-trimester combined screening, we examine the proportion of different chromosomal abnormalities and their distribution of risk for trisomies 21, 18 and 13 from the combined test. The objective of the study is to estimate the proportion of clinically significant chromosomal abnormalities that could be missed, firstly, if the combined test was to be replaced by cfDNA testing as the primary method of screening for trisomies 21, 18 and 13 and, secondly, if the combined test was retained as the first-line method of screening and cfDNA testing was introduced contingent on the results of the combined test.

Methods

Population Having Invasive Karyotyping

The data for this study were derived from singleton pregnancies undergoing CVS following screening for trisomies 21, 18 and 13 by the combination of maternal age, fetal NT and FHR and maternal serum-free β-hCG and PAPP-A at 11–13 weeks' gestation [2]. Women were counselled as to the results of the screening test and those choosing invasive testing had CVS and full karyotyping from cultured chorionic villi. We searched our database to identify all singleton pregnancies with first-trimester combined screening and fetal karyotyping between July 1999 and July 2013 at King's College Hospital, London and the Fetal Medicine Centre, London, UK.

The results of full karyotype by cytogenetic analysis of samples from CVS and any further investigations including amniocentesis, disomy studies and parental karyotyping were classified as normal or abnormal. The normal group included cases of 46,XY and 46,XX, normal variants, balanced inherited and de novo rearrangements, inherited marker chromosomes and confined placental mosaicism. The abnormal group was further classified into trisomies 21, 18 or 13, monosomy X, other sex chromosome aneuploidies, triploidy and other. The other chromosomal abnormalities included trisomies 8, 9, 16 or 22, deletions or duplications and mosaic trisomies, deletions, duplications or sex chromosome aneuploidies; two independent clinical geneticists subdivided these into high, low or unknown risk of adverse clinical outcome.

Population Undergoing Combined Screening

The potential ability to detect different types of chromosomal abnormalities was examined if cfDNA testing for trisomies 21, 18 and 13 was introduced as a primary method of screening or as a contingent test on the basis of results from primary screening by the combined test. In these calculations we used the data derived from routine screening for trisomies 21, 18 and 13 by a combination of maternal-age, fetal NT, FHR, free β-hCG and PAPP-A at 11^{+0}–13^{+6} weeks' gestation [3]. The pregnancies had screening at King's College Hospital, London, University College Hospital, London and Medway Maritime Hospital, UK, between March 2006 and May 2012. Women considering their risks to be high were offered invasive fetal karyotyping.

Statistical Analysis

Data regarding continuous variables, such as maternal age, fetal CRL, fetal NT thickness, FHR and serum-free β-hCG and PAPP-

A, were expressed as median and interquartile range in the normal and each of the aneuploid groups. Medians were compared using the Mann-Whitney U test with post hoc Bonferroni correction to adjust the significance level for multiple comparisons. Data regarding categorical variables were expressed as number with percentage and were compared between the groups using χ^2 test with Yate's continuity correction.

In the population having invasive karyotyping, in the normal and each of the chromosomal abnormality groups, odds ratios with 95% confidence intervals (CI) were calculated for the proportion of patients with risk for trisomy 21 or risk for trisomies 18 or 13 ≥1:100 compared to those with risk <1:100, as well as in those with a fetal NT thickness ≥3.5 mm compared to those with NT <3.5 mm.

In the population having combined screening, the observed proportion of normal and trisomic pregnancies with fetal NT thickness ≥3.5 mm and ≥4.0 mm were calculated. We also estimated the proportions with risks of ≥1:10, ≥1:20, ≥1:30, ≥1:40, ≥1:50 and ≥1:100 after standardization so that they relate to the pregnant population of England and Wales in 2011 [3].

The potential ability to detect different types of chromosomal abnormalities was examined if cfDNA testing for trisomies 21, 18 and 13 was introduced as a primary method of screening or as a contingent test on the basis of results from primary screening by the combined test (fig. 1, 2). In the first strategy, there is primary screening by cfDNA testing for trisomies 21, 18 and 13 with respective DR of 99, 96.8 and 92.1%, at combined total FPR of 0.4% and no reporting of result rate of 2% [4]. Invasive testing is carried out in women with a screen-positive result from cfDNA testing. In women with no result from cfDNA testing, the results of the combined test are considered and invasive testing is carried out for those with a risk for trisomy 21 or a risk for trisomies 18 or 13 of ≥1:100. In the second strategy, there is primary screening by the combined test and invasive testing is carried out if the risk for trisomy 21 or the risk for trisomies 18 or 13 is ≥1:10, ≥1:20, ≥1:30, ≥1:40, ≥1:50 or ≥1:100 (fig. 2). In addition, cfDNA testing is carried out for those with an intermediate risk between the cut-off for invasive testing and 1:1,000 (or 1:2,500).

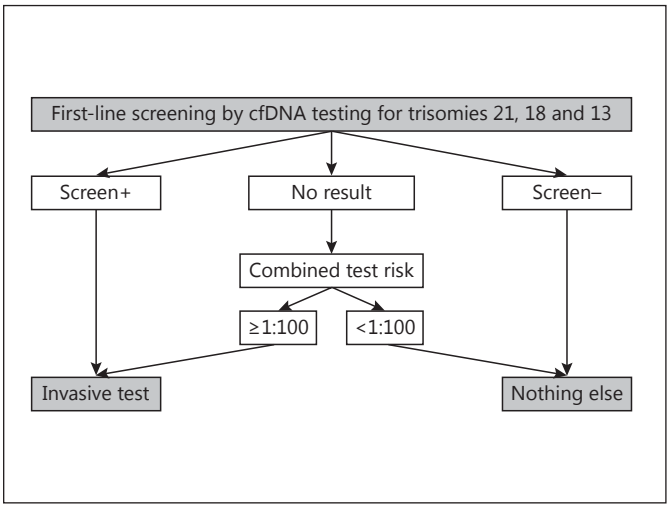

Fig. 1. First-line screening by cfDNA testing is carried out in all pregnancies. In those with a positive result invasive testing is performed and in those with a negative result there is no further testing. In the group of women with no result from cfDNA testing, the results of the combined test are considered and invasive testing is carried out for those with a risk for trisomy 21, 18 or 13 ≥1:100.

