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Screening for trisomy 21 by maternal age, fetal nuchal translucency thickness, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A

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KEYWORDS: first-trimester screening; free β-hCG; nuchal translucency; PAPP-A; trisomy 21

ABSTRACT

Objectives *To derive a model and examine the performance of first-trimester combined screening by maternal age, fetal nuchal translucency (NT) thickness and maternal serum free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A).*

Methods *Prospective combined screening for trisomy 21 was carried out at 11 + 0 to 13 + 6 weeks in 56 771 singleton pregnancies, including 56 376 cases with a normal karyotype or delivery of a phenotypically normal baby (unaffected group) and 395 cases with trisomy 21. The blood test and ultrasound scan were carried out in the same visit. In each case the maternal age-related risk for trisomy 21 at term was calculated and adjusted according to the gestational age at the time of screening to derive the* a-priori *risk. The measured NT was transformed into a likelihood ratio using the mixture model of NT distributions. The measured free β-hCG and PAPP-A were converted into a multiple of the median (MoM) for gestational age, adjusted for maternal weight, ethnicity, smoking status, method of conception and parity, and a likelihood ratio was subsequently calculated. The likelihood ratios for NT and for the biochemical markers were multiplied by the* a-priori *risk to derive the patient-specific risk. Detection rates and false-positive rates were calculated by taking the proportions with risks above a given risk threshold after adjustment for maternal age according to the distribution of pregnancies in England and Wales in 2000–2002. These standardized rates were compared with detection and false-positive rates estimated using Monte Carlo methods to sample from the modeled Gaussian distributions.*

Results *The performance of screening based on the model was in good agreement with that observed in our population. In a strategy for first-trimester combined screening where the blood test and scan are carried out in the same visit it was estimated that, for false-positive rates of 3% and 5%, the detection rates were 92% and 94%, respectively, at 11 weeks, 85% and 90% at 12 weeks, and 79% and 83% at 13 weeks. In an alternative strategy, with the blood taken at 10 weeks and the measurement of NT performed at 12 weeks, the estimated detection rates were 94% and 96% for false-positive rates of 3% and 5%, respectively.*

Conclusions *The aim of the first-trimester scan is not just to screen for trisomy 21 but also to diagnose an increasing number of fetal malformations. In this respect the ability to visualize fetal anatomy is better at 12–13 weeks than at 11 weeks. Consequently, the ideal gestation for combined testing in the same visit would be 12 weeks. An alternative strategy, with the blood taken at 10 weeks and the measurement of NT performed at 12 weeks, is associated with higher detection rates of trisomy 21. However, the cost of two-stage screening would be higher and, in addition, the potential advantage in terms of detection rate may be eroded by the likely increased non-compliance with the additional step. Copyright © 2008 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Effective screening for trisomy 21 is provided by assessment of a combination of maternal age, fetal nuchal translucency (NT) thickness, and maternal serum

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free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11 to 13 + 6 weeks of gestation[1,2]. Prospective studies have demonstrated that, for a false-positive rate of 5%, combined screening can identify about 90% of affected fetuses[3,4]. In the assessment of patient-specific risks, the *a-priori* maternal age-related risk is multiplied by likelihood ratios, determined from the deviation of the measured NT, free β-hCG and PAPP-A from the respective expected median.

The traditional approaches to quantifying the deviation in the measured NT from the normal median for crown–rump length (CRL) have been either by subtraction (delta NT method) or by division (multiples of the median (MoM) method). However, we have recently proposed a new approach based on the observation that in both trisomy 21 and unaffected pregnancies fetal NT follows two distributions, one which is CRL dependent and another which is CRL independent[5]. In this mixture model the proportions of trisomy 21 and unaffected fetuses that follow the CRL-independent distribution are 95% and 5%, respectively. The detection rate of a screening policy based on maternal age and the mixture model of fetal NT was 80% for a 5% false-positive rate[5].

