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First-trimester contingent screening for trisomy 21 by biomarkers and maternal blood cell-free DNA testing

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KEYWORDS: β-human chorionic gonadotropin; combined screening; nuchal translucency; pregnancy-associated plasma protein-A; risk cut-off

ABSTRACT

Objective *To define risk cut-offs with corresponding detection rates (DR) and false-positive rates (FPR) in screening for trisomy 21 using maternal age and combinations of first-trimester biomarkers in order to determine which women should undergo contingent maternal blood cell-free (cf) DNA testing.*

Methods *From singleton pregnancies undergoing screening for aneuploidies at three UK hospitals between March 2006 and May 2012, we analyzed prospectively collected data on the following biomarkers: fetal nuchal translucency thickness (NT) and ductus venosus pulsatility index for veins (DV-PIV) at 11 + 0 to 13 + 6 weeks' gestation and serum free β-human chorionic gonadotropin (β-hCG), pregnancy-associated plasma protein-A (PAPP-A), placental growth factor (PlGF) and alpha-fetoprotein (AFP) at 8 + 0 to 13 + 6 weeks. Estimates of risk cut-offs, DRs and FPRs were derived for combinations of biomarkers and these were used to define the best strategy for contingent cfDNA testing.*

Results *In contingent screening, detection of 98% of fetuses with trisomy 21 at an overall invasive testing rate < 0.5% can be potentially achieved by offering cfDNA testing to about 36%, 21% and 11% of cases identified by first-line screening using the combined test alone, using the combined test with the addition of serum PlGF and AFP and using the combined test with the addition of PlGF, AFP and DV-PIV, respectively.*

Conclusions *Effective first-trimester screening for trisomy 21, with DR of 98% and invasive testing rate < 0.5%, can be potentially achieved by contingent screening incorporating biomarkers and cfDNA testing.*

INTRODUCTION

Analysis of cell-free (cf) DNA in maternal blood can detect more than 99% of cases of trisomy 21 for a false-positive rate (FPR) of about 0.1%[1–13]. This is far superior to the best of the currently available methods of screening: the first-trimester combination of maternal age, fetal nuchal translucency thickness (NT) and maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A), with a detection rate (DR) of about 90% for a FPR of 5%[14]. Consequently, there will be widespread uptake of cfDNA testing in routine clinical practice, either as a first-line method of screening or contingent on the results of the combined test at 11–13 weeks' gestation.

Contingent screening could lead to a very high DR and very low invasive testing rate at a considerably lower cost than would be possible using cfDNA testing as a first-line method of screening. This 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 for prevention, of a wide range of pregnancy complications, including preterm birth and pre-eclampsia[15]. There is some evidence that the performance of the combined test can be improved by the addition of the fetal ductus venosus pulsatility index for veins (DV-PIV), which is also useful in screening for cardiac defects, serum placental growth factor (PlGF), which is also useful in screening for pre-eclampsia and fetal growth restriction, and serum alpha-fetoprotein (AFP), which is also useful in screening for preterm birth[16–23].

The objective of this study was to define risk cut-offs, with corresponding DRs and FPRs, for first-line screening for trisomy 21, using maternal age and combinations of fetal NT, DV-PIV and serum free β-hCG, PAPP-A, PlGF

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and AFP as the basis for defining the group that should undergo cfDNA testing.

METHODS

Study population

In this study we present the results of an analysis of prospectively collected data including fetal NT and DV-PIV at 11 + 0 to 13 + 6 weeks' gestation and serum free β-hCG, PAPP-A, PlGF and AFP at 8 + 0 to 13 + 6 weeks from singleton pregnancies undergoing screening for aneuploidies at King's College Hospital, London, University College London Hospital, London and Medway Maritime Hospital, Gillingham, UK, between March 2006 and May 2012. The measurements of serum PAPP-A and free β-hCG at 8 + 0 to 10 + 6 weeks and serum PlGF and AFP at 8 + 0 to 13 + 6 weeks were part of a research study which was approved by the appropriate hospital ethics committee.

Maternal weight and height, measured at the time of blood sampling, demographic characteristics, ultrasonographic measurements and biochemical results were recorded in computer databases. Patients were asked to complete a questionnaire on maternal age, racial origin (Caucasian, African, South Asian, East Asian or mixed), method of conception (spontaneous or assisted conception requiring the use of ovulation drugs or *in-vitro* fertilization), cigarette smoking during pregnancy (yes or no), pre-existing diabetes mellitus (Type 1, Type 2 or no) and obstetric history including parity. The questionnaire was then reviewed by a doctor together with the patient. Blood samples were analyzed within 10 min of collection for measurement of serum free β-hCG, PAPP-A, PlGF and AFP using automated machines that provide reproducible results (DELFIA Xpress system, PerkinElmer Life and Analytical Sciences, Waltham, MA, USA). Ultrasound examination was carried out transabdominally and included measurement of fetal crown–rump length (CRL) to determine gestational age[24], examination of fetal anatomy for diagnosis of major fetal defects and measurement of fetal NT thickness and DV-PIV[14,25].

The patient-specific risks for trisomies 21, 18 and 13 were estimated from a combination of maternal age, fetal NT and serum free β-hCG and PAPP-A[26]. Women considering their risks to be high were offered chorionic villus sampling (CVS) or amniocentesis for fetal karyotyping. Karyotype results and details on pregnancy outcomes were added to the database when they became available.

