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Prospective validation of first-trimester combined screening for trisomy 21

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KEYWORDS: combined screening; first trimester; trisomy 21

ABSTRACT

Objective *To examine the performance of the new algorithm in screening for trisomy 21 by a combination of maternal age, fetal nuchal translucency (NT) and maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A).*

Methods *This was a prospective screening study for trisomy 21 in singleton pregnancies at 11 + 0 to 13 + 6 weeks of gestation using an algorithm combining maternal age, fetal NT thickness based on the mixture model for the assessment of NT, and maternal serum free β-hCG and PAPP-A based on a multiple regression model for the assessment of serum biochemistry. The NT measurements were performed by 60 operators who had obtained The Fetal Medicine Foundation certificate of competence in the 11–13-week scan.*

Results *The study population consisted of 19 614 pregnancies with a normal karyotype or delivery of a phenotypically normal baby (euploid group) and 122 cases of trisomy 21. In the euploid fetuses the NT was above the previously defined 50th, 95th and 99th centiles in 10 033 (51.2%), 618 (3.2%) and 123 (0.6%) cases and the respective values for trisomy 21 were 117 (95.9%), 94 (77.0%) and 57 (46.7%). The median fetal NT was within 0.1 mm of the expected in 47 (78.3%) of the 60 sonographers and within 0.2 mm in all. In the euploid fetuses the median free β-hCG was 1.0 (range, 0.1–29.4) multiples of the median (MoM) and the median PAPP-A was 1.0 (range, 0.2–3.3) MoM. The median MoM values were 1.0 or close to 1.0 MoM for each subgroup of pregnancy characteristics, including gestations of 11, 12 and 13 weeks, maternal weight of < 60 kg, 60–80 kg and > 80 kg, different ethnic origins, cigarette smokers and non-smokers, natural conception and* in vitro *fertilization. For a false-positive rate of 3%, the detection rate of trisomy 21 in screening by maternal age and fetal NT was 81% (95% CI, 73–89%), by maternal age and maternal serum biochemistry it was 63% (95% CI, 56–72%) and by combined screening based on maternal age, fetal NT and maternal serum biochemistry it was 90% (95% CI, 84–96%).*

Conclusion *This study has validated the new risk algorithm and demonstrated that in combined screening for trisomy 21 based on maternal age, fetal NT and free β-hCG and PAPP-A the detection rate is about 90% for a 3% false-positive rate. Copyright © 2009 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Effective screening for trisomy 21 can be achieved using a combination of 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) at 11–13 weeks of gestation[1,2]. In the assessment of patient-specific risks the *a-priori* maternal age-related risk is multiplied by likelihoods, determined from the deviation of the measured NT, free β-hCG and PAPP-A from the respective expected median.

We have recently proposed a new approach for quantifying the deviation in the measured NT from the normal, which is based on the observation that in both trisomy 21 and unaffected pregnancies, fetal NT follows two distributions, one that is dependent on crown–rump length (CRL) and another that is independent of CRL[3]. In this mixture model the proportions of trisomy 21 and unaffected fetuses that follow the CRL-independent distribution are about 95% and 5%, respectively. Similarly, in terms of biochemical testing we have recently proposed a new approach for quantifying the deviation of the measured free β-hCG and PAPP-A from the respective expected medians. We used multiple regression analysis to define the contribution of maternal and fetal characteristics, such as gestational age, maternal weight, ethnicity,

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smoking status, method of conception, parity and number of fetuses, that influence the measured concentration of free β-hCG and PAPP-A, and the interaction between these covariates[4]. We then estimated that with the new approaches for the assessment of fetal NT and serum biochemistry a strategy of first-trimester combined screening in a population with the maternal age distribution of pregnancies in England and Wales[5] would detect 85% and 90% of trisomy 21 pregnancies at false-positive rates of 3% and 5%, respectively[1].

The aim of this prospective study of more than 20 000 pregnancies was to examine the performance of the new algorithm in screening for trisomy 21 by a combination of maternal age, fetal NT and maternal serum free β-hCG and PAPP-A.

