Ultrasound Obstet Gynecol 2009; **33**: 512–517
Published online 31 March 2009 in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/uog.6330

Ductus venosus Doppler in screening for trisomies 21, 18 and 13 and Turner syndrome at 11–13 weeks of gestation

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KEYWORDS: chromosomal abnormality; ductus venosus; first trimester; screening; trisomy 21

ABSTRACT

Objectives *To investigate the performance of first-trimester screening for aneuploidies by including assessment of ductus venosus flow in the combined test of maternal age, fetal nuchal translucency thickness, fetal heart rate, and serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A.*

Methods *Screening by the combined test was performed in singleton pregnancies, including 19 614 with euploid fetuses, 122 with trisomy 21, 36 with trisomy 18, 20 with trisomy 13 and eight with Turner syndrome. In all cases the a-wave in the fetal ductus venosus flow was assessed. We examined the performance of two screening strategies: first, assessment of the a-wave in all patients and, second, first-stage screening using the combined test in all patients followed by second-stage assessment of the a-wave only in those with an intermediate risk of one in 51 to one in 1000 after the first stage*

Results *Reversed a-wave was observed in 3.2% of the euploid fetuses, and in 66.4%, 58.3%, 55.0% and 75.0% of fetuses with trisomies 21, 18 and 13 and Turner syndrome, respectively. Inclusion of ductus venosus flow in all pregnancies would detect 96%, 92%, 100% and 100% of trisomies 21, 18 and 13 and Turner syndrome, respectively, at a false-positive rate of 3%. The same detection rates were achieved with the two-stage strategy at a false-positive rate of 2.6%, in which it was necessary to assess the ductus venosus in only 15% of the total population.*

Conclusions *Assessment of ductus venosus flow improves the performance of first-trimester screening for aneuploidies.* *Copyright © 2009 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

A high proportion of fetuses with trisomy 21 and other chromosomal abnormalities have increased impedance to flow in the ductus venosus at 11–13 weeks of gestation. In the combined data from seven studies, abnormal ductal blood flow was observed in 5.2% of euploid fetuses, and 70.8%, 89.3%, 81.8% and 76.9% of fetuses with trisomies 21, 18 and 13 and Turner syndrome, respectively (Table 1)[1–7]. There is uncertainty about whether the incidence of abnormal ductal blood flow is associated with the other first-trimester sonographic and biochemical markers of chromosomal abnormalities, and the extent to which assessment of the ductus venosus would improve the performance of combined first-trimester screening.

Screening for trisomy 21 by 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 is associated with a detection rate of about 90% for a false-positive rate of 5%[8,9]. A beneficial consequence of screening for trisomy 21 is the early diagnosis of other major chromosomal abnormalities, including trisomies 18 and 13 and Turner syndrome. Although all four chromosomal abnormalities are associated with increased fetal NT there are some differences in their distribution of maternal age, serum PAPP-A and serum free β-hCG. We have recently reported the development of specific algorithms for trisomy 21, trisomy 18 and trisomy 13, which, in addition to maternal age, fetal NT and serum free β-hCG and PAPP-A, also use fetal heart rate (FHR)[10]. When all three algorithms are used in combination the detection rates of trisomies 21, 18 and 13 and Turner syndrome are 91%, 97%, 94%

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Accepted: 7 January 2009

Table 1 Studies reporting on the incidence of abnormal flow in the ductus venosus in the first trimester in euploid fetuses, and in those with trisomies 21, 18 and 13, Turner syndrome and other chromosomal abnormalities