In biochemical testing it is necessary to make adjustments in the measured maternal serum concentration of free β-hCG and PAPP-A to correct for certain maternal and pregnancy characteristics. Essentially, each measured level is first converted to a multiple of the expected normal median (MoM) specific to a pregnancy of the same gestational age, maternal weight, ethnicity, smoking status, method of conception and parity, as well as the machine and reagents used for the assays. In a recent study of 491 pregnancies with trisomy 21 and 96 803 unaffected pregnancies we used multiple regression analysis to define the contribution of maternal variables that influence the measured concentration of free β-hCG and PAPP-A, and the interaction between these covariates[6]. The traditional method of sequential adjustment for each individual parameter fails to take into account the interaction between the covariates[7]. The detection rate of a screening policy based on maternal age and the two biochemical markers was 65% for a 5% false-positive rate[6].

In this study of more than 56 000 normal pregnancies and 395 cases with trisomy 21 we used the mixture model for fetal NT[5] and the new estimates for free β-hCG and PAPP-A based on the multiple regression approach[6] to examine the performance of first-trimester combined screening by maternal age, fetal NT, and maternal serum free β-hCG and PAPP-A.

METHODS

At the Fetal Medicine Centre, London, screening for trisomy 21 is carried out by a combination of maternal age, fetal NT thickness, and maternal serum free β-hCG and PAPP-A in a one-stop-clinic for first-trimester assessment of risk at 11 + 0 to 13 + 6 weeks of gestation[3]. Transabdominal ultrasound examination is performed to diagnose any major fetal defects, and for measurement of CRL and fetal NT thickness[1]. The Kryptor system (Brahms AG, Berlin, Germany) is used to measure PAPP-A and free β-hCG. Maternal demographic characteristics, ultrasonographic measurements and biochemical results are recorded in a computer database. Karyotype results and details of pregnancy outcomes are added to the database as soon as they become available. A search of the database was done to identify all singleton pregnancies in which first-trimester combined screening was carried out from July 1999 to July 2007.

Statistical analysis

The following steps were taken. First, the maternal age-related risk for trisomy 21 at term was calculated and adjusted according to the gestational age at the time of screening[8,9]. Second, the measured NT was transformed into a likelihood ratio using the mixture model of NT distributions[5]. Third, the measured free β-hCG and PAPP-A were converted into a MoM for gestational age, adjusted for maternal weight, ethnicity, smoking status, method of conception and parity, and subsequently a likelihood ratio was calculated from the fitted bivariate Gaussian distributions in trisomy 21 and unaffected pregnancies. Fourth, the likelihood ratios for NT and for the biochemical markers were multiplied by the age-related risk at the time of screening in each case. Fifth, detection rates and false-positive rates were calculated by taking the proportions with risks above a given risk threshold after adjustment for maternal age, according to the distribution of pregnancies in England and Wales in 2000–2002[10]. These standardized rates were compared with detection and false-positive rates estimated using Monte Carlo methods to sample from the modeled Gaussian distributions[5,6].

RESULTS

The search of the database identified 60 172 singleton pregnancies. In 3053 (5.1%) cases the outcome or one of the covariates were not available and in 348 (0.6%) cases there was a chromosomal abnormality other than trisomy 21. Thus, our study population consisted of 56 376 pregnancies with a normal karyotype or delivery of a phenotypically normal baby (unaffected group) and 395 cases with trisomy 21. The characteristics of the study population are summarized in Table 1. According to the maternal age distribution of our population and the gestational age at the time of screening we would have expected 367 (95% prediction interval, 329–405) cases with trisomy 21[9].

The distribution of NT in trisomy 21 fetuses is shown in Figure 1. In about 5% of cases the NT followed the CRL-dependent distribution of unaffected pregnancies, whereas in 95% of cases the NT did not change with gestation and in this group the mean (SD) NT was 3.4 (1.6) mm. Contour plots for free β-hCG and PAPP-A in trisomy 21 and unaffected pregnancies are shown in Figure 2. In the unaffected pregnancies the median free β-hCG was 1.0 (range, 0.03–30.4) MoM and the median

Table 1 Characteristics of the study population of 56 771 women

Parameter	*Median (range) or* n *(%)*
Maternal characteristics	
Age (years)	35.4 (14.1–52.5)
Weight (kg)	63.6 (34.0–165.0)
Spontaneous conception	54 135 (95.4)
Smoker	2566 (4.5)
Ethnicity	
Caucasian	50 708 (89.3)
Afro-Caribbean	2430 (4.3)
East Asian	640 (1.1)
South Asian	2218 (3.9)
Mixed	775 (1.4)
Gestational age	
11 + 0 to 11 + 6 weeks	5546 (9.8)
12 + 0 to 12 + 6 weeks	31 883 (56.2)
13 + 0 to 13 + 6 weeks	19 342 (34.1)
Crown–rump length (mm)	62.9 (45.0–84.0)
Karyotype	
Normal	56 376 (99.3)
Trisomy 21	395 (0.7)

