

Tricuspid regurgitation in screening for trisomies 21, 18 and 13 and Turner syndrome at 11 + 0 to 13 + 6 weeks of gestation

K. O. KAGAN*†, C. VALENCIA*, P. LIVANOS*, D. WRIGHT‡ and K. H. NICOLAIDES*

*Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London and ‡Department of Mathematics and Statistics, University of Plymouth, Plymouth, UK and †Department of Obstetrics and Gynaecology, University of Tuebingen, Tuebingen, Germany

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

ABSTRACT

Objective To investigate the performance of first-trimester screening for aneuploidies by including assessment of tricuspid blood flow in the combined test of maternal age, fetal nuchal translucency (NT) thickness, fetal heart rate (FHR) and serum free β -human chorionic gonadotropin (β -hCG) and pregnancy-associated plasma protein A (PAPP-A).

Method Screening by the combined test was performed in singleton pregnancies, including 19 614 with chromosomally normal fetuses or the delivery of a phenotypically normal baby (euploid group), 122 with trisomy 21, 36 with trisomy 18, 20 with trisomy 13 and eight with Turner syndrome. In all cases tricuspid flow was assessed to determine if there was tricuspid regurgitation. We examined the performance of two screening strategies: firstly, assessment of tricuspid flow in all patients and secondly, first-stage screening using the combined test in all patients followed by second-stage assessment of tricuspid flow only in those with an intermediate risk of 1 in 51 to 1 in 1000 after the first stage.

Results Tricuspid regurgitation was observed in 0.9% of the euploid fetuses and 55.7%, 33.3% and 30% of the fetuses with trisomies 21, 18 and 13, respectively, and in 37.5% of those with Turner syndrome. In a screening policy based on maternal age, fetal NT, FHR, serum free β -hCG and PAPP-A, for a fixed false positive rate of 3% the standardized detection rates were 91% for trisomy 21 and 100% for trisomy 18, trisomy 13 and Turner syndrome. Assessment of tricuspid flow in all pregnancies would increase the detection rate of trisomy 21 to 96%, and the detection rates of trisomy 18, trisomy 13 and Turner syndrome would be 92%, 100% and 100%,

respectively. The same detection rates were achieved with the two-stage strategy – in which it was necessary to assess tricuspid flow in only 15% of the total population – at a false positive rate of 2.4%.

Conclusions Assessment of tricuspid flow improves the performance of first-trimester screening for trisomy 21. Copyright © 2008 ISUOG. Published by John Wiley & Sons, Ltd.

INTRODUCTION

Tricuspid regurgitation, as determined by pulsed wave Doppler ultrasonography, is a common finding in trisomic fetuses at 11 + 0 to 13 + 6 weeks' gestation^{1,2}. A study assessing fetuses before karyotyping by chorionic villus sampling reported tricuspid regurgitation in 67.5% of 114 fetuses with trisomy 21, 33.3% of 42 fetuses with trisomy 18 and 4.4% of 1323 euploid fetuses³.

We have recently reported the development of specific algorithms for first-trimester screening for trisomy 21, trisomy 18 and trisomy 13, based on maternal age, fetal nuchal translucency (NT), fetal heart rate (FHR) and maternal serum free β -human chorionic gonadotropin (β -hCG) and pregnancy-associated plasma protein A (PAPP-A) (combined test)^{4,5}. When all three algorithms are used the estimated detection rates of trisomies 21, 18 and 13 are 91%, 97% and 94%, respectively, for an overall false positive rate of 3.1%^{4,5}.

The aims of this study were to derive a specific algorithm that incorporates assessment of tricuspid blood flow into the combined first-trimester screening test and to examine the performance of such an algorithm in screening for trisomies 21, 18 and 13 and Turner syndrome. We

Correspondence to: Prof. K. H. Nicolaides, Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, Denmark Hill, London SE5 9RS, UK (e-mail: fmf@fetalmedicine.com)

Accepted: 19 September 2008

examined the performance of two screening strategies: firstly, assessment of tricuspid flow in all patients and secondly, first-stage screening using the combined test followed by second-stage assessment of tricuspid flow only in those with an intermediate risk for trisomies 21, 18 and 13 and Turner syndrome of between 1 in 51 and 1 in 1000.

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 to 13 + 6 weeks of gestation^{6,7}. Transabdominal ultrasound examination was performed to diagnose any major fetal defects and for measurement of fetal crown-rump length (CRL), NT thickness and FHR⁶. Assessment of tricuspid blood flow was also routinely performed by sonographers who had received the appropriate Fetal Medicine Foundation Certificates of Competence³. Automated machines that provide reproducible results within 30 min were used to measure PAPP-A and free β -hCG (Delfia Express System, Perkin Elmer, Waltham, MA, USA).

