Ultrasound Obstet Gynecol (2010)
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.7663

Two-stage first-trimester screening for trisomy 21 by ultrasound assessment and biochemical testing

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KEYWORDS: ductus venosus flow; nasal bone; nuchal translucency; screening for trisomy 21; tricuspid regurgitation

ABSTRACT

Objectives *This study was carried out to examine the performance of a contingent policy in first-trimester screening for trisomy 21, in which the estimated risk was first derived by a combination of maternal age, fetal nuchal translucency (NT) thickness, presence/absence of the nasal bone, blood flow in the ductus venosus or flow across the tricuspid valve, and biochemical testing was carried out only in those who were found to have an intermediate risk. We also examined the performance of a policy in which the estimated risk was first derived by a combination of maternal age and biochemical testing, and ultrasound examination was carried out only in those with an intermediate risk.*

Methods *The data for this study were derived from prospective screening for trisomy 21 in singleton pregnancies, using, as markers, a combination of maternal age, fetal NT thickness and maternal-serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A), in a one-stop clinic for first-trimester assessment of risk at 11 + 0 to 13 + 6 weeks of gestation. Assessment of the fetal nasal bone, ductus venosus flow and tricuspid flow were also routinely performed by appropriately trained sonographers. The performance of different screening policies was examined.*

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. The best performance was achieved by a contingent policy in which first-stage screening was based on maternal age, fetal NT thickness and either tricuspid valve or ductus venosus blood flow, followed by biochemical testing only those with an intermediate risk, of 1 in 51 to 1 in 1000 (which constituted about 20% of the total). The performance of contingent screening in which first-stage testing relies on biochemistry was poorer than when first-stage screening was performed by ultrasound examination because, in order to achieve the same detection rate, the false-positive rate was twice as high.*

Conclusion *Effective first-trimester screening for trisomy 21 can be achieved by a contingent policy in which first-stage testing is based on ultrasound examination and second-stage biochemical testing is carried out in only 20% of the patients. Copyright © 2010 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, maternal-serum free β-human chorionic gonadotropin (β-hCG) and pregnancy associated plasma protein-A (PAPP-A) at 11–13 weeks of gestation. The detection rate of fetuses with trisomy 21 is about 90%, and the false-positive rate is 5%[1].

A further improvement in the screening performance can be achieved by including assessment of the nasal bone and the blood flow in the ductus venosus and across the tricuspid valve. Absence of the nasal bone, reversed a-wave in the ductus venosus and tricuspid regurgitation are observed in about 60, 65 and 55% of fetuses with trisomy 21 and in 2.6, 3.2 and 0.9%, respectively, of euploid fetuses. Assessment of each of these ultrasound markers can be incorporated into first-trimester combined screening that already uses maternal age, fetal NT thickness and maternal-serum free β-hCG

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Accepted: 7 April 2010

and PAPP-A as markers, resulting in an improvement in the screening effectiveness, giving a detection rate of 93–96% and a false-positive rate of 2.5%. A similar effectiveness of screening can be achieved by a contingent policy in which combined screening is offered to all cases, and ultrasound assessment of additional markers is carried out only in the 15% of the total population whose estimated risk after combined screening is between 1 in 51 and 1 in 1000[2–4].

The aim of this study was to examine the effectiveness of an alternative policy in which the estimated risk was first derived by a combination of maternal age, measurement of fetal NT thickness and ultrasound assessment of the nasal bone, blood flow in the ductus venosus or flow across the tricuspid valve, and in which biochemical testing was carried out only in those with an intermediate risk. We also examined the effectiveness of a policy in which the estimated risk was first derived by a combination of maternal age and biochemical testing and in which ultrasound examination was carried out only in those with an intermediate risk.

METHODS

The data for this study were derived from prospective screening 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[5,6]. Transabdominal ultrasound examination was performed to diagnose any major fetal defects and to measure the fetal crown–rump length (CRL) and NT thickness. Assessment of the fetal nasal bone, ductus venosus flow and tricuspid flow were also routinely performed by sonographers who had received the appropriate Fetal Medicine Foundation Certificates of Competence[7–11]. Automated machines that provide reproducible results within 30 min were used to measure PAPP-A and free β-hCG (Delfia Express System; PerkinElmer, Waltham, MA, USA). Maternal demographic characteristics, ultrasound measurements and biochemical results were recorded in a computer database. Karyotype results and details of pregnancy outcomes were added to the database as soon as they became available. A search of the database was performed to identify all singleton pregnancies in which first-trimester combined screening was carried out from January 2006 to May 2007.

