Nuchal fold thickness, nasal bone absence or hypoplasia, ductus venosus reversed flow and tricuspid valve regurgitation in screening for trisomies 21, 18 and 13 in the early second trimester

A. GEIPEL, A. WILLRUTH, J. VIETEN, U. GEMBRUCH and C. BERG
Department of Obstetrics and Prenatal Medicine, University of Bonn, Bonn, Germany

KEYWORDS: aneuploidy; ductus venosus; first trimester; genetic sonogram; nasal bone; nuchal fold; second trimester; tricuspid regurgitation; trisomy 21

ABSTRACT

Objective To investigate the performance of nuchal fold thickness, nasal bone hypoplasia, reversed flow in the ductus venosus and tricuspid valve regurgitation in the prediction of fetal aneuploidies in the early second trimester.

Methods This was a prospective study of 870 fetuses at 14 + 0 to 17 + 6 weeks of gestation, performed from 2005 to 2007. In all cases we assessed classical structural anomalies, second-trimester markers of aneuploidy including nuchal fold thickness and nasal bone length, as well as ductus venosus blood flow pattern and tricuspid valve regurgitation.

Results The study group included 37 fetuses with trisomy 21, eight with trisomy 18 and four with trisomy 13. Nasal bone hypoplasia was the single most sensitive parameter to identify fetuses with trisomy 21. Independent from maternal age, screening by assessment of nuchal fold and nasal bone identified 64.9% of cases with trisomy 21 and 66.7% of cases with trisomy 18/13 (false-positive rate (FPR), 5.8%). By including ductus venosus and tricuspid flow evaluation, the detection rate increased to 75.7% for trisomy 21 and 83.3% for trisomy 18/13 (FPR, 10.8%). Identification of fetuses with structural abnormalities combined with assessment of all four markers under investigation raised the detection rate of trisomy 21 to 83.9% and that of trisomy 18/13 to 100%. The sensitivity of classical second-trimester markers was 62.2% for trisomy 21 and 70.6% for other autosomal aneuploidies (FPR, 11.3%).

Conclusion The combination of assessment of nuchal fold thickness, nasal bone hypoplasia, ductus venosus reversed flow and tricuspid regurgitation in the early second trimester is associated with a higher detection rate of autosomal trisomies compared with classical second-trimester marker screening. Copyright © 2010 ISUOG. Published by John Wiley & Sons, Ltd.

INTRODUCTION

Prenatal screening for chromosomal abnormalities has become standard practice in many countries worldwide. Although first-trimester ultrasound and biochemical screening are utilized increasingly, second-trimester risk evaluation is a frequent demand of prenatal ultrasound screening in many obstetric units. Since 1985, a number of second-trimester ultrasound findings have been reported to be associated with an increased risk for detection of fetal aneuploidy. Indicators of aneuploidy include structural defects, biometric discrepancies and variations in normal anatomy, the latter being so called ‘markers’. Among them, a thickened nuchal fold (NF) is considered to be one of the most sensitive and specific individual markers. However, various studies have shown that the best combination of sensitivity and specificity is achieved by using markers as a cluster rather than individually. A multicenter study addressing the efficacy of the second-trimester genetic sonogram in high-risk pregnancies reported a combined sensitivity of 71.6% for fetal Down syndrome. While congenital heart defects are the most common structural anomalies in fetuses with aneuploidy, they are frequently
under-detected in screening studies. Therefore incorporation of fetal echocardiography including color Doppler ultrasound has been proposed to identify a higher proportion of affected fetuses, for example by identifying tricuspid regurgitation (TR) 8.

First-trimester aneuploidy screening using nuchal translucency (NT) measurements and maternal blood specimens is a highly effective screening method 9. Detection rates for Down syndrome and other aneuploidies approach 80–90% for a false-positive rate (FPR) of 5% in quality-controlled settings 9,10. Inclusion of additional components such as assessment of nasal bone (NB), tricuspid blood flow and ductus venosus waveform increases the detection rate for trisomy 21 to as much as 92–96%, with a FPR of 3%10–12.

Based on the performance of increased NT thickness, ductus venosus negative a-wave, TR and hypoplastic NB in various first-trimester screening studies 9–12, the aim of our study was to investigate whether this cluster of markers also contributes to the prediction of fetal aneuploidies when used in the early second trimester, the time at which women usually attend for genetic amniocentesis.