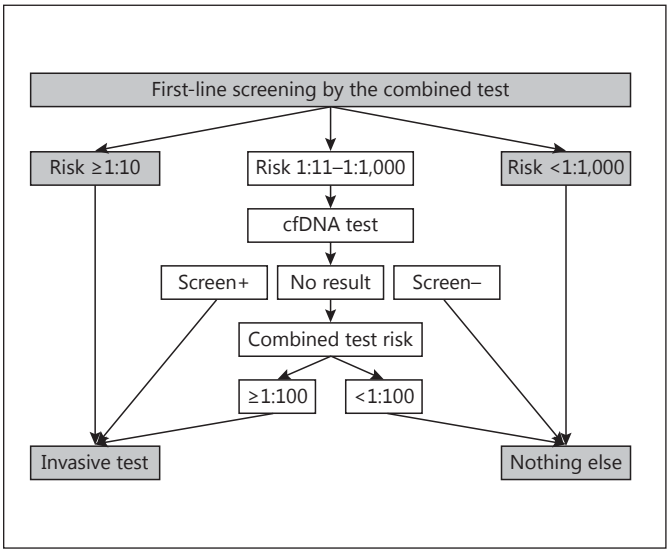

Fig. 2. First-line screening by the combined test is carried out in all pregnancies. In those with a risk for trisomies 21, 18 or 13 ≥1:10 invasive testing is performed and in those with a risk <1:1,000 there is no further testing. In women with risk between 1:11 and 1:1,000 cfDNA testing is carried out. In those with a positive cfDNA result invasive testing is performed and in those with a negative result there is no further testing. In the group of women with no result from cfDNA testing, the results of the combined test are considered and invasive testing is carried out for those with a risk for trisomy 21, 18 or 13 ≥1:100.

Results

Characteristics of the Study Population Having Invasive Karyotyping

The study population consisted of 14,684 singleton pregnancies and on the basis of the CVS karyotype and the results of any necessary further investigations, 12,654 (86.2%) were classified as normal and 2,030 (13.8%) as abnormal (table 1). In the abnormal group, 79.2% cases were trisomies 21, 18 or 13, 8.4% were sex chromosome aneuploidies, 3.9% were triploidies and 8.5% were other chromosomal abnormalities. The other abnormalities were subdivided into those at high, low and unknown risk of adverse clinical outcome. The 134 cases of other chromosomal abnormalities at high risk of adverse clinical outcome included deletions or duplications (n = 56), tri-

Table 1. Median (IQR) of maternal age, fetal CRL, fetal NT thickness, FHR and serum-free β-hCG and PAPP-A according to fetal karyotype

Aneuploidy	n	Age, years	CRL, mm	NT, mm	FHR, bpm	PAPP-A, MoM	Free β-hCG, MoM
Normal in screened population	73,964	31.2 (26.7–35.1)	63.1 (58.1–68.7)	1.8 (1.5–2.1)	159 (155–164)	1.023 (0.700–1.452)	0.977 (0.665–1.473)
Pregnancies having invasive testing							
Normal	12,654	37.4 (33.6–40.3)	64.0 (58.2–70.7)	2.0 (1.6–2.5)	160 (155–165)	0.840 (0.524–1.272)	1.226 (0.776–2.019)
Trisomies 21, 18 or 13							
Trisomy 21	1,089	38.7 (35.9–41.0)[a,b]	65.0 (59.1–71.5)[a,b]	3.6 (2.7–5.1)[a,b]	161 (155–166)[a,b]	0.557 (0.364–0.855)[a,b]	2.243 (1.505–3.306)[a,b]
Trisomy 18	379	38.9 (35.4–41.6)[a,b]	55.8 (50.9–62.0)[a,b]	4.8 (2.4–7.2)[a,b]	160 (155–166)	0.187 (0.112–0.306)[a,b]	0.218 (0.132–0.355)[a,b]
Trisomy 13	140	37.2 (33.6–39.6)[a]	58.6 (53.5–64.7)[a,b]	4.8 (2.5–6.9)[a,b]	180 (172–184)[a,b]	0.287 (0.182–0.403)[a,b]	0.526 (0.356–0.783)[a,b]
Sex chromosome aneuploidies							
Monosomy X	134	32.2 (27.6–35.5)[b]	61.4 (55.7–66.6)[a,b]	8.9 (6.9–10.3)[a,b]	170 (163–178)[a,b]	0.450 (0.281–0.621)[a,b]	1.169 (0.663–1.834)
47,XXX or 47,XXY, or 47,XYY	36	38.4 (34.7–41.6)[a]	64.7 (57.8–68.6)	2.1 (1.8–3.5)[a]	158 (155–162)	0.783 (0.566–1.152)	0.923 (0.533–1.374)
Triploidy							
69,XXX or 69,XXY, or 69,XYY	79	34.1 (29.9–36.8)[a,b]	53.9 (50.0–59.1)[a,b]	1.5 (1.2–2.2)[b]	157 (151–162)[a,b]	0.079 (0.043–0.295)[a,b]	0.170 (0.083–4.477)[a,b]
Diandric	23	34.5 (29.8–35.7)[b]	61.2 (53.4–63.5)[b]	3.6 (2.2–7.0)[a,b]	161 (151–167)	0.813 (0.501–1.815)	9.462 (7.049–18.010)[a,b]
Digynic	56	33.5 (30.0–36.9)[a,b]	52.3 (48.7–57.0)[a,b]	1.3 (1.2–1.6)[a,b]	157 (152–160)[a,b]	0.058 (0.039–0.091)[a,b]	0.107 (0.058–0.210)[a,b]
All other aneuploidies							
High risk of adverse outcome	134	35.4 (31.8–39.3)[a,b]	61.4 (55.5–67.1)[b]	2.2 (1.8–3.6)[a,b]	160 (155–167)	0.526 (0.322–0.835)[a,b]	1.292 (0.645–2.019)
Low risk of adverse outcome	22	35.6 (34.1–38.7)[a]	65.0 (58.8–74.6)	2.3 (1.8–3.2)[a]	161 (157–170)	0.855 (0.438–1.201)	1.551 (0.734–3.185)
Unknown risk of adverse outcome	17	38.7 (34.3–41.9)[a]	62.8 (60.0–68.8)	2.1 (1.7–2.7)	161 (155–167)	0.478 (0.271–0.726)[a,b]	1.708 (1.291–3.014)[a]