The same database was used previously as a developmental dataset to incorporate assessment of the nasal bone, flow across the tricuspid valve and the ductus venosus blood flow into the risk algorithm[2–4].

Statistical analysis

The performance of five screening policies was assessed as follows.

First policy. Screening using the markers maternal age, fetal NT thickness[12] and nasal bone[2], ductus venosus[3] or tricuspid blood flow[4] in all patients.

Second policy. Screening using the markers maternal age, fetal NT thickness, maternal-serum free β-hCG and PAPP-A[13], and nasal bone, ductus venosus or tricuspid blood flow in all patients.

Third policy. First-stage screening using maternal age and fetal NT thickness as markers. Patients with a risk of 1 in 50 or more were considered to be screen positive and those with a risk of less than 1 in 1000 were screen negative. The patients with intermediate risk, of 1 in 51 to 1 in 1000, underwent a second-stage screening with free β-hCG and PAPP-A assessment, which modified their first-stage risk. If the adjusted risk was 1 in 100 or more the patients were considered to be screen positive, and those with a risk of less than 1 in 100 were considered to be screen negative.

Fourth policy. First-stage screening using the markers maternal age, fetal NT thickness and nasal bone, tricuspid blood flow or ductus venosus flow. Patients with a risk of 1 in 50 or more were considered to be screen positive and those with a risk of less than 1 in 1000 were considered to be screen negative. The patients with an intermediate risk, of 1 in 51 to 1 in 1000, underwent second-stage screening with free β-hCG and PAPP-A assessment, which modified their first-stage risk. If the adjusted risk was 1 in 100 or more the patients were considered to be screen positive and those with a risk of less than 1 in 100 were considered to be screen negative.

Fifth policy. First-stage screening using the markers maternal age and maternal-serum free β-hCG and PAPP-A. Patients with a risk of 1 in 50 or more were considered to be screen positive and those with a risk of less than 1 in 1000 were considered to be screen negative. Patients with an intermediate risk of 1 in 51 to 1 in 1000 underwent second-stage screening using, as markers, fetal NT alone or fetal NT with nasal bone, ductus venosus or tricuspid blood flow, which modified their first-stage risk. If the adjusted risk was 1 in 100 or more the patients were considered to be screen positive and those with a risk of less than 1 in 100 were considered to be screen negative.

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 (Office for National Statistics, 2000–2002)[14].

RESULTS

Study population

The database search identified 21 141 singleton pregnancies, including 122 with fetal trisomy 21. In 1298 (6.1%) cases either the pregnancy outcome or one of the covariates was missing, and in 107 (0.5%) cases a chromosomal

abnormality, other than trisomy 21, was present. Therefore, 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 maternal age, CRL, distribution of NT thickness, free β-hCG and PAPP-A, and the prevalence of absent nasal bone, reversed a-wave in the ductus venosus and tricuspid regurgitation in the euploid and trisomy 21 pregnancies are shown in Table 1.

According to the maternal[15] and gestational age[16] distribution of the study population, 105 (95% prediction interval, 85–125) cases with trisomy 21 were expected to be identified by screening.

Screening by ultrasound markers in all patients

Screening for trisomy 21 using the markers maternal age and fetal NT, with a risk cut-off of 1 in 100, identified 76% of fetuses with trisomy 21 for a false-positive rate of 1.9% (Table 2). Examination of the nasal bone in all cases increased the detection rate to 83% and the false-positive rate to 2.9%. Examination of either the ductus venosus flow or the tricuspid flow in all cases increased the detection rate to 85% and the false-positive rate to 2.7%.

Screening by ultrasound markers and serum biochemistry in all patients

Screening for trisomy 21 using the markers maternal age, fetal NT and maternal-serum free β-hCG and PAPP-A, with a risk cut-off of 1 in 100, identified 89% of fetuses with trisomy 21 and gave a false-positive rate of 2.3% (Table 2). Additional examination of nasal bone, ductus venosus flow or tricuspid flow in all cases was associated with the respective detection and false-positive rates of 91% and 2.5%, 96% and 2.5%, and 96% and 2.6%.