We compared the distribution of biomarkers and risks in pregnancies with trisomy 21, diagnosed by cytogenetic analysis of CVS or amniocentesis samples prenatally or neonatal blood postnatally, with those in pregnancies unaffected by this aneuploidy. The unaffected group included pregnancies that were euploid or that resulted in the birth of phenotypically normal neonates.

Statistical analysis

Each measured value of free β-hCG, PAPP-A, PlGF and AFP in the trisomy 21 and unaffected pregnancies was expressed as a multiple of the normal median (MoM) after adjustment for those characteristics found to provide a substantial contribution to the log-transformed value[18,22,27]. Multivariate Gaussian distributions were fitted to the joint distribution of log-MoM values for free β-hCG, PAPP-A, PlGF and AFP[18,22,27]. The likelihoods of pregnancies unaffected or affected by trisomy 21 for given NT and given DV-PIV were obtained from the mixture model for NT and DV-PIV, respectively[16,28]. Likelihoods for unaffected and trisomy 21 pregnancies were computed under the assumptions of conditional independence, given outcome, between the three components (i) NT, (ii) DV-PIV and (iii) biochemical markers. Distributional characteristics of biomarker values in trisomy 21 and unaffected pregnancies in the screening population will be the subject of another publication.

For a given risk cut-off and maternal age, risks were computed using Bayes theorem to combine the likelihoods with the maternal age-specific prior risk of trisomy 21 at 12.5 weeks' gestation[29]. The resultant risks were compared with the risk cut-off to obtain an age-specific DR for each year of maternal age from 12 to 50 years. All likelihoods were used for each maternal age. The weighted average of these age-specific rates was then computed to produce a standardized DR. The weights used were obtained from the maternal age distribution of trisomy 21 pregnancies in England and Wales in 2011 at 12.5 weeks' gestation. This distribution was obtained from the maternal-age distribution of England and Wales in 2011[30] and the gestational and maternal age-specific risk of trisomy 21[29]. Similarly, standardized FPRs were computed by obtaining the likelihoods in unaffected pregnancies and then applying these to each year of maternal age from 12 to 50 years to estimate the age-specific FPRs. These were then weighted according to the maternal age distribution of unaffected pregnancies in England and Wales in 2011[30]. Empirical estimates of performance were obtained using likelihoods for the sample data. Modeled performance was obtained using likelihoods from simulated data from the fitted model. Samples of 100 000 unaffected and trisomy 21 pregnancies were used in these simulations.

Statistical software package R was used for data analyses[31].

RESULTS

Characteristics of the study population

We obtained measurements of NT, DV-PIV and serum biochemistry in 93 545 consecutive singleton pregnancies undergoing routine screening in the first trimester. We excluded 6304 cases because they had missing outcome data, the fetal karyotype was not known and the pregnancies resulted in termination, miscarriage or stillbirth or there was an aneuploidy other than trisomy 21. The study population of 87 241 cases included 324 cases of trisomy 21 and 86 917 unaffected pregnancies with normal fetal karyotype or the birth of a

phenotypically normal neonate. All measurements of NT, DV-PIV, serum PAPP-A and free β-hCG were obtained prospectively. Serum PlGF and serum AFP were measured prospectively in some pregnancies and retrospectively in nested case–control studies in others (Table 1). The characteristics of the trisomy 21 and unaffected groups are presented in Table 2. The observed number of cases of trisomy 21 is consistent with that expected, 333.2 ($P = 0.61$), given the maternal and gestational age distribution of the cohort. Models were fitted to the data and used to produce estimates of screening performance.

Performance of screening for trisomy 21 by combinations of biomarkers

Modeled standardized DRs and FPRs in screening for trisomy 21 by maternal age in combination with serum biochemistry, fetal NT and DV-PIV are shown in Tables 3–5.

Table 1 Number of cases in which biomarker data were available for use in deriving algorithm for trisomy 21 and modeled performance of screening

Biomarker	*Unaffected* (n = *86 917*)	*Trisomy 21* (n = *324*)
Nuchal translucency	86 913	323
Ductus venosus PIV	86 733	320
Fetal heart rate	86 440	317
Serum PAPP-A	73 964	303
Serum free β-hCG	73 964	303
Serum PlGF		
Prospective	19 445	78
Nested case–control	8850	60
Serum AFP		
Prospective	6404	26
Nested case–control	2744	39

β-hCG, beta-human chorionic gonadotropin; PAPP-A, pregnancy-associated plasma protein-A; PIV, pulsatility index for veins; PlGF, placental growth factor.

Table 3 gives the FPRs and risk cut-offs to achieve DRs ranging between 90% and 99%. To achieve a DR of 98%, the estimated FPR in screening by fetal NT and serum free β-hCG and PAPP-A was 25.5%; this was reduced to 17.2% if the biochemical test included PlGF and AFP, and further reduced to 7.9% with the inclusion of DV-PIV.

Table 4 gives the estimated FPRs and DRs at fixed risk cut-offs ranging from 1:100 to 1:8000. The empirical FPRs and DRs were similar to the modeled ones, as shown in Figure 1. At a risk cut-off of 1:100 at 12.5 weeks' gestation, which corresponds to the recommendations of the UK National Screening Committee as the cut-off for offering invasive testing, the FPR and DR in screening by fetal NT and serum free β-hCG and PAPP-A were 2.1% and 85.2%, respectively. If the biochemical test included PlGF and AFP, the FPR was reduced to 1.8% and the DR increased to 87.9%, and inclusion of DV-PIV further improved the performance of screening, with a FPR of 1.3% and DR of 92.4%. When screening by fetal NT and serum free β-hCG and PAPP-A at a risk cut-off of 1:3000, the DR was about 98% for a FPR of 24%.

