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First-Trimester Contingent Screening for Trisomies 21, 18 and 13 by Biomarkers and Maternal Blood Cell-Free DNA Testing

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Key Words
Biomarkers · First-line screening · First-trimester contingent screening · Maternal blood cfDNA testing · Trisomies 21, 18 and 13

Abstract
Objective: To examine potential performance of screening for trisomies by cell-free (cf) DNA testing in maternal blood contingent on results of first-line testing by combinations of fetal translucency thickness (NT), fetal heart rate (FHR), ductus venosus pulsatility index (DV PIV), and serum-free β-human chorionic gonadotropin (β-hCG), pregnancy-associated plasma protein-A (PAPP-A), placental growth factor (PLGF) and α-fetoprotein (AFP). ***Methods:*** Performance was estimated for firstly, screening by cfDNA in all pregnancies and secondly, cfDNA testing contingent on results of first-line testing by combinations of ultrasound and biochemical markers. ***Results:*** In first-line screening by cfDNA testing, the detection rate for trisomy 21 and trisomies 18 or 13 would be 99 and 96%, respectively, after invasive testing in 1% of the population. In contingent screening, a detection rate of 98% for trisomy 21 and 96% for trisomy 18 or 13, at an invasive testing rate of 0.7%, can be achieved by carrying out cfDNA testing in about 35, 20 and 11% of cases identified by first-line screening with the combined test alone (age, NT, FHR, β-hCG, PAPP-A), the combined test plus PLGF and AFP and the combined test plus PLGF, AFP and DV PIV, respectively. ***Conclusions:*** Effective first-trimester screening for trisomies can be achieved by contingent screening incorporating biomarkers and cfDNA testing.

Introduction

First-trimester screening for trisomies 21, 18 and 13 by a combination of maternal age, fetal nuchal translucency thickness (NT), fetal heart rate (FHR) and serum-free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) can detect about 90% of cases with trisomy 21 and 95% of those with trisomies 18 and 13, at a false positive rate (FPR) of about 5% [1, 2]. The performance of first-trimester screening for these trisomies can be improved by expanding the combined test to include measurement of fetal ductus venosus pulsatility index for veins (DV PIV) and serum placental growth factor (PLGF) and α-fetoprotein (AFP) [3] (table 1).

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Table 1. Modelled detection rates of trisomy 21 (T21) and trisomies 18 or 13 (T18/13) and FPRs in first-trimester screening for fetal trisomies by the algorithm for trisomy 21 and the algorithms for trisomies 18 and 13 using various combinations of biomarkers at fixed risk cut-offs

Risk cut-off for T21 (1:x) and T18/13 (1:x)	NT, FHR, PAPP-A, β-hCG			NT, FHR, PAPP-A, β-hCG, AFP, PLGF			NT, FHR, PAPP-A, β-hCG, AFP, PLGF, DV PIV		
	FPR	DR T21	DR T18/13	FPR	DR T21	DR T18/13	FPR	DR T21	DR T18/13
100	2.2	87.0	91.8	2.0	89.4	93.0	1.3	93.3	95.4
200	3.9	90.4	94.3	3.5	92.3	95.2	2.2	95.1	96.8
300	5.4	92.1	95.5	4.7	93.8	96.2	3.0	96.0	97.4
400	6.7	93.2	96.2	5.9	94.7	96.8	3.7	96.5	97.8
500	7.9	94.0	96.7	6.9	95.3	97.2	4.3	96.9	98.1
1,000	13.0	96.1	97.9	11.1	97.0	98.3	6.9	97.9	98.7
1,500	17.2	97.0	98.5	14.5	97.8	98.7	8.9	98.4	99.0
2,000	20.8	97.6	98.8	17.2	98.2	99.0	10.6	98.6	99.2
2,500	23.9	98.0	99.0	19.6	98.5	99.1	12.2	98.8	99.3
3,000	26.6	98.3	99.1	21.7	98.7	99.3	13.5	98.9	99.4
3,500	29.0	98.5	99.2	23.6	98.9	99.4	14.8	99.1	99.5
4,000	31.3	98.7	99.3	25.3	99.0	99.4	15.9	99.1	99.5
5,000	35.2	98.9	99.4	28.4	99.2	99.5	18.0	99.3	99.6
6,000	38.7	99.1	99.5	31.1	99.3	99.6	19.9	99.4	99.7
7,000	41.7	99.2	99.6	33.5	99.4	99.6	21.5	99.4	99.7
8,000	44.5	99.3	99.6	35.5	99.5	99.7	23.0	99.5	99.7

Rates (in percent) are standardized so that they relate to a population with the maternal age distribution of pregnancies in England and Wales in 2011 and the estimated prevalence of trisomies 21, 18 and 13 at 12.5 weeks' gestation [3].