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 at 11 + 0 to 13 + 6 weeks of gestation[2,5]. Transabdominal ultrasound examination was performed to diagnose any major fetal defects and for measurement of fetal CRL, NT thickness and fetal heart rate. Automated machines that provide reproducible results within 30 min were used to measure PAPP-A and free β-hCG (Kryptor System, Berlin, Germany and Delfia Express System, Perkin Elmer, Waltham, USA).

Maternal demographic characteristics, ultrasonographic measurements and biochemical results were recorded in a computer database. Karyotype results and details of 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 January 2006 to May 2007.

The NT measurement was performed by 60 operators who had obtained The Fetal Medicine Foundation certificate of competence in the 11–13-week scan. There is no overlap between the dataset used in this study and the original datasets that were used to estimate the covariates for the mixture model and the estimates for the multiple regression approach for free β-hCG and PAPP-A.

Statistical analysis

Patient-specific risks were computed by multiplying each age-related risk for trisomy 21 with the likelihood for fetal NT and the combined likelihood for maternal serum free β-hCG and PAPP-A[3,4]. Crude detection rates and false-positive rates were calculated by taking the proportions with risks above a given risk threshold. Maternal age-specific detection and false-positive rates were then produced, and adjusted according to the maternal age distribution of pregnancies in England and Wales in 2000–2002[6]. We used 'bootstrapping' techniques with 100 bootstrap replications to compute the 95% confidence intervals[7].

RESULTS

Study population

Screening with the new algorithm was carried out in 21 141 singleton pregnancies. However, 1405 (6.6%) cases were excluded from further analysis because in 920 (4.4%) cases it was not possible to determine the fetal karyotype because the pregnancies were lost to follow-up; 190 (0.9%) cases resulted in a miscarriage without further karyotyping; in 188 (0.9%) cases one of the covariates was missing; and in 107 (0.5%) cases there was a chromosomal abnormality other than trisomy 21. Thus, our study population consisted of 19 614 pregnancies with a normal karyotype or delivery of a phenotypically normal baby (euploid group) and 122 cases of trisomy 21. The characteristics of the study population are summarized in Table 1.

Fetal nuchal translucency

In the euploid fetuses the NT was above the previously defined 50th, 95th and 99th centiles[3] in 10 033 (51.2%), 618 (3.2%) and 123 (0.6%) cases, respectively. The respective values for trisomy 21 were 117 (95.9%), 94 (77.0%) and 57 (46.7%) (Figure 1).

The mixture model with the previously defined covariates[3] was in good agreement with the NT distribution of euploid and aneuploid fetuses. In euploid pregnancies, 7% of the cases followed the CRL-independent distribution, with a median NT of 2.1 mm. In trisomy 21 pregnancies, 94% of the cases followed the CRL-independent distribution, with a median NT of 3.4 mm.

The 60 sonographers each carried out between 30 and 1201 (median, 183) measurements of fetal NT. The

Table 1 Characteristics of the study group

Parameter	*Value*
Maternal age (years, median (range))	34.5 (14.1–50.1)
Maternal weight (kg, median (range))	64.0 (34.0–165.0)
Spontaneous conception (*n* (%))	18 938 (96.0)
Smoker (*n* (%))	1142 (5.8)
Ethnicity (*n* (%))	
Caucasian	15 796 (80.0)
Afro-Caribbean	2144 (10.9)
East Asian	271 (1.4)
South Asian	1027 (5.2)
Mixed	498 (2.5)
Gestational age (*n* (%))	
11 + 0 to 11 + 6 weeks	1453 (7.4)
12 + 0 to 12 + 6 weeks	11 461 (58.1)
13 + 0 to 13 + 6 weeks	6822 (34.6)
Crown–rump length (mm, median (range))	63 (45.0–84.0)
Karyotype (*n* (%))	
Euploid	19 614 (99.4)
Trisomy 21	122 (0.6)
Total (*n* (%))	19 736 (100.0)