Table 5 gives the performance of screening by various combinations of biomarkers according to maternal age at a fixed risk cut-off of 1:3000. For all methods of screening, with increasing maternal age there was an increase in both DR and FPR (Figure 2).

Performance of contingent screening in the detection of trisomy 21

In a population with the maternal age distribution of trisomy 21 and unaffected pregnancies in England and Wales in 2011, the estimated prevalence of trisomy 21 at 12.5 weeks' gestation is 1:340[29,30]. In such a population of 100 000 singleton pregnancies, the expected number of cases of trisomy 21 is 294.

Table 2 Characteristics of study population of singleton pregnancies affected by trisomy 21 and unaffected pregnancies

Characteristic	*Unaffected* (n = *86 917*)	*Trisomy 21* (n = *324*)
Maternal age (years)	31.2 (26.7–35.1)	37.9 (34.6–40.2)
Maternal weight (kg)	65.5 (58.9–75.5)	65.0 (60.0–74.0)
Spontaneous conception	83 875 (96.5)	294 (90.7)
Smoker	8662 (10.0)	26 (8.0)
Racial origin		
Caucasian	65 221 (75.0)	270 (83.3)
Afro-Caribbean	13 002 (15.0)	35 (10.8)
South Asian	4389 (5.0)	8 (2.5)
East Asian	2216 (2.5)	7 (2.2)
Mixed	2089 (2.4)	4 (1.2)
Crown–rump length (mm)	63.1 (58.1–68.7)	63.8 (58.5–70.0)
Nuchal translucency (mm)	1.8 (1.5–2.1)	3.5 (2.4–5.0)
Ductus venosus pulsatility index	1.059 (0.950–1.160)	1.561 (1.210–1.995)
Serum PAPP-A (MoM)	1.023 (0.700–1.452)	0.545 (0.351–0.829)
Serum free β-hCG (MoM)	0.977 (0.665–1.473)	2.036 (1.395–2.957)
Serum PlGF (MoM)	1.002 (0.784–1.285)	0.667 (0.524–0.843)
Serum AFP (MoM)	0.986 (0.740–1.333)	0.778 (0.536–1.021)

Data are given as median (interquartile range) or *n* (%). AFP, alpha-fetoprotein; β-hCG, beta-human chorionic gonadotropin; PAPP-A, pregnancy-associated plasma protein-A; PlGF, placental growth factor.

Table 3 Modeled performance of screening by various combinations of biomarkers at fixed detection rates (DR)

	DR 90%		DR 95%		DR 96%		DR 97%		DR 98%		DR 99%	
Method of screening	*FPR (%)*	*Cut-off*	*FPR (%)*	*Cut-off*	*FPR (%)*	*Cut-off*	*FPR (%)*	*Cut-off*	*FPR (%)*	*Cut-off*	*FPR (%)*	*Cut-off*
PAPP-A, free β-hCG, PlGF and AFP	13.1	390	23.1	930	26.7	1200	31.4	1600	38.0	2408	49.1	4500
PAPP-A, free β-hCG and PlGF	14.8	410	24.9	930	28.4	1200	33.0	1600	39.4	2366	50.0	4400
PAPP-A, free β-hCG and AFP	19.2	540	32.6	1200	37.1	1500	42.8	2000	50.6	2768	62.7	4700
PAPP-A and free β-hCG	20.9	550	34.3	1200	38.8	1500	44.4	1900	52.1	2708	63.8	4600
NT	19.2	950	40.8	2400	48.1	3000	57.0	3800	68.2	4962	81.3	6300
NT and DV-PIV	4.5	260	14.2	1400	19.1	2100	26.6	3300	38.3	5529	58.6	10 000
NT, PAPP-A, free β-hCG, PlGF and AFP	2.6	150	7.1	620	9.2	890	12.2	1400	17.2	2408	26.8	5500
NT, PAPP-A, free β-hCG and PlGF	3.2	180	8.3	680	10.5	960	13.7	1500	18.9	2526	29.1	5800
NT, PAPP-A, free β-hCG and AFP	3.6	220	9.9	860	12.7	1200	16.9	1900	23.7	3208	36.6	7000
NT, PAPP-A and free β-hCG	4.3	250	11.2	920	14.3	1300	18.6	1900	25.5	3251	38.2	6900
NT, DV-PIV, PAPP-A, free β-hCG, PlGF and AFP	0.8	50	2.5	260	3.4	420	5.0	740	7.9	1498	14.8	4200
NT, DV-PIV, PAPP-A, free β-hCG and AFP	1.1	70	3.5	370	4.8	590	7.0	1000	11.0	2085	20.3	5800
NT, DV-PIV, PAPP-A, free β-hCG and PlGF	0.9	61	3.0	310	4.1	500	5.9	850	9.2	1698	16.8	4700
NT, DV-PIV, PAPP-A and free β-hCG	1.3	82	4.0	420	5.5	660	7.8	1100	12.2	2215	22.1	6000

Rates were standardized so that they relate to the pregnant population of England and Wales in 2011.[30] AFP, alpha-fetoprotein; β-hCG, beta-human chorionic gonadotropin; DV-PIV, ductus venosus pulsatility index for veins; FPR, false-positive rate; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein-A; PlGF, placental growth factor.