IQR = Interquartile range; MoM = multiple of the median. [a] Significant difference from median of the normal group of the screened population. [b] Difference from median of the normal group of the pregnancies having invasive testing. [a,b] Comparison of medians (Mann-Whitney U test with post hoc Bonferroni correction for multiple comparisons: significant difference if $p < 0.005$).

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Table 2. Comparison of prevalence of chromosomal abnormalities in the groups with estimated risk for trisomy 21 or trisomies 18 or 13 of ≥1:100 vs. <1:100 and fetal NT thickness of ≥3.5 mm vs. <3.5 mm in the population having invasive karyotyping

Fetal karyotype	n	Estimated risk for trisomy 21 or trisomies 18 and 13			NT thickness		
		≥1:100	<1:100	odds ratio (95% CI)	≥3.5 mm	<3.5 mm	odds ratio (95% CI)
All	14,684	6,498	8,186		2,069	12,615	
Normal	12,654	4,641 (71.42)	8,013 (97.89)	30.2 (25.7–35.5)*	977 (47.22)	11,677 (92.56)	0.07 (0.06–0.08)*
Trisomies 21, 18 or 13							
Trisomy 21	1,089	1,014 (15.60)	75 (0.92)	24.3 (19.2–30.8)*	583 (28.18)	506 (4.01)	9.39 (8.24–10.70)*
Trisomy 18	379	370 (5.69)	9 (0.11)	58.3 (30.1–113.1)*	231 (11.16)	148 (1.17)	10.59 (8.56–13.09)*
Trisomy 13	140	134 (2.06)	6 (0.07)	29.3 (12.9–66.4)*	88 (4.25)	52 (0.41)	10.73 (7.59–15.17)*
Sex chromosome aneuploidies							
Monosomy X	134	131 (2.01)	3 (0.04)	57.3 (18.2–180.0)*	126 (6.09)	8 (0.06)	102.19 (49.93–209.15)*
47,XXX or 47,XXY, or 47,XYY	36	19 (0.29)	17 (0.21)	1.41 (0.73–2.72)	11 (0.53)	25 (0.20)	2.69 (1.32–5.48)*
Triploidy							
69,XXX or 69,XXY, or 69,XYY	79	72 (1.11)	7 (0.09)	13.2 (6.1–28.8)*	13 (0.63)	66 (0.52)	1.20 (0.66–2.18)
Diandric	23	21 (0.32)	2 (0.02)	13.3 (3.1–56.8)*	13 (0.63)	10 (0.08)	7.97 (3.49–18.20)*
Digynic	56	51 (0.78)	5 (0.06)	13.0 (5.2–32.7)*	0	56 (0.44)	–
Other aneuploidies							
High risk of adverse outcome	134	91 (1.40)	43 (0.53)	2.7 (1.9–3.9)*	35 (1.69)	99 (0.78)	2.18 (1.48–3.21)*
Low risk of adverse outcome	22	13 (0.20)	9 (0.11)	1.8 (0.8–4.3)	4 (0.19)	18 (0.14)	1.36 (0.46–4.01)
Unknown risk of adverse outcome	17	13 (0.20)	4 (0.05)	4.1 (1.3–12.6)	1 (0.05)	16 (0.13)	0.38 (0.05–2.87)

Values are numbers (with percentages in parentheses) or odds ratios (with 95% CI in parentheses). * $p < 0.001$.

somies 8, 9, 16 or 22 (n = 21), mosaic deletions or duplications (n = 17), mosaic sex aneuploidies (n = 7), mosaic trisomies 18 or 21 (n = 14) and mosaic trisomies 2, 4, 5, 7, 8, 9, 10, 12, 15, 16, 17, 20, or 22 (n = 19).

In the 1,831 clinically significant chromosomal abnormalities in the group with risk for trisomies 21, 18 and 13 of ≥1:100, the contribution of trisomies 21, 18 or 13, sex chromosome aneuploidies, triploidy and other abnormalities at high risk for adverse clinical outcome was 82.9, 8.2, 3.9 and 5.0%, respectively (table 2.).

Biomarker Levels and Risks from the Combined Test in the Population Having Invasive Karyotyping

In the invasive karyotyping group, the distribution of maternal age, fetal NT, FHR and serum-free β-hCG and PAPP-A in the normal fetuses and in each of the chromosomal abnormality groups are shown in table 1. The medians from each abnormal group were compared with the median of the normal group of both the screened population [3] and those having invasive testing. Compared to marker levels in the normal groups, in trisomy 21 there was higher maternal age, fetal NT, FHR and free β-hCG and lower PAPP-A; in trisomy 18 there was higher maternal age and fetal NT and lower free β-hCG and PAPP-A; in trisomy 13 there was higher maternal age, fetal NT and FHR and lower free β-hCG and PAPP-A; in monosomy X there was higher fetal NT and FHR and lower PAPP-A; in diandric triploidy there was higher fetal NT and free β-hCG, whereas in digynic triploidy there was lower fetal NT, FHR, free β-hCG and PAPP-A, and in other chromosomal abnormalities at high risk of adverse outcome there was higher maternal age, fetal NT and lower PAPP-A. In some cases there were different marker levels in the aneuploid groups when they were compared to levels in the normal pregnancies of the screened and the invasively tested groups. For example, the median maternal age in the monosomy X group was not significantly different from the age of the normal group in the screened population but was significantly lower than in the normal group of the invasive testing cases.

