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Screening for trisomy 18 by maternal age, fetal nuchal translucency, free β-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 18

ABSTRACT

Objectives *To derive a model and examine the performance of first-trimester screening for trisomy 18 by maternal age, fetal nuchal translucency (NT) thickness, and maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A).*

Methods *Prospective combined screening for trisomy 21 was performed at 11 + 0 to 13 + 6 weeks in 56 893 singleton pregnancies, including 56 376 cases of euploid fetuses, 395 with trisomy 21 and 122 with trisomy 18. The measured free β-hCG and PAPP-A were converted into a multiple of the median (MoM) and then into likelihood ratios (LR). Similarly, the measured NT was transformed into LRs using the mixture model of NT distributions. In each case the LRs for NT and the biochemical markers were multiplied by the age and gestation-related risk to derive the risk for trisomy 21 and trisomy 18. Detection rates (DRs) and false-positive rates (FPRs) were calculated by taking the proportions with risks above a given risk threshold.*

Results *In screening with the algorithm for trisomy 21, at a FPR of 3%, the estimated DRs of trisomies 21 and 18 were 89% and 82%, respectively. The use of an algorithm for trisomy 18 identified 93% of affected fetuses at a FPR of 0.2%. When the algorithm for trisomy 21 was used and screen positivity was fixed at a FPR of 3%, and in addition the algorithm for trisomy 18 was used and screen positivity was fixed at a FPR of 0.2%, the overall FPR was 3.1% and the DRs of trisomies 21 and 18 were 90% and 97%, respectively.*

Conclusions *A beneficial side effect of first-trimester combined screening for trisomy 21 is the detection of a high proportion of fetuses with trisomy 18. If an algorithm for trisomy 18 in addition to the one for trisomy 21 is used, more than 95% of trisomy 18 fetuses can be detected with a minor increase of 0.1% in the overall FPR. Copyright © 2008 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Effective screening for trisomy 21 is provided by a combination of maternal age, fetal nuchal translucency (NT) thickness, serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11 + 0 to 13 + 6 weeks of gestation, with a detection rate of about 90% for a false-positive rate of 5%[1,2]. A beneficial consequence of screening for trisomy 21 is the early diagnosis of trisomy 18, which is the second most common chromosomal abnormality. At 11 + 0 to 13 + 6 weeks the relative prevalence of trisomy 18 to trisomy 21 is one to three[1].

Trisomies 21 and 18 are associated with increased maternal age, increased fetal NT and decreased maternal serum PAPP-A but, in trisomy 21, serum free β-hCG is increased whereas in trisomy 18 it is decreased[1–3]. 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. In biochemical testing, 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[4].

In terms of NT the traditional approaches to quantifying the deviation in the measured value from the

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normal median for crown–rump length (CRL) have been either by subtraction (delta NT method) or by division (multiple of the median (MoM) method). However, we have recently proposed a new approach based on the observation that fetal NT follows two distributions in both trisomic and unaffected pregnancies, one of which is CRL dependent and the other CRL independent[5]. In this mixture model the proportion of trisomy 21, trisomy 18 and unaffected fetuses that follow the CRL-independent distribution is 95%, 71% and 5%, respectively. The median CRL-independent NT was 2.0 mm in the euploid group, 3.4 mm in trisomy 21 and 5.5 mm in trisomy 18.

In this study we investigate the performance in the detection of trisomy 18 of specific algorithms for trisomy 21 and for trisomy 18 based on the respective *a-priori* maternal age-related risk, distribution of NT according to the mixture model, and distribution of serum free β-hCG and PAPP-A MoMs.

METHODS

This was a prospective screening study for trisomy 21 in singleton pregnancies 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 (OSCAR) at 11 + 0 to 13 + 6 weeks of gestation[2]. Transabdominal ultrasound examination was performed to diagnose any major fetal defects and for measurement of CRL and fetal NT thickness[1]. Automated machines that provide reproducible results within 30 min were used to measure PAPP-A and free β-hCG (Kryptor system, Brahms AG, Berlin, Germany; or Delfia Express System, Perkin Elmer, Waltham, MA, USA). Maternal demographic characteristics, ultrasononographic measurements and biochemical results were recorded in a computer database. Karyotype results and details on pregnancy outcomes were added into the database as soon as they became 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 May 2007.