Maternal demographic characteristics, ultrasonographic 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 done to identify all singleton pregnancies in which first-trimester combined screening was carried out from January 2006 to May 2007.

The presence or absence of tricuspid regurgitation was determined by pulsed wave Doppler during fetal quiescence. A sample volume of 2.0–3.0 mm was positioned across the tricuspid valve in an apical four-chamber view such that the angle to the direction of flow was less than 20°. A minimum of three attempts were made since the regurgitation jet, when present, may vary its direction towards the right atrium. Tricuspid regurgitation was diagnosed if it was found during at least half of the systole and with a velocity of over 80 cm/s, since aortic or pulmonary arterial blood flow at this gestation can produce a maximum velocity of 50 cm/s.

Statistical analysis

First, we estimated the risk for trisomy 21, trisomy 18, trisomy 13 and Turner syndrome by the combined test based on maternal age, fetal NT, FHR, free β -hCG and PAPP-A⁵. Then this risk was modified by the findings from assessment of tricuspid blood flow. In order to do this we used multiple logistic regression analysis to model the conditional probability of tricuspid regurgitation given fetal karyotype, fetal NT, free β -hCG and PAPP-A and covariates representing ethnicity and maternal smoking status. Thirdly, Bayes' theorem was applied to produce

risks for trisomy 21, trisomy 18, trisomy 13 and Turner syndrome.

We examined the performance of two screening strategies: first, assessment of tricuspid flow in all patients and second, first-stage screening using the combined test followed by second-stage assessment of tricuspid flow only in those with an intermediate risk for trisomies 21, 18 and 13 and Turner syndrome of between 1 in 51 and 1 in 1000. Screening performance was assessed by calculating the proportions with risks above a given threshold after adjustment for maternal age according to the distribution of pregnancies in England and Wales in 2000–2002⁸.

RESULTS

Study population

The search of the database identified 21 141 singleton pregnancies. In 1110 (5.3%) cases the outcome was not available, in 188 (0.9%) cases one of the covariates was missing and in 43 (0.2%) cases there was a chromosomal abnormality other than trisomy 21, 18 or 13 or Turner syndrome. Thus, our study population consisted of 19 614 pregnancies with a normal karyotype or delivery of a phenotypically normal baby (euploid group), 122 cases of trisomy 21, 36 cases of trisomy 18, 20 cases of trisomy 13 and eight cases of Turner syndrome. The characteristics of the study population are summarized in Table 1.

Table 1 Characteristics of the study population

Characteristic	Median (range) or n (%)
Maternal characteristics	
Age (years)	34.5 (14.1–50.1)
Weight (kg)	64.0 (34.0–165.0)
Spontaneous conception	19 038 (96.2)
Smoker	1145 (5.8)
Ethnicity	
Caucasian	15 850 (80.1)
Afro-Caribbean	2148 (10.8)
East Asian	271 (1.4)
South Asian	1031 (5.2)
Mixed	500 (2.5)
Gestational age	
11 + 0 to 11 + 6 weeks	1477 (7.5)
12 + 0 to 12 + 6 weeks	11 495 (58.1)
13 + 0 to 13 + 6 weeks	6828 (34.5)
Crown-rump length (mm)	63 (45.0–84.0)
Karyotype	
Normal karyotype	19 614 (99.1)
Trisomy 21	122 (0.6)
Trisomy 18	36 (0.2)
Trisomy 13	20 (0.1)
Turner syndrome	8 (0.04)
Total	19 800 (100.0)

Table 2 Fetal nuchal translucency, fetal heart rate, serum pregnancy-associated plasma protein-A (PAPP-A) and serum free β -human chorionic gonadotropin (β -hCG) in chromosomally normal and abnormal fetuses