The prevalence of chromosomal abnormalities in the groups with estimated risk for trisomy 21 or trisomies 18 or 13 of ≥1:100 versus <1:100 and fetal NT thickness of ≥3.5 mm versus <3.5 mm is shown in table 2. In those with risk ≥1:100 or NT ≥3.5 mm the prevalence of trisomies 21, 18 and 13, monosomy X, triploidy and other abnormalities at high risk for adverse outcome, but not in other sex chromosome aneuploidies or other abnormalities at low risk of adverse outcome, was significantly higher than in those with risk <1:100 or NT <3.5 mm. For example, the odds ratio for monosomy X in pregnancies with risk ≥1:100, compared to those with risk <1:100, was 57.3 and the respective value for those of other chromosomal abnormalities at high risk of adverse outcome was 2.7.

The proportions of different karyotype groups with risks from the combined test with the algorithm for trisomy 21 and the algorithm for trisomies 18 and 13 of ≥1:10, ≥1:20, ≥1:30, ≥1:40, ≥1:50 and ≥1:100 and fetal NT of ≥3.5 mm and ≥4.0 mm are summarized in table 3. The proportions with risk ≥1:10 were 93.3% for monosomy X, 16.7% for other sex chromosome aneuploidies, 26.6% for triploidy and 35.1% for other abnormalities at high risk of adverse outcome. Similarly, the proportions with NT ≥3.5 mm were 94.0% for monosomy X, 30.6% for other sex chromosome aneuploidies, 16.5% for triploidy and 26.1% for other abnormalities at high risk of adverse outcome (table 3).

Chromosomal Abnormalities in the Screened Population Undergoing Combined Testing

The study population of 74,561 singleton pregnancies included 597 (0.8%) with chromosomal abnormalities and 73,964 unaffected pregnancies with normal fetal karyotype or the birth of a phenotypically normal neonate. The abnormal group consisted of trisomy 21 (n = 303), trisomy 18 (n = 114), trisomy 13 (n = 39), monosomy X (n = 44), other sex chromosome aneuploidies (n = 18), triploidy (n = 28) and other chromosomal abnormalities (n = 51: 36 high, 10 low and 5 unknown risk of adverse clinical outcome).

In the subgroup of 3,947 pregnancies with risk for trisomies 21, 18 and 13 of ≥1:100, there were 520 (13.2%) clinically significant chromosomal abnormalities. The proportions of trisomies 21, 18 or 13, sex chromosome aneuploidies, triploidy and other abnormalities at high risk of adverse clinical outcome were 80.6, 10.6, 4.8 and 4.0%, respectively (table 4).

Estimated Detection of 47,XXY, 47,XYY or 47,XXX by the Combined Test

In the screening study of 74,561 pregnancies, 18 cases of 47,XXY, 47,XYY or 47,XXX (0.024% or 1:4,167) were identified. The risk for trisomies 21, 18 or 13 from the combined test was ≥1:100 in 12 (66.7%) of the 18 cases of 47,XXY, 47,XYY or 47,XXX. However, this high DR is likely to be an overestimate because most of the sex chromosome aneuploidies, unlike trisomy 21, would not have been detected by clinical examination at birth.

Table 3. Proportion of different chromosomal abnormalities with risks from the combined test with the algorithm for trisomy 21 and the algorithm for trisomies 18 and 13 at ≥1:10, ≥1:20, ≥1:30, ≥1:40, ≥1:50 and ≥1:100 and fetal NT thickness at ≥3.0 mm, ≥3.5 mm and ≥4.0 mm in the population having invasive karyotyping

Abnormal fetal karyotype	n	Estimated risk for trisomy 21 or trisomies 18 and 13						NT thickness		
		≥1:10	≥1:20	≥1:30	≥1:40	≥1:50	≥1:100	≥4.0 mm	≥3.5 mm	≥3.0 mm
Monosomy X	134	125 (93.3)	126 (94.0)	127 (94.8)	128 (95.5)	129 (96.3)	131 (97.8)	124 (92.5)	126 (94.0)	127 (94.8)
47,XXX or 47,XXY, or 47,XYY	36	6 (16.7)	9 (25.0)	12 (33.3)	14 (38.9)	15 (41.7)	19 (52.8)	6 (16.7)	11 (30.6)	12 (33.3)
69,XXX or 69,XXY, or 69,XYY	79	21 (26.6)	30 (38.0)	38 (48.1)	47 (59.5)	54 (68.4)	72 (91.1)	11 (13.9)	13 (16.5)	14 (17.7)
Others at high risk of adverse outcome	134	47 (35.1)	61 (45.5)	75 (56.0)	78 (58.2)	80 (59.7)	91 (67.9)	30 (22.4)	35 (26.1)	45 (33.6)

Values are numbers (with percentages in parentheses).

Table 4. Proportion of different chromosomal abnormalities with risks from the combined test with the algorithm for trisomy 21 and the algorithm for trisomies 18 and 13 at ≥1:10, ≥1:20, ≥1:30, ≥1:40, ≥1:50 and ≥1:100 and fetal NT thickness at ≥3.5 mm and ≥4.0 mm in the screened population of 74,561 pregnancies undergoing routine first trimester combined testing