Figure 3 illustrates the potential consequence, in such a population, of first-line screening by cfDNA testing, assuming that the 99 706 pregnancies unaffected by trisomy 21 are actually euploid. If cfDNA testing fails to provide a result in about 4% of cases[32] and in those with a result the DR is 99.5% and FPR 0.1%, there will be a positive result in 281 of the trisomy 21 and 96 of the unaffected pregnancies that would require CVS for diagnosis of the true karyotype. In the cases with no result from cfDNA testing, the combined test can be carried out and CVS should be performed in those with estimated risk of 1:100 or higher, including 2.1% of the unaffected group and 85.2% of the trisomy 21 group. Such a policy would lead to the diagnosis of 99% of the cases of trisomy 21 and the need for invasive testing in 0.47% of the total population.

Figure 4 illustrates the potential consequence, in a similar population of 100 000 singleton pregnancies, of first-line screening by the combined test. If the risk cut-off of 1:3000 is used for the offer of cfDNA testing and the uptake is 100%, then the test will be carried out in 24.3% of the unaffected pregnancies and 97.9% of those with trisomy 21 (see Table 4). If cfDNA testing fails to provide a result in 4% of cases[32] and in those with a result the DR is 99.5% and FPR 0.1%, there will be a positive result in 275 of the trisomy 21 and 23 of the unaffected pregnancies that would require CVS for diagnosis of the true karyotype. In the cases with no result from cfDNA testing, CVS can be carried out in those with estimated risk from the combined test of 1:100 or higher, which includes 8.6% (2.1% of the 24.3% with combined test risk of 1:3000 or higher) of the unaffected group and 87.0% (85.2% of the 97.9% with combined test risk of 1:3000 or higher) of the trisomy 21 group. Such a policy would lead to the diagnosis of 96.9% of the cases of trisomy 21 and the need for invasive testing in 0.39% of the total population.

Table 6 summarizes the overall cfDNA testing rate, DR and invasive testing rate for contingent first-trimester screening of trisomy 21. In contingent screening, a DR of 98% in fetuses with trisomy 21 at an overall invasive testing rate < 0.5% can be achieved by offering the cfDNA test to 35.8%, 21.4% and 11.2% of cases identified by first-line screening using the combined test alone, using the combined test with the addition of serum PlGF and AFP and using the combined test with the addition of PlGF, AFP and DV-PIV, respectively.

DISCUSSION

Principal findings of this study

In this study we have defined risk cut-offs with corresponding DRs and FPRs for first-line screening for trisomy 21, using maternal age and combinations of fetal NT, DV-PIV and serum free β-hCG, PAPP-A, PlGF and AFP as the basis of defining the group for cfDNA testing.

The results demonstrate that in contingent screening, a DR of 98% in fetuses with trisomy 21, at an overall invasive testing rate < 0.5%, could be achieved by offering

Table 4 Modeled detection rates (DR) and false-positive rates (FPR) in first-trimester screening for trisomy 21 by various combinations of biomarkers at fixed risk cut-offs

	Nuchal translucency with:					
	PAPP-A and β-hCG		*PAPP-A, β-hCG, AFP and PlGF*		*PAPP-A, β-hCG, AFP, PlGF and DV-PIV*	
Risk cut-off (1:x)	*FPR (%)*	*DR (%)*	*FPR (%)*	*DR (%)*	*FPR (%)*	*DR (%)*
100	2.1	85.2	1.8	87.9	1.3	92.4
500	7.2	92.9	6.1	94.4	3.9	96.3
1000	11.9	95.3	9.9	96.3	6.1	97.5
1500	15.7	96.4	12.9	97.2	8.0	98.0
2000	19.0	97.1	15.4	97.7	9.5	98.3
2500	21.8	97.5	17.5	98.1	10.9	98.6
3000	24.3	97.9	19.5	98.3	12.2	98.7
3500	26.6	98.1	21.2	98.5	13.3	98.9
4000	28.7	98.3	22.7	98.7	14.3	99.0
5000	32.4	98.6	25.6	98.9	16.2	99.1
6000	35.6	98.9	28.0	99.1	17.9	99.2
7000	38.4	99.0	30.1	99.2	19.5	99.3
8000	41.0	99.1	32.1	99.3	20.9	99.4

Rates were standardized so that they relate to the pregnant population of England and Wales in 2011. AFP, alpha-fetoprotein; β-hCG, beta-human chorionic gonadotropin; DV-PIV, ductus venosus pulsatility index for veins; PAPP-A, pregnancy-associated plasma protein-A; PlGF, placental growth factor.