METHODS

This prospective study was performed from 2005 to 2007 at the Department of Obstetrics and Prenatal Medicine of the University of Bonn, a German tertiary referral center. A total of 936 fetuses with targeted ultrasound examination between 14 + 0 and 17 + 6 weeks of gestation were identified from the perinatal database. The gestational age was verified according to crown–rump length measurement in the first trimester. After exclusion of 33 monochorionic twin pairs with twin-to-twin transfusion syndrome, the study group comprised 870 fetuses (756 singletons and 114 multichorionic multiples). Indications for referral were maternal age ≥ 35 years (57.9%) and other anamnestic risks (9.2%), suspected abnormalities in preceding examinations (20.6%), abnormal karyotype (4.9%), abnormal serum screens (2.2%), multiple pregnancy (3.8%) and maternal anxiety or wish for targeted ultrasound examination (1.4%). Therefore, the studied population represents a high-risk collective. Pregnancy outcome was obtained from routine newborn examination at the delivery units.

Appropriately trained and experienced specialists examined all patients, using high-resolution ultrasound equipment (IU22, Philips Healthcare, Hamburg, Germany or Voluson E8, GE Medical Systems, Solingen, Germany). This second-trimester ultrasound scan included detailed anatomical evaluation and fetal echocardiography as well as screening for markers and structural abnormalities known to be associated with fetal aneuploidy. The following markers were investigated: NB hypoplasia, defined as either absent NB or NB length < 0.75 multiples of the median (MoM) for gestational age; NF thickness ≥ 5 mm; TR; abnormal ductus venosus waveform (negative a-wave); renal pyelectasis ≥ 4 mm; choroid plexus cysts; echogenic bowel; echogenic intracardiac focus; short femur; short humerus; ventriculomegaly (8–12 mm); and single umbilical artery.

The fetal NB was examined as described previously 13. Briefly, the facial profile was obtained in the midsagittal plane and the NB was identified by sliding the transducer sideways. Measurements were taken at the level of the synostosis with an angle of insonation close to 45° or 135°. Following the findings of Odibo et al. 14 we defined NB hypoplasia as length < 0.75 MoM for gestational age. NF measurements were obtained from a transverse view of the fetal head that included the cerebellum, occipital bone and cavum septi pellucidi, slightly below the biparietal diameter. The calipers were placed from the outer edge of the occipital bone to the outer edge of the skin 15. Fetal echocardiography incorporated the four-chamber view with outflow tracts and the three-vessels and trachea view. Color Doppler was applied additionally. To screen for TR, an apical four-chamber view with an angle of insonation < 30° to the interventricular septum was chosen and pulsed wave Doppler was used to confirm blood flow velocities > 100 cm/s across the valve 16. Ductus venosus Doppler recordings were obtained during fetal quiescence by color flow mapping near its origin at the umbilical vein, with an insonation angle < 30°. Abnormal ductus venosus blood flow was defined as reversed velocities during atrial contraction (a-wave) 17. Markers were assessed individually and in clusters in such a way that at least two of the markers were present.

Statistical analysis included descriptive statistics and Fisher’s exact test. Continuous variables are expressed as mean ± SD. P < 0.05 was considered statistically significant.

RESULTS

In total 870 fetuses were evaluated. Their mean gestational age was 16 + 0 (range, 14 + 0 to 17 + 6) weeks. The mean ± SD maternal age was 34.3 ± 5.3 (range, 15–49) years and 96% of the mothers were Caucasian.

Sonographic findings were normal in 67.6% (588/870) and abnormal in 32.4% (282/870) of fetuses. Of the latter group, 61.0% (172/282) presented with markers and 39.0% (110/282) with structural abnormalities. Invasive testing was performed in 429 (49.3%) cases. There were 62 (7.1%) cases of aneuploidy in the study population, including 37 cases of trisomy 21 (two diagnosed postnatally), eight of trisomy 18, four each of trisomy 13 and triploidy, three of microdeletion 22q11, two of 47,XXX, one of Turner syndrome and three with miscellaneous anomalies. Sonographic abnormalities were present in 93.5% (58/62) of these cases.

Of fetuses with Down syndrome, 32.4% presented with a thickened NF, 45.9% with NB absence or hypoplasia, 27.0% with TR and 24.3% with an abnormal ductus venosus blood flow pattern, with respective FPRs of 2.7%, 3.2%, 4.6% and 1.6% (Table 1). While NB hypoplasia was the most sensitive single marker, abnormal ductus venosus blood flow was the most specific one. Values
Table 1 Incidence of markers and structural abnormalities in chromosomally normal and abnormal fetuses