Table 1 Maternal age, crown–rump length, fetal nuchal translucency (NT) thickness, maternal serum pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) and prevalence of absent nasal bone, reversed a-wave in the ductus venosus and tricuspid regurgitation in euploid and trisomy 21 pregnancies

	Euploid (n = *19 614*)	*Trisomy 21* (n = *122*)
Maternal age (years)	34.4 (14.1–50.1)*	39.5 (19.6–47.2)*
Crown–rump length (mm)	63.2 (45.0–84.0)	63.1 (47.4–84.0)
Deviation in fetal NT (mm)	0.1 (−1.0 to 8.5)*	1.4 (−0.4 to 11.2)*
Serum PAPP-A (MoM)	1.0 (0.2–3.3)*	0.5 (0.06–2.2)*
Serum free β-hCG (MoM)	1.0 (0.1–29.4)*	2.0 (0.1–7.0)*
Absent nasal bone	513 (2.6)*	73 (59.8)*
Reversed a-wave in ductus venosus	622 (3.2)*	81 (66.4)*
Tricuspid regurgitation	181 (0.9)*	68 (55.7)*

Data expressed as median (range) or as *n* (%). *Significant differences. Test statistics: maternal age, PAPP-P multiples of the median (MoM) and free β-hCG MoM: *t*-test, $P < 0.0001$; crown–rump length: *t*-test, $P = 0.624$; Delta NT: Mann–Whitney *U*-test, $P < 0.0001$; absent nasal bone, reversed a-wave in ductus venosus and tricuspid regurgitation: chi-square test, $P < 0.0001$.

Table 2 Effectiveness of screening, with a risk cut-off of 1 in 100, for different policies incorporating maternal age (MA), fetal nuchal translucency (NT) thickness, maternal serum-free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A), fetal nasal bone, ductus venosus flow and flow across the tricuspid valve

Screening policy	*Euploid* (n = *19 614*)	*Trisomy 21* (n = *122*)
MA and fetal NT thickness	1.9	76
MA and serum biochemistry	4.8	62
MA, NT and serum biochemistry	2.3	89
MA, NT and fetal nasal bone	2.9	83
MA, NT and ductus venosus flow	2.7	85
MA, NT and flow across the tricuspid valve	2.7	85
MA, NT, serum biochemistry and fetal nasal bone	2.5	91
MA, NT, serum biochemistry and ductus venosus flow	2.5	96
MA, NT, serum biochemistry and flow across the tricuspid valve	2.6	96

Data are expressed as percentages, which were standardized to the MA distribution of pregnancies in England and Wales in 2000–2002[14]. β-hCG and PAPP-A are combined as serum biochemistry.

Contingent policy with fetal NT in the first stage and biochemical testing in the second stage

A contingent policy comprising first-stage screening using the markers maternal age and fetal NT thickness, and second-stage screening using the markers free β-hCG and PAPP-A in those with an intermediate first-stage risk of 1 in 51 to 1 in 1000, produced the following results (also shown in Table 3). After first-stage screening the risk was 1 in 50 or more in 1.4% of the euploid pregnancies and in 75% of the trisomy 21 pregnancies, and 1 in 51 to 1 in 1000 in 28.3% of the euploid pregnancies and in 23% of the trisomy 21 pregnancies. With a final risk cut-off of 1 in 100, the overall detection rate for trisomy 21 was 89% and the false-positive rate was 3.0%.

Contingent policy with ultrasound markers in the first stage and biochemical testing in the second stage

A contingent policy comprising first-stage screening using the markers maternal age, fetal NT thickness and one of the additional ultrasound markers, followed by second-stage screening using the markers free β-hCG and PAPP-A in those with an intermediate first-stage risk of 1 in 51 to 1 in 1000, produced the following results (also shown in Table 3).

First, after first-stage screening using the markers maternal age, fetal NT thickness and nasal bone, the risk was 1 in 50 or more in 1.3% of the euploid pregnancies and in 73% of the trisomy 21 pregnancies, and 1 in 51 to 1 in 1000 in 21.0% of the euploid pregnancies and