Table 5 Modeled detection rates (DR) and false-positive rates (FPR) in first-trimester screening for fetal trisomy 21 by various combinations of biomarkers according to maternal age (MA) at a fixed risk cut-off of 1:3000

	Nuchal translucency with:					
	PAPP-A and β-hCG		*PAPP-A, β-hCG, AFP and PlGF*		*PAPP-A, β-hCG, AFP, PlGF and DV-PIV*	
MA (years)	*FPR (%)*	*DR (%)*	*FPR (%)*	*DR (%)*	*FPR (%)*	*DR (%)*
20	13.5	93.8	11.3	95.2	6.8	96.7
25	14.8	94.3	12.3	95.6	7.4	97.0
30	19.7	95.7	16.0	96.7	9.7	97.6
31	21.6	96.2	17.5	97.0	10.6	97.8
32	24.0	96.6	19.2	97.4	11.7	98.1
33	27.0	97.1	21.3	97.8	13.0	98.3
34	30.6	97.6	23.9	98.1	14.7	98.5
35	34.6	98.0	27.0	98.5	16.6	98.7
36	39.3	98.4	30.5	98.7	18.9	98.9
37	44.4	98.8	34.4	99.0	21.6	99.1
38	50.0	99.1	38.5	99.2	24.7	99.3
39	55.7	99.3	43.1	99.4	28.3	99.5
40	61.6	99.5	47.9	99.6	32.3	99.6
41	67.5	99.7	52.8	99.7	36.6	99.7
42	73.2	99.8	57.7	99.8	41.1	99.8
43	78.5	99.9	62.4	99.8	45.9	99.8
44	83.2	99.9	66.9	99.9	51.0	99.9
45	87.2	99.9	71.4	99.9	55.8	99.9

AFP, alpha-fetoprotein; β-hCG, beta-human chorionic gonadotropin; DV-PIV, ductus venosus pulsatility index for veins; PAPP-A, pregnancy-associated plasma protein-A; PlGF, placental growth factor.

the cfDNA test to about 36%, 21% and 11% of cases identified by first-line screening using the combined test alone, using the combined test with the addition of serum PlGF and AFP and using the combined test with the addition of PlGF, AFP and DV-PIV, respectively (Table 6). An increase in DR from 98% to 99% would require a larger than 10% increase in the number of cases requiring cfDNA testing, irrespective of the first-line method of screening.

The methodology for deriving the overall rates of cfDNA testing, detection and invasive testing in contingent first-trimester screening for trisomy 21 (Table 6) is well illustrated in Figure 4, allowing easy derivation of the appropriate rates should the assumptions concerning cfDNA testing (DR of 99.5%, FPR of 0.1%, no result rate of 4% and 100% utilization) change.

The estimates in our model are based on a population with maternal age distribution in England and Wales in

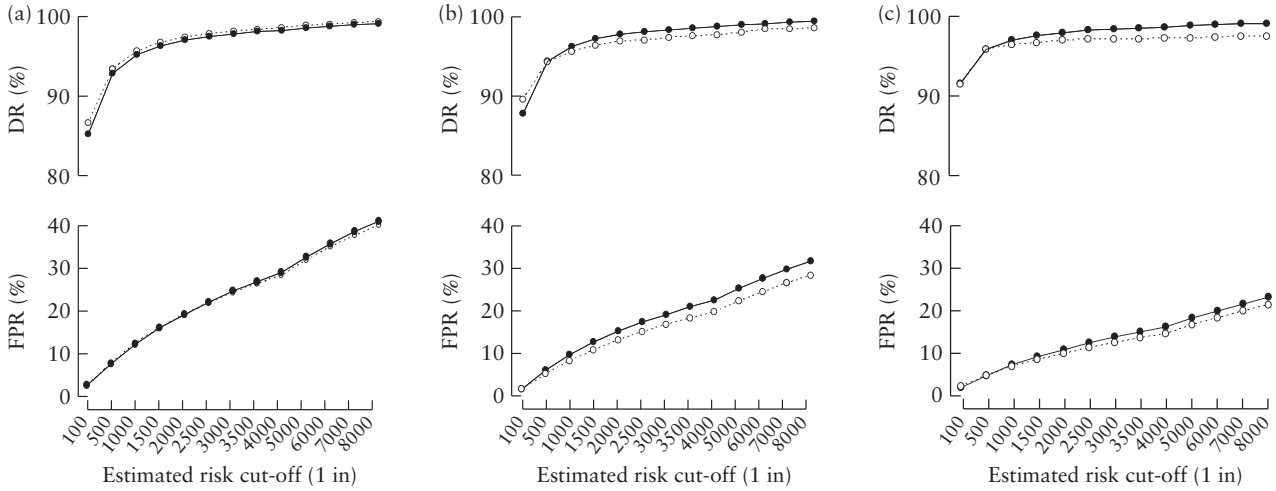

Figure 1 Modeled (—●—) and empirical (--○-) detection rate (DR) (upper lines) and false-positive rate (FPR) (lower lines) in screening for trisomy 21 by combined test (maternal age, fetal nuchal translucency thickness and serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A) (a), combined test with addition of serum placental growth factor (PlGF) and alpha-fetoprotein (AFP) (b) and combined test with addition of ductus venosus pulsatility index and serum PlGF and AFP (c).

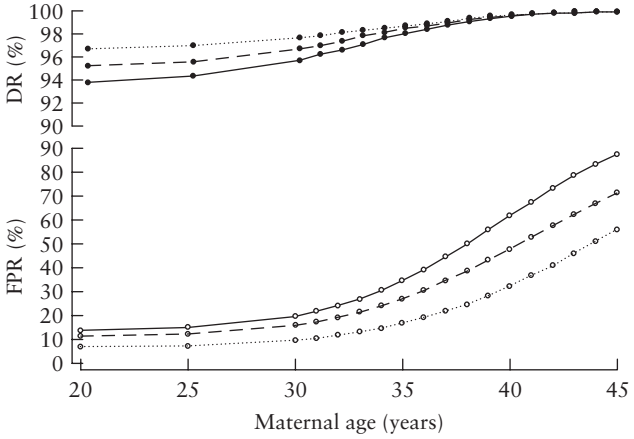

Figure 2 Modeled detection rates (DR) (●) and false-positive rates (FPR) (○) in first-trimester screening for fetal trisomy 21 by various combinations of biomarkers (combined test (——); combined test with addition of serum placental growth factor (PlGF) and alpha-fetoprotein (AFP) (– –); and combined test with addition of ductus venosus pulsatility index and serum PlGF and AFP (.....)) according to maternal age at a fixed risk cut-off of 1:3000.