Reference	n	*Normal*	*Trisomy 21*	*Trisomy 18*	*Trisomy 13*	*Turner syndrome*	*Other*
Matias *et al.* 1998[1]	486	13/423 (3.1)	35/38 (92.1)	12/12 (100)	5/7 (71.4)	2/3 (66.7)	3/3 (100)
Antolin *et al.* 2001[2]	924	39/911 (4.3)	5/7 (71.4)	3/3 (100)	—	1/1 (100)	1/2 (50.0)
Murta *et al.* 2002[3]	372	7/343 (2.0)	18/18 (100)	1/1 (100)	2/2 (100)	2/2 (100)	3/6 (50.0)
Zoppi *et al.* 2002[4]	325	38/292 (13.0)	14/20 (70.0)	6/7 (85.7)	1/1 (100)	1/3 (33.3)	1/2 (50.0)
Borrell *et al.* 2003[5]*	3382	162/3249 (5.0)	36/48 (75.0)	—	—	—	—
Toyama *et al.* 2004[6]	1097	69/1075 (6.4)	5/7 (71.4)	3/5 (60.0)	1/1 (100)	4/4 (100)	2/5 (40.0)
Prefumo *et al.* 2005[7]*	572	26/497 (5.2)	18/47 (38.3)	—	—	—	—
Total	7158	354/6790 (5.2)	131/185 (70.8)	25/28 (89.3)	9/11 (81.8)	10/13 (76.9)	10/18 (55.6)

Values are *n* (%). *These papers do not provide specific data for other chromosomal abnormalities.

and 100%, respectively, for an overall false-positive rate of 3.1%[10].

The aims of this study were, first, to derive a specific algorithm that combines assessment of ductus venosus flow with maternal age, fetal NT, FHR and maternal serum free β-hCG and PAPP-A and, second, to examine the performance of such an algorithm in screening for trisomies 21, 18 and 13 and Turner syndrome. We examined the performance of two screening strategies: first, integrated first-trimester screening including assessment of the ductus venosus in all patients and, second, first-stage screening of all patients using maternal age, fetal NT, FHR and maternal serum free β-hCG and PAPP-A followed by second-stage assessment of ductus venosus flow only in those with an intermediate risk of one in 51 to one in 1000 after the first stage.

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[8,9]. Transabdominal ultrasound examination was performed to diagnose any major fetal defects and for measurement of fetal crown–rump length (CRL), NT and FHR[10]. Ductus venosus blood flow velocity waveforms were also routinely obtained by sonographers who had received the appropriate Fetal Medicine Foundation Certificate of Competence in this assessment[11]. Automated machines that provide reproducible results within 30 min were used to measure PAPP-A and free β-hCG (Delfia Xpress System, Perkin Elmer, Waltham, MA, USA).

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

In the ductus venosus studies the following criteria were fulfilled[11]: (1) the examinations were undertaken during fetal quiescence; (2) the magnification of the image was such that the fetal thorax and abdomen occupied the whole screen; (3) a right ventral mid-sagittal view of the fetal trunk was obtained and color flow mapping was used to demonstrate the umbilical vein, ductus venosus and fetal heart; (4) the pulsed Doppler sample was small (0.5–1.0 mm) to avoid contamination from the adjacent veins and it was placed in the aliasing area, which is the portion immediately above the umbilical sinus; (5) the insonation angle was less than 30°; (6) the filter was set at a low frequency (50–70 Hz) to allow visualization of the whole waveform; and (7) the sweep speed was high (2–3 cm/s) so that the waveforms were widely spread, allowing better assessment of the a-wave. Waveforms were assessed qualitatively, and considered to be abnormal if the a-wave was reversed and normal if it was present or absent (Figure 1).

Statistical analysis

The performance of contingent screening was assessed by applying the findings of ductus venosus Doppler imaging to the group with a total risk for trisomies 21, 18 and 13 and Turner syndrome of between one in 51 and one in 1000 based on the combined test including maternal age, fetal NT, FHR, free β-hCG and PAPP-A[10]. Screen positivity was defined as either a risk of one in 50 or higher on the basis of the combined test, or a risk of one in 100 or higher after inclusion of ductus venosus flow in the intermediate group with risks between one in 51 and one in 1000.