Recent evidence suggests that analysis of cell-free (cf) DNA in maternal blood can detect more that 99% of cases of trisomy 21, about 98% of trisomy 18, and 92% of trisomy 13, with respective FPRs of 0.1, 0.1 and 0.3% [4–21]. However, cfDNA testing is expensive and we proposed that widespread uptake of the test into routine clinical practice is likely to be contingent on the results of the combined test at 11–13 weeks' gestation, rather than as a primary method of screening [22]. Such a strategy would also retain the advantages of first-trimester testing by ultrasound and biochemistry, including accurate pregnancy dating, early detection of many major fetal defects and prediction, with the potential of prevention, of a wide range of pregnancy complications, including preterm birth and preeclampsia [23]. In contingent screening, detection of 98% of fetuses with trisomy 21 at an overall invasive testing rate of less than 0.5% could potentially be achieved by offering cfDNA testing to about 35, 20 and 10% of cases identified by first-line screening with the combined test alone, the combined test with the addition of serum PLGF and AFP and the combined test with the addition of PLGF, AFP and fetal DV PIV, respectively [22].

The objective of this study is to examine the potential performance of screening for trisomies 21, 18 and 13 by a strategy of cfDNA testing in maternal blood contingent on the results of first-line testing by maternal age and combinations of fetal NT, DV PIV and serum-free β-hCG, PAPP-A, PLGF and AFP.

Methods

The modeled performance of screening for trisomy 21 and trisomies 18 or 13, in a population with the maternal age distribution of pregnancies in England and Wales in 2011 [24, 25], with the combined test alone, the combined test with the addition of serum PLGF and AFP and the combined test with the addition of PLGF, AFP and DV PIV, was obtained from a previous publication and shown in table 1 [3]. In such a population the estimated prevalence of trisomies 21, 18 and 13 at 12.5 weeks' gestation is 1:340, 1:813 and 1:2,555, respectively [24, 25]. In 100,000 singleton pregnancies the expected number of cases of trisomies 21, 18 and 13 is 294, 123 and 39, respectively. For the purpose of this study it is assumed that the 99,544 pregnancies unaffected by trisomies 21, 18 or 13 are euploid.

The potential performance of screening for trisomies 21, 18 and 13, including detection rate (DR) and invasive testing rate, was estimated for two policies: firstly, first-line screening by cfDNA testing in maternal blood and secondly, cfDNA testing contingent on