2011, when the median age was 29.3 years. However, we provide data for each age between 20 and 45 years to facilitate application of the models to other populations (Table 5). For all methods of screening, with increasing maternal age there was an increase in both DR and FPR. For example, if there was a policy of combined screening followed by cfDNA testing in those with a risk of 1:3000 or higher, more than 60% of women aged 40 years or older would end up having the cfDNA test.

Limitations of the study

This study was confined to the prediction of trisomy 21 because, first, in the last 40 years this has been the sole or main factor in defining strategies of screening for aneuploidies and, second, we wanted to minimize the complexity of the model that would arise from inclusion of other aneuploidies. Similarly, we assumed that the only factor defining the decision for or against invasive testing was the estimated risk for trisomy 21, which is unlikely to be true. Trisomy 21 accounts for only half of the clinically significant aneuploidies associated with increased fetal NT and therefore invasive testing should be considered in the presence of high NT even if screening suggests that the risk for trisomy 21 is low[33,34]. Additionally, some women in the high-risk group want to avoid an invasive test and some in the low-risk group still desire to have a diagnostic test to provide certainty of exclusion not only of trisomy 21 but also of other aneuploidies[35].

We derived data for NT, DV-PIV, free β-hCG and PAPP-A from more than 85 000 prospectively screened pregnancies, including more than 300 cases of trisomy 21. The study population for PlGF was more than 25 000, including 138 cases of trisomy 21, but for AFP we examined fewer than 10 000 pregnancies, including only 65 cases of trisomy 21. Consequently, because of the relatively limited data available, the modeled measures of screening performance, especially at very high DRs, are somewhat speculative and may be biased optimistically[36]. Evidence that the true DR is within 1% of an estimated DR of 98% would require investigation of more than 1000 cases of trisomy 21 and, therefore, a study population of more than 300 000 pregnancies. Since a prospective study of such magnitude is unlikely to be carried out before the routine introduction of screening for trisomy 21 by cfDNA testing contingent on the results of the combined test, it is important that the performance of the test after implementation is monitored closely.

The model assumes that cfDNA testing can detect 99.5% of cases of trisomy 21 with a FPR of 0.1%. These assumptions were made on the basis of published studies, derived mainly from the investigation of high-risk pregnancies[1–13]. The ability to detect aneuploidy with cfDNA is dependent upon assay precision and fetal DNA

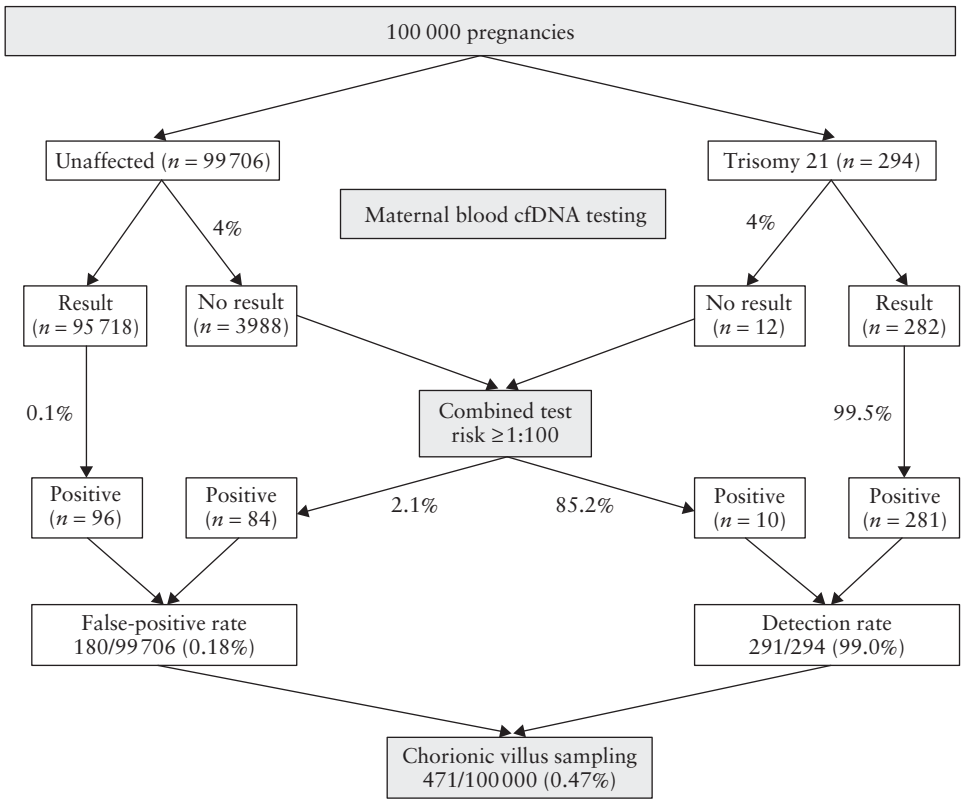

Figure 3 First-line screening by cell-free (cf) DNA testing in a population of 100 000 pregnancies, including 294 with trisomy 21. Chorionic villus sampling is carried out in those with a positive cfDNA result and, if cfDNA testing fails to provide a result, in those with a combined test risk of 1:100 or higher. Such a policy would lead to diagnosis of 99% of cases of trisomy 21 and require invasive testing in 0.47% of total population.