Statistical analysis

The following steps were taken. First, the maternal age-related risks for trisomy 21 and trisomy 18 at term were calculated, and adjusted according to the gestational age at the time of screening[6–8]. Second, the measured NT was transformed into likelihood ratios for trisomy 21 and trisomy 18 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, parity and machine for the assays, and likelihood ratios were subsequently calculated from the fitted bivariate Gaussian distributions of trisomy 21 and unaffected pregnancies and trisomy 18 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, because the median maternal age of our study population (35 years) was higher than in the general population (29 years)[9].

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 226 (0.4%) cases there was a chromosomal abnormality other than trisomy 21 or trisomy 18. Thus, our study population consisted of 56 376 pregnancies with a normal karyotype or delivery of a phenotypically normal baby (unaffected group), 395 cases of trisomy 21 and 122 cases of trisomy 18. The characteristics of the study population are summarized in Table 1.

The distribution of NT in the trisomy 18 fetuses is shown in Figure 1. According to the fitted mixture model[5], in an estimated 29% of cases NT followed the CRL-dependent distribution of unaffected pregnancies. In 71% of cases the NT was independent of gestation and the median was 5.5 mm. A scatter diagram of free β-hCG and PAPP-A MoM in the trisomy 18 pregnancies is shown in Figure 2. Fitted contours containing 90% of the fitted Gaussian distributions for trisomy 18, trisomy 21 and unaffected pregnancies are superimposed on the scatter diagram.

In unaffected pregnancies the median free β-hCG was 1.0 (range, 0.03–30.4) MoM and the median 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, and in the trisomy 18 pregnancies the

Table 1 Characteristics of the study population ($n = 56\,893$)

Parameter	*Median (range) or* n *(%)*
Maternal characteristics	
Age (years)	35.4 (14.1–52.5)
Weight (kg)	63.6 (34–165)
Spontaneous conception	54 251 (95.4)
Smoker	2582 (4.5)
Ethnicity	
Caucasian	50 815 (89.3)
Afro-Caribbean	2437 (4.3)
East Asian	642 (1.1)
South Asian	2223 (3.9)
Mixed	776 (1.4)
Gestational age	
11 + 0 to 11 + 6 weeks	5612 (9.9)
12 + 0 to 12 + 6 weeks	31 929 (56.1)
13 + 0 to 13 + 6 weeks	19 352 (34.0)
Crown–rump length (mm)	62.8 (45.0–84.0)
Karyotype	
Normal	56 376 (99.1)
Trisomy 21	395 (0.7)
Trisomy 18	122 (0.2)

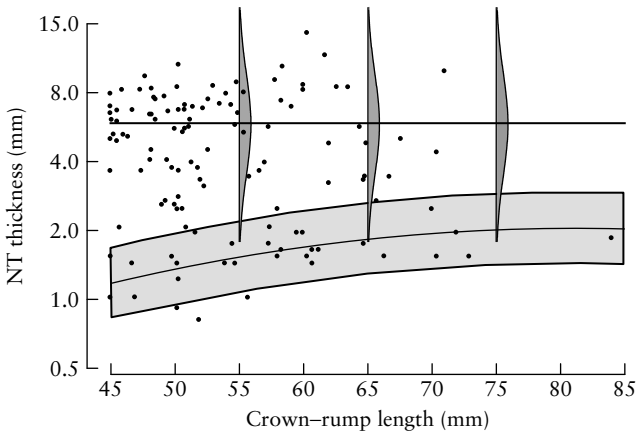

Figure 1 Distribution of nuchal translucency (NT) thickness with crown–rump length (CRL) in trisomy 18 fetuses. In 29% of fetuses with trisomy 18, the NT follows the CRL-dependent distribution, which applies to 95% of chromosomally normal fetuses (light shaded area; median, 5th and 95th centiles), and in 71% of cases the NT does not change with gestation and the mean is 5.5 mm (dark shaded bells represent the mean, 5th and 95th centiles).