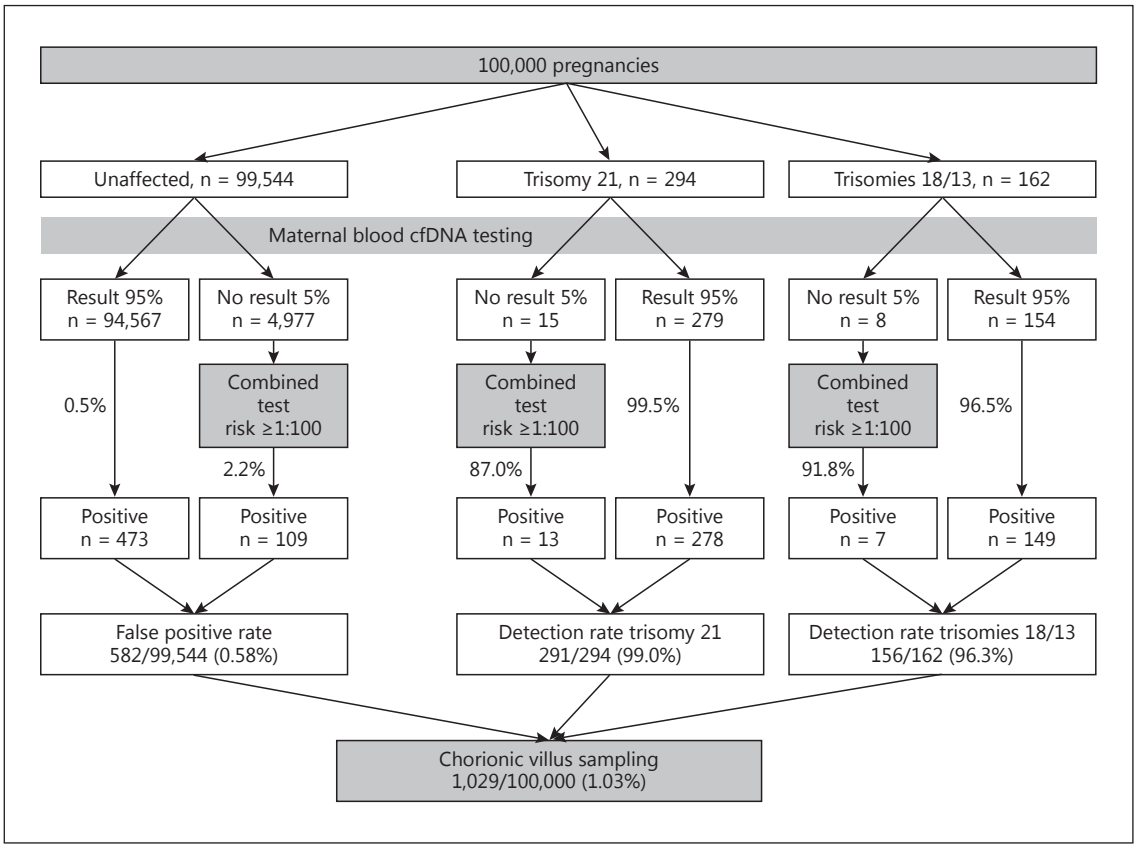

Fig. 1. First-line screening by cfDNA testing in a population of 100,000 pregnancies including 294 with trisomy 21, 162 with trisomy 18 or 13, and 99,544 unaffected by trisomies 21, 18 or 13. Invasive testing is carried out in those with a positive cfDNA result and in those with estimated combined test risk for trisomy 21 and trisomies 18 or 13 of 1:100 or higher if cfDNA testing fails to provide a result. On the assumption that cfDNA testing does not provide a risk for trisomies in 5% of cases and the FPR is 0.5%, the estimated DR for trisomy 21 would be 99.0%, the DR for trisomies 18 or 13 would be 96.3%, and the need for invasive testing would be 1.03%.

the results of first-line testing by maternal age and combinations of fetal NT, FHR, DV PIV and serum-free β-hCG, PAPP-A, PLGF and AFP.

In the first policy, all pregnancies have cfDNA testing and in those with a positive result for any one of the three trisomies invasive testing is carried out. In those where cfDNA testing fails to give a risk for trisomies the combined test is performed and if the risk for trisomy 21 and trisomies 18 or 13 is 1:100 or higher, invasive testing is also carried out. In the second policy, all pregnancies have the combined test or the combined test with the addition of serum PLGF and AFP or the combined test with the addition of PLGF, AFP and fetal DV PIV. If the estimated risk for trisomy 21 and trisomies 18 or 13 from these tests is above a given cut-off then cfDNA testing is undertaken and invasive testing is performed if the cfDNA test is positive for any of the three trisomies.

In the estimates of performance of screening the following assumptions were made: (a) cfDNA testing fails to give a risk for trisomies in between 1 and 10% of cases, (b) in cases with a cfDNA result, the combined FPR for the three trisomies is between 0.1 and 1%, (c) the DR of cfDNA testing is 99.5% for trisomy 21 and 96.5% for trisomies 18 and 13 (the DR for trisomy 18 is 98% and for trisomy 13 is 92% and the proportion of trisomy 18 to 13 at 11–13 weeks' gestation is 3:1) [4–20], (d) in the combined test, at a risk cut-off of 1:100 for trisomy 21 and trisomies 18 or 13, the FPR is 2.2%, the DR for trisomy 21 is 87.0% and the DR for trisomies 18 or 13 is 91.8% (see table 1) [3], and (e) in the combined test the performance of screening is improved by the addition of fetal DV PIV and serum PLGF and AFP (see table 1).

