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Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening

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KEYWORDS: ductus venosus; free β-human chorionic gonadotropin; nasal bone; nuchal translucency; pregnancy-associated plasma protein-A; screening; tricuspid regurgitation; trisomy 21

ABSTRACT

Objectives *To evaluate the performance of first-trimester 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). In addition, the potential impact of a new individual risk-orientated two-stage approach to first-trimester screening was examined.*

Methods *First-trimester combined screening for trisomy 21 was carried out in 75 821 singleton pregnancies with live fetuses at 11 + 0 to 13 + 6 gestational weeks. The detection and false-positive rates for different risk cut-offs were calculated. To examine the potential impact of an individual risk-orientated two-stage approach to first-trimester screening it was assumed that, after first-trimester combined screening, chorionic villus sampling (CVS) would be performed in all patients with a risk estimate of 1 in 100 or more and in none of those with a risk estimate of less than 1 in 1000. Those in the intermediate-risk category, with a risk estimate of between 1 in 101 and 1 in 1000, would have further assessment of risk by first-trimester ultrasound examination to determine presence/absence of the nasal bone, presence/absence of tricuspid regurgitation or normal/abnormal Doppler velocity waveform in the ductus venosus, and CVS would be performed if their adjusted risk became 1 in 100 or more.*

Results *Fetal NT and maternal serum free β-hCG and PAPP-A were successfully measured in all cases. The median maternal age was 31 (range, 13–49) years, the median gestation at screening was 12 (range, 11 + 0 to 13 + 6) weeks and the median fetal crown–rump length was 62 (range, 45–84) mm. Chromosomal abnormalities were identified in 544 pregnancies, including 325 cases of trisomy 21. The estimated risk for trisomy 21 was 1 in 300 or greater in 5.2% of normal pregnancies, in 92.6% of those with trisomy 21, in 88.5% of those with trisomy 18 or 13 and in 85.6% of those with other chromosomal defects. The detection rates for trisomy 21 were about 75% and 80% for respective false-positive rates of 1% and 2%. In the proposed individual risk-orientated two-stage screening for a risk cut-off of 1 in 100 the total false-positive rate would vary with the method used for the second stage of screening from 2.1% for absence of the nasal bone to 2.7% for increased impedance in the ductus venosus and 2.7% for tricuspid regurgitation and the respective detection rates would be 92.0%, 94.2% and 91.7%.*

Conclusions *First-trimester combined screening for trisomy 21 is associated with a detection rate of about 90% for a false-positive rate of 5%. Individual risk-orientated two-stage screening for trisomy 21 can potentially identify, in the first trimester of pregnancy, more than 90% of affected fetuses for a false-positive rate of 2–3%.* *Copyright © 2005 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Prospective studies have demonstrated that the most effective method of screening for trisomy 21 is provided by

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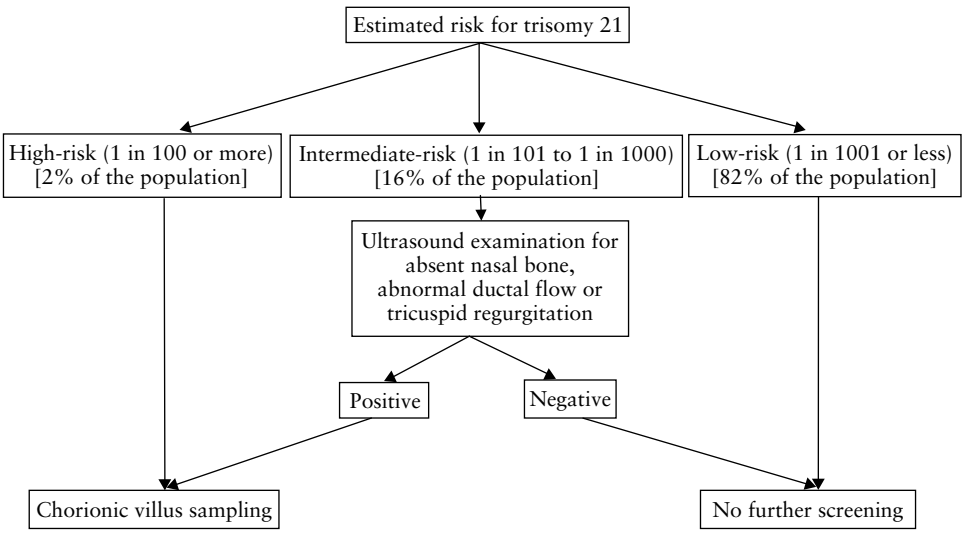

Figure 1 Screening by maternal age, fetal nuchal translucency and maternal serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A.