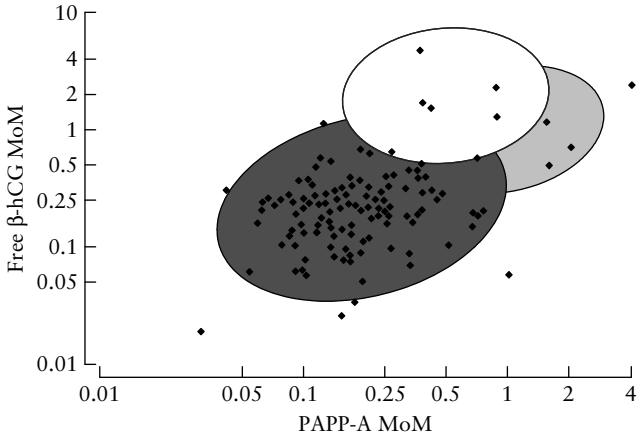

Figure 2 Distribution of multiple of the median (MoM) values of serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) in normal fetuses (light-shaded ellipse), in trisomy 21 fetuses (unshaded ellipse) and in trisomy 18 fetuses (dark-shaded ellipse containing 90% of cases). The individual diamonds are the values of the trisomy 18 fetuses.

median free β-hCG was 0.2 (range, 0.02–4.7) MoM and the median PAPP-A was 0.2 (range 0.03–4.1) MoM. Details of the biochemical parameter estimates of trisomy 18 are shown in Table 2. In the trisomy 18 pregnancies there was no significant association between log MoM free β-hCG and gestational age ($P = 0.879$), or between log MoM PAPP-A and gestational age ($P = 0.900$).

Table 3 shows detection rates for trisomy 18 for given false-positive rates using the trisomy 18 algorithm for maternal age and NT, maternal age and biochemistry, and for the combination of maternal age, NT and biochemistry. For a false-positive rate of 0.1% the respective detection rates were 56%, 66% and 88%. With combined screening using the algorithm for trisomy 18 the detection rate of trisomy 18 was 93% and 97% at respective false-positive rates of 0.2% and 0.3%.

Performance of screening for trisomy 21 and trisomy 18 by combined screening with maternal age, fetal NT and maternal serum biochemistry using both algorithms independently from each other is compared in Figure 3 and Table 4. At a 3% false-positive rate the estimated detection rates of trisomy 21 and trisomy 18 using our previously reported algorithm for trisomy 21[10] were 89% and 82%, respectively.

Table 5 shows the total false-positive rate and detection rate of trisomy 18 in screening for trisomy 18 by the combined use of the algorithm for trisomy 21[10] and the algorithm for trisomy 18. When screen positivity was defined by a 3% false-positive rate using the algorithm for trisomy 21[10], and in addition by a 0.2% false-positive rate using the algorithm for trisomy 18, then the detection rate of trisomy 18 was 97% for a total false-positive rate of 3.1%. The false-positive rate of 0.2% with the algorithm of trisomy 18 refers to a risk cut-off of one in 50.

DISCUSSION

This study shows that screening for trisomy 21 by maternal age, fetal NT, free β-hCG and PAPP-A identifies about 90% of fetuses with trisomy 21 and 82% of fetuses with trisomy 18 for a false-positive rate of 3%. The use of a specific risk algorithm for trisomy 18 identifies 93% of affected fetuses at a false-positive rate of 0.2%.

Three previous studies proposed the use of a specific risk algorithm for trisomy 18 based on maternal age, fetal NT, free β-hCG and PAPP-A[3,11,12]. The first study examined fetal NT and serum metabolites in 50 cases of trisomy 18 and 947 controls, and developed an algorithm for trisomy 18. This identified 86% of the affected fetuses at a false-positive rate of 0.5%[3]. A second study

Table 2 Biochemical parameter estimates and correlation for cases with trisomy 18 ($n = 122$)

	Estimate (95% CI)	
Parameter	*Normal karyotype*	*Trisomy 18 karyotype*
Mean log MoM free β-hCG	0 (–)	−0.6668 (−0.7123 to −0.6213)
Mean log MoM PAPP-A	0 (–)	−0.7149 (−0.7549 to −0.6749)
SD of log MoM free β-hCG	0.2544 (0.2529 to 0.2559)	0.3723 (0.3188 to 0.4454)
SD of log MoM PAPP-A	0.2203 (0.2190 to 0.2216)	0.3307 (0.2832 to 0.3957)
Correlation	0.2143 (0.2064 to 0.2222)	0.3860 (0.1683 to 0.5677)

The estimates for unaffected pregnancies are from our previous study of 96 717 cases[4]. β-hCG, β-human chorionic gonadotropin; MoM, multiples of the median; PAPP-A, pregnancy-associated plasma protein-A.