Results

First-Line Screening by cfDNA Testing

Figure 1 illustrates the potential consequence of first-line screening by cfDNA testing in a population of 100,000 singleton pregnancies, including 294 with trisomy 21, 162 with trisomy 18 or 13 and 99,544 unaffected by trisomies 21, 18 or 13. On the assumption that cfDNA testing does

Table 2. Estimated detection rate of trisomies 21, 18 and 13 and invasive testing rate in first-line screening by cfDNA testing in maternal blood

Failure rate of cfDNA testing, %	Detection rate, %		Invasive testing rate, %
	trisomy 21	trisomies 18/13	
1	99.7	96.3	0.96
2	99.3	96.3	0.98
3	99.3	96.9	1.00
4	99.0	96.9	1.01
5	99.0	96.3	1.03
6	99.0	96.3	1.05
7	98.6	96.3	1.06
8	98.6	96.3	1.08
9	98.6	96.3	1.10
10	98.3	96.3	1.11

The detection and invasive testing rates are examined in relation to the failure rate of cfDNA testing to provide a risk for trisomies. If cfDNA testing fails to provide a result then the combined test is carried out. Invasive testing is performed in (a) those with a positive cfDNA result and in (b) those with estimated combined test risk for trisomy 21 and trisomies 18 or 13 of 1:100 or higher. These rates apply to the maternal age distribution of pregnancies in England and Wales in 2011. The expected number of cases of trisomies 21, 18 or 13 at 12.5 weeks' gestation are 294 and 162 per 100,000 pregnancies, respectively [25, 26].

not provide a risk for trisomies in 5% of cases and the total FPR is 0.5%, the estimated DR for trisomy 21 would be 99.0%, the DR for trisomies 18 or 13 would be 96.3% and the need for invasive testing would be 1.03% of the total population, including 0.58% of unaffected pregnancies.

If the combined FPR from cfDNA testing for trisomies 21, 18 and 13 was lower or higher than the assumed 0.5% the DR of these trisomies would not be affected but the invasive testing rate would change. For example, if the total FPR was 0.1% the rate of invasive testing would be 0.65% and this would increase to 1.5% if the total FPR was 1%.

In table 2 we report the DR of trisomies 21, 18 and 13 and the invasive testing rate for a range of failure rates of cfDNA testing to provide a risk for trisomies from 1 to 10%.

cfDNA Testing Contingent on the Results of Combined Ultrasound and Serum Biochemistry

Figure 2 illustrates the potential consequence of first-line screening in the population by the combined test and cfDNA testing in those with a risk for trisomy 21 or trisomies 18 or 13 of 1:2,500 or higher. Invasive testing is carried out in (a) those with a positive cfDNA result and (b) in those with a combined test risk of 1:100 or higher if cfDNA testing fails to provide a result. On the assumption that cfDNA testing does not provide a risk for trisomies in 5% of cases and the FPR is 0.5%, the estimated DR for trisomy 21 would be 96.9%, the DR for trisomies 18 or 13 would be 95.1% and the need for invasive testing would be 0.66%.

Table 3 summarizes the overall cfDNA testing rate, DR and invasive testing rate in first-trimester screening of trisomies 21, 18 and 13 by cfDNA testing contingent on the results of first-line screening by maternal age and combinations of fetal NT, DV PIV and serum-free β-hCG, PAPP-A, PLGF and AFP. In contingent screening, detection of 98% of fetuses with trisomy 21 and about 96% of fetuses with trisomy 18 or 13, at an overall invasive testing rate of 0.6–0.7%, can be achieved by carrying out cfDNA testing in 35.5, 20.0 and 11.0% of cases identified by first-line screening with the combined test alone, the combined test with the addition of serum PLGF and AFP and the combined test with the addition of PLGF, AFP and DV PIV, respectively.

If the combined FPR from cfDNA testing for trisomies 21, 18 and 13 was lower or higher than the assumed 0.5% the DR of these trisomies would not be affected but the invasive testing rate would change. For example, if screening was carried out as indicated in figure 2 but the total FPR was 0.1% the rate of invasive testing would be 0.57% and this would increase to 0.78% if the total FPR was 1%.

In figure 2 we assumed that cfDNA testing fails to provide a risk for trisomies in 5% of cases. In table 4 we report the DR of trisomies 21, 18 and 13 and the invasive testing rate for a range of failure rates of cfDNA testing from 1 to 10%.