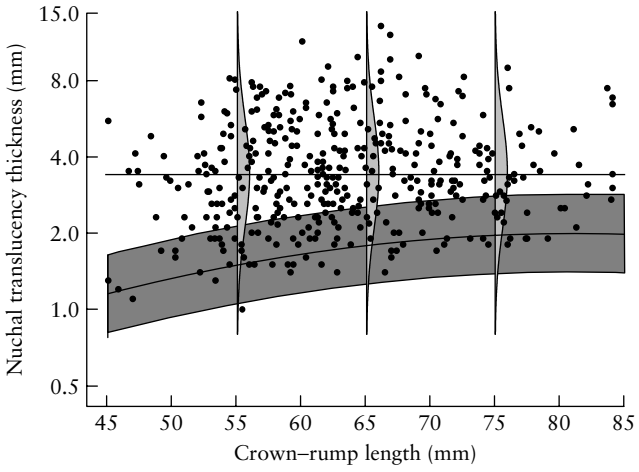

Figure 1 Distribution of nuchal translucency thickness with respect to crown–rump length (CRL) in trisomy 21 fetuses. In this mixture model the nuchal translucency thickness in 5% of cases follows the CRL-dependent distribution (dark shaded area with median and 5th and 95th centiles) and in 95% of cases nuchal translucency does not change with gestation and the mean is 3.4 mm (light shaded bells).

PAPP-A was 1.0 (range, 0.02–7.9) MoM. In the trisomy 21 pregnancies the median free β-hCG was 2.0 (range, 0.1–11.3) MoM and the median PAPP-A was 0.5 (range, 0.05–2.2) MoM. In the trisomy 21 pregnancies there was a significant increase in log MoM PAPP-A ($P < 0.0001$) and log MoM free β-hCG ($P = 0.039$) with gestation (Figure 3).

Performance of screening for trisomy 21 by maternal age alone, maternal age and serum biochemistry, maternal age and fetal NT, and combined screening is compared in Figure 4. For a 5% false-positive rate the respective detection rates were 30%, 65%, 80% and 91%. The performance of screening for different risk cut-offs varied with maternal age (Figure 5). For example, at a risk cut-off

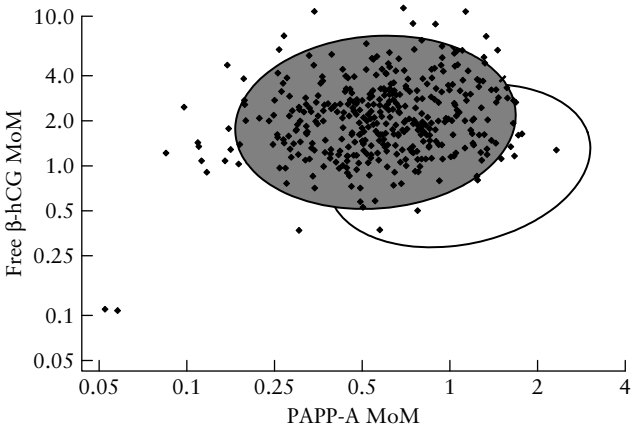

Figure 2 Distribution of multiples of the median (MoM) values for free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) in chromosomally normal pregnancies (90% contour, unshaded) and in trisomy 21 pregnancies (90% contour, shaded). Raw data for our sample of 395 cases of trisomy 21 are plotted (♦).

of 1 in 100, the detection rates for a 20-, 30- and 40-year-old woman were 72%, 77% and 92%, respectively, for a false-positive rate of 1.1%, 1.9% and 9.9%, respectively.