Cut-off	Normal (n = 73,964)	Trisomy 21 (n = 303)	Trisomies 18/13 (n = 153)	Monosomy X		47,XXY, 47,XYY or 47,XXX		Triploidy (n = 28)	Other high-risk adverse outcome	
				observed (n = 44)	adjusted (n = 50)	observed (n = 18)	adjusted (n = 149)		observed (n = 36)	adjusted (n = 162)
Risk ≥1:10	*392 (0.5%)*	*228 (75.2%)*[a]	*116 (75.8%)*[a]	*37 (84.1%)*	*37 (74.0%)*	*5 (27.8%)*	*5 (3.4%)*	*8 (28.6%)*	*11 (30.3%)*	*11 (6.8%)*
Risk ≥1:20	837 (1.1%)	247 (81.5%)	130 (85.0%)	40 (90.9%)	40 (80.0%)	7 (38.9%)	7 (4.7%)	10 (35.7%)	13 (36.1%)	13 (8.0%)
Risk ≥1:30	1,198 (1.6%)	254 (83.8%)	133 (86.9%)	41 (93.2%)	41 (82.0%)	8 (44.4%)	8 (5.4%)	12 (42.9%)	18 (50.0%)	18 (11.1%)
Risk ≥1:40	1,537 (2.1%)	258 (85.1%)	136 (88.9%)	42 (95.5%)	42 (84.0%)	10 (55.6%)	10 (6.7%)	15 (53.6%)	20 (55.6%)	20 (12.3%)
Risk ≥1:50	1,898 (2.6%)	262 (86.5%)	137 (89.5%)	42 (95.5%)	42 (84.0%)	11 (61.1%)	11 (7.4%)	18 (64.3%)	20 (55.6%)	20 (12.3%)
Risk ≥1:100	3,427 (4.6%)	275 (90.8%)	144 (94.1%)	43 (97.7%)	43 (86.0%)	12 (66.7%)	12 (8.1%)	25 (89.3%)	21 (58.3%)	21 (13.0%)
NT ≥4.0 mm	191 (0.3%)	126 (41.6%)	84 (54.9%)	40 (90.9%)	40 (80.0%)	4 (22.2%)	4 (2.7%)	3 (10.7%)	7 (19.4%)	7 (4.3%)
NT ≥3.5 mm	*373 (0.5%)*	*164 (54.1%)*	*90 (58.8%)*	*41 (93.2%)*	*41 (82.0%)*	*7 (38.9%)*	*7 (4.7%)*	*4 (14.3%)*	*8 (22.2%)*	*8 (4.9%)*

[a] A policy of selecting the high-risk group for invasive testing based on the combined risk of ≥1:10 vs. fetal NT thickness ≥3.5 mm (in italics), would have the same false-positive rate of 0.5%, significantly higher detection rate of trisomies 21, 18 and 13, but no significant differences in the detection of other chromosomal abnormalities.

A study involving karyotyping of umbilical cord blood obtained from 34,910 live births reported that the combined birth prevalence of 47,XXY, 47,XYY and 47,XXX was about 1:500 [8]. There is some evidence suggesting that the rate of intrauterine lethality of these aneuploidies is not higher than in euploid fetuses [9]. Therefore, on the assumption that their prevalence at 11–13 weeks' gestation is similar to that in live births, our population of 74,561 pregnancies would contain 149 cases of these aneuploidies. Consequently, combined screening for trisomies 21, 18 and 13 at a risk of ≥1:100 would detect only 8.1% (12 of the expected 149 cases in the screened population) rather than 66.7% (12 of the detected 18 cases).

On the assumption that our population of 74,561 pregnancies contained 149 cases of 47,XXY, 47,XYY or 47,XXX, the proportion with risk for trisomies 21, 18 or 13 of ≥1:10, ≥1:20, ≥1:30, ≥1:40, ≥1:50 or ≥1:100 would be 3.4, 4.7, 5.4, 6.7, 7.4 and 8.1%, respectively, and those with fetal NT of ≥4 mm or ≥3.5 mm would be 2.7 and 4.7%, respectively (table 4).

Estimated Detection of Monosomy X by the Combined Test

In the screening study of 74,561 pregnancies, 44 cases of monosomy X (0.06% or 1:1,667) were identified. The risk for trisomies 21, 18 or 13 from the combined test was ≥1:100 in 43 (97.7%) of the cases of monosomy X. However, as in the case of the other sex chromosome aneuploidies, this high DR is likely to be an overestimate because many of the affected cases would not have been detected by clinical examination at birth.

Monosomy X, with an estimated prevalence of 1:1,500 at 12 weeks and 1:4,200 at 40 weeks, is associated with a high rate of intrauterine lethality of about 65% between 12 and 40 weeks [10]. It could therefore be assumed that at 12 weeks' gestation our population of 74,561 pregnancies would contain 50 cases of monosomy X; in the absence of prenatal diagnosis and selective termination, 32 would be expected to die in utero and only 18 to be liveborn. Combined screening for trisomies 21, 18 and 13 at a risk of ≥1:100 would detect only 86% (43 of the expected 50 cases in the screened population) rather than 97.7% (43 of the detected 45 cases).

Fetal NT is very high in most of the detected fetuses and intrauterine lethality is known to increases with high NT. It is therefore conceivable that most of the detected cases would have died in utero. On the extreme assumption that all 32 of the estimated intra-uterine deaths are from those with a risk of ≥1:100, then the detection of potential live births by the combined test could be as low as 61.1% (11 of 18).

On the assumption that our population of 74,561 pregnancies contained 50 cases of monosomy X, the proportion with risk for trisomies 21, 18 or 13 of ≥1:10, ≥1:20, ≥1:30, ≥1:40, ≥1:50 or ≥1:100 would be 74.0, 80.0, 82.0, 84.0, 84.0 and 86.0%, respectively, and those with fetal NT of ≥4 mm or ≥3.5 mm would be 80.0 and 82.0%, respectively (table 4).

Estimated Detection of Triploidy by the Combined Test

In the screening study of 74,561 pregnancies, 28 cases of triploidy were identified. Previous studies estimated that the prevalence of this aneuploidy, which is not related to maternal age and is associated with a very high rate of intrauterine lethality, decreases from about 1:1,000 at 10 weeks to 1:3,500 at 12 weeks, 1:10,000 at 14 weeks and 0% at 40 weeks [9, 11]. It is therefore likely that we have identified most if not all affected cases in the study population and in about 90% the estimated risk for trisomies 21, 18 or 13 was >1:100.

In the 28 cases of triploidy, the proportion with risk for trisomies 21, 18 or 13 of ≥1:10, ≥1:20, ≥1:30, ≥1:40, ≥1:50 or ≥1:100 were 28.6, 35.7, 42.9, 53.6, 64.3 and 89.3%, respectively, and those with fetal NT of ≥4 mm or ≥3.5 mm were 10.7 and 14.3%, respectively (table 4).

