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First-Trimester Screening for Trisomies 21, 18 and 13 by Ultrasound and Biochemical Testing

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Key Words

Combined test · Aneuploidies · First trimester · Nuchal translucency thickness · Ductus venosus pulsatility index for veins · Serum free β-hCG · Pregnancy-associated plasma protein A · Placental growth factor · α-Fetoprotein

Abstract

Objective: To examine the performance of screening for trisomies 21, 18 and 13 at 11–13 weeks' gestation using specific algorithms for these trisomies based on combinations of fetal nuchal translucency thickness (NT), fetal heart rate (FHR), ductus venosus pulsatility index for veins (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:*** Model-based estimates of screening performance were produced for the distribution of maternal ages in England and Wales in 2011, and prospectively collected data on fetal NT, FHR, DV PIV, β-hCG, PAPP-A, PLGF and AFP from singleton pregnancies undergoing aneuploidy screening. ***Results:*** In screening by NT, FHR, free β-hCG and PAPP-A, using specific algorithms for trisomy 21 and trisomies 18 and 13 at the risk cutoff of 1:100, the estimated detection rate (DR) was 87.0% for trisomy 21 and 91.8% for trisomies 18 and 13, at a false-positive rate (FPR) of 2.2%. Addition of PLGF, AFP and DV PIV increased the DR to 93.3% for trisomy 21 and 95.4% for trisomies 18 and 13 and reduced the FPR to 1.3%. ***Conclusions:*** Effective screening for trisomies can be achieved using specific algorithms based on NT, FHR, DV PIV, β-hCG, PAPP-A, PLGF and AFP.

Introduction

First-trimester screening for trisomy 21 by a combination of maternal age, fetal nuchal translucency thickness (NT) and serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A) can detect about 90% of affected pregnancies at a false-positive rate (FPR) of about 5% [1]. A beneficial consequence of screening for trisomy 21 is the early diagnosis of trisomies 18 and 13, which are the second and third most common chromosomal abnormalities, with a relative prevalence to trisomy 21 at 11–13 weeks' gesta-

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tion of 1:3 and 1:7, respectively [2, 3]. We have previously reported that since all three trisomies are similar in being associated with increased maternal age, increased fetal NT and decreased serum PAPP-A, screening using the algorithm for trisomy 21 can detect about 90% of cases of trisomy 21 and 75% of cases of trisomies 18 and 13, at an FPR of about 5% [4]. However, with the use of specific algorithms for each trisomy, which incorporate not only their similarities but also their differences in biomarker pattern, including high serum free β-hCG in trisomy 21 and low levels in trisomies 18 and 13 and high fetal heart rate (FHR) in trisomy 13, it is possible to increase the detection rate (DR) of trisomies 18 and 13 to about 95% with a small increase in the overall FPR from 5 to 5.2% [4].

Recent evidence suggests that the performance of the combined test in screening for trisomy 21 can be improved with the addition of serum placental growth factor (PLGF) and α-fetoprotein (AFP) and fetal ductus venosus pulsatility index for veins (DV PIV) [5–8].

The objective of this study is to examine the performance of screening for trisomies 21, 18 and 13 using specific algorithms for these trisomies based on maternal age and combinations of fetal NT, DV PI, FHR and serum free β-hCG, PAPP-A, PLGF and AFP.

Methods

Study Population

We present the results of analysis of prospectively collected data on fetal NT, FHR and DV PIV at 11^{+0}–13^{+6} weeks' gestation and serum free β-hCG, PAPP-A, PLGF and AFP (DELFIA Xpress system, PerkinElmer Life and Analytical Sciences, Waltham, Mass., USA) at 8^{+0}–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, UK, between March 2006 and May 2012. Gestational age was determined from the fetal crown-rump length [9]. The patient-specific risks for trisomies 21, 18 and 13 were estimated from a combination of maternal age, fetal NT, FHR and serum free β-hCG and PAPP-A [4]. Women considering their risks to be high were offered chorionic villus sampling (CVS) or amniocentesis for fetal karyotyping.