Discussion

In first-trimester screening for fetal trisomies by a combination of maternal age, fetal NT and FHR and serum-free β-hCG and PAPP-A, about 90% of fetuses with trisomy 21 and 94% with trisomies 13 and 18 can be detected for an overall FPR of 4% [3] (table 1). The performance of screening can improve further, with increase in DR and decrease in FPR, by expanding the combined test to include measurements of fetal DV PIV and serum PLGF and AFP. Measurement of serum PLGF and AFP can be performed in the same sample and by the same automated machines used for free β-hCG and PAPP-A at little extra cost. Additionally, these metabolites are useful

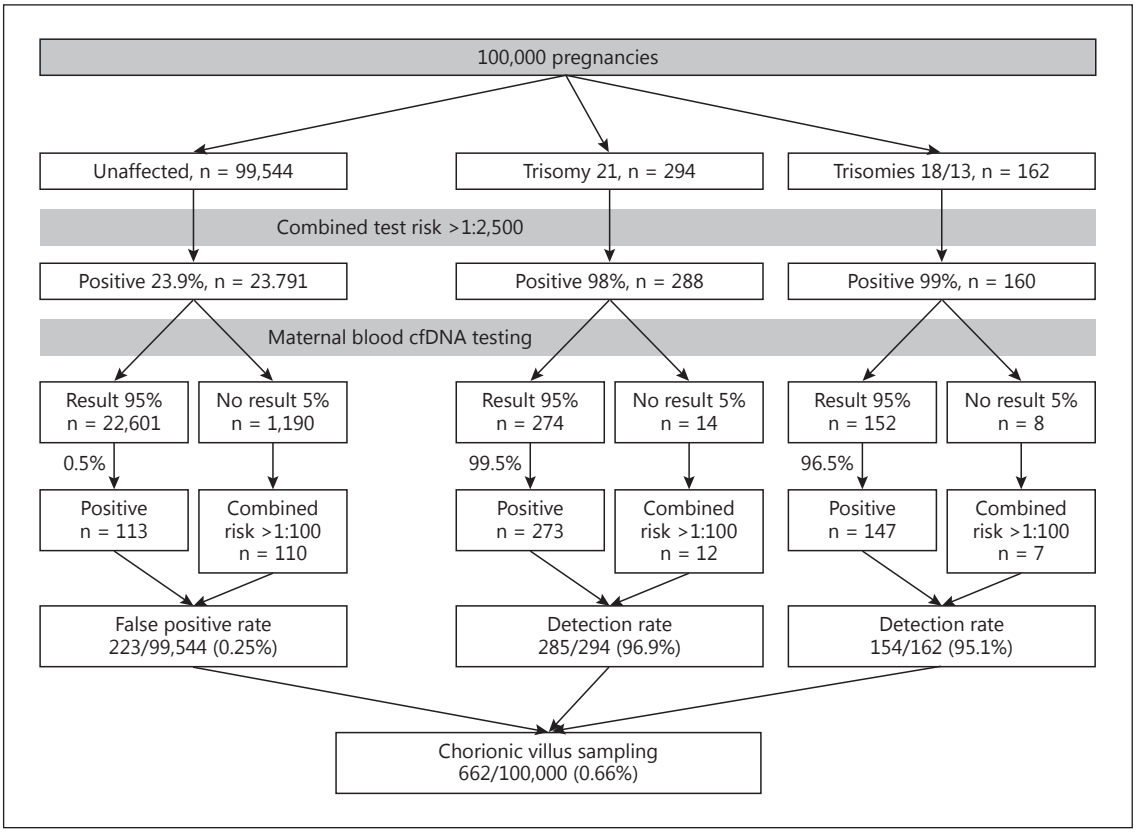

Fig. 2. Contingent screening by the combined test and cfDNA testing in those with a risk of 1:2,500 or higher in a population of 100,000 pregnancies including 294 with trisomy 21, 162 with trisomy 18 or 13, and 99,544 unaffected by trisomies 21, 18 or 13. Invasive testing is carried out in those with a positive cfDNA result and in those with a combined test risk of 1:100 or higher if cfDNA testing fails to provide a result. On the assumption that cfDNA testing does not provide a risk for trisomies in 5% of cases and the FPR is 0.5%, the estimated DR for trisomy 21 would be 96.9%, the DR for trisomies 18 or 13 would be 95.1%, and the need for invasive testing would be 0.66%.

in first-trimester screening for preeclampsia, fetal growth restriction and preterm birth [26–28]. Measurement of DV PIV can improve the performance of screening for trisomies considerably and it is also useful in screening for major cardiac defects [29–31]. However, competence in this examination requires extensive experience in the 11- to 13-week scan and specific training [32]; the test is at present carried out in specialist centres and is unlikely to be used universally in first-line screening.