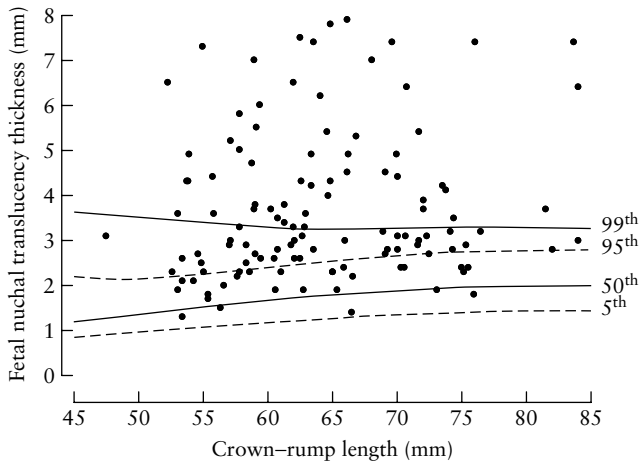

Figure 1 Distribution of fetal nuchal translucency (NT) thickness according to crown–rump length in fetuses with trisomy 21. For comparison, the 5th, 50th, 95th and 99th centiles of fetal NT of euploid fetuses[3] are shown.

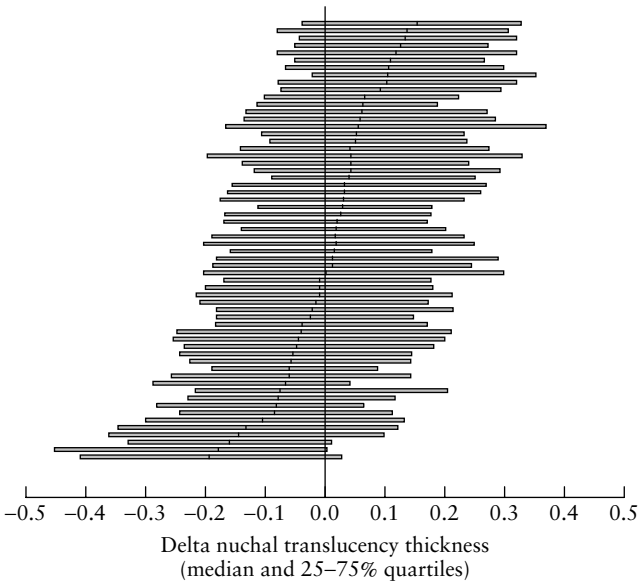

Figure 2 Distribution (median and 25–75% quartiles) of delta fetal nuchal translucency thickness of the 60 sonographers who contributed to the study.

median fetal NT was within 0.1 mm of the expected in 47 (78.3%) of the sonographers, within 0.15 mm in 57 (95.0%) and within 0.2 mm in all (Figure 2). The median 25–75% interquartile range of delta NT (difference between expected and observed measurement) was 0.39 (range, 0.28–0.55) mm. The number of ultrasound examinations of each operator did not show a significant effect on the median delta NT nor on the 25–75% interquartile range of each operator (delta NT: $P = 0.066$, $r = 0.520$; 25–75% interquartile range: $P = 0.962$, $r = 0.005$).

Maternal serum biochemistry

In the euploid fetuses the median free β-hCG was 1.0 (range, 0.1–29.4) multiples of the median (MoM) and the median PAPP-A was 1.0 (range, 0.2–3.3) MoM. The respective values for the trisomy 21 fetuses were 2.0 (range, 0.1–7.0) MoM and 0.5 (range, 0.06–2.2) MoM. Figure 3 shows the median MoM values for free β-hCG and PAPP-A at 11, 12 and 13 weeks' gestation, for different ethnic origin, for cigarette smokers vs. non-smokers, for natural conception vs. *in vitro* fertilization (IVF) and for maternal weight. All median MoM values are 1.0 or close to 1.0 MoM. Figure 4 gives the contour plots of the euploid pregnancies and the individual free β-hCG and PAPP-A MoMs in the trisomy 21 pregnancies.