Table 3 Detection rate of trisomy 18 for given false-positive rates (FPR) using the trisomy 18 algorithm for maternal age and fetal nuchal translucency (NT), maternal age and biochemistry, and for the combination of maternal age, NT and biochemistry

	Detection rate (%)		
FPR (%)	*NT*	*Free β-hCG and PAPP-A*	*Combined*
0.1	56	66	88
0.2	61	74	93
0.3	65	77	97
0.4	67	79	97
0.5	68	80	97

Adjustment for maternal age was done according to the distribution of pregnancies in England and Wales in 2000–2002[9].
β-hCG, β-human chorionic gonadotropin; PAPP-A, pregnancy-associated plasma protein-A.

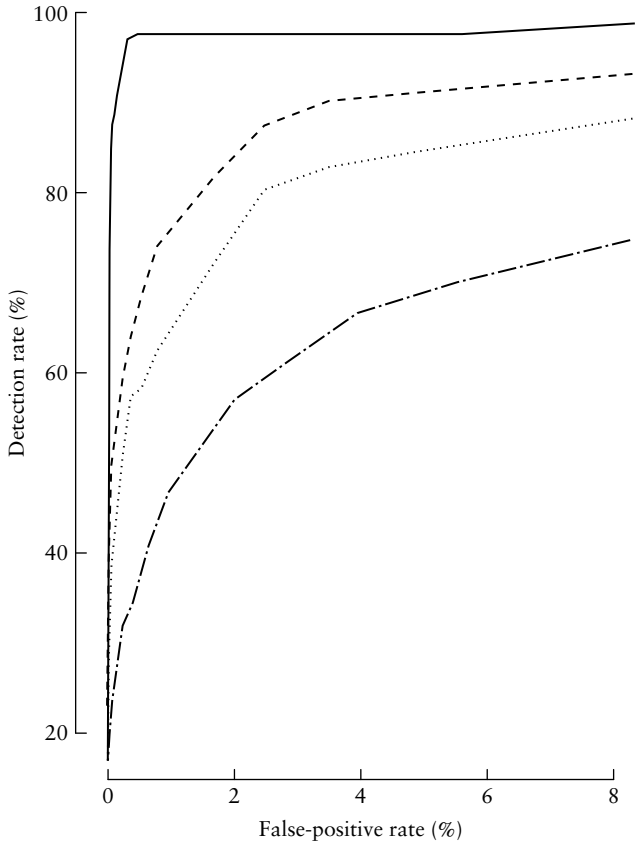

Figure 3 Receiver–operating characteristics curves for the performance of screening for trisomy 21 (T21) and trisomy 18 (T18) by maternal age, fetal nuchal translucency thickness, and serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A, using the algorithm for T21[10] and the algorithm for T18. Curves are shown for T18 by T18 algorithm (———), T21 by T21 algorithm (- - - -), T18 by T21 algorithm (·····) and T21 by T18 algorithm (—·—·—·—).

examined 59 cases of trisomy 18 and 45 cases of trisomy 13, and reported a combined trisomy 18/13 algorithm. It was estimated that 95% of affected fetuses would be detected at a false-positive rate of 0.3%[11]. In the third study first-trimester combined screening for trisomy 21 was performed in 35 974 pregnancies including 28 cases

Table 4 Detection rates for fixed false-positive rates (FPR) for each chromosomal abnormality using the algorithms for trisomy 21 and trisomy 18 based on maternal age, fetal nuchal translucency and serum biochemistry