Characteristic	Median (range)
Crown-rump length (mm)	
Normal karyotype	63.2 (45.0 to 84.0)
Trisomy 21	63.1 (47.4 to 84.0)
Trisomy 18	55.1 (45.0 to 70.4)
Trisomy 13	57.0 (45.5 to 82.9)
Turner syndrome	62.0 (45.0 to 69.7)
Observed deviation from expected fetal nuchal translucency (mm)	
Normal karyotype	0.1 (-1.0 to 8.5)
Trisomy 21	1.4 (-0.4 to 11.2)
Trisomy 18	2.6 (-0.4 to 9.3)
Trisomy 13	3.1 (0.0 to 6.3)
Turner syndrome	8.5 (1.5 to 10.4)
PAPP-A (MoM)	
Normal karyotype	1.0 (0.2 to 3.3)
Trisomy 21	0.5 (0.06 to 2.2)
Trisomy 18	0.2 (0.03 to 3.9)
Trisomy 13	0.3 (0.1 to 0.6)
Turner syndrome	0.5 (0.3 to 0.8)
Free β -hCG (MoM)	
Normal karyotype	1.0 (0.1 to 29.4)
Trisomy 21	2.0 (0.1 to 7.0)
Trisomy 18	0.2 (0.02 to 4.8)
Trisomy 13	0.4 (0.2 to 1.1)
Turner syndrome	1.2 (0.3 to 2.0)
Observed deviation from expected fetal heart rate (beats per min)	
Normal karyotype	-0.1 (-32.4 to 45.4)
Trisomy 21	0.6 (-21.6 to 17.8)
Trisomy 18	-3.4 (-17.4 to 10.5)
Trisomy 13	18.4 (10.5 to 32.0)
Turner syndrome	2.1 (-3.9 to 9.5)

MoM, multiples of the median.

Fetal NT, FHR and maternal serum biochemistry

The distributions of fetal NT, FHR and maternal serum free β -hCG and PAPP-A in fetuses with trisomies 21, 18 and 13 and Turner syndrome are shown in Table 2.

Tricuspid blood flow

Tricuspid regurgitation was observed in 0.9% (181 of 19 614) of the euploid fetuses, 55.7% (68 of 122), 33.3% (12 of 36) and 30% (6 of 20) of the fetuses with trisomies

21, 18 and 13, respectively, and in 37.5% (3 of 8) of fetuses with Turner syndrome.

Multiple logistic regression analysis demonstrated statistically significant effects on the prevalence of tricuspid regurgitation from fetal NT, smoking, maternal weight and fetal karyotype ($P < 0.01$), but not CRL ($P = 0.73$), ethnicity ($P = 0.07$), maternal age ($P = 0.11$), serum free β -hCG ($P = 0.59$), PAPP-A ($P = 0.16$), or FHR ($P = 0.23$). The fitted model is shown in Table 3.

Risk distribution and test performance

The total risk for trisomies 21, 18 and 13 and Turner syndrome, according to maternal age, fetal NT, FHR, serum PAPP-A and serum free β -hCG and after standardization for the maternal age distribution of pregnancies in England and Wales in 2000–2002, was 1 in 50 or higher in 1.5% of the euploid pregnancies and in 85%, 88%, 100% and 100% of those with trisomies 21, 18 and 13 and Turner syndrome, respectively. Total risks of 1 in 51 to 1 in 1000 were found in 14.7% of the euploid pregnancies and in 13%, 12%, 0% and 0% of those with trisomies 21, 18 and 13 and Turner syndrome, respectively. Total risks of less than 1 in 1000 were found in 83.8% of the euploid pregnancies and in 1.2%, 0%, 0% and 0% of those with trisomies 21, 18 and 13 and Turner syndrome, respectively.

The performance of screening is shown in Tables 4 and 5. In a screening policy based on maternal age, fetal NT, FHR, serum free β -hCG and PAPP-A, for a fixed false positive rate of 3% the standardized detection rates were 91% for trisomy 21 and 100% for trisomy 18, trisomy 13 and Turner syndrome. Assessment of tricuspid flow in all pregnancies would increase the detection rate of trisomy 21 to 96%, and the detection rates for trisomy 18, trisomy 13 and Turner syndrome would be 92%, 100% and 100%, respectively (Table 4).

A contingent policy (where screen positivity is defined as either a first-stage total risk of 1 in 50 or higher based on maternal age, fetal NT, FHR, serum PAPP-A and serum free β -hCG or a risk of 1 in 100 or higher after assessment of tricuspid flow in those cases where the first-line sum risk is between 1 in 51 and 1 in 1000) would detect 95.6% of all cases of trisomy 21 for a false positive rate of 2.4%, and the respective detection rates for trisomy 18, trisomy 13 and Turner syndrome would be 91.7%, 100% and 100% (Table 5).