Estimated Detection of Other Chromosomal Abnormalities at High Risk of Adverse Outcome by the Combined Test

In the screening study of 74,561 pregnancies, 36 cases of other chromosomal abnormalities at high risk of adverse outcome were identified and in 21 (58.3%) of these the risk for trisomies 21, 18 or 13 was ≥1:100. There are no population-based data on the prevalence of these abnormalities at 12 weeks' gestation or in live births and it is therefore impossible to derive an accurate estimate of what is the proportion of affected cases that is represented by our 36 detected cases.

In our invasive karyotyping population of 14,684 pregnancies, there were 134 such chromosomal abnormalities and their prevalence in the group with risk for trisomies 21, 18 or 13 of ≥1:100 was 2.7 times higher than in those with a risk of <1:100. On the assumption that this relative proportion would be also true for our screened population, then the total population of 74,561 pregnancies would contain 162 other abnormalities at high risk of adverse outcome. Consequently, combined screening for trisomies 21, 18 and 13 at a risk of ≥1:100 would de-

tect only 13.0% (21 of the expected 162 cases in the screened population) rather than 58.3% (21 of the detected 36 cases).

We estimated that the proportion of other chromosomal abnormalities at high risk of adverse outcome with risk for trisomies 21, 18 or 13 of ≥1:10, ≥1:20, ≥1:30, ≥1:40, ≥1:50 or ≥1:100 would be 6.8, 8.0, 11.1, 12.3, 12.3 and 13.0%, respectively, and those with fetal NT of ≥4 mm or ≥3.5 mm would be 4.3 and 4.9%, respectively (table 4). In the euploid group the prevalence of risk ≥1:10 and NT ≥3.5 mm was the same at 0.5%. A policy of selecting the high-risk group for invasive testing based on the combined risk of ≥1:10 versus fetal NT thickness ≥3.5 mm would have the same FPR of 0.5%, significantly higher DR of trisomy 21 ($p < 0.0001$) and trisomies 18 and 13 ($p = 0.0023$), but no significant differences in the detection of monosomy X ($p = 0.469$), other sex chromosome aneuploidies ($p = 0.769$), triploidy ($p = 0.329$) or other abnormalities at high risk of adverse outcome ($p = 0.637$).

Implications of Universal Screening by the Combined Test for Trisomies 21, 18 and 13

In this approach combined screening is carried out in all pregnancies and invasive testing is performed in women with a risk for trisomy 21 or a risk for trisomies 18 or 13 of ≥1:100. We estimated that in a population with the maternal age distribution of pregnancies in England and Wales in 2011, such a policy would lead to an invasive testing rate of 2.6% and the detection of about 87% of cases of trisomy 21 and 92% of trisomies 18 and 13 [3]. In this study, we estimated that such a policy would also detect 86% of cases of monosomy X, 8.1% of other sex chromosome aneuploidies, 89.3% of triploidies and 13.0% of other chromosomal abnormalities at high risk of adverse outcome (table 5).

Implications of Universal Screening by cfDNA Testing for Trisomies 21, 18 and 13

In this approach cfDNA testing for trisomies 21, 18 and 13 is carried out in all pregnancies and invasive testing is performed in women with a screen-positive result (fig. 1). In the 2% of women with no result from cfDNA testing, the results of the combined test are considered and invasive testing is carried out for those with a risk for trisomy 21 or a risk for trisomies 18 or 13 of ≥1:100. Such a policy would lead to an invasive testing rate of 0.9% and the detection of 98.6% of cases of trisomy 21 and 95.7% of trisomies 18 and 13, but none of the other chromosomal abnormalities.

Table 5. Estimated detection rates of different chromosomal abnormalities by a strategy of first-line screening by the combined test and the use of algorithms for trisomy 21 and trisomies 18 or 13 in a population with the maternal age distribution of pregnancies in England and Wales in 2011 [3]

Strategy	Combined screening		cfDNA, %	Invasive test, %	Detection rate, %					
	high risk	intermediate risk			trisomy 21	trisomies 18/13	monosomy X[a]	other sex aneuploidy[a]	triploidy[a]	other high-risk adverse outcome[a]
Current combined screening with invasive testing for risk ≥1:100			–	2.6	87.0	91.8	86.0	8.1	89.3	13.0
Universal cfDNA testing			100	0.9	98.6	95.7	–	–	–	–
Universal combined screening	risk ≥1:10	risk 1:11 – 1:1,000	12.8	0.8	95.9	97.5	74.0	3.4	28.6	6.8
cfDNA test: intermediate risk	risk ≥1:20	risk 1:21 – 1:1,000	12.5	1.1	95.9	97.5	80.0	4.7	35.7	8.0
Invasive test:	risk ≥1:30	risk 1:31 – 1:1,000	12.3	1.3	96.3	97.5	82.0	5.4	42.9	11.1
• High risk	risk ≥1:40	risk 1:41 – 1:1,000	12.0	1.6	96.3	97.5	84.0	6.7	53.6	12.3
• Positive cfDNA test	risk ≥1:50	risk 1:51 – 1:1,000	11.8	1.8	96.3	97.5	84.0	7.4	64.3	12.3
• No result from cfDNA test and risk of 1:100 from combined test	risk ≥1:100	risk 1:101 – 1:1,000	10.8	2.9	96.3	98.1	86.0	8.1	89.3	13.0
	risk ≥1:10	risk 1:11 – 1:2,500	23.6	0.9	97.3	98.1	74.0	3.4	28.6	6.8
	risk ≥1:20	risk 1:21 – 1:2,500	23.4	1.2	97.6	98.1	80.0	4.7	35.7	8.0
	risk ≥1:30	risk 1:31 – 1:2,500	23.1	1.5	97.6	98.1	82.0	5.4	42.9	11.1
	risk ≥1:40	risk 1:41 – 1:2,500	22.9	1.7	98.0	98.1	84.0	6.7	53.6	12.3
	risk ≥1:50	risk 1:51 – 1:2,500	22.7	2.0	98.0	98.1	84.0	7.4	64.3	12.3
	risk ≥1:100	risk 1:101 – 1:2,500	21.6	3.2	98.0	98.8	86.0	8.1	89.3	13.0

The high-risk group, defined by risk cut-offs for trisomies 21, 18 and 13, has invasive testing. The intermediate-risk group has cfDNA testing for trisomies 21, 18 and 13. Also provided are the estimates from a policy of universal screening by cfDNA testing and the current method of combined screening followed by invasive testing in those with a risk of ≥1:100. The risks for sex chromosome aneuploidies, triploidy and other abnormalities at high risk of adverse outcome were assumed to be unrelated to maternal age. [a] See table 4.