In this study, we examine the distribution of biomarkers and risks in pregnancies with trisomies 21, 18 and 13 diagnosed by cytogenetic analysis of CVS or amniocentesis samples prenatally or neonatal blood, and those unaffected by these aneuploidies. The unaffected group included pregnancies that were euploid or resulted in the birth of phenotypically normal neonates.

Statistical Analysis

Each measured value of free β-hCG, PAPP-A, PLGF and AFP in trisomy 21, trisomy 18, trisomy 13 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 [5, 6, 10]. Heart rate (bpm) was expressed as a deviation from expected value (Δ) obtained from a multiple regression model on gestational age, maternal weight, ethnicity, smoking and diabetes. Multivariate Gaussian distributions were fitted to the joint distribution of log MoM values for free β-hCG. PAPP-A, PLGF and AFP and ΔFHR for unaffected, trisomy 21, trisomy 18 and trisomy 13 pregnancies. Distributions of NT (log transformed) and DV PIV were obtained from the mixture model for NT and DV PIV, respectively [7, 11]. Likelihoods for unaffected, trisomy 21, trisomy 18 and trisomy 13 pregnancies were computed under the assumptions of conditional independence between the three components (a) NT, (b) DV PIV and (c) FHR and biochemical markers.

Bayes theorem was used to compute risks by combining the likelihoods for the biomarkers with the maternal age-specific prior risk of trisomy 21, trisomy 18 and trisomy 13 at 12.5 weeks' gestation [12]. The resultant risks were compared with the risk cutoff to obtain an age-specific DR for each year of maternal age from 12 to 50. 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 pregnancies in England and Wales in 2011 at 12.5 weeks' gestation [13]. This distribution was obtained from the maternal age distribution of England and Wales in 2011 and the gestational and maternal age specific risk of each trisomy [12, 13]. 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 [13]. Empirical estimates of performance were obtained using likelihoods for the sample data. Modelled performance was obtained using likelihoods from simulated data from the fitted model. Samples of 100,000 unaffected, trisomy 21 and trisomy 18 or 13 pregnancies were used in these simulations.

The statistical software package R was used for data analyses [14].

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 6,137 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 trisomies 21, 18 and 13. The study population of 87,408 cases included 324 cases of trisomy 21, 125 of trisomy 18, 42 of trisomy 13 and 86,917 unaffected pregnancies with normal fetal karyotype or the birth of a phenotypically normal neonate. All measurements of NT, DV PIV and serum PAPP-A and free β-hCG were obtained prospectively. Serum

Table 1. Population used for deriving the algorithm for trisomy 21 and the modeled performance of screening by the first-trimester combined test

Marker	Unaffected (n = 86,917)	Trisomy 21 (n = 324)	Trisomy 18 (n = 125)	Trisomy 13 (n = 42)
NT	86,913	323	124	42
DV PIV	86,733	320	122	42
FHR	86,440	317	122	41
Serum PAPP-A	73,964	303	114	39
Serum free β-hCG	73,964	303	114	39
Serum PLGF				
Prospective	19,445	78	26	5
Nested case control	8,850	60	27	6
Serum AFP				
Prospective	6,404	26	11	2
Nested case control	2,744	39	7	12