In combined screening the detection rates for fixed false-positive rates observed in our population, after adjustment for maternal age according to the distribution of pregnancies in England and Wales in 2000–2002, were compared with the values derived from the modeled Gaussian distributions (Table 2). These values indicate that the performance of screening based on the model is in good agreement with that observed in our population. This is further highlighted by the data presented in Table 3, where for each range of estimated risks according to the model there is good agreement with the number of observed cases of trisomy 21. Table 4 shows the modeled detection and false-positive rates for different risk cut-offs. The risk was 1 in 200 or higher in 89% of trisomy 21 and in 4.6% of the unaffected pregnancies. Table 5 shows the estimated detection rates of trisomy 21 with a policy of biochemical testing and ultrasound scanning carried out at two separate visits, with the first done at 10 or 11 weeks and the second at 12–13 weeks.

DISCUSSION

This study shows that screening by maternal age, fetal NT, free β-hCG and PAPP-A identifies about 90% of all pregnancies with trisomy 21 for a false-positive rate of 5%, and that screening at 11 weeks of gestation performs substantially better than at 13 weeks. It also demonstrates that there is good agreement between the estimated risks derived from the model and the results in our study population.

In the calculation of patient-specific risk for trisomy 21 the maternal age-related *a-priori* risk is multiplied by the likelihood ratios for the fetal NT and maternal serum biochemical markers. Consequently, for the same measurements of NT, free β-hCG and PAPP-A the final