Trisomy 21 algorithm[10]			*Trisomy 18 algorithm*		
	Detection rate (%)			*Detection rate (%)*	
FPR (%)	*Trisomy 21*	*Trisomy 18*	*FPR (%)*	*Trisomy 21*	*Trisomy 18*
1	78	67	0.1	20	88
2	84	77	0.2	28	93
3	89	82	0.3	32	97
4	90	83	0.4	33	97
5	91	84	0.5	35	97

Table 5 Overall false-positive rate (FPR) and detection rate (in parentheses) of trisomy 18 in screening for trisomy 18 by the combined use of the algorithm for trisomy 21 and the algorithm for trisomy 18

Algorithm for trisomy 21: FPR (%)	*Algorithm for trisomy 18: FPR (risk cut-off)*				
	0.1% (1 in 21)	*0.2% (1 in 50)*	*0.3% (1 in 80)*	*0.4% (1 in 110)*	*0.5% (1 in 138)*
1.0	1.1 (94)	1.1 (95)	1.2 (97)	1.3 (97)	1.4 (97)
2.0	2.0 (96)	2.1 (97)	2.2 (99)	2.3 (99)	2.4 (99)
3.0	3.0 (96)	3.1 (97)	3.2 (99)	3.3 (99)	3.4 (99)
4.0	4.0 (96)	4.1 (97)	4.2 (99)	4.2 (99)	4.3 (99)
5.0	5.0 (96)	5.1 (97)	5.2 (99)	5.2 (99)	5.3 (99)

When screen positivity was defined by a 3% FPR using the algorithm for trisomy 21[10] and in addition by a 0.2% FPR using the algorithm for trisomy 18, then the detection rate of trisomy 18 was 97% for a total FPR of 3.1%. The FPR of 0.2% with the algorithm of trisomy 18 refers to a risk cut-off of one in 50.

of trisomy 18. It was estimated that with the use of a trisomy 18 algorithm 79% of affected fetuses would be detected at false-positive rate of 0.3%[12].

The National Screening Committee in the UK recommended that a screening test for trisomy 21 should provide a detection rate of at least 75% for a false-positive rate of 3% or less[13]. In our study the detection rate of 75% was achieved at a false-positive rate of less than 1%, and at a false-positive rate of 3% the detection rate was 90%. A beneficial side effect of first-trimester combined screening for trisomy 21 is the identification of a high proportion of fetuses with trisomy 18, with a detection rate of 82% at a false-positive rate of 3%.

The majority of fetuses with trisomy 18 die *in utero* and the relative prevalence of trisomy 18 to trisomy 21 at 12 weeks is one to three, compared with one to 12 at birth[8]. Those born alive usually die within a few days and certainly within the first year of postnatal life[14]. It could therefore be argued that it is unnecessary to subject women to the difficult decisions regarding invasive testing and ultimately pregnancy termination in an affected pregnancy that would otherwise have ended in spontaneous miscarriage or early postnatal death. Alternatively, it

could be argued that, because many trisomy 18 fetuses can be identified during the second trimester by the presence of multiple sonographic features, women could have the option of second-trimester termination of pregnancy and avoid the risk of invasive testing if the result of first-trimester screening proves to be false positive.

However, most women prefer screening to be performed in the first rather than in the second trimester, and the provision of a high-quality first-trimester screening service significantly enhances the autonomy of pregnant women[15–17]. Additionally, termination of pregnancy is much safer in the first than in the second trimester[18]. As far as the false-positive rate is concerned, this study has demonstrated that, with the addition of only 0.1% to the overall rate, more than 95% of trisomy 18 fetuses can be identified by a first-trimester screening program for trisomy 21 with the inclusion of an algorithm for trisomy 18. In contrast, there is no algorithm for second-trimester sonographic screening and, as many of the affected fetuses present only a few of about 20 markers, the false-positive rate is likely to be substantially higher than 0.1%.

In first-trimester screening for trisomy 21 by maternal age, fetal NT, free β-hCG and PAPP-A the performance of the test is improved by the inclusion of additional sonographic markers, such as absent nasal bone, wide frontomaxillary facial angle, reversed end-diastolic flow in the ductus venosus and tricuspid regurgitation[19–22]. These markers are also commonly found in fetuses with trisomy 18[21–24], but the effect of their inclusion in screening for trisomy 21 on the performance of screening for trisomy 18 remains to be determined.

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