Table 2. Characteristics of the study population

Characteristic	Unaffected (n = 86,917)	Trisomy 21 (n = 324)	Trisomy 18 (n = 125)	Trisomy 13 (n = 42)
Maternal age, years	31.2 (26.7–35.1)	37.9 (34.6–40.2)	37.5 (32.8–41.1)	34.5 (28.8–37.8)
Maternal weight, kg	65.5 (58.9–75.5)	65.0 (60.0–74.0)	66.8 (59.5–76.4)	68.5 (60.0–77.2)
Spontaneous conception	83,875 (96.5)	294 (90.7)	108 (86.4)	38 (90.5)
Smoker	8,662 (10.0)	26 (8.0)	11 (8.8)	2 (4.8)
Racial origin				
Caucasian	65,221 (75.0)	270 (83.3)	91 (72.8)	35 (83.3)
Afro-Caribbean	13,002 (15.0)	35 (10.8)	23 (18.4)	5 (11.9)
South Asian	4,389 (16.8)	8 (2.5)	5 (4.0)	2 (4.8)
East Asian	2,216 (2.5)	7 (2.2)	2 (1.6)	0 (0)
Mixed	2,089 (2.7)	4 (1.2)	4 (1.2)	0 (0)
Crown-rump length, mm	63.1 (58.1–68.7)	63.8 (58.5–70.0)	55.0 (51.1–60.1)	57.6 (53.5–61.5)
NT, mm	1.8 (1.5–2.1)	3.5 (2.4–5.0)	5.1 (2.2–7.3)	3.9 (2.1–6.4)
FHR, bpm	159 (155–164)	161 (155–166)	158 (154–164)	179 (172–184)
DV PIV	1.059 (0.950–1.160)	1.561 (1.210–1.995)	1.73 (1.320–2.290)	1.50 (1.170–1.950)
Serum PAPP-A, MoM	1.023 (0.700–1.452)	0.545 (0.351–0.829)	0.208 (0.114–0.344)	0.270 (0.162–0.405)
Serum free β-hCG, MoM	0.977 (0.665–1.473)	2.036 (1.395–2.957)	0.209 (0.135–0.305)	0.506 (0.403–0.874)
Serum PLGF, MoM	1.002 (0.784–1.285)	0.667 (0.524–0.843)	0.573 (0.392–0.704)	0.459 (0.415–0.624)
Serum AFP, MoM	0.986 (0.740–1.333)	0.778 (0.536–1.021)	0.843 (0.636–1.485)	0.843 (0.581–1.082)

Data are presented as n (%) or median (IQR).

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 trisomic and unaffected groups are presented in table 2. The observed number of cases of trisomies 21, 18 and 13 are consistent with the respective expected numbers of 333.2 ($p = 0.61$), 136.5 ($p = 0.33$) and 43.5 ($p = 0.82$) given the maternal and gestational age distribution of the cohort. Models were fitted to the data and used to produce estimates of screening performance. The prospective data were generally consistent with our previous publications [4, 7, 10, 11]. Parameters relating to PLGF and AFP are presented in table 3. Serum PLGF in trisomy 21, 18 and 13 pregnancies was significantly lower than in unaffected pregnancies ($p < 0.0001$). Serum AFP was significantly reduced in trisomy 21 ($p < 0.0001$) but not in trisomy 18 or 13 ($p > 0.05$).

Table 3. Distributional parameter estimates, with median and 95% confidence interval, for maternal serum AFP and PLGF

Serum metabolite	Unaffected	Trisomy 21	Trisomy 18	Trisomy 13
Median MoM				
AFP	1.000	0.74765 (0.672 to 0.8318)	0.9030 (0.8101 to 1.0066)	0.8177 (0.6475 to 1.0326)
PLGF	1.000	0.65554 (0.6131 to 0.7009)	0.5358 (0.4807 to 0.5973)	0.5204 (0.4120 to 0.6571)
Standard deviations of log_{10}, MoM				
AFP	0.1878 (0.1844 to 0.1913)	0.1960 (0.1603 to 0.2503)	0.2509 (0.1749 to 0.4254)	0.1863 (0.1244 to 0.3479)
PLGF	0.1711 (0.1693 to 0.1729)	0.1543 (0.1337 to 0.1817)	0.2023 (0.1615 to 0.2679)	0.1951 (0.1245 to 0.4089)
Correlations between log_{10} (MoM) values				
AFP and PAPP-A	–0.0314 (–0.0519 to –0.0109)	0.2205 (–0.0247 to 0.4407)	–0.3048 (–0.6755 to 0.189)	–0.1562 (–0.6342 to 0.4082)
AFP and free β-hCG	0.0068 (–0.0137 to 0.0273)	–0.0112 (–0.2544 to 0.2333)	–0.4258 (–0.7446 to 0.0513)	–0.1501 (–0.6305 to 0.4134)
AFP and ΔFHR	–0.0173 (–0.0378 to 0.0032)	0.0897 (–0.1577 to 0.3265)	0.0534 (–0.424 to 0.5076)	–0.0537 (–0.5681 to 0.4909)
PLGF and PAPP-A	0.3248 (0.3143 to 0.3352)	0.1620 (–0.0072 to 0.3221)	0.2882 (0.0137 to 0.5223)	0.1044 (–0.5286 to 0.6628)
PLGF and free β-hCG	0.1296 (0.1181 to 0.1411)	0.1300 (–0.0398 to 0.2925)	0.1100 (–0.1708 to 0.3742)	–0.0519 (–0.6600 to 0.5973)
PLGF and delta FHR	–0.0783 (–0.09 to –0.0666)	–0.2508 (–0.4061 to –0.0815)	0.0942 (–0.1921 to 0.3657)	–0.4614 (–0.8454 to 0.2371)
PLGF and AFP	–0.1021 (–0.125 to –0.0791)	0.0726 (–0.253 to 0.3834)	–0.1525 (–0.6679 to 0.4618)	–0.1021[a]