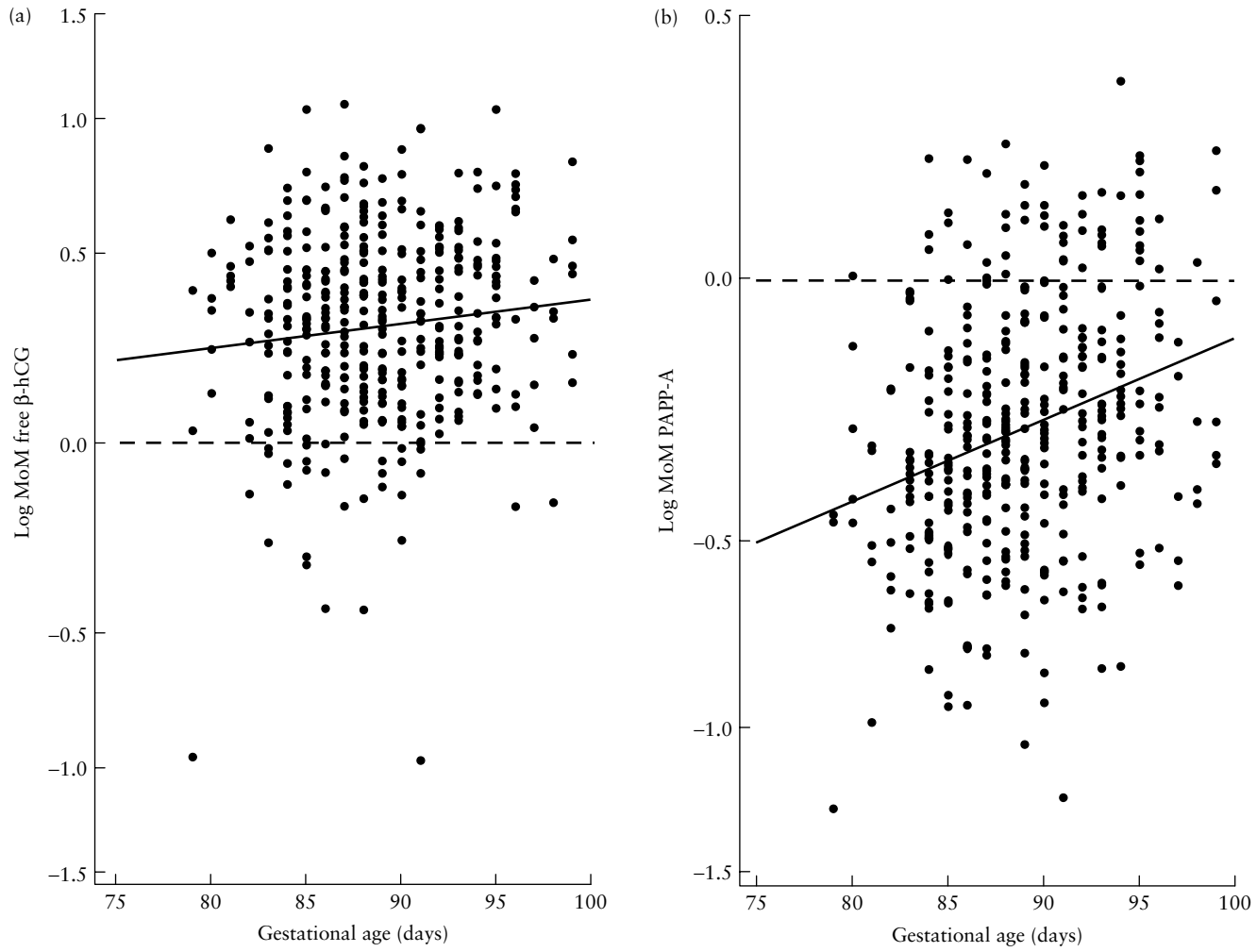

Figure 3 Individual values and regression lines (———) of log multiples of the median (MoM) free beta-human chorionic gonadotropin (β-hCG) (a) and pregnancy-associated plasma protein-A (PAPP-A) (b) in trisomy 21 pregnancies with respect to gestational age. The dashed line is the regression line in normal pregnancies.

Table 2 Detection rates of trisomy 21 for given false-positive rates observed in our population, after adjustment for maternal age according to the distribution of pregnancies in England and Wales in 2000–2002, compared with values derived from the modeled Gaussian distributions

	Detection rate (%)							
False-positive rate (%)	*Overall* (n = *395*)		*11 weeks* (n = *28*)		*12 weeks* (n = *223*)		*13* + weeks (n = *144*)	
	Observed	*Modeled*	*Observed*	*Modeled*	*Observed*	*Modeled*	*Observed*	*Modeled*
1	78	75	78	83	84	76	77	69
2	84	81	81	88	88	83	84	75
3	89	84	90	92	91	85	86	79
4	90	86	95	93	91	88	89	81
5	91	89	96	94	92	90	90	83

risk will vary with maternal age, and for the same risk cut-off both the detection and false-positive rates increase with maternal age (Figure 5). This information, which is often requested by patients, may be useful in counseling. Furthermore, it is essential in comparing overall detection and false-positive rates between centers or against a national standard because the overall performance depends on the maternal age distribution of the screened population.

The National Screening Committee in the UK recommended that a screening test for trisomy 21 should provide an overall detection rate of at least 75% for a false-positive rate of 3% or less[11]. In our study both the modeled and observed detection rate of 75% was achieved at a false-positive rate of 1%. At a false-positive rate of 3% the observed and modeled detection rates were 89% and 84%, respectively. These detection rates are higher than the respective 53% and 60% achieved by the triple

and quadruple second-trimester biochemical tests[12]. Furthermore, the performance of our combined model is substantially superior to that in two previous multicenter studies in the UK and USA[13,14]. The Serum, Urine and Ultrasound Screening Study (SURUSS) recruited 47 053 pregnancies, including 101 with trisomy 21, but NT was measured in only 39 983 cases, including 85 with trisomy 21; free β-hCG and PAPP-A were measured retrospectively in stored samples from 98 trisomy 21 pregnancies and 1090 matched unaffected controls[13]. The modeled detection rates from combined screening were 68%, 79% and 84% for respective false-positive rates of 1%, 3% and 5%. Similarly, in the First- and Second-Trimester Evaluation of Risk (FASTER) study, 38 167 pregnancies were recruited, including 117 with trisomy 21; free β-hCG and PAPP-A were measured retrospectively in stored samples from 79 trisomy 21 pregnancies and 395 matched unaffected controls[14]. The modeled detection rates at 12 weeks were 72% and 85% for respective false-positive rates of 1% and 5%.