Table 6 Overall cell-free (cf) DNA testing rate, detection rate (DR) and rate of invasive testing (IR) by chorionic villus sampling in contingent first-trimester screening for trisomy 21

	NT, PAPP-A, β-hCG			*NT, PAPP-A, β-hCG, PlGF, AFP*			*NT, DV-PIV, PAPP-A, β-hCG, PlGF, AFP*		
Risk cut-off	*cfDNA (%)*	*DR (%)*	*IR (%)*	*cfDNA (%)*	*DR (%)*	*IR (%)*	*cfDNA (%)*	*DR (%)*	*IR (%)*
100	2.3	84.7	0.34	2.1	87.4	0.33	1.6	92.2	0.32
500	7.5	92.2	0.36	6.4	93.9	0.35	4.2	95.9	0.34
1000	12.1	94.6	0.37	10.2	95.6	0.36	6.4	96.9	0.34
1500	15.9	95.6	0.38	13.1	96.6	0.37	8.3	97.3	0.35
2000	19.2	96.3	0.39	15.6	96.9	0.37	9.8	97.6	0.35
2500	22.0	96.9	0.39	17.7	97.3	0.38	11.2	98.0	0.35
3000	24.5	96.9	0.39	19.7	97.6	0.38	12.5	98.0	0.35
3500	26.8	96.9	0.39	21.4	98.0	0.38	13.6	98.3	0.35
4000	28.9	97.3	0.40	22.9	98.0	0.38	14.5	98.3	0.36
5000	32.6	97.6	0.40	25.8	98.3	0.39	16.4	98.3	0.36
6000	35.8	98.0	0.41	28.2	98.3	0.39	18.1	98.6	0.36
7000	38.6	98.0	0.41	30.3	98.6	0.39	19.7	98.6	0.36
8000	41.2	98.0	0.41	32.3	98.6	0.39	21.1	98.6	0.36

All women had first-line screening by various combinations of ultrasound and serum biomarkers and those with estimated risk above certain cut-offs had cfDNA testing. Invasive testing was carried out if the cfDNA test was positive and in the cases with no result from cfDNA testing but with estimated risk from first-line screening of 1:100 or higher. In these calculations it was assumed that cfDNA testing failed to provide a result in 4% of cases, and that in those with a result, DR was 99.5% and FPR was 0.1%. AFP, alpha-fetoprotein; β-hCG, beta-human chorionic gonadotropin; DV-PIV, ductus venosus pulsatility index for veins; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein-A; PlGF, placental growth factor.

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[9].

Another assumption of the study is that in 4% of cases the cfDNA test would fail to provide a result. This was based on our findings from clinical implementation of cfDNA testing at 10 weeks' gestation[32]. In about half of