[a] There were only 3 cases of trisomy 13 in the data set with measurements of both PAPP-A and AFP and the correlation was assumed to be the same as that in the unaffected group.

Table 4. Modeled detection rates of trisomy 21 and trisomies 18 or 13 and FPR in first-trimester screening for fetal trisomies by the algorithm for trisomy 21 using various combinations of biomarkers at fixed risk cutoffs

Risk cutoff for T21 (1:x)	NT, PAPP-A, β-hCG			NT, PAPP-A, β-hCG, AFP, PLGF			NT, 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	2.0	86.4	65.8	1.9	89.1	66.0	1.2	93.1	76.7
200	3.5	89.9	69.8	3.2	92.0	70.4	2.0	94.9	80.6
300	4.7	91.7	71.9	4.3	93.5	72.7	2.7	95.8	82.7
400	5.8	92.9	73.3	5.3	94.5	74.2	3.3	96.4	84.1
500	6.8	93.7	74.4	6.2	95.2	75.4	3.9	96.8	85.2
1,000	11.3	95.9	77.7	9.9	96.9	78.8	6.2	97.8	88.1
1,500	15.0	96.9	79.8	12.9	97.6	80.8	8.1	98.2	89.6
2,000	18.1	97.5	81.3	15.4	98.1	82.1	9.7	98.5	90.6
2,500	20.8	97.9	82.5	17.6	98.4	83.2	11.1	98.7	91.3
3,000	23.3	98.2	83.5	19.5	98.6	84.1	12.3	98.8	91.8
3,500	25.5	98.4	84.4	21.3	98.8	84.8	13.4	99.0	92.3
4,000	27.5	98.6	85.1	22.8	98.9	85.5	14.5	99.1	92.6
5,000	31.2	98.8	86.4	25.7	99.1	86.5	16.4	99.2	93.2
6,000	34.4	99.0	87.4	28.1	99.3	87.4	18.1	99.3	93.6
7,000	37.1	99.2	88.2	30.2	99.4	88.1	19.6	99.4	94.0
8,000	39.6	99.3	89.0	32.1	99.4	88.7	21.0	99.4	94.3

Rates are standardized so that they relate to the pregnant population of England and Wales in 2011 [13].

Performance of Screening for Trisomies 21, 18 and 13 by the Algorithm for Trisomy 21

Modelled standardized DRs for trisomies 21, 18 and 13 and FPRs in screening for trisomy 21 by maternal age in combination with serum biochemistry, fetal NT and DV PIV are shown in table 4.

At a risk cutoff of 1:100 at 12.5 weeks' gestation, which according to the recommendations of the UK National Screening Committee is the cutoff for offering invasive testing, the FPR and DR of trisomy 21 in screening by fetal NT and serum free β-hCG and PAPP-A were 2.0 and 86.4%, respectively. If the biochemical test included PLGF and

Table 5. Modeled detection rates of trisomy 21 and trisomies 18 or 13 and FPR 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 cutoffs

Risk cutoff 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 are standardized so that they relate to the pregnant population of England and Wales in 2011 [13].