Table 3 Distribution of risk and effectiveness of contingent screening

		First-stage screening: distribution of risks			*Second-stage screening: risk ≥ 1 in 100*		
First-stage screening	*Karyotype*	*≥ 1 in 50*	*1 in 51 to 1 in 1000*	*< 1 in 1000*	*Screening method*	*Additional screen positive*	*Total screen positive*
MA and fetal NT	Euploid	1.4	28.3	70.3	Free β-hCG and PAPP-A	1.6	3.0
	Trisomy 21	75	23	2		14	89
MA, fetal NT and nasal bone	Euploid	1.3	21.0	77.7	Free β-hCG and PAPP-A	1.3	2.6
	Trisomy 21	73	24	3		17	90
MA, fetal NT and ductus venosus flow	Euploid	1.4	22.2	76.4	Free β-hCG and PAPP-A	1.3	2.7
	Trisomy 21	79	21	0		17	96
MA, fetal NT and tricuspid flow	Euploid	1.4	20.9	77.7	Free β-hCG and PAPP-A	1.2	2.6
	Trisomy 21	82	15	3		12	94
MA, free β-hCG and PAPP-A	Euploid	4.1	42.5	53.4	Fetal NT	0.9	5.0
	Trisomy 21	63	35	2		27	90
MA, free β-hCG and PAPP-A	Euploid	4.1	42.5	53.4	Fetal NT and nasal bone	1.1	5.2
	Trisomy 21	63	35	2		29	92
MA, free β-hCG and PAPP-A	Euploid	4.1	42.5	53.4	Fetal NT and ductus venosus	1.2	5.3
	Trisomy 21	63	35	2		32	95
MA, free β-hCG and PAPP-A	Euploid	4.1	42.5	53.4	Fetal NT and tricuspid flow	1.0	5.1
	Trisomy 21	63	35	2		32	95

Data are expressed as percentages, which were adjusted according to the maternal age distribution of pregnancies in England and Wales in 2000–2002[14]. In the first stage, screening was performed by maternal age (MA), fetal nuchal translucency (NT) thickness and additional ultrasound markers. Patients with a risk of 1 in 50 or higher were considered as screen positive and those with a risk of less than 1 in 1000 were considered screen negative. Patients with an intermediate risk, of 1 in 51 to 1 in 1000, underwent second-stage screening with free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A), which modified their first-stage risk. If the adjusted risk was 1 in 100 or more the patients were considered as screen positive and those with a risk of less than 1 in 100 were considered screen negative. The overall screen-positive rates are listed in the last column.

in 24% of the trisomy 21 pregnancies. With a final risk cut-off of 1 in 100, the overall detection rate for trisomy 21 was 90% and the false-positive rate was 2.6%.

Second, after first-stage screening using the markers maternal age, fetal NT thickness and ductus venosus blood flow, the risk was 1 in 50 or more in 1.4% of the euploid pregnancies and in 79% of the trisomy 21 pregnancies, and 1 in 51 to 1 in 1000 in 22.2% of the euploid pregnancies and in 21% of the trisomy 21 pregnancies. With a final risk cut-off of 1 in 100, the overall detection rate for trisomy 21 was 96% and the false-positive rate was 2.7%.

Third, after first-stage screening using the markers maternal age, fetal NT thickness and tricuspid flow, the risk was 1 in 50 or more in 1.4% of the euploid pregnancies and in 82% of the trisomy 21 pregnancies and 1 in 51 to 1 in 1000 in 20.9% of the euploid pregnancies and in 15% of the trisomy 21 pregnancies. With a final risk cut-off of 1 in 100, the overall detection rate for trisomy 21 was 94% and the false-positive rate was 2.6%.

Contingent policy with biochemical testing in the first stage and ultrasound markers in the second stage

A contingent policy comprising first-stage screening using the markers maternal age and maternal-serum free β-hCG and PAPP-A, followed by second-stage screening with fetal NT thickness and one of the additional ultrasound markers for those with an intermediate first-stage risk of 1 in 51 to 1 in 1000, produced the following results (also shown in Table 3). After first-stage screening using the markers maternal age and maternal-serum free β-hCG and PAPP-A, the risk was 1 in 50 or more in 4.1% of the euploid pregnancies and in 63% of the trisomy 21 pregnancies and 1 in 51 to 1 in 1000 in 42.5% of the euploid pregnancies and in 35% of the trisomy 21 pregnancies. After second-stage screening with fetal NT as a marker and a final risk cut-off of 1 in 100, the overall detection rate for trisomy 21 was 90% and the false-positive rate was 5.0%. After second-stage screening with fetal NT and either nasal bone, ductus venosus flow or tricuspid flow as markers, the respective overall detection and false-positive rates were 92% and 5.2%, 95% and 5.3%, and 95% and 5.1%, respectively.

DISCUSSION

The findings of this study suggest that effective first-trimester screening for trisomy 21 can be provided by the combination of maternal age and the ultrasonographic measurement of fetal NT. At a risk cut-off of 1 in 100, the detection rate of trisomy 21 is about 75%, with a false-positive rate of about 2%. The effectiveness of screening can be improved further by the additional examination for the absence or presence of the fetal nasal bone and even more by the Doppler assessment of blood flow across the tricuspid valve or blood flow in the ductus venosus, which increased the detection rate to about 85% and gave a false-positive rate of 2.7%.