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) concentration at 11 + 0 to 13 + 6 weeks' gestation. It was shown that for a false-positive rate of 5% the detection rate of trisomy 21 by this method is about 90%, which is superior to the 30% achieved by maternal age alone or the 65% by maternal age and second-trimester serum biochemistry[1–4]. With the advent of rapid immunoassays, it has become possible to provide pretest counseling, highly accurate and reproducible biochemical testing of the mother, ultrasound examination of the fetus and posttest counseling of a combined risk estimate, all within a 1-h visit to a multidisciplinary, one-stop clinic for assessment of risk for fetal anomalies.

This prospective study of more than 75 000 pregnancies examined further the effectiveness of first-trimester combined screening for trisomy 21. In addition, it examined the potential impact of a new individual risk-orientated two-stage approach to screening. Studies from specialist centers have demonstrated that, in addition to NT, other highly sensitive and specific first-trimester sonographic markers of trisomy 21 are absence of the nasal bone, increased impedance to flow in the ductus venosus and tricuspid regurgitation[5–7]. We propose that after combined fetal NT and maternal serum free β-hCG and PAPP-A screening, patients are assigned into a high-risk category with a risk estimate of 1 in 100 or more, a low-risk category with a risk estimate of less than 1 in 1000 or an intermediate-risk category with a risk estimate of between 1 in 101 and 1 in 1000 (Figure 1). Patients in the high-risk category are offered karyotyping by chorionic villus sampling (CVS) and those in the low-risk category are reassured that their fetus is unlikely to be chromosomally abnormal. Those in the intermediate-risk category have further assessment of risk by first-trimester ultrasound examination to determine presence/absence of the nasal bone, normal/abnormal Doppler velocity waveform in the ductus venosus or presence/absence of tricuspid regurgitation, and CVS is offered if their adjusted risk becomes 1 in 100 or more.

METHODS

All women booked for maternity care at the following UK hospitals were offered screening for trisomy 21 by a combination of fetal NT and maternal serum free β-hCG and PAPP-A at 11 + 0 to 13 + 6 weeks' gestation: Harold Wood Hospital, Romford (between June 1998 and December 2003), King George Hospital, Goodmayes (between July 2001 and December 2003), Kent and Canterbury Hospital, Canterbury (between July 2002 and December 2003), William Harvey Hospital, Ashford (between July 2002 and December 2003), Queen Elizabeth The Queen Mother's Hospital, Margate (between July 2002 and December 2003), King's College Hospital, London (between January 1999 and February 2000) and those attending The Fetal Medicine Centre, London (between July 1999 and December 2003). Women received an information leaflet about the service and gave details about their demographic characteristics and medical history, which were entered into a computer database. The maternal serum free β-hCG and PAPP-A were measured using the Kryptor analyzer (Brahms AG, Berlin, Germany) and an ultrasound examination was carried out to measure the fetal NT and crown–rump length (CRL) and to diagnose any major defects. All scans were carried out by sonographers who had obtained The Fetal Medicine Foundation Certificate of Competence in the 11–14-week scan (www.fetalmedicine.com).

The patients were counseled with regard to their combined estimated risk and the available options for the subsequent management of the pregnancy, including CVS or amniocentesis. They were informed that a risk of 1 in 300 or more was generally considered to be high. They were also informed that the procedure-related

risk of miscarriage from CVS or amniocentesis was 1%. Data on pregnancy outcome were obtained from the cytogenetics laboratories, the patients themselves, their general practitioners or the maternity units in which they delivered.

Patient-specific risks were calculated by a multivariate approach using population parameters established in the authors' retrospective study and the maternal age- and gestation-related risk of trisomy 21 at the time of screening[1,8]. Essentially, the maternal age-related risk was multiplied with each likelihood ratio (LR) derived from the fetal NT and maternal weight-adjusted serum free β-hCG and PAPP-A. The maximum and minimum LRs allowed were 0.12 and 55 for NT, 0.018 and 7.138 for each metabolite and 0.1 and 80 for the combined sonographic and biochemical markers.

The detection rate of chromosomal defects and false-positive rate of the screening test for different risk categories were calculated.