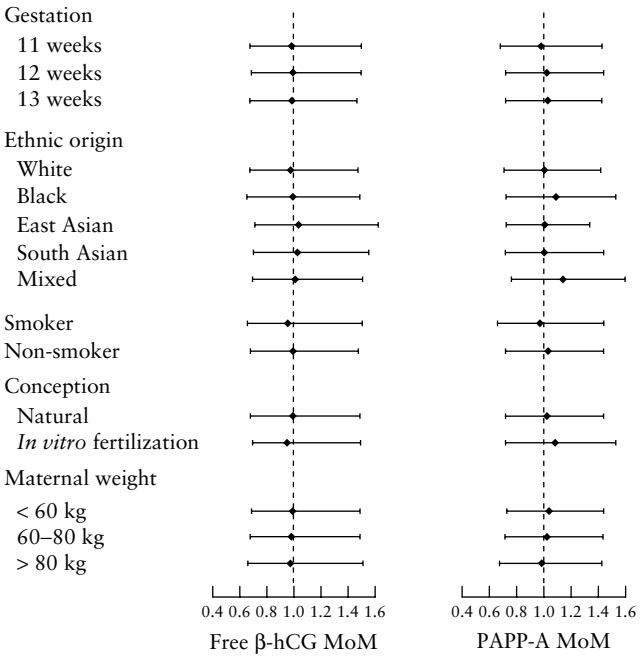

Figure 3 Distribution (median and 25–75% quartiles) of maternal serum free β-human chorionic gonadotropin (β-hCG) multiples of the median (MoM) and pregnancy-associated plasma protein-A (PAPP-A) MoM according to gestational age at the time of screening, maternal ethnic origin, smoking status, mode of conception and maternal weight.

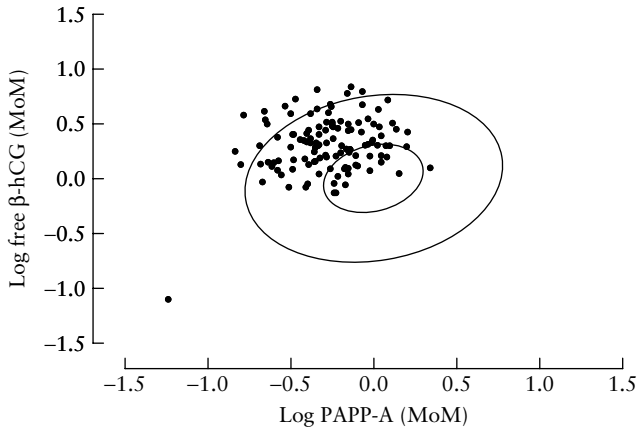

Figure 4 Distribution of multiples of the median (MoM) values of free β-human chorionic gonadotropin (β-hCG) and of pregnancy-associated plasma protein-A (PAPP-A) in fetuses with trisomy 21 compared to the 90% and 50% contours of the euploid fetuses.

Screening performance

The detection rates with 95% confidence intervals for given false-positive rates in screening for trisomy 21 by fetal NT, maternal serum free β-hCG and PAPP-A and by a combination of maternal age, fetal NT and serum biochemistry are shown in Table 2. For a false-positive rate of 3%, the detection rate of trisomy 21 in screening by maternal age and fetal NT was 81% (95% CI, 73–89%), by maternal age and maternal serum biochemistry it was 63% (95% CI, 56–72%) and by combined screening based on maternal age, fetal NT and maternal serum biochemistry it was 90% (95% CI, 84–96%).

The false-positive and detection rates of trisomy 21 for given risk cut-offs in screening for trisomy 21 by fetal NT, maternal serum free β-hCG and PAPP-A and by a combination of maternal age, fetal NT and serum biochemistry are shown in Table 3. For a risk cut-off of 1 : 150 at the time of screening, the false-positive and detection rates by combined screening were 3.1% (95% CI, 2.8–3.3%) and 91% (95% CI, 84–98%), respectively.

Table 4 compares the expected and observed number of cases with trisomy 21 according to the estimated risk range.