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 96% with trisomies 13 and 18 could be detected at an overall invasive testing rate of 1% (fig. 1). This constitutes a major improvement in the performance of screening compared to that achieved by the combined test.

These estimates on performance of universal screening by cfDNA are based on a series of assumptions. The first assumption is that cfDNA testing can detect 99.5% of cases of trisomy 21, 98% of trisomy 18 and 92% of trisomy 13, with respective FPRs of 0.1, 0.1 and 0.3%. These are the summary values of published studies which mainly examined high-risk pregnancies and although the total number of cases of trisomies 21 and 18 is several hundreds that of cases of trisomy 13 is only 82. The ability to detect aneuploidy with cfDNA is dependent upon assay precision and fetal DNA percentage in the sample rather than the prevalence of the disease in the study population and it is therefore likely that the test will perform equally well in low-risk pregnancies. In the case of trisomy 13, individual studies vary in their reported DR from 78 to 100%. The second assumption is that the failure rate of

Table 3. Overall cfDNA testing, detection rate and invasive testing rate (in percent) in contingent first-trimester screening for trisomies 21, 18 and 13

Risk cut-off	NT, FHR, PAPP-A, β-hCG				NT, FHR, PAPP-A, β-hCG, PLGF, AFP				NT, FHR, DV PIV, PAPP-A, β-hCG, PLGF, AFP			
	cfDNA	DR T21	DR T18/13	CVS	cfDNA	DR T21	DR T18/13	CVS	cfDNA	DR T21	DR T18/13	CVS
100	2.6	86.7	88.9	0.52	2.4	89.1	90.1	0.52	1.7	92.9	92.6	0.49
200	4.3	90.1	91.4	0.54	3.9	91.8	92.0	0.54	2.6	94.9	93.8	0.51
300	5.8	91.5	92.6	0.56	5.1	93.2	93.2	0.55	3.4	95.6	94.4	0.51
400	7.1	92.5	93.2	0.56	6.3	93.9	93.8	0.56	4.1	96.3	94.4	0.52
500	8.3	93.2	93.8	0.57	7.3	94.6	93.8	0.56	4.7	96.3	95.1	0.52
1,000	13.4	95.6	95.1	0.61	11.5	96.3	95.1	0.59	7.3	97.3	95.7	0.54
1,500	17.6	96.3	95.1	0.63	14.9	97.3	95.7	0.61	9.3	97.6	95.7	0.55
2,000	21.2	96.6	95.1	0.65	17.6	97.6	95.7	0.62	**11.0**	**98.0**	**96.3**	**0.56**
2,500	24.2	96.9	95.1	0.66	**20.0**	**98.0**	**96.3**	**0.64**	12.6	98.0	96.3	0.57
3,000	26.9	97.3	95.7	0.68	22.1	98.0	95.7	0.65	13.9	98.3	96.3	0.57
3,500	29.3	97.6	95.7	0.69	23.9	98.3	95.7	0.66	15.2	98.3	96.3	0.58
4,000	31.6	97.6	95.7	0.70	25.6	98.3	95.7	0.66	16.3	98.3	96.3	0.59
5,000	**35.5**	**98.0**	**95.7**	**0.72**	28.7	98.6	95.7	0.68	18.4	98.6	96.3	0.60
6,000	39.0	98.0	95.7	0.74	31.4	98.6	95.7	0.69	20.3	98.6	96.9	0.61
7,000	42.0	98.3	95.7	0.75	33.8	98.3	95.7	0.70	21.9	98.6	96.9	0.61
8,000	44.8	98.3	95.7	0.76	35.8	98.6	96.3	0.71	23.4	99.0	96.9	0.62

All women have first-line screening by various combinations of ultrasound and serum biomarkers using specific algorithms for each of the trisomies and those with estimated risk for trisomy 21 or trisomies 18 and 13 above certain cut-offs have cfDNA testing. Invasive testing is carried out if (a) the cfDNA test is positive and (b) in cases with no result from cfDNA testing the estimated risk from first-line screening is 1:100 or higher. In these calculations it is assumed that cfDNA testing fails to provide a risk for trisomies in 5% of cases, and in those with a result the total FPR for the three trisomies is 0.6% and the detection rate is 99.5% for trisomy 21 and 96.5% for trisomies 18 and 13. Values in bold typeface denote the optimal detection and invasive testing rate.