Table 3 Fitted logistic regression model for presence of tricuspid regurgitation

	Coefficient	Standard error	z	P	Odds ratio (95% CI)
Constant	-4.50084	0.41541	-10.83	< 0.0001	
Nuchal translucency (mm)	0.44818	0.07738	5.79	< 0.0001	1.565 (1.345–1.822)
Smoker/non-smoker	0.78978	0.22838	3.46	0.0005	2.203 (1.408–3.447)
Maternal weight (kg)	-0.01613	0.00602	-2.68	0.0073	0.984 (0.972–0.996)
Trisomy 21/euploid	4.14098	0.24253	17.07	< 0.0001	62.864 (38.080–101.123)
Trisomy 13/euploid	2.50798	0.58557	4.28	< 0.0001	12.280 (3.897–38.694)
Trisomy 18/euploid	2.73503	0.47767	5.73	< 0.0001	15.410 (6.042–39.302)
Turner syndrome/euploid	0.60994	1.04721	0.58	0.5603	1.840 (0.236–14.332)

Table 4 Detection rates for given false positive rates (FPR) in screening by maternal age, fetal nuchal translucency, fetal heart rate, maternal serum free β -human chorionic gonadotropin and pregnancy-associated plasma protein-A with and without assessment of tricuspid blood flow (TBF) in all pregnancies

FPR (%)	Detection rate							
	Trisomy 21 (n = 122)		Trisomy 18 (n = 36)		Trisomy 13 (n = 20)		Turner syndrome (n = 8)	
	Without TBF	With TBF	Without TBF	With TBF	Without TBF	With TBF	Without TBF	With TBF
1.0	80	92	84	84	100	100	100	100
2.0	86	95	88	88	100	100	100	100
3.0	91	96	100	92	100	100	100	100
4.0	93	96	100	92	100	100	100	100
5.0	94	96	100	92	100	100	100	100
2.4	96		92		100		100	

For comparison, the last row gives the FPRs and detection rates of contingent screening whereby the tricuspid flow is assessed only in the subgroup with an intermediate risk. All percentages are standardized to the maternal age distribution of pregnancies in England and Wales in 2000–2002⁸.

Table 5 Distribution of risk and effectiveness of contingent screening with tricuspid blood flow assessment

Fetal karyotype	First-stage screening			Second-stage screening	Total (%)
	≥ 1 in 50 (%)	1 in 51 to 1 in 1000 (%)	< 1 in 1000 (%)	> 1 in 100 (%)	
Euploid	1.5	14.7	83.8	0.9	2.4
Trisomy 21	85.3	13.4	1.2	10.3	95.6
Trisomy 18	88.5	11.5	0.0	3.3	91.7
Trisomy 13	100	0.0	0.0	0.0	100.0
Turner syndrome	100	0.0	0.0	0.0	100.0

In the first stage the patients are divided into three risk categories after screening by maternal age, fetal nuchal translucency, fetal heart rate, maternal serum free β -human chorionic gonadotropin and pregnancy-associated plasma protein-A. Patients with a risk of 1 in 50 or more are considered to be screen positive and those with a risk of less than 1 in 1000 are screen negative. Patients with an intermediate risk of 1 in 51 to 1 in 1000 have second-stage screening with tricuspid blood flow assessment, which modifies their risk. If the adjusted risk is 1 in 100 or more the patients are considered to be screen positive and those with a risk of less than 1 in 100 are screen negative. The last column lists the overall detection and false positive rates. All percentages are adjusted according to the maternal age distribution of pregnancies in England and Wales in 2000–2002⁸.

DISCUSSION

Effective first-trimester screening for trisomy 21 is provided by a combination of maternal age, fetal NT thickness, FHR and maternal serum free β -hCG and PAPP-A with an estimated detection rate of 91% at a false positive rate of 3%⁵. The findings of this prospective screening study demonstrate that tricuspid regurgitation at 11 + 0 to 13 + 6 weeks is found in about 1% of euploid fetuses, in 56% of fetuses with trisomy 21 and in about one third of fetuses with trisomy 18, trisomy 13 or Turner syndrome. This association between chromosomal abnormalities and tricuspid regurgitation is compatible with findings in an echocardiographic study in mice at 11–14 days' gestation. Tricuspid regurgitation was found in 25% of 20 embryos with trisomy 20 (which is the animal model of human trisomy 21), but in none of 129 euploid embryos⁹.