Table 2 Detection rates (95% CI) of trisomy 21 for given false-positive rates in screening for trisomy 21 by fetal nuchal translucency (NT) thickness, maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) and by a combination of maternal age, fetal NT and serum biochemistry

	Detection rate (%) for screening by:		
False-positive rate (%)	*Fetal nuchal translucency*	*Free β-hCG and PAPP-A*	*Combined screening*
1	70 (60–79)	42 (32–51)	83 (76–90)
2	77 (68–86)	54 (46–63)	84 (70–91)
3	81 (73–89)	63 (56–72)	90 (84–96)
4	82 (74–90)	65 (58–73)	92 (86–98)
5	82 (74–90)	67 (59–74)	93 (87–99)

All rates standardized according to maternal age distribution of pregnancies in England and Wales in 2000–2002[6].

DISCUSSION

The findings of this prospective study demonstrate that screening for trisomy 21 at 11–13 weeks' gestation by a combination of maternal age, fetal NT and maternal serum free β-hCG and PAPP-A identifies about 90% of affected pregnancies at a false-positive rate of 3%. These results are compatible with the predicted performance of the new algorithm based on the mixture model for assessment of NT and the multiple regression model for assessment of serum biochemistry.

Fetal NT remains the single most effective marker in screening for trisomy 21. During the development process of the new risk algorithm for fetal NT, it became obvious that neither the delta NT (subtraction of expected from observed measurement) nor the MoM (division of observed by expected measurement) approach was appropriate in quantifying the deviation of an observed to the expected NT measurement[3]. We have therefore proposed a new approach based on the observation that in both trisomy 21 and unaffected pregnancies fetal NT follows two distributions, one that is dependent on CRL and one that is independent of CRL[3]. In this mixture model the proportions of trisomy 21 and unaffected fetuses that follow the CRL-independent distribution are 95% and 5%, respectively. The findings of this prospective screening study provide support for the validity of the proposed mixture model of NT distribution. Screening for trisomy 21 by fetal NT identified 81% of affected pregnancies at a false-positive rate of 3%.

Effective screening for chromosomal abnormalities utilizing the measurement of fetal NT necessitates appropriate training of sonographers, adherence to a standard ultrasound technique and regular audit of their results. As demonstrated by the distribution of NT measurements of the 60 sonographers involved in this study, the median NT of the individual operators was within 0.15 mm of the expected in 95% of cases and within 0.2 mm in all. We have previously reported that such small deviations in median NT do not have an adverse effect on the performance of screening[8]. In contrast, larger deviations in median NT have a substantial adverse effect on the performance of screening. One approach in correcting for deviations in median NT is to use operator-specific

Table 3 False-positive rate (FPR) and detection rate (DR) of trisomy 21 for given risk cut-offs in screening for trisomy 21 by fetal nuchal translucency (NT) thickness, maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) and by a combination of maternal age, fetal NT and serum biochemistry

	Fetal nuchal translucency		*Free β-hCG and PAPP-A*		*Combined screening*	
Risk cut-off	*FPR (% (95% CI))*	*DR (% (95% CI))*	*FPR (% (95% CI))*	*DR (% (95% CI))*	*FPR (% (95% CI))*	*DR (% (95% CI))*
1 : 50	1.1 (0.9–1.2)	75 (66–83)	2.4 (2.2–2.5)	61 (52–69)	1.2 (1.0–1.3)	83 (74–92)
1 : 100	1.9 (1.7–2.1)	76 (69–83)	4.8 (4.6–5.0)	62 (59–74)	2.3 (2.0–2.5)	89 (81–96)
1 : 150	2.7 (2.4–3.0)	80 (73–87)	6.8 (6.5–7.1)	74 (67–80)	3.1 (2.8–3.3)	91 (84–98)
1 : 200	3.6 (3.2–3.9)	82 (75–88)	8.7 (8.4–9.0)	77 (71–84)	3.9 (3.6–4.1)	92 (85–99)
1 : 250	4.4 (4.1–4.7)	82 (76–89)	10.6 (10.3–10.9)	79 (73–86)	4.6 (4.3–4.9)	93 (86–100)
1 : 300	5.3 (4.9–5.6)	82 (76–89)	12.1 (11.7–12.4)	83 (76–89)	5.3 (5.0–5.6)	93 (86–100)
1 : 1000	19.2 (18.6–19.7)	97 (93–100)	28.7 (28.2–29.3)	88 (84–92)	13.1 (12.6–13.6)	97 (92–100)

All rates standardized according to maternal age distribution of England and Wales in 2000–2002[6].