In order to modify the risk from the combined test on the basis of the findings of ductus venosus Doppler imaging we used multiple logistic regression to model the

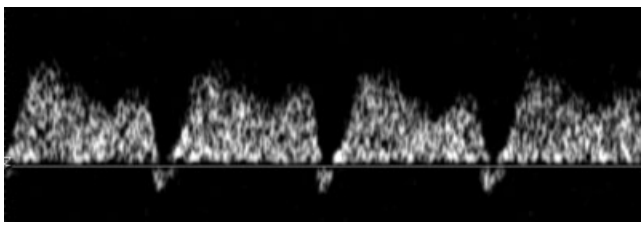

Figure 1 Reversed a-wave in the ductus venosus in a fetus with trisomy 21 at 12 weeks of gestation.

conditional probability of reversed a-wave in the ductus venosus given fetal karotype, fetal NT, free β-hCG and PAPP-A, and covariates representing ethnicity and maternal smoking status. Bayes' theorem was applied to produce risks of trisomy 21, trisomy 18, trisomy 13 and Turner syndrome. This enabled us to examine the performance of a screening policy where combined test and ductus venosus Doppler imaging is used in all pregnancies.

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 2000–2002 (Office for National Statistics, 2000–2002)[12].

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 trisomies 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 2.

Fetal nuchal translucency, heart rate 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 3.

Table 2 Characteristics of 19 800 patients

Variable	*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	19 614 (99.1)
Trisomy 21	122 (0.6)
Trisomy 18	36 (0.2)
Trisomy 13	20 (0.1)
Turner syndrome	8 (0.04)

Table 3 Crown–rump length (CRL), fetal nuchal translucency thickness (NT), fetal heart rate (FHR), serum pregnancy-associated plasma protein-A (PAPP-A) and serum free β-human chorionic gonadotropin (β-hCG) in chromosomally normal and abnormal fetuses

Variable	*Median (range)*
CRL (mm)	
Normal karyotype	63.2 (45.0–84.0)
Trisomy 21	63.1 (47.4–84.0)
Trisomy 18	55.1 (45.0–70.4)
Trisomy 13	57.0 (45.5–82.9)
Turner syndrome	62.0 (45.0–69.7)
Deviation from expected fetal NT (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 (0.2–3.3)
Trisomy 21	0.5 (0.06–2.2)
Trisomy 18	0.2 (0.03–3.9)
Trisomy 13	0.3 (0.1–0.6)
Turner syndrome	0.5 (0.3–0.8)
Free β-hCG (MoM)	
Normal karyotype	1.0 (0.1–29.4)
Trisomy 21	2.0 (0.1–7.0)
Trisomy 18	0.2 (0.02–4.8)
Trisomy 13	0.4 (0.2–1.1)
Turner syndrome	1.2 (0.3–2.0)
Deviation from expected FHR (bpm)	
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.

Ductus venosus flow

Reversed a-wave in the ductus venosus flow was observed in 3.2% (622/19 614) of the euploid fetuses, in 66.4% (81/122), 58.3% (21/36) and 55.0% (11/20) of fetuses with trisomies 21, 18 and 13, respectively, and in 75.0% (6/8) of fetuses with Turner syndrome.

Logistic regression analysis demonstrated highly significant ($P < 0.0001$) effects on the prevalence of reversed a-wave from maternal Afro-Caribbean ethnicity, fetal NT, fetal CRL, serum PAPP-A and fetal karyotype (Table 4). The effects of maternal age, weight, smoking status and serum free β-hCG were not significant ($P > 0.05$). FHR had a small but significant effect ($P = 0.002$) and was negatively associated with reversed DV flow.