The performance of combined screening for trisomy 21 decreased with gestation and, for a 3% false-positive rate, the estimated detection rate was 92%, 85% and 79% at 11, 12 and 13 weeks, respectively. This is a consequence of both fetal NT and serum PAPP-A. In the mixture model of NT the overlap between the CRL-dependent and CRL-independent distribution increases with gestation, and therefore the separation between trisomy 21 and unaffected pregnancies decreases. Similarly, the separation in maternal serum PAPP-A between trisomy 21 and unaffected pregnancies decreases with gestation. Although there is an increase in the separation for free β-hCG with gestation this is considerably less than the decrease observed for PAPP-A. The implications of these findings are that, in terms of the performance of screening for trisomy 21 in a set-up in which biochemical testing and the ultrasound scan are carried out in the same visit, the best gestational age is 11 weeks. However, the aim of the first-trimester scan is not just to screen for trisomy 21 but also to diagnose an increasing number of fetal malformations, and in this respect the ability to visualize fetal anatomy is better at 12–13 weeks than at 11 weeks[15]. Consequently, the ideal gestation for combined testing in the same visit would be 12 weeks when, for false-positive rates of 3% and 5%, the estimated detection rates of trisomy 21 are 85% and 90%, respectively.

An alternative strategy for first-trimester combined screening is for biochemical testing and ultrasound scanning to be carried out in two separate visits, with the

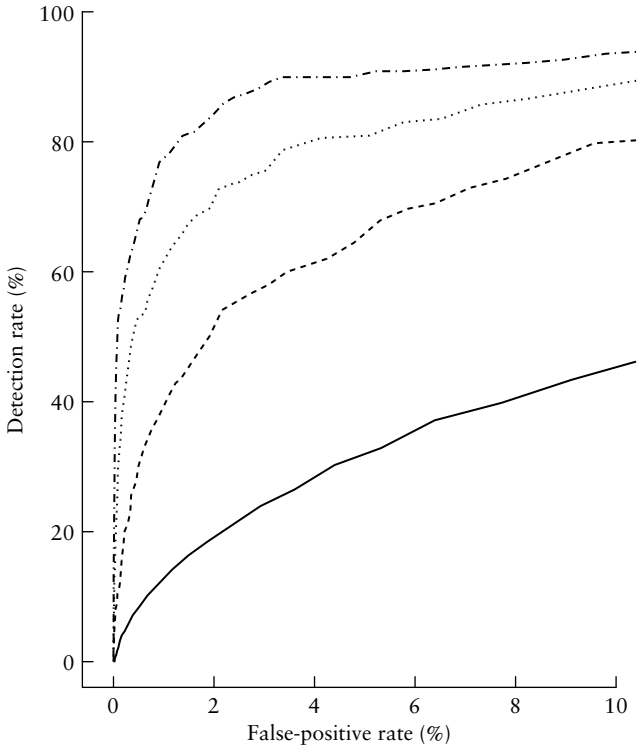

Figure 4 Receiver–operating characteristics curves for the performance of screening for trisomy 21 by maternal age alone (———); maternal age, serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A (- - - -); maternal age and fetal nuchal translucency thickness (·····); and combined screening (·–·–·–).