Table 4 Accuracy of estimated risk for trisomy 21 by a combination of maternal age, fetal nuchal translucency thickness and maternal serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A

Estimated risk for trisomy 21 (median (range))	*Trisomy 21* (n (%))	*Unaffected* (n (%))	*Observed risk*
1 in 4 (≥ 1 in 10)	88 (72.1)	109 (0.6)	1 in 2
1 in 53 (1 in 11 to 1 in 100)	24 (19.7)	644 (3.3)	1 in 28
1 in 172 (1 in 101 to 1 in 250)	5 (4.1)	834 (4.3)	1 in 167
1 in 574 (1 in 251 to 1 in 1000)	3 (2.5)	2502 (12.8)	1 in 835
1 in 8480 (< 1 in 1000)	2 (1.6)	15 525 (79.1)	1 in 7764

medians[9]. However, large deviations in median NT are likely to be the consequence of a faulty technique in ultrasound scanning with a large overlap in NT measurements between euploid and trisomy 21 fetuses. In such cases the operators require retraining in the correct methodology of measuring NT rather than the false reassurance provided by the calculation of operator-specific medians.

In biochemical testing we used multiple regression analysis to correct the measured free β-hCG and PAPP-A concentrations for maternal and pregnancy characteristics[4]. At a given concentration of free β-hCG and PAPP-A the MoM value increases with maternal weight from about 0.7 MoM if the weight is 45 kg to 1.5 MoM if the weight is 90 kg. Similarly, the MoM value is influenced by fetal CRL, and at a given concentration of PAPP-A it decreases from about 2.0 MoM at a CRL of 45 mm to 1.0 MoM at a CRL of 65 mm, whereas the values for free β-hCG are 0.8 MoM and 1.2 MoM, respectively. In Black compared with White women PAPP-A is 57% higher and free β-hCG is 12% higher, in cigarette smokers there is a decrease in both PAPP-A and free β-hCG – by 17% and 4%, respectively – and in pregnancies conceived by IVF there is a 10% decrease in PAPP-A and a 9% increase in free β-hCG. Failure to adjust for these variations has a major effect on the overall performance of screening and the patient-specific risks. When the appropriate corrections are applied the MoM values for free β-hCG and PAPP-A in the unaffected population should always be around 1.0 MoM. As demonstrated in our study, correction for the maternal and pregnancy characteristics by our previously derived algorithm resulted in values of about 1.0 MoM for both free β-hCG and PAPP-A in each subgroup of the unaffected pregnancies. Screening for trisomy 21 by serum biochemistry identified 63% of affected pregnancies at a false-positive rate of 3%.

The data on detection and false-positive rates are useful in comparing different methods of screening and allowing healthcare planners to develop governmental and insurance company policies, whereby a risk cut-off for invasive testing is defined with the aim of maximizing cost-effectiveness. However, in the management of each pregnancy the objective of screening is to provide parents with an accurate individualized assessment of their risk for trisomy 21 on which they will base their decision in favor of, or against, invasive testing. As demonstrated in this study the new algorithm provided accurate patient-specific risks and in each range of estimated risks for trisomy 21 by combined screening there was good agreement between the expected and observed number of affected fetuses.

In summary, in this study we have validated the new risk algorithm and demonstrated that in combined screening for trisomy 21 based on maternal age, fetal NT, free β-hCG and PAPP-A the detection rate is about 90% for a 3% false-positive rate.

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