Estimation of the potential impact of individual risk-orientated two-stage screening

In this estimate it was assumed that, after first-trimester combined screening, CVS would be performed in all patients with a risk estimate of 1 in 100 or more and in none of those with a risk estimate of less than 1 in 1000. Those in the intermediate-risk category (i.e. a risk estimate of between 1 in 101 and 1 in 1000) had further assessment of risk by first-trimester ultrasound examination for presence/absence of the nasal bone, presence/absence of tricuspid regurgitation or normal/abnormal Doppler velocity waveform in the ductus venosus, and CVS was performed if their adjusted risk became 1 in 100 or more.

At the scan at 11 + 0 to 13 + 6 weeks, trisomy 21 is associated with absence of the nasal bone, increased impedance to flow in the ductus venosus and tricuspid regurgitation. In the combined data from nine studies, the nasal bone was absent in 176/12 652 (1.4%) chromosomally normal fetuses and in 274/397 (69.0%) fetuses with trisomy 21 and therefore the LR for absent nasal bone was 49.3[5,9–16]. In the combined data from six studies, abnormal ductal flow was observed in 273/5462 (5.0%) chromosomally normal fetuses and in 108/131 (82.4%) fetuses with trisomy 21 and therefore the LR for abnormal ductal flow was 16.5[6,17–21]. In our ongoing studies there was tricuspid regurgitation in 72/1394 (5.2%) chromosomally normal fetuses and in 109/162 (67.3%) fetuses with trisomy 21 and therefore the LR for tricuspid regurgitation was 12.9.

In the intermediate-risk category, the same proportion of absence of the nasal bone, abnormal ductal flow and tricuspid regurgitation was assigned to trisomy 21 and chromosomally normal fetuses as reported in the above studies. Since the LR for each of these markers is at least 10, then all fetuses with these markers would have a readjustment of their risk to at least 1 in 100. The estimated new detection and false-positive rates for a risk cut-off of 1 in 100 were then calculated.

Table 1 Estimated risk for trisomy 21 of 1 in 300 or greater based on the combination of maternal age, fetal nuchal translucency and maternal serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A in the chromosomally normal and abnormal pregnancies

Fetal karyotype	n	*Estimated risk of ≥ 1 in 300* (n *(%))*
Normal	75 277	3909 (5.2)
Trisomy 21	325	301 (92.6)
Trisomy 18 or 13	122	108 (88.5)
Turner syndrome	38	33 (86.8)
Triploidy	28	27 (96.4)
Other*	31	23 (74.2)
Total	75 821	4401 (5.8)

*Deletions, partial trisomies, unbalanced translocations or sex chromosome aneuploidies.

RESULTS

First-trimester combined screening for trisomy 21 by fetal NT and maternal serum free β-hCG and PAPP-A was carried out in 78 428 singleton pregnancies with live fetuses at 11 + 0 to 13 + 6 (median, 12) weeks' gestation. Pregnancy outcome, including karyotype results or the birth of a phenotypically normal baby, was obtained from 75 821 cases. A total of 2607 cases were excluded from further analysis because the fetal karyotype was not known and they resulted in spontaneous fetal loss or termination of pregnancy ($n = 490$) or were lost to follow-up ($n = 2117$).

The median maternal age of the 75 821 cases was 31 (range, 13–49) years, the median gestation at screening was 12 (11 + 0 to 13 + 6) weeks and the median fetal CRL was 62 (range, 45–84) mm. Chromosomal abnormalities were identified in 544 pregnancies including 325 cases of trisomy 21 (Table 1).

The estimated risk for trisomy 21 based on maternal age, fetal NT and maternal serum free β-hCG and PAPP-A was 1 in 300 or greater in 5.2% of normal pregnancies, in 92.6% of those with trisomy 21, in 88.5% of those with trisomy 18 or 13 and in 85.6% of those with other chromosomal defects (Table 1). The detection rates of trisomy 21 and other chromosomal defects and the false-positive rates for different estimated risk cut-offs after screening by a combination of maternal age, fetal NT and maternal serum free β-hCG and PAPP-A are shown in Table 2. The detection and false-positive rates, in addition to the rates of abnormal results per 1000 invasive tests for each risk category, are given in Table 3.