AFP, the FPR was reduced to 1.9% and DR increased to 89.1%, and inclusion of DV PIV improved the performance of screening further with FPR of 1.2% and DR of 93.1%.

The algorithm for trisomy 21, at a risk cutoff of 1:100 at 12.5 weeks' gestation, also identified 65.8% of cases of trisomy 18 or 13 in screening by fetal NT and serum free β-hCG and PAPP-A. The DR was 66.0% with inclusion of PLGF and AFP and 76.7% with inclusion of DV PIV.

Performance of Screening for Trisomies 21, 18 and 13 by the Combined Algorithms for Trisomies 21, 18 and 13

Modelled standardized DRs for trisomies 21, 18 and 13 and FPRs in screening by the algorithms for trisomies 21, 18 and 13 are shown in table 5, and these are compared with the empirical rates in figures 1–3.

In screening by fetal NT, FHR and serum free β-hCG and PAPP-A, at a risk cutoff of 1:100 for trisomy 21 at 12.5 weeks' gestation with the algorithm for trisomy 21 and risk cutoff of 1:100 for trisomies 18 and 13 using the algorithms for trisomy 18 and trisomy 13, the total FPR was 2.2%, and the DR of trisomy 21 and trisomies 18 and 13 were 87.0 and 91.8%, respectively. If the biochemical test included PLGF and AFP, the FPR was reduced to 2.0%, and DRs increased to 89.4% for trisomy 21 and 93.0% for trisomies 18 and 13. Inclusion of DV PIV improved the performance of screening further with FPR of 1.3% and DRs of 93.3% for trisomy 21 and 95.4% for trisomies 18 and 13.

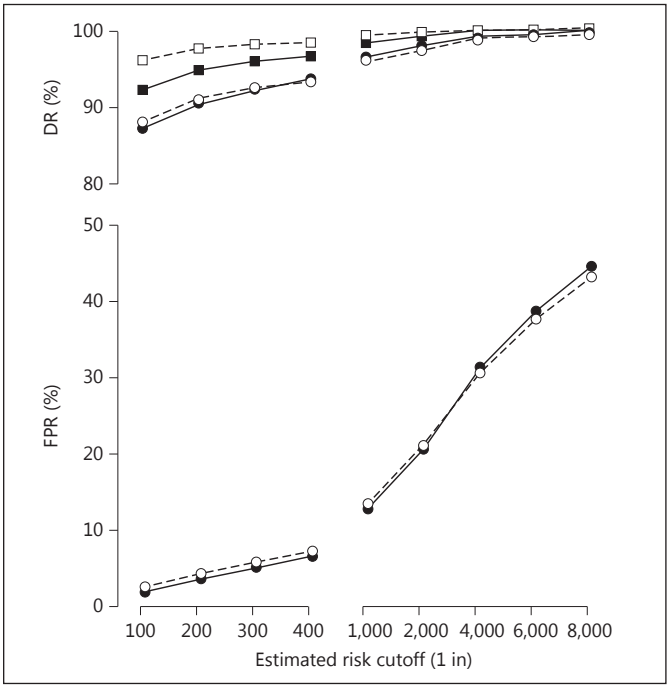

Fig. 1. Modelled and empirical FPR (modelled: solid lines and black circles, empirical: interrupted lines and open circles) and DR of trisomy 21 (modelled: solid lines and black circles, empirical: interrupted lines and open circles) and trisomy 18 or 13 (modelled: solid lines and black squares, empirical: interrupted lines and open squares) in screening for these trisomies by specific algorithms incorporating maternal age, fetal NT, FHR and serum free β-hCG and PAPP-A.