Risk distribution and test performance

The total risk for trisomies 21, 18, 13 and Turner syndrome, according to maternal age, fetal NT, FHR, serum PAPP-A and serum free β-hCG, after standardization for the maternal age distribution of pregnancies in England and Wales in 2000–2002, was one in 50 or higher in 1.5% of the euploid pregnancies, and in

Table 4 Fitted logistic regression model for presence of reversed ductus venosus flow

Parameter	*Coefficient*	*Standard error*	Z	P	*OR (95% CI)*
Constant	−1.73803	0.33612	−5.17	< 0.0001	
Nuchal translucency (mm)	0.53657	0.05760	9.31	< 0.0001	1.71 (1.53–1.91)
Crown–rump length (mm)	−0.04447	0.00543	−8.19	< 0.0001	0.96 (0.95–0.97)
Log PAPP-A MoM	−0.87863	0.16062	−5.47	< 0.0001	0.42 (0.30–0.57)
Trisomy 21/euploid	3.11399	0.22851	13.63	< 0.0001	22.51 (14.38–35.23)
Trisomy 13/euploid	1.66997	0.54401	3.07	0.0021	5.31 (1.83–15.43)
Trisomy 18/euploid	1.77208	0.43844	4.04	< 0.0001	5.88 (2.49–13.89)
Turner/euploid	0.78711	1.11791	0.70	0.4814	2.20 (0.25–19.65)
Black/White ethnicity	1.07125	0.09719	11.02	< 0.0002	2.92 (2.41–3.53)

MoM, multiples of the median; OR, odds ratio; PAPP-A, pregnancy-associated plasma protein-A.

Table 5 Detection rates for given false-positive rates (FPRs) in screening by maternal age, fetal nuchal translucency thickness (NT), fetal heart rate, maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A), with and without ductus venosus (DV) Doppler, standardized to the maternal age distribution of pregnancies in England and Wales in 2000–2002[12]

	Detection rate (%)							
	Trisomy 21		*Trisomy 18*		*Trisomy 13*		*Turner syndrome*	
FPR (%)	*Without DV*	*With DV*	*Without DV*	*With DV*	*Without DV*	*With DV*	*Without DV*	*With DV*
1.0	80	88	84	84	100	100	100	100
2.0	86	94	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	97	100	92	100	100	100	100
2.6	96		92		100		100	

The last row gives the results of contingent screening in which DV Doppler is carried out only in those with risk estimates of one in 51 to one in 1000 after first-line screening by maternal age, fetal NT, fetal heart rate and maternal serum free β-hCG and PAPP-A.

Table 6 Distribution of risk and effectiveness of contingent screening

	First stage			*Second stage*	
Fetal karyotype	*≥ 1 in 50 (%)*	*1 in 51 to 1 in 1000 (%)*	*< 1 in 1000 (%)*	*≥ 1 in 100 (%)*	*Total (%)*
Euploid	1.5	14.7	83.8	1.1	2.6
Trisomy 21	85.3	13.4	1.2	10.5	95.9
Trisomy 18	88.5	11.5	0.0	3.2	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 thickness, fetal heart rate, maternal serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A. The patients with a risk of one in 50 or more are considered to be screen positive and those with a risk of less than one in 1000 are screen negative. The patients with an intermediate risk of one in 51 to one in 1000 have second-stage screening with fetal ductus venosus Doppler which modifies their risk. If the adjusted risk is one in 100 or more the patients are considered to be screen positive and those with a risk of less than one in 100 are screen negative. The last column lists the overall detection rates at a false-positive rate of 2.6%. All percentages are adjusted according to the maternal age distribution of pregnancies in England and Wales in 2000–2002[12].

85.3%, 88.5%, 100% and 100% of those with trisomies 21, 18 and 13 and Turner syndrome, respectively. Total risks of one in 51 to one in 1000 were found in 14.7% of the euploid pregnancies, and 13.4%, 11.5%, 0% and 0% of those with trisomies 21, 18 and 13 and Turner syndrome, respectively. Total risks of less than one in 1000 were found in 83.8% of the euploid pregnancies, and 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 5 and 6. For a fixed false-positive rate of 3% the standardized detection rate was 91% for trisomy 21, whereas for trisomy 18, trisomy 13 and Turner syndrome it was 100%. Assessment of the ductus venosus flow in all pregnancies would increase the detection rate of trisomy

21 to 96%, and those for trisomy 18, trisomy 13 and Turner syndrome would be 92%, 100% and 100%.