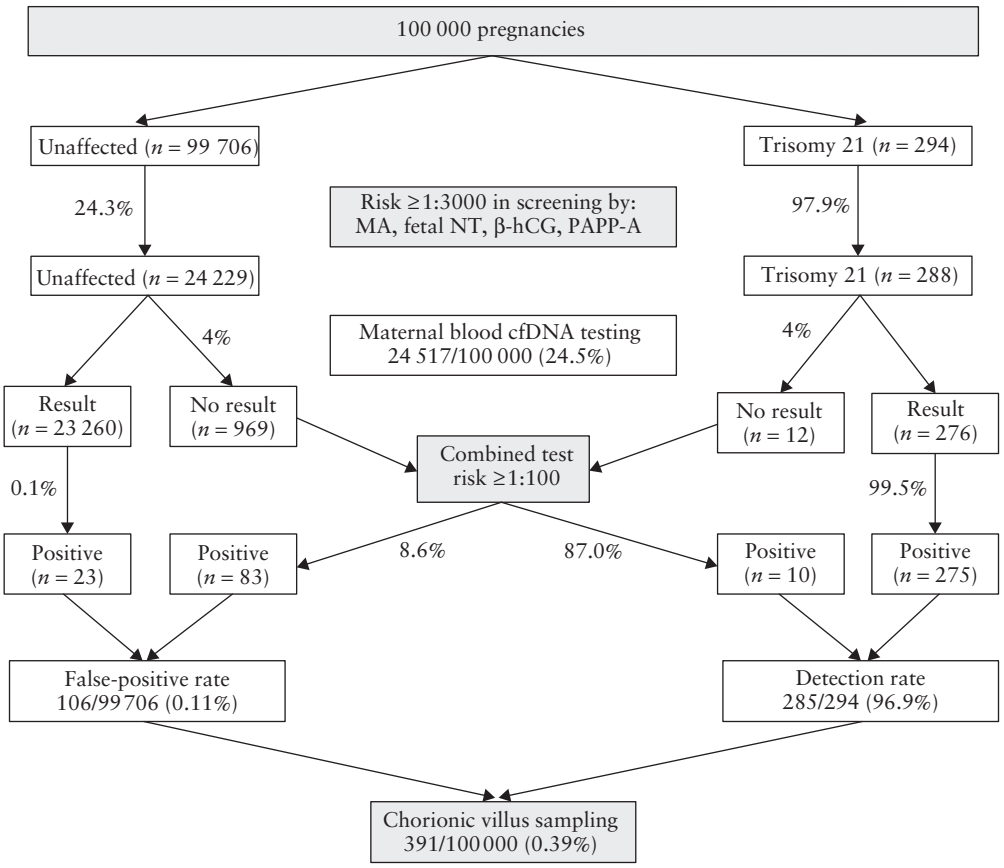

Figure 4 Contingent screening by combined test and cell-free (cf) DNA testing in pregnancies with a risk of 1:3000 or higher in a population of 100 000, including 294 with trisomy 21. Chorionic villus sampling is carried out in those with a positive cfDNA result and, if cfDNA testing fails to provide a result, in those with a combined test risk of 1:100 or higher. Such a policy would lead to diagnosis of 96.9% of cases of trisomy 21 and require invasive testing in 0.39% of total population.

such cases a result was obtained after repeat sampling, but in the study we assumed that the test would not be repeated and that a decision in favor or against invasive testing would instead be based on the result of first-line screening.

Content of first-line screening

Measurement of serum PlGF and AFP can be performed in the same sample and by the same automated machines as those used for free β-hCG and PAPP-A at little extra cost. The finding, in this prospective screening study, that inclusion of these metabolites in the combined test would substantially increase the DR and reduce the FPR, is compatible with predictions from previous prospective and retrospective case–control studies[18,22,37]. For example, a DR of 90% in cases of trisomy 21 can be achieved with a 40% reduction in FPR, from 4.3% to 2.6% (Table 3). Additionally, these metabolites are useful in first-trimester screening for pre-eclampsia, fetal growth restriction and preterm birth[19–21,23].

It could be argued that a first-trimester biochemistry-only test is a viable alternative to the combined test in screening for trisomy 21 because it avoids the need to measure fetal NT, which requires training, certification of competence and external quality assurance to maintain reliable standards. However, such argument is flawed because an ultrasound scan underpins all methods of screening, with a minimal expectation of demonstrating whether the fetus is alive, diagnosing multiple pregnancies and providing an accurate measure of gestational age, which is critical in the interpretation of biochemical results. Consequently, the need for certification of competence and external quality assurance remains, even if the only aim of the scan is measurement of fetal CRL[38].

Fetal NT is an essential part of screening for aneuploidies with and without the use of cfDNA testing. As shown in this study, a DR of 90% in cases of trisomy 21 can be achieved at a FPR of 13.1% with the first-trimester serum quad test (free β-hCG, PAPP-A, PlGF and AFP), whereas with inclusion of fetal NT there is a five-fold reduction in FPR to 2.6% (Table 3). Similarly, if contingent screening is to be used with the aim of achieving a DR of 98%, cfDNA testing should be offered to 20% more of the population when first-line screening is based on serum biochemistry alone than when fetal NT is included. Additionally, fetal NT is a marker not only of trisomy 21 but also of many other clinically significant aneuploidies, cardiac defects, skeletal dysplasias and several genetic syndromes[39]. Similarly, an ultrasound scan at 11–13 weeks is not just for measurement of CRL and

NT; it is also used for detailed examination of the fetus and the early diagnosis of major fetal abnormalities[40]. The great improvement in screening for some aneuploidies achieved by the introduction of cfDNA testing could be lost if this test was considered to be an alternative rather than complementary to the existing strategy of first-trimester ultrasound and biochemistry.

The findings of this study demonstrate that incorporating measurement of DV-PIV in the combined test can improve the performance of screening considerably. For example, a DR of 90% in cases of trisomy 21 can be achieved with a 70% reduction in FPR (from 4.3% to 1.3%, Table 3), and in contingent screening aiming for a DR of 98%, inclusion of DV-PIV in first-line testing could reduce the need for the cfDNA test by about 10%. However, sonographers with prior extensive experience in the 11–13-week scan require an average of 80 examinations before they can achieve a high level of competence in Doppler assessment of the DV and therefore this examination is confined to specialist centers[25].

Health economic assessment

In this study we did not undertake a formal health economic assessment of different strategies for detection of trisomy 21, but certain conclusions can be drawn. The first-trimester scan is essential for pregnancy care and it is therefore an integral part of any strategy of screening for aneuploidies. Consequently, the basis for any health economic assessment would be, first, the relative cost of invasive testing and components of biochemical screening compared with cfDNA testing and, second, the DR of trisomy 21.

At present, the cost of cfDNA testing is comparable to that of invasive testing and the substantial reduction of the latter by introduction of the former would be cost neutral, but with the major advantage of avoidance of miscarriage from unnecessary invasive tests.

While the other major advantage of cfDNA testing is improvement in DR, the cost of a strategy of universal screening by the cfDNA test, which could potentially lead to a DR of 99%, is likely to be considerably higher than that of the proposed approach of using cfDNA testing contingent on the results of first-trimester ultrasound and biochemical screening.

Conclusions

Screening for trisomy 21 by cfDNA testing contingent on the results of an expanded combined test would retain the advantages of the current method of screening, but with a simultaneous major increase in DR and decrease in the rate of invasive testing.

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