Implications of Screening by the Combined Test and cfDNA Testing for the Intermediate-Risk Group

In this strategy, there is primary screening by the combined test and invasive testing is carried out if the risk for trisomy 21 or the risk for trisomies 18 or 13 is ≥1:10, ≥1:20, ≥1:30, ≥1:40, ≥1:50 or ≥1:100 (fig. 2). In addition, cfDNA testing is carried out for those with an intermediate risk between the cut-off for invasive testing and 1:1,000 (or 1:2,500). Invasive testing is carried out, firstly, in those with a high risk, secondly, in those with a positive result from cfDNA testing and, thirdly, in those with no result from cfDNA testing and a risk of ≥1:100 from the combined test. The results of this approach are summarized in table 5.

The rate of invasive testing and DR for trisomies 21, 18 and 13 increases with the risk cut-off that defines the high-risk group. The only cut-off that retains the invasive testing rate to a level similar to that of universal screening by cfDNA testing (less than 1%) is 1:10.

A policy of first-line screening by the combined test followed by invasive testing in those with risk ≥1:10 and cfDNA testing in those with risk of 1:11–1:1,000 would lead to detection of 95.9% of cases of trisomy 21 and 97.5% of trisomies 18 and 13, with cfDNA testing in 12.8% of the population and a total invasive testing rate of 0.8%. Offering cfDNA testing to those with risk of 1:11–1:2,500 would improve the DR of trisomy 21 to 97.3% and of trisomies 18 and 13 to 98.1%, but would double the rate of cfDNA testing to 23.6% (table 5). These policies could also potentially detect 74.0% of cases of monosomy X, 3.4% of other sex chromosome aneuploidies, 28.6% of triploidies and 6.8% of other chromosomal abnormalities at high risk of adverse outcome.

The proposed new strategy, compared to the current one of combined screening followed by invasive testing for those with a risk of ≥1:100, has a lower invasive testing rate, higher DR for trisomies 21, 18 and 13, but lower DR for other chromosomal abnormalities.

Discussion

Principal Findings of This Study

This study in pregnancies undergoing CVS for fetal karyotyping after first-trimester combined screening for trisomies 21, 18 and 13 has demonstrated three findings. Firstly, trisomies 21, 18 and 13 account for about 80% of the detected clinically significant chromosomal abnormalities. Secondly, the distribution of some or all marker levels, including maternal age, fetal NT, FHR and serum free β-hCG and PAPP-A, in the various abnormalities are significantly different from those in the normal pregnancies. Thirdly, the prevalence of trisomies 21, 18 and 13, monosomy X, triploidy and other abnormalities at high risk of adverse outcome is higher in the group with estimated risk for trisomies 21, 18 or 13 of ≥1:100 compared to those with risk of <1:100, and also in those with fetal NT ≥3.5 mm compared to those with NT <3.5 mm. Consequently, these aneuploidies are preselected, to varying degrees, by the first-trimester combined test.

In sex chromosome aneuploidies, other than monosomy X, and other chromosomal abnormalities at low-risk of adverse outcome the distribution of marker levels and risks were similar to those of the euploid group undergoing invasive testing. This suggests that these abnormalities are not over-represented in the pregnancies identified by the combined test as being at high risk for trisomies 21, 18 and 13.

In the case of trisomies 21, 18 and 13, extensive studies have established the relationship of their prevalence with maternal age and gestational age and have defined their characteristic first-trimester biomarker profile [1]. Data concerning other chromosomal abnormalities are limited. Our study has, firstly, shown that the prevalence of monosomy X, triploidy and other aneuploidies at high risk of adverse outcome is higher in the screen-positive group for trisomies 21, 18 and 13 than in the screen-negative group and, secondly, provided some data on the first-trimester biomarker profile for these aneuploidies.

Our data have also highlighted, through the example of sex chromosome aneuploidies, two important aspects of screening for chromosomal abnormalities; firstly, the need for ascertainment of all affected cases in the population examined and, secondly, definition of the potential impact of prenatal diagnosis on the prevalence of a given abnormality in live births. Unlike the situation with trisomies 21, 18 and 13, most neonates with sex chromosome aneuploidies and those in the heterogeneous group classified as other chromosomal abnormalities at high risk of adverse clinical outcome are often phenotypically normal. Consequently, studies that do not involve karyotyping of the whole population will inevitably underestimate the true prevalence of these abnormalities and overestimate the potential sensitivity of a prenatal screening test. As illustrated in our study, the erroneous conclusion could be reached that screening for trisomies 21, 18 and 13 by the combined test and carrying out an invasive test for those with a risk of ≥1:100 could identify 67% of fetuses with 47,XXY, 47,XYY or 47,XXX, 98% of those with monosomy X and 58% of those with other abnormalities at high risk of adverse outcome, when the true sensitivity may be as low as 8, 86 and 13%, respectively.