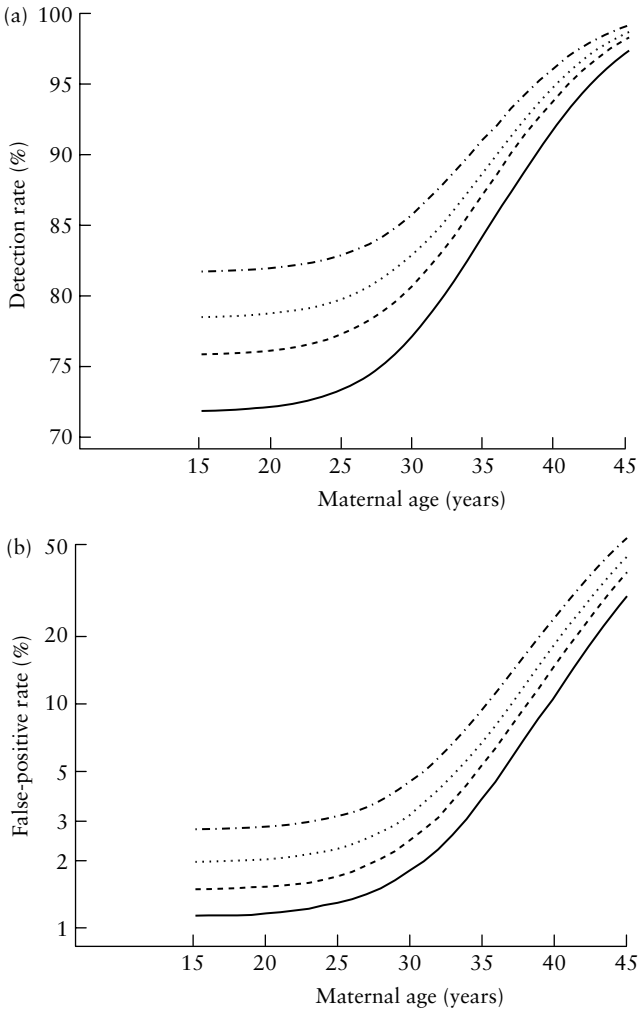

Figure 5 Performance of combined first-trimester screening for trisomy 21. Modeled detection rate (a) and false-positive rate (b) for risk cut-offs of 1 in 300 (·–·–·–), 1 in 200 (·····), 1 in 150 (- - - -) and 1 in 100 (———) in relation to maternal age.

Table 3 Accuracy of estimated risk of trisomy 21 by a combination of maternal age, fetal nuchal translucency thickness, and maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A

Estimated risk of trisomy 21		*Trisomy 21*	*Unaffected*	*Observed*
Range	*Median*	(n *(%)*)	(n *(%)*)	*risk*
≥ 1 in 10	1 in 5	273 (69.1)	340 (0.6)	1 in 2
1 in 11 to 1 in 50	1 in 28	60 (15.2)	909 (1.6)	1 in 16
1 in 51 to 1 in 100	1 in 74	16 (4.1)	947 (1.7)	1 in 60
1 in 101 to 1 in 250	1 in 173	15 (3.8)	2305 (4.1)	1 in 155
1 in 251 to 1 in 1000	1 in 579	19 (4.8)	7362 (13.1)	1 in 388
1 in 1001 to 1 in 5000	1 in 2443	10 (2.5)	16 685 (29.6)	1 in 1 670
< 1 in 5000	1 in 16 144	2 (0.5)	27 828 (49.4)	1 in 13 915

Table 4 Modeled detection rates (DRs) and false-positive rates (FPRs) for given risk cut-offs for trisomy 21

	Total		*11 weeks*		*12 weeks*		*13 weeks*	
Risk cut-off	*DR (%)*	*FPR (%)*	*DR (%)*	*FPR (%)*	*DR (%)*	*FPR (%)*	*DR (%)*	*FPR (%)*
1 in 20	71	0.6	78	0.6	71	0.6	62	0.5
1 in 50	79	1.4	86	1.4	79	1.4	71	1.3
1 in 100	84	2.6	90	2.5	85	2.5	77	2.5
1 in 150	87	3.6	92	3.5	87	3.5	81	3.6
1 in 200	89	4.6	93	4.3	89	4.5	83	4.7
1 in 300	91	6.3	95	5.8	91	6.1	86	6.7
1 in 1000	95	15.9	98	13.3	96	15.2	93	17.9

first done at 10–11 weeks and the second at 12–13 weeks. For false-positive rates of 3% and 5%, the estimated detection rates of combined testing with the blood taken at 10 weeks and the measurement of NT performed at 12 weeks are 94% and 96%, respectively. The cost and patient acceptability of the two alternative policies of first-trimester testing will depend on the existing infrastructure of antenatal care. The potential advantage of two-stage screening in terms of detection rate may be eroded by the likely increased non-compliance with the additional step. Another element to be considered in terms of alternative policies of early screening is the contribution of additional sonographic markers, such as absent nasal bone, wide frontomaxillary facial angle, reversed end-diastolic flow in the ductus venosus and tricuspid regurgitation, which have already been incorporated into routine practice in some specialist centers[3,16–19].

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Table 5 Estimated detection rates of trisomy 21 from different policies on screening according to the timing of biochemical testing and ultrasound scanning

	Detection rate (%)						
	Scan and blood at same visit			*Scan 12 weeks*		*Scan 13 weeks*	
False-positive rate (%)	*11 weeks*	*12 weeks*	*13 weeks*	*Blood 10 weeks*	*Blood 11 weeks*	*Blood 10 weeks*	*Blood 11 weeks*
1	83	76	69	88	81	85	78
2	88	83	75	92	87	90	83
3	92	85	79	94	89	92	86
4	93	88	81	95	91	94	88
5	94	90	83	96	92	95	90
10	97	94	89	98	96	97	94

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