The prevalence of tricuspid regurgitation is affected not only by the fetal karyotype but also by maternal smoking (being higher in smokers); fetal NT (it increases

with increasing NT); and maternal weight (it decreases with increasing maternal weight). We used logistic regression analysis to take into account these factors in the development of an algorithm for the calculation of risks for chromosomal abnormalities. The relation between the prevalence of tricuspid regurgitation with high fetal NT is the most likely explanation for the lower prevalence of tricuspid regurgitation in euploid fetuses in this screening study (0.9%) compared to our previous report in pregnancies undergoing chorionic villus sampling because of increased estimated risk for trisomy 21 (4.4%)³. The association between tricuspid regurgitation and increased NT in both chromosomally abnormal and euploid fetuses may be mediated by the coincidence of cardiac defects, as well as increased preload and increased afterload².

Inclusion of tricuspid blood flow assessment in first-trimester combined screening increased the detection rate for trisomy 21 from 91 to 96% for the same false positive rate of 3%, with simultaneous detection of

nearly all cases of trisomy 18, trisomy 13 and Turner syndrome. Assessment of tricuspid flow and accurate diagnosis of tricuspid regurgitation can be performed by sonographers trained in fetal echocardiography. The process of training involves both theoretical and practical examinations in which the sonographer must demonstrate their ability to identify the normal and abnormal anatomy of the heart and to obtain standard views of the four chambers, outflow tracts, arterial duct and aortic arch on cross-sectional imaging, and to use color-flow mapping and pulsed wave Doppler in the assessment of the fetal heart. At present there is a limited number of sonographers competent in these techniques. Consequently, assessment of tricuspid flow need not be undertaken in all pregnancies undergoing routine first-trimester combined screening. As shown in our study a contingent policy in which tricuspid flow is assessed only in the 15% of the total population with an intermediate risk (between 1 in 51 and 1 in 1000) after combined testing would detect 96% of fetuses with trisomy 21 and nearly all cases of trisomy 18, trisomy 13 and Turner syndrome for a total false positive rate of 2.4%. Although the model used for biochemistry, FHR and NT thickness was obtained from an independent data set⁴, the logistic regression model used for tricuspid regurgitation was fitted and tested on the same data and the results will be biased towards superior performance. There is therefore a need for further independent validation.

There is a theoretical risk of thermal damage to the developing fetus from the use of color and pulsed Doppler examination. However, such theoretical risk only applies to transvaginal sonography before 10 weeks' gestation, and in any case there is no epidemiological or other evidence to support such an assertion¹⁰. 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.

In conclusion, assessment of tricuspid flow at 11 + 0 to 13 + 6 weeks by sonographers with appropriate training and certification of their competence in performing such a

scan improves the performance of first-trimester screening for trisomy 21.

ACKNOWLEDGMENT

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

REFERENCES

1. Huggon IC, DeFigueiredo DB, Allan LD. Tricuspid regurgitation in the diagnosis of chromosomal anomalies in the fetus at 11–14 weeks of gestation. *Heart* 2003; **89**: 1071–1073.
2. Faiola S, Tsoi E, Huggon IC, Allan LD, Nicolaides KH. Likelihood ratio for trisomy 21 in fetuses with tricuspid regurgitation at the 11 to 13 + 6-week scan. *Ultrasound Obstet Gynecol* 2005; **26**: 22–27.
3. Falcon O, Faiola S, Huggon I, Allan L, Nicolaides KH. Fetal tricuspid regurgitation at the 11 + 0 to 13 + 6-week scan: association with chromosomal defects and reproducibility of the method. *Ultrasound Obstet Gynecol* 2006; **27**: 609–612.
4. Kagan KO, Wright D, Baker A, Sahota D, Nicolaides KH. Screening for trisomy 21 by maternal age, fetal NT, free β -hCG and PAPP-A. *Ultrasound Obstet Gynecol* 2008; **31**: 618–624.
5. Kagan KO, Wright D, Valencia C, Maiz N, Nicolaides KH. Screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency, fetal heart rate, free beta-hCG and pregnancy-associated plasma protein-A. *Hum Reprod* 2008; **23**: 1968–1975.
6. Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. *Lancet* 1998; **352**: 343–346.
7. Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. 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. *Ultrasound Obstet Gynecol* 2005; **25**: 221–226.
8. Office for National Statistics. Birth Statistics. Review of the Registrar General on births and patterns of family building in England and Wales. 2000–2002. Series FM1, No. 29–31. Stationery Office: London.
9. Gui Y, Linask K, Khowsathit P, Huhta J. Doppler echocardiography of normal and abnormal embryonic mouse heart. *Pediatr Res* 1996; **40**: 633–642.
10. Campbell S, Platt L. The publishing of papers on first-trimester Doppler. *Ultrasound Obstet Gynecol* 1999; **14**: 159–160.