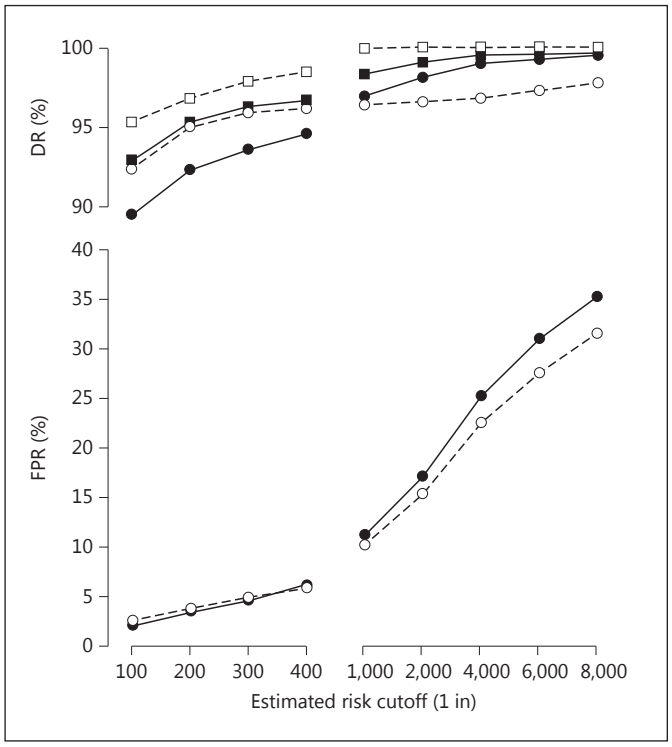

Fig. 2. Modelled and empirical FPR (modelled: solid lines and black circles, empirical: interrupted lines and open circles) and DR of trisomy 21 (modelled: solid lines and black circles, empirical: interrupted lines and open circles) and trisomy 18 or 13 (modelled: solid lines and black squares, empirical: interrupted lines and open squares) in screening for these trisomies by specific algorithms incorporating maternal age, fetal NT, FHR and serum free β-hCG, PAPP-A, PLGF and AFP.

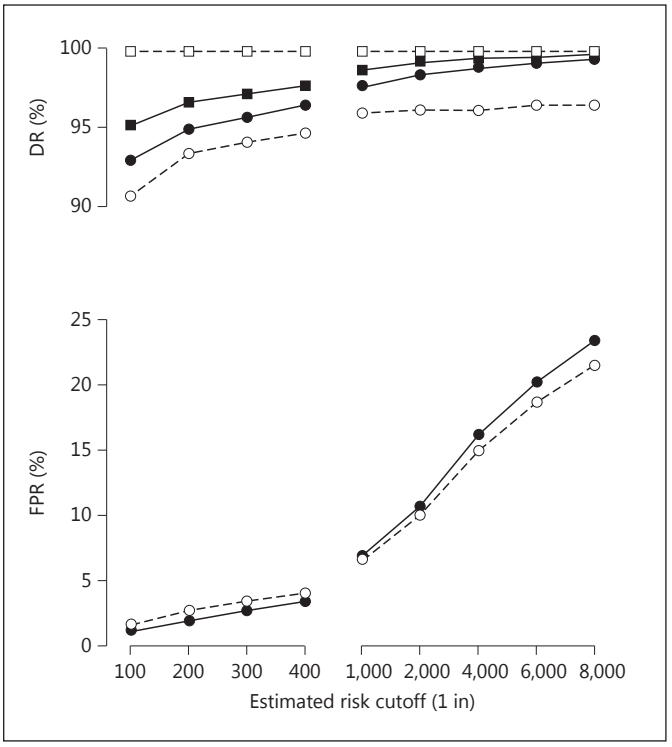

Fig. 3. Modelled and empirical FPR (modelled: solid lines and black circles, empirical: interrupted lines and open circles) and DR of trisomy 21 (modelled: solid lines and black circles, empirical: interrupted lines and open circles) and trisomy 18 or 13 (modelled: solid lines and black squares, empirical: interrupted lines and open squares) in screening for these trisomies by specific algorithms incorporating maternal age, fetal NT, FHR, DV PIV and serum free β-hCG, PAPP-A, PLGF and AFP.