Potential impact of individual risk-orientated two-stage screening

In the prospective study of screening by a combination of maternal age, fetal NT and maternal serum biochemistry at 11 + 0 to 13 + 6 weeks' gestation, the estimated risk for trisomy 21 was 1 in 100 or more in 1.9% (1439/75 277) of the chromosomally normal fetuses and in 81.5%

Table 2 Detection rates of trisomy 21 and other chromosomal defects and false-positive rates for different estimated risk cut-offs in screening for trisomy 21 by the combination of maternal age, fetal nuchal translucency and maternal serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A

		Fetal karyotype (n *(%))*				
	Total (n *(%))*	*Normal*	*Abnormal*			
Estimated risk for trisomy 21	(n = *75 821)*	(n = *75 277)*	*All* (n = *544)*	*Trisomy 21* (n = *325)*	*Trisomy 18 or 13* (n = *122)*	*Other* (n = *97)*
≥ 1 in 50	1128 (1.5)	737 (1.0)	391 (71.9)	244 (75.1)	91 (74.6)	56 (57.7)
≥ 1 in 100	1863 (2.5)	1439 (1.9)	424 (77.9)	265 (81.5)	96 (78.7)	63 (64.9)
≥ 1 in 150	2485 (3.3)	2036 (2.7)	449 (82.5)	274 (84.3)	102 (83.6)	73 (75.3)
≥ 1 in 200	3124 (4.1)	2662 (3.5)	462 (84.9)	281 (86.5)	104 (85.2)	77 (79.4)
≥ 1 in 300	4401 (5.8)	3909 (5.2)	492 (90.4)	301 (92.6)	108 (88.5)	83 (85.6)
≥ 1 in 500	7149 (9.4)	6647 (8.8)	502 (92.3)	308 (94.8)	109 (89.3)	85 (87.6)
≥ 1 in 1000	14 104 (18.6)	13 582 (18.0)	522 (96.0)	315 (96.9)	115 (94.3)	92 (94.8)
≥ 1 in 2000	25 835 (34.1)	25 301 (33.6)	534 (98.2)	320 (98.5)	120 (98.4)	94 (96.9)
≥ 1 in 5000	47 651 (62.8)	47 107 (62.6)	544 (100)	325 (100)	122 (100)	97 (100)
1 in > 5000	28 170 (37.2)	28 170 (37.4)	—	—	—	—

Table 3 Screen-positive rates (false- and true-positive rates) and detection rates for each estimated risk category in screening for trisomy 21 by a combination of maternal age, fetal nuchal translucency and maternal serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A

		Fetal karyotype (n *(%))*					
	Total (n *(%))*	*Normal*	*Abnormal*				*Abnormal fetuses* per 1000 invasive tests*
Estimated risk for trisomy 21	(n = *75 821*)	(n = *75 277*)	*All* (n = *544*)	*Trisomy 21* (n = *325*)	*Trisomy 18 or 13* (n = *122*)	*Other* (n = *97*)	
1 in 2 to 1 in 100	1863 (2.5)	1439 (1.9)	424 (77.9)	265 (81.5)	96 (78.7)	63 (64.9)	228
1 in 101 to 1 in 200	1261 (1.7)	1223 (1.6)	38 (7.0)	16 (4.9)	8 (6.6)	14 (14.4)	30
1 in 201 to 1 in 300	1277 (1.7)	1247 (1.7)	30 (5.5)	20 (6.2)	4 (3.3)	6 (6.2)	23
1 in 301 to 1 in 500	2748 (3.6)	2738 (3.6)	10 (1.8)	7 (2.2)	1 (0.8)	2 (2.1)	4
1 in 501 to 1 in 1000	6955 (9.2)	6935 (9.2)	20 (3.7)	7 (2.2)	6 (4.9)	7 (7.2)	3
1 in 1001 to 1 in 5000	33 547 (44.2)	33 525 (44.5)	22 (4.0)	10 (3.1)	7 (5.7)	5 (5.2)	< 1
1 in > 5000	28 170 (37.2)	28 170 (37.4)	—	—	—	—	—

*Trisomy 21 and other chromosomal defects.

(265/325) with trisomy 21 (Table 3). The estimated risk was less than 1 in 1000 in 82% (61 695/75 277) of the chromosomally normal fetuses and in 3.1% (10/325) with trisomy 21. The estimated risk was between 1 in 101 and 1 in 1000 in 16.1% (12 143/75 277) of the chromosomally normal fetuses and in 15.4% (50/325) with trisomy 21.