Table 4. Estimated detection rate of trisomies 21, 18 and 13 and invasive testing rate in screening by cfDNA testing in maternal blood contingent on the results of first-line screening by various combinations of ultrasound and serum biomarkers

Failure rate of cfDNA testing, %	NT, FHR, PAPP-A, β-hCG			NT, FHR, PAPP-A, β-hCG, PLGF, AFP			NT, FHR, DV PIV, PAPP-A, β-hCG, PLGF, AFP		
	detection rate, %		invasive testing rate, %	detection rate, %		invasive testing rate, %	detection rate, %		invasive testing rate, %
	trisomy 21	trisomies 18/13		trisomy 21	trisomies 18/13		trisomy 21	trisomies 18/13	
1	97.6	95.1	0.58	98.3	95.7	0.56	98.3	95.7	0.52
2	97.3	95.7	0.60	98.0	95.7	0.58	98.3	95.7	0.53
3	97.3	95.7	0.62	98.0	96.3	0.60	98.0	96.3	0.54
4	97.3	95.7	0.64	98.0	96.3	0.62	98.0	96.3	0.55
5	96.9	95.1	0.66	98.0	96.3	0.64	98.0	96.3	0.57
6	96.9	95.1	0.68	97.6	95.7	0.65	98.0	96.3	0.58
7	96.9	95.1	0.70	97.6	95.7	0.67	98.0	96.3	0.59
8	96.6	95.1	0.72	97.6	95.7	0.69	98.0	95.7	0.60
9	96.6	95.1	0.74	97.6	95.7	0.71	98.0	95.7	0.61
10	96.6	95.1	0.76	97.3	95.7	0.73	97.6	95.7	0.63

The detection and invasive testing rates are examined in relation to the failure rate of cfDNA testing to provide a risk for trisomies. In first-line screening the risk cut-off of 1:2,500 for trisomy 21 or trisomies 18 and 13 is used for offering cfDNA testing. Invasive testing is carried out if (a) the cfDNA test is positive and (b) in cases with no result from cfDNA testing the estimated risk from first-line screening is 1:100 or higher. These rates apply to the maternal age distribution of pregnancies in England and Wales in 2011. The expected number of cases of trisomies 21, 18 or 13 at 12.5 weeks' gestation are 294 and 162 per 100,000 pregnancies, respectively [25, 26].

cfDNA testing to provide a result is 5%. This is based on our finding from clinical implementation of cfDNA testing at 10 weeks' gestation [33]. In about half of such cases a result is obtained after repeat sampling but in the study we assumed that the test will not be repeated but rather a decision in favor or against invasive testing will be based on the result of the combined test. If the failure rate was reduced to 1%, the DR of trisomy 21 could improve from 99 to 99.7% (table 2). The third assumption is that invasive testing is carried out in firstly, those with a positive result from cfDNA testing and secondly, those where cfDNA testing fails to give a risk for trisomies and the combined test risk is 1:100 or higher. However, in practice it is likely that some women in the low-risk group from cfDNA testing would still desire to have a diagnostic test to provide certainty of exclusion of trisomies 21, 18 and 13 but also of other aneuploidies. This is particularly important in cases with fetal abnormalities and those with high NT.

The alternative to universal screening by the cfDNA test is a strategy of cfDNA testing contingent on the results of first-line screening by ultrasound and biochemical testing. This approach retains the major advantages of cfDNA testing in increasing DR and decreasing FPR, but at considerably lower cost than offering cfDNA testing to the whole population. In contingent screening, detection of 98% of fetuses with trisomy 21 and about 96% of fetuses with trisomies 18 or 13, at an overall invasive testing rate of less than 1%, can be achieved by carrying out cfDNA testing in about 35, 20 and 10% of cases identified by first-line screening with the combined test alone, the combined test with the addition of serum PLGF and AFP, and the combined test with the addition of PLGF, AFP and DV PIV, respectively. If the desired DR of trisomy 21 was to be reduced from 98 to 97 or 96% and the first-line method of screening was the combined test the proportion of the population requiring cfDNA testing could be reduced from about 35–25 and 15%, respectively.

The proposed strategy of screening for trisomies 21, 18 and 13 by cfDNA testing contingent on the results of first-trimester ultrasound and biochemistry testing can substantially improve the performance of screening for these trisomies but also retain the advantages of the combined test. These include firstly, diagnosis of aneuploidies within the first trimester with the option for earlier and safer termination of pregnancy, and secondly, early detection of major defects and prediction of a wide range of pregnancy complications which allows for earlier therapeutic intervention and better pregnancy management [23].

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