Table 6 gives the performance of screening by various combinations of biomarkers according to maternal age at a fixed risk cutoff of 1:100 for trisomy 21 with the algorithm for trisomy 21 and risk cutoff of 1:100 for trisomies 18 and 13 using the algorithms for trisomy 18 and trisomy 13. Table 7 gives the same values for risk cutoff of 1:2,500 for each algorithm. For all methods of screening with increasing maternal age, there was an increase in both DR and FPR.

Discussion

Principal Findings of This Study

This study has shown that first-trimester screening by a combination of maternal age, fetal NT and serum free β-hCG and PAPP-A and the use of a risk algorithm for trisomy 21, detects about 90% of fetuses with trisomy 21 for an FPR of about 4.0% (risk cutoff of 1 in 250). This screen-positive group also includes 70% of those with trisomies 18 or 13. When FHR is taken into account and specific risks for trisomies 18 and 13, in addition to that for trisomy 21 are also used, about 90% of fetuses with trisomy 21 and 95% with trisomies 13 and 18 can be detected for the same overall FPR of 4%.

Inclusion of serum PLGF and AFP and DV PIV in the combined test would improve the performance of screening for all three trisomies with increase in DR and decrease in FPR. The results for trisomy 21 in combined screening with the addition of PLGF and AFP are compatible with the prediction from previous prospective and retrospective case control studies [5, 6, 15]. This study has shown that measurement of PLGF and AFP is also useful in screening for trisomies 18 and 13. It is therefore likely

Table 6. Modeled detection rates and FPR in first-trimester screening for fetal trisomies 21, 18 and 13 by various combinations of biomarkers according to maternal age at a fixed risk cutoff of 1:100 for trisomy 21 with the algorithm for trisomy 21 and risk cutoff of 1:100 for trisomies 18 and 13 using the algorithms for trisomy 18 and trisomy 13

Maternal age, years	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, %
20	0.9	76	84	0.9	80	85	0.7	87	91
25	1.0	77	85	1.0	81	86	0.8	88	91
30	1.3	80	87	1.2	83	88	1.0	89	93
31	1.5	81	88	1.5	84	89	1.1	90	93
32	1.7	82	89	1.7	85	90	1.3	91	94
33	2.1	83	89	2.0	86	91	1.5	91	94
34	2.5	84	90	2.4	87	91	1.7	92	95
35	2.9	85	91	2.8	89	92	2.0	93	95
36	3.6	86	92	3.4	90	93	2.3	94	96
37	4.4	88	93	4.2	91	94	2.8	94	96
38	5.5	89	94	5.1	92	95	3.3	95	97
39	6.8	90	95	6.3	93	96	3.9	96	97
40	8.7	92	96	7.6	93	96	4.6	96	98
41	11.0	93	96	9.5	94	97	5.5	97	98
42	13.6	94	97	11.6	95	98	6.7	97	98
43	16.8	95	97	13.9	96	98	8.3	98	99
44	20.8	96	98	16.7	97	98	10.0	98	99
45	25.4	97	98	20.3	98	99	12.1	98	99

Table 7. Modeled detection rates and FPR in first-trimester screening for fetal trisomies 21, 18 and 13 by various combinations of biomarkers according to maternal age at a fixed risk cutoff of 1:2,500 for trisomy 21 with the algorithm for trisomy 21 and risk cutoff of 1:2,500 for trisomies 18 and 13 using the algorithms for trisomy 18 and trisomy 13

Maternal age, years	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, %
20	13.0	94	97	11.1	96	98	6.8	97	98
25	14.4	95	97	12.1	96	98	7.4	97	98
30	19.0	96	98	16.0	97	97	9.7	98	99
31	21.1	96	98	17.5	97	97	10.5	98	99
32	23.3	97	98	19.4	98	98	11.7	98	99
33	26.1	97	99	22.0	98	98	13.1	98	99
34	29.8	98	99	24.7	98	98	14.7	99	99
35	33.8	98	99	27.5	99	99	16.7	99	99
36	38.8	98	99	30.9	99	99	19.1	99	99
37	44.2	99	99	34.7	99	99	21.9	99	>99
38	50.1	99	99	39.1	99	99	25.2	99	>99
39	56.2	99	>99	43.9	>99	>99	28.7	>99	>99
40	62.9	>99	>99	48.6	>99	>99	32.5	>99	>99
41	69.2	>99	>99	54.0	>99	>99	36.7	>99	>99
42	75.5	>99	>99	59.1	>99	>99	41.3	>99	>99
43	81.4	>99	>99	64.5	>99	>99	46.3	>99	>99
44	86.8	>99	>99	69.3	>99	>99	51.8	>99	>99
45	91.1	>99	>99	74.2	>99	>99	57.2	>99	>99