A contingent policy (where screen positivity is defined as either a first-stage total risk of one in 50 or higher based on maternal age, fetal NT, FHR, serum PAPP-A and serum free β-hCG, or a risk of one in 100 or higher after assessment of the a-wave in the ductus venosus flow in those cases where the first-line total risk is between one in 51 and one in 1000) would detect 96% of all cases with trisomy 21 for a false-positive rate of 2.6%, and the respective detection rates for trisomy 18, trisomy 13 and Turner syndrome would be 92%, 100% and 100%, respectively (Table 6).

DISCUSSION

The findings of this prospective screening study demonstrate that reversed a-wave in the ductus venosus at 11–13 weeks is found in about 3% of euploid fetuses, in 65% of fetuses with trisomy 21, in about 55% of those with trisomies 13 and 18, and in 75% of those with Turner syndrome. Inclusion of ductus venosus flow in first-trimester screening by maternal age, fetal NT, FHR and maternal serum free β-hCG and PAPP-A would detect about 96% of trisomy 21 fetuses at a false-positive rate of about 2.5%.

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 applies only to transvaginal sonography before 10 weeks and in any case there is no epidemiological or other evidence to support such an assertion[13]. 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 prevalence of reversed flow in the ductus venosus during atrial contraction (a-wave) is affected not only by the fetal karyotype but also by maternal ethnicity (being higher in black than in white women); it is also inversely related to fetal CRL and serum PAPP-A and increases with fetal NT. 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. Reversed flow in the venous system is observed when atrial contraction occurs against a ventricle of high end-diastolic pressure. There are three factors that may explain the CRL-related decrease in the prevalence of reversed a-wave: first, improved ventricular filling and decreased myocardial stiffness with advancing gestation, second, decrease in placental resistance and therefore cardiac afterload and, third, improvement in renal function to counteract any tendency to fluid retention[14–17]. Similarly, the association between the prevalence of reversed a-wave with low PAPP-A and black ethnicity may be explained by increased cardiac afterload due to impaired placentation and therefore increased placental resistance. We have reported previously that low PAPP-A and black ethnicity are associated with increased risk of fetal growth restriction, development of pre-eclampsia and fetal death[18–20]. The association between reversed a-wave and increased NT may be explained by the coincidence of cardiac defects or transient cardiac dysfunction.

Effective first-trimester screening for chromosomal abnormalities is provided by a combination of maternal age, fetal NT and maternal serum free β-hCG and PAPP-A[10]. The estimated detection rate of trisomy 21 was 91% for a false-positive rate of 3.1%[10]. As demonstrated in this study, assessment of ductus venosus flow improves the performance of first-trimester combined screening by increasing the estimated detection rate to about 96% and reducing the false-positive rate to about 2.5%. We investigated two strategies for assessment of ductus venosus flow. In the first approach the ductus venosus can be examined in all cases with the advantage of not only improving the performance of screening for chromosomal abnormalities, but also identifying pregnancies at increased risk of fetal cardiac defects and fetal death[18]. Because assessment of ductus venosus flow is time consuming and requires appropriately trained sonographers, the alternative strategy is to reserve this examination for the subgroup of pregnancies with an intermediate risk (between one in 51 and one in 1000) after combined fetal NT, FHR, free β-hCG and PAPP-A screening, which constitutes only one sixth of the total population. Similar two-stage strategies have been advocated for assessment of flow across the tricuspid valve and in examination for the presence or absence of the nasal bone[9,21,22].

Sonographers undertaking risk assessment by Doppler examination of the ductus venosus should receive appropriate training and certification of their competence in performing such a scan, and should adhere to a series of strict criteria for obtaining the appropriate waveform. We have previously shown that sonographers with extensive experience in the 11–13-week scan require an average of 80 examinations to achieve this level of competence[11].

ACKNOWLEDGMENT

This study was supported by a grant from The Fetal Medicine Foundation (UK charity number 1037116).

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