<table>
<thead>
<tr>
<th>Chromosomal defect</th>
<th>Trisomy 21 (n = 37)</th>
<th>Other* (n = 17)</th>
<th>Euploidy (n = 808)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marker</td>
<td>(n (%))</td>
<td>LR+</td>
<td>LR−</td>
</tr>
<tr>
<td>Thickened nuchal fold</td>
<td>12 (32.4)</td>
<td>11.9</td>
<td>0.69</td>
</tr>
<tr>
<td>Nasal bone absence/hypoplasia</td>
<td>17 (45.9)</td>
<td>14.3</td>
<td>0.59</td>
</tr>
<tr>
<td>Tricuspid regurgitation</td>
<td>10 (27.0)</td>
<td>5.9</td>
<td>0.76</td>
</tr>
<tr>
<td>Abnormal ductus venosus</td>
<td>9 (24.3)</td>
<td>15.1</td>
<td>0.77</td>
</tr>
<tr>
<td>Classical second-trimester markers†</td>
<td>23 (62.2)</td>
<td>5.5</td>
<td>0.43</td>
</tr>
<tr>
<td>Structural abnormalities</td>
<td>16 (43.2)</td>
<td>4.8</td>
<td>0.62</td>
</tr>
<tr>
<td>Normal ultrasound scan</td>
<td>2 (5.4)</td>
<td>0.1</td>
<td>3.4</td>
</tr>
</tbody>
</table>

*Trisomy 13, trisomy 18, triploidy, Turner syndrome. Five fetuses with miscellaneous aneuploidies and structural abnormalities were not included in this table. †Mild ventriculomegaly (8–12 mm), shortened femur/humerus, echogenic intracardiac focus, echogenic bowel, pyelectasis, single umbilical artery, choroid plexus cysts. LR+, positive likelihood ratio; LR−, negative likelihood ratio.

Table 2 Effectiveness of screening for autosomal trisomies with combined assessment of different markers

<table>
<thead>
<tr>
<th>Markers</th>
<th>Trisomy 21 (n = 37)</th>
<th>Trisomy 18/13 (n = 12)</th>
<th>Euploidy (n = 808)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF and/or NB</td>
<td>24 (64.9)</td>
<td>8 (66.7)</td>
<td>47 (5.8)</td>
</tr>
<tr>
<td>NF and/or NB and/or DV</td>
<td>27 (73.0)</td>
<td>10 (83.3)</td>
<td>58 (7.2)</td>
</tr>
<tr>
<td>NF and/or NB and/or TR</td>
<td>26 (70.3)</td>
<td>8 (66.7)</td>
<td>76 (9.4)</td>
</tr>
<tr>
<td>NF and/or NB and/or TR and/or DV</td>
<td>28 (75.7)</td>
<td>10 (83.3)</td>
<td>87 (10.8)</td>
</tr>
<tr>
<td>NF and/or NB and/or TR and/or DV and/or SA</td>
<td>31 (83.8)</td>
<td>12 (100)</td>
<td>143 (17.7)</td>
</tr>
<tr>
<td>NF and/or NB and/or TR and/or DV and/or AMA</td>
<td>34 (91.9)</td>
<td>11 (91.7)</td>
<td>444 (55.0)</td>
</tr>
</tbody>
</table>

AMA, advanced maternal age (≥ 35 years); NF, thickened nuchal fold; NB, nasal bone absence/hypoplasia; SA structural abnormalities; TR, tricuspid regurgitation; DV, abnormal ductus venosus waveform.

for other chromosomal defects are presented in Table 1. Cardiac abnormalities were observed in 43.2% of fetuses with trisomy 21, 75% of fetuses with trisomy 18/13 and 3.3% (27/808) of euploid fetuses. There was an abnormal ductus venosus blood flow pattern in 13 euploid fetuses, five of which had congenital heart disease. There was a reversed a-wave in the ductus venosus in 18.5% (5/27) of fetuses with non-chromosomal cardiac defects. Of the 37 euploid cases with TR, three had cardiac disease.

Screening based on a combination of increased NF and/or hypoplastic or absent NB as the two strongest individual markers yielded a detection rate of 64.9% for trisomy 21 and 66.7% for trisomy 18/13 (FPR, 5.8%) (Table 2). The addition of further markers (abnormal ductus venosus blood flow, TR) increased the detection rate to 75.7% and 83.3% for trisomy 21 and trisomy 18/13, respectively, but also doubled the FPR to 10.8%. Identification of fetuses with structural abnormalities combined with assessment of all four markers under investigation raised the detection of trisomy 21 to 83.8% and that of trisomy 18/13 to 100%. In contrast, screening by classical second-trimester markers, excluding NF and NB evaluation, identified 62.2% of fetuses with trisomy 21 and 70.6% of fetuses with other aneuploidies (Table 1).

DISCUSSION

The findings of our prospective study demonstrate the feasibility of using NF thickness, NB absence or hypoplasia, reversed flow in the ductus venosus and TR in a cluster to screen for aneuploidies in the early second trimester. By assessing these four markers, 75.7% of fetuses with trisomy 21 and 83.3% of fetuses with trisomy 18/13 were identified. Although our detection rate for trisomy 21 was lower compared to the results of previous first-trimester screening studies10–12, it was still higher compared with our results reached by the evaluation of classical second-trimester markers. Higher detection rates were achievable if not only markers but also structural anomalies were assessed, which identified 83.8% of cases with trisomy 21 and all cases with trisomy 18/13. These findings are consistent with other second-trimester screening studies3,18.