that inclusion of PLGF and AFP, which can be measured in the same sample and by the same automated machines used for free β-hCG and PAPP-A at little extra cost, would be beneficial in screening for trisomies. There is also evidence that serum PLGF and AFP are useful in first-trimester screening for preeclampsia, fetal growth restriction and preterm birth [16–18]. Similarly, measurement of DV PIV can improve the performance of screening for trisomies and it is also useful in screening for major cardiac defects [7, 19, 20].

The estimates in our model are based on a population with the maternal age distribution in England and Wales in 2011 when the median age was 29.3 years [13]. However, we provide data for each age between 20 and 45 years to allow the readers to apply the models to their own population. For all methods of screening with increasing maternal age, there is an increase in both DR and FPR.

Limitations of the Study

We derived data for NT, FHR and DV PIV from more than 85,000 prospectively screened pregnancies, and serum free β-hCG and PAPP-A from more than 70,000 pregnancies. These included more than 300 cases of trisomy 21 and more than 100 cases of trisomy 18, but only 39 of trisomy 13. The study population for PLGF was more than 25,000, including 138 cases of trisomy 21, 53 of trisomy 18 but only 11 of trisomy 13. For AFP, we examined less than 10,000 pregnancies and only 65 cases of trisomy 21, 18 of trisomy 18 and 14 of trisomy 13. Consequently, because of the relatively limited data available, the modelled measures of screening performance are subject to a high degree of uncertainty due to sampling and non-sampling errors that are not easily quantified. However, the consistency between the modelled and empirical rates is reassuring.

Clinical Implications of the Study

The UK National Screening Committee recommends that all pregnant women should be offered first-trimester combined screening for trisomy 21, and invasive testing should be considered at a risk cutoff of 1:100 [21]. This policy, in a population with the maternal age distribution in England and Wales in 2011, would detect about 86% of pregnancies with trisomy 21 and 66% with trisomies 18 and 13, at an FPR of 2.0% (table 4). Screening by the combined algorithms for the three trisomies would increase the DR to 87% for trisomy 21 and 92% for trisomies 18 and 13, with a small increase in the FPR to 2.2% (table 5). Further improvement in the performance of screening could be achieved by the addition of serum PLGF and AFP and DV PIV to the combined test with an increase in DR to 93% for trisomy 21 and 95% for trisomies 18 and 13, with a major decrease in the FPR to 1.3%.

Recent evidence suggests that analysis of cell-free DNA (cfDNA) in maternal blood can detect about 99% of cases of trisomy 21, about 97% of trisomy 18 and about 92% of trisomy 13, with respective FPRs of 0.08, 0.15 and 0.20% [22]. 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 [23]. Such 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 [24]. In contingent screening, the risk cutoff for cfDNA testing would be set at such a level as to maximize the overall DR of trisomies and minimize the proportion of the population requiring the cfDNA test.

In contingent screening, DR of 98% for trisomy 21 and 96% for trisomies 18 and 13, at an invasive testing rate of 0.7%, can be achieved by carrying out cfDNA testing if the risk cutoff from the combined test is 1:5,000, which is found in about 35% of the population [23]. The same performance of screening can potentially be achieved by offering cfDNA testing if the risk cutoff from first-line screening with the combined test plus PLGF and AFP is 1:2,500, which is found in about 20% of the population. If first-line screening is by the combined test plus PLGF, AFP and DV PIV, cfDNA testing could be carried out in only those with a risk cutoff of 1:2,000, which is found in about 10% of the population.

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