The study also showed that the best performance of first-trimester screening is achieved if, in addition to maternal age and fetal NT, maternal serum biochemical testing and Doppler assessment of flow in the ductus venosus and across the tricuspid valve are included. At a risk cut-off of 1 in 100, the detection rate of trisomy 21 is about 95% with a false-positive rate of 2.5%. This degree of screening effectiveness is achieved by a policy in which biochemical testing is undertaken in all cases or by a contingent policy in which first-stage screening is based on maternal age, fetal NT and either tricuspid or ductus venosus flow, and biochemical testing is then performed in only those with an intermediate risk, constituting about 20% of the total.

In this study we examined the performance of different strategies of first-trimester screening for trisomy 21 by using the risk cut-off of 1 in 100 rather than the previously used cut-off of 1 in 300. This new cut-off is now recommended by the National Screening Committee in England, which has highlighted that the objective of new methods of screening should not only be an increase in the detection rate but also a reduction in the false-positive rate and a consequent decrease in unnecessary invasive tests[17].

A clear implication of our study results is that first-trimester screening using the markers maternal age and fetal NT detects about 75% of trisomy 21 pregnancies. The detection rate is improved to about 95% by the inclusion of assessment of blood flow in the ductus venosus or across the tricuspid valve and maternal serum biochemical testing. Although screening for the additional sonographic markers or biochemical testing can be performed in all cases, similar results are achieved by a contingent policy in which either of the two is reserved for only 20% of the population.

An alternative first-trimester contingent screening policy consists of performing maternal serum biochemistry testing in all pregnancies followed by measurement of fetal NT thickness only in those with an intermediate risk after biochemical testing. Previous studies examining this policy reported estimated detection rates of 80–90%, false-positive rates of 4–6% and measurement of fetal NT in only 20–30% of cases[18–21]. Similarly, in our study the estimated detection and false-positive rates were 90% and 5%, respectively, with measurement of fetal NT performed in about 40% of cases.

The major advantages of choosing ultrasound assessment rather than biochemical testing as a first-stage policy are as follows: first, there is a substantial reduction in the cost of screening because measurement of maternal-serum free β-hCG and PAPP-A is undertaken in only 20% of pregnancies, rather than in all pregnancies; second, the Doppler studies can be carried out in the same ultrasound examination as for measurement of fetal NT; and, third, reversed a-wave in the ductus venosus or tricuspid regurgitation are not only useful in screening for trisomy 21 and other major chromosomal abnormalities but can also identify pregnancies at increased risk for cardiac defects and adverse pregnancy outcome[22,23]. The disadvantage is that Doppler assessment of tricuspid and ductus venosus flow can be time-consuming and requires appropriately trained sonographers[10,11]. There is a theoretical risk of thermal damage to the developing fetus from the use of color and pulsed Doppler examinations. However, such theoretical risks only apply to transvaginal sonography performed before 10 weeks of gestation and in any case there is no epidemiological or other evidence to support such an assertion[24]. In our study the ultrasound examinations were performed transabdominally after 11 weeks and we used the as low as reasonably achievable (ALARA) principle, with output settings of the machines resulting in thermal index and mechanical index values below 0.6. The advantage of biochemical testing as a first-stage policy relies on its apparent simplicity. However, interpretation of biochemical results necessitates accurate ultrasonographic measurement of fetal CRL and therefore an ultrasound examination cannot be avoided. In both our study and in previous studies, the fetal CRL was measured by appropriately trained sonographers during assessment of fetal NT. It would be wrong to assume that the motivation of sonographers and the accuracy in measuring CRL would remain as high if the scans were carried out purely to measure the CRL and not to examine the fetus. Additionally, the performance of contingent screening in which first-stage testing relies on biochemistry is poorer than that in which first-stage screening relies on ultrasound because, in order to achieve the same detection rate, the false-positive rate is twice as high.

Effective first-trimester screening for trisomy 21 can be achieved by a contingent policy in which first-stage testing is based on the markers maternal age, fetal NT thickness and assessment of flow in the ductus venosus or across the tricuspid valve, and second-stage biochemical testing is carried out in only 20% of the patients.

ACKNOWLEDGMENT

The study was supported by a grant from The Fetal Medicine Foundation (UK Charity No: 1037116).

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