A policy of universal screening by cfDNA testing for trisomies 21, 18 and 13 would lead to an invasive testing rate of about 1% and the detection of more than 98% of cases of trisomy 21 and about 96% of trisomies 18 and 13, but none of those with sex chromosome aneuploidies, triploidies or other abnormalities at high risk of adverse outcome. In contrast, a policy of first-line screening by the combined test followed by invasive testing in those with risk ≥1:10 and cfDNA testing in those with risk of 1:11–1:1000 would also lead to an invasive testing rate of about 1% and potentially detect 96% of cases of trisomy 21 and 97.5% of trisomies 18 and 13. Such a policy could also detect about 86% of cases of monosomy X, half of other sex chromosome aneuploidies, half of other chromosomal abnormalities at high risk of adverse outcome, and one third of triploidies, which are currently detected by screening with the combined test and invasive karyotyping for those with a risk of ≥1:100.

Comparison of the Findings with Previous Studies in the Literature

In our population undergoing invasive karyotyping there were 1,831 clinically significant chromosomal abnormalities with risk for trisomies 21, 18 and 13 ≥1:100. The contribution of trisomies 21, 18 or 13, sex chromosome aneuploidies, triploidy and other abnormalities at high risk of adverse clinical outcome was 82.9, 8.2, 3.9 and 5.0%, respectively. Similarly, in our screened population the subgroup of 3,947 pregnancies with risk for trisomies 21, 18 and 13 ≥1:100 included 520 with clinically significant chromosomal abnormalities; the contribution of trisomies 21, 18 or 13, sex chromosome aneuploidies, triploidy and other abnormalities at high risk of adverse clinical outcome was 80.6, 10.6, 4.8 and 4.0%, respectively. The high contribution of trisomies 21, 18 or 13 is not surprising since both the markers selected and the algorithms used for screening were specifically targeting these three trisomies.

In contrast to our findings, other studies reported that the contribution of trisomies 21, 18 or 13 was lower and that of other chromosomal abnormalities was higher. In a multicentre study in the USA where invasive testing for a variety of indications was carried out in 4,406 pregnancies, including 420 with clinically significant chromosomal abnormalities, the respective contributions of the various abnormalities were 75.5, 13.6, 4.0 and 6.9% [11]. A European registry of chromosomal abnormalities diagnosed prenatally or within 1 year of postnatal life included 10,024 clinically significant abnormalities, and the contribution of trisomies 21, 18 or 13, sex chromosome aneuploidies, triploidy and other abnormalities was 73.2, 12.5, 3.0 and 11.4%, respectively [12]. A study of 96,416 pregnancies undergoing CVS or amniocentesis for advanced maternal age (≥35 years) or maternal anxiety (<35 years) identified 1,381 chromosomal abnormalities at high or intermediate risk of abnormal fetal phenotype; the contribution of trisomies 21, 18 or 13, monosomy X, triploidy and other abnormalities was 69.5, 2.1, 1.2 and 27.2%, respectively [13].

The discrepancy in results between studies inevitably reflects differences in indications and gestational age at invasive testing, as well as laboratory techniques used for the analysis of samples and interpretation of clinical significance of uncommon chromosomal abnormalities. Our findings are likely to be the most relevant in examining the potential impact of introducing cfDNA testing for trisomies 21, 18 and 13 on the diagnosis of other clinically significant chromosomal abnormalities that are currently detected by a policy of screening by the combined test for trisomies 21, 18 and 13 and undertaking invasive karyotyping in the high-risk group.

Limitations of the Study

The main limitation of our screening study relates to ascertainment of pregnancy outcome, especially for the group classified as euploid, which was essentially based on the absence of any suspicious clinical findings in the neonatal period. In the case of sex chromosome aneuploidies we estimated the potential impact of such ascertainment bias. However, in the case of other abnormalities, both for those at high risk of adverse outcome and more so for those at low risk, it is impossible in the absence of karyotyping all neonates to define their true prevalence; it is likely that this has been considerably underestimated and the ability of the combined test to detect them has been overestimated.

The estimates we derived on the prevalence of other chromosomal abnormalities at high risk of adverse outcome are based on assumptions that will be difficult to validate.

Implications for Practice

The performance of screening for trisomies 21, 18 and 13 by cfDNA analysis of maternal blood is superior to that of the combined test [4]. However, the test is expensive and it is therefore unlikely that it would be used for routine screening of the whole population. We have previously suggested that the best model of screening is to offer cfDNA testing contingent on the results of first-line screening by the combined test [14]. In this study, we extend the concept of contingent screening in proposing that following combined testing the population is divided into a very high-risk group, an intermediate-risk group

and a low-risk group. In this model it is proposed that, firstly, invasive testing is carried out in all cases in the very high-risk group and, secondly, cfDNA testing is carried out in the intermediate-risk group followed by invasive testing for those with a screen-positive result.

The proposed new strategy, compared to the current one of combined screening followed by invasive testing for those with a risk of ≥1:100, would require cfDNA testing for less than 15% of the population and would lead to a higher DR for trisomies 21, 18 and 13 with a lower invasive testing rate of <1%. Such a policy would also potentially detect most of the cases of monosomy X and between half and one third of the few other clinically significant chromosomal abnormalities that are currently detected by invasive testing if the risk for trisomies 21, 18 or 13 from the combined test is ≥1:100.

The objective of screening for trisomy 21 over the last 4 decades has been to increase the DR and decrease the rate of unnecessary invasive tests. Such a decrease in invasive testing would inevitably reduce the coincidental detection of other chromosomal abnormalities. In the case of trisomies 18 and 13, research led to the development of an algorithm that relies on the same biomarkers as in screening for trisomy 21 and reliably detects more than 90% of affected cases with no or only a minor increase in the overall rate of invasive testing. Consequently, inclusion of trisomies 18 and 13, which are lethal, in prenatal screening should not be controversial. However, the extent to which we should be screening the pregnant population for other chromosomal abnormalities necessitates consideration among a wide range of health care providers and patient groups on whether the individual conditions fulfil established criteria for such screening [15]. In the meantime, individual patients requesting information on all chromosomal abnormalities could be advised that neither the combined test nor cfDNA testing can achieve this objective and that the investigation of choice is invasive testing for fetal karyotyping and microarray analysis.

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