In the intermediate-risk category absence of the nasal bone, abnormal ductal flow and tricuspid regurgitation was expected to be found in 171 (1.4%), 608 (5.0%) and 631 (5.2%) of the 12 143 chromosomally normal fetuses and in 34 (68.0%), 41 (82.0%) and 33 (66.0%) of the 50 trisomy 21 fetuses, respectively, and in all these cases the adjusted risk would become at least 1 in 100. Therefore, in this proposed individual risk-orientated two-stage screening for a risk cut-off of 1 in 100 the total false-positive rate would vary with the method used for the second stage of screening from 2.1% (1439 + 171/75 277) for absence of the nasal bone, 2.7% (1439 + 608/75 277) for increased impedance in the ductus venosus and 2.7% (1439 + 631/75 277) for tricuspid regurgitation (Table 4). Similarly, the detection rates would vary from 92.0% (265 + 34/325) for absence of the nasal bone, 94.2% (265 + 41/325) for increased impedance in the ductus venosus and 91.7% (265 + 33/325) for tricuspid regurgitation.

DISCUSSION

The findings of this prospective study of screening for trisomy 21, by a combination of maternal age, fetal NT and maternal serum free-β-hCG and PAPP-A at 11 + 0 to 13 + 6 weeks' gestation, confirm that first-trimester screening is associated with a detection rate of about 90% for a false-positive rate of 5% and detection rates of about 75% and 80% for respective false-positive rates of 1%

Table 4 Potential detection rates of trisomy 21 and false-positive rates in the proposed individual risk-orientated two-stage screening with a risk cut-off of 1 in 100. In this population there were 325 fetuses with trisomy 21 and 75 277 chromosomally normal fetuses

Method of second-stage screening	*Detection rate* (n *(%)*)	*False-positive rate* (n *(%)*)
Nasal bone examination	299 (92.0)	1610 (2.1)
Ductus venosus Doppler	306 (94.2)	2047 (2.7)
Tricuspid valve Doppler	298 (91.7)	2070 (2.7)

and 2%. The results also demonstrate that this method of screening is effective in identifying a high proportion of not only trisomy 21 but all major chromosomal defects, including trisomies 18 and 13, triploidy, sex chromosome aneuploidies, deletions and unbalanced translocations.

In the proposed individual risk-orientated two-stage screening the patients were subdivided into a high-risk group, requiring invasive testing, a low-risk group, which could be reassured that an abnormality is unlikely, and an intermediate-risk group, in which further non-invasive testing can be used to refine the risk further. This approach is compatible with the basic principles of clinical practice in all fields of medicine. For example, in the great majority of patients presenting with abdominal pain the correct diagnosis of the presence or absence of a serious problem is reached after history taking and clinical examination. In a minority of cases a series of further investigations of increasing sophistication may be necessary before the correct diagnosis is made.

The high-risk group of patients, with an estimated risk for trisomy 21 of 1 in 100 or more, constituted 2.5% of the total and contained 81.5% of all cases of trisomy 21 and 72.6% of all other chromosomal defects. In this group, about 1 in 4 fetuses was chromosomally abnormal. Since the procedure-related risk of miscarriage from amniocentesis or transabdominal CVS is about 1%[22,23], in this high-risk group there are more than 20 abnormalities diagnosed for each procedure-related miscarriage. In contrast, the low-risk group of patients, with an estimated risk for trisomy 21 of less than 1 in 1000, constituted about 80% of the total and contained only 4% of all chromosomal defects. In this low-risk group, only 1 in 2800 fetuses was chromosomally abnormal and had this group been subjected to invasive testing there would have been 28 procedure-related miscarriages for each chromosomal defect that was diagnosed.

In the intermediate-risk group of patients, further assessment of risk can be undertaken by more detailed sonographic examination of the fetus at 11 + 0 to 13 + 6 weeks' gestation. Extensive studies have demonstrated that absence of the nasal bone, increased impedance to flow in the ductus venosus or tricuspid regurgitation are highly sensitive and specific markers of trisomy 21. However, accurate examination for these markers is time consuming and requires highly skilled operators and at present it is unlikely that this assessment will be incorporated into the routine first-trimester scan. However, it could be used in specialist centers to re-evaluate the risk in patients with intermediate risk after screening by fetal NT and maternal serum biochemistry. In the reassessment of such fetuses it would be necessary to undertake an examination for only one of the three markers, which may vary with the skill of the individual sonographer and fetal position.

Individual risk-orientated two-stage screening for trisomy 21 can potentially identify, in the first trimester of pregnancy, more than 90% of affected fetuses for a false-positive rate of 2–3%.

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