NF thickness is one of the earliest and most sensitive and specific sonographic markers which can be used to adjust the risk for Down syndrome5. Interestingly, a recent study investigating the possible correlation between first-trimester NT and second-trimester NF measurements found no correlation in either Down syndrome or unaffected pregnancies19. Using NF alone
with a commonly used threshold of 5 or 6 mm, reported detection rates for trisomy 21 vary widely, from 4% to 35%.\textsuperscript{20–22} A NF of 5 mm at 14–16 weeks was considered by Borrell et al.\textsuperscript{23} to be $\pm 2.5$ SD of the normal distribution. Increased NF identified 32.4% of fetuses with Down syndrome in our study, which is on the upper edge of reported sensitivities. This could be for two reasons: first, the lower cut-off of 5 mm; second, our gestational age being <19 weeks, which might have the effect of finding more affected fetuses with this often transient finding than would be identified later in gestation.

Similar to the first-trimester evaluation, NB assessment is being included increasingly in second-trimester studies and has been described as being one of the strongest markers. In contrast to the first trimester when screening is performed to determine the presence or absence of the NB, in the second trimester NB evaluation additionally includes assessment of its length. In our study there was absent or hypoplastic NB in 45.9% of fetuses with trisomy 21 and 47.1% of fetuses with other aneuploidies. Besides structural anomalies, hypoplastic NB was the most sensitive single marker in our study, superior to NF evaluation. Furthermore, NF and NB in combination were able to identify 64.9% of cases with trisomy 21 and 66.7% of cases with trisomy 18/13. Different methods to define NB hypoplasia have been reported, including fixed cut-offs, the use of percentiles for gestation and the use of ratios such as biparietal diameter/NB length\textsuperscript{14}. More recently, using MoM (NB length $<0.75$ MoM or $<0.70$ MoM) has been proposed as the most efficient means of discriminating between trisomy 21 and normal fetuses\textsuperscript{14,26}. The reported prevalence of NB absence or hypoplasia at 14–25 weeks’ gestation ranges from 43.5% to 100% in fetuses with trisomy 21 and 0.4% to 6% in euploid fetuses\textsuperscript{14,25–27}. This marker therefore appears to maximize the performance of second-trimester screening.

The prevalence of TR on second-trimester fetal echocardiography has been reported as 6.2% in euploid fetuses and is often regarded as a physiological and transient finding\textsuperscript{28}. However, it might be also a sign of cardiac failure or malformation. In our study, TR was found in 4.6% of euploid fetuses, 2.7% of fetuses affected with Down syndrome and 29.4% of fetuses with other chromosomal defects. TR was also described in 28.8% of fetuses with trisomy 21 and 22.3% of fetuses with other chromosomal abnormalities by DeVore\textsuperscript{18,29}. Compared with our findings, slightly higher rates of TR in cases with trisomy 21, 18 and 13 have been reported in the first trimester (55.7%, 33.3% and 30%, respectively)\textsuperscript{11}. Furthermore, cardiac abnormalities have been reported in up to 40% of euploid fetuses with TR and increased NT\textsuperscript{30,31}.

An abnormal ductus venous blood flow pattern in the first trimester is associated with a higher risk for trisomy 21, other chromosomal abnormalities, cardiac defects and fetal death, especially if this is in association with increased NT\textsuperscript{12}. The prevalence in the first trimester of reversed a-wave has been reported as 64% and 68% in cases of trisomy 21 and trisomy 18/13, respectively\textsuperscript{12}, these rates being about three times greater for trisomy 21 and slightly greater for trisomy 18/13 compared with our early second-trimester values. Assessment of tricuspid flow and ductus venous blood flow pattern as a component of specific first-trimester risk evaluation has been shown to improve the performance of screening for trisomies\textsuperscript{1,12}. In our second-trimester evaluation, incorporation of these two parameters also increased the detection rate, to 75.7% for trisomy 21 and 83.3% for trisomies 18/13; however, it also doubled the FPR, to 10.8%.

Potential limitations of this study are the inclusion of mainly high-risk patients and the small sample size, the inclusion of fetuses with chromosomal anomalies known at the time of ultrasound evaluation as well as the unknown percentage of women with a normal first-trimester screening result. The combination of markers in such a way that at least two were present increased the sensitivity of screening, but decreased its specificity. Individual risk assessment would be more accurate if calculated likelihood ratios were used in a step-wise manner.

In conclusion, the findings of our study demonstrate that, compared with using classical second-trimester markers, the assessment of a cluster of NF thickness, NB absence or hypoplasia, ductus venosus flow pattern and TR is associated with a higher sensitivity to detect fetuses with trisomy 21 and other chromosomal anomalies. Among these markers, hypoplastic or absent NB was the single most effective parameter and should be incorporated into second-trimester screening.

REFERENCES