Nuchal translucency beyond Down syndrome screening
Timmerman, E.

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Low-resistance hepatic artery flow in first-trimester fetuses: an ominous sign

C.M. Bilardo, E. Timmerman, P.G. Robles de Medina and S.A. Clur

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ABSTRACT

Objective Low-resistance hepatic artery (HA) flow has been reported in severely growth-restricted fetuses. The same finding has been incidentally observed in first trimester fetuses with enlarged nuchal translucency (NT). The aim of this study was to investigate HA flow in first-trimester fetuses.

Methods Crown–rump length (CRL), NT, ductus venosus (DV) pulsatility index for veins (PIV) and HA pulsatility index (PI) were measured prospectively in fetuses at increased risk on first-trimester assessment for aneuploidy and in a control group of low-risk fetuses. Outcome of pregnancy was known in all cases. Independent sample t-test was used for intergroup comparison.

Results NT, DV-PIV and HA-PI were measured prospectively in 59 fetuses. Thirty-four had an enlarged NT and underwent karyotyping, which was abnormal in 16 cases (trisomy 21, n = 12; trisomy 18, n = 3; 47,XXY, n = 1). Two pregnancies were terminated in view of fetal anomalies. In three other infants an abnormality was confirmed after birth (Noonan syndrome, unspecified genetic syndrome and cardiac defect). The remaining 13 fetuses with enlarged NT and the 25 with normal NT had an uneventful pregnancy outcome. HA-PI was significantly and inversely correlated with NT and DV-PIV. Mean HA-PI was significantly lower in fetuses with adverse outcome (chromosomal anomalies 1.60; chromosomally normal fetuses with adverse outcome 1.66) than in controls (2.03).

Conclusions Low-resistance HA flow can be observed in first-trimester fetuses and, based on its association with adverse outcome, it can be regarded as an ominous sign.
INTRODUCTION

While investigating ductus venosus (DV) flow in severely growth-restricted fetuses, a strong arterial signal can sometimes be recorded on the reverse channel. This signal arises from the hepatic artery (HA), a vessel situated in close proximity to the DV (Figure 1). From studies in second- and third-trimester growth-restricted fetuses it is known that the flow in this vessel increases in conditions of redistribution of the fetal circulation driven by hypoxemia 1–3. This mechanism has been called the hepatic arterial buffer response (HABR) 3. The underlying mechanism of the HABR is a compensatory one, aiming to maintain blood supply to the liver when there is increased shunting of umbilical venous blood through the DV. In postnatal life the HABR operates in conditions of reduced portal flow such as in anemia, hypovolemia or liver cirrhosis 4–6.

While investigating DV flow in first-trimester fetuses at increased risk of aneuploidy we incidentally observed that in particularly severe circumstances (hydrops, large nuchal translucency (NT)) a similarly strong arterial signal can be detected on the reverse channel of the DV Doppler flow spectrum 7. The aim of this study was to prospectively investigate blood flow in this vessel and to attempt to understand the underlying pathophysiological mechanism of HABR in first-trimester fetuses.

METHODS

Ultrasound and Doppler investigations at the Fetal Medicine Unit of the Academic Medical Centre were performed by two operators (C.M.B., P.G.R.) using General Electric Voluson 730 Expert and E8 ultrasound machines (GE Healthcare Ultrasound, Milwaukee, WI, USA). All ultrasound examinations were performed transabdominally after 11 weeks’
gestation with use of the ‘as low as reasonably achievable’ (ALARA) principle, with the output settings of the ultrasound equipment resulting in thermal index and mechanical index values below 0.6.

In all cases the crown–rump length (CRL) and NT thickness were measured and a survey of fetal anatomy was carried out. DV flow was evaluated and the pulsatility index for veins (PIV) was measured as previously described. The high-pass filter was kept as low as possible and the sample gate narrowed.

In first-trimester fetuses the HA is seen in continuity with the celiac artery, branching off the aorta and running in the supero-anterior direction towards the DV, with which it comes into close contact (Figure 2). After recording the DV waveform the sample gate can be slightly moved towards the descending thoracic aorta so that HA waveforms can be visualized simultaneously on the reverse channel (Figure 3). The sample gate can also be moved in such a way as to obtain only waveforms from the DV (Figure 3b) or from the HA (Figure 3c). In all cases the pulsatility index (PI) of the HA waveforms was measured using the automated system of the ultrasound equipment after manual tracing of at least three waveforms. Doppler tracings and measurements were stored digitally.

From January 2008 to November 2009 C.M.B. attempted to record and measure HA flow prospectively in all fetuses referred in view of an increased first-trimester risk prior to

Figure 2  a. Longitudinal ultrasound view of the trunk of a 12-week fetus showing the umbilical vein, the ductus venosus and the descending thoracic aorta on color flow mapping.

b. The hepatic artery (arrowhead) is seen as the vessel coming into close contact with the ductus venosus (*) and in continuity with the celiac artery, arising as the first anterior branch from the descending aorta. The hepatic artery forms a triangle with the descending aorta and the ductus venosus.

c. Normal hepatic artery waveform of a 13-week fetus.
to karyotyping. Measurements in low-risk first trimester fetuses were performed by P.G.R. on women undergoing the combined test at our institution. Few women without specific *a priori* risk factors undergo NT screening at our institution, the majority being referred for fetal assessment and karyotyping in view of an increased NT, combined test risk of $\geq 1:200$ or fetal anomalies.

In all cases at increased risk for aneuploidy karyotyping was performed by chorionic villus sampling. In continuing pregnancies repeat detailed scans were carried out. Pregnancy outcome was obtained from hospital notes and follow-up forms filled in by parents.

**Statistical analysis**

The independent samples $t$-test was used for intergroup comparison. Correlations were assessed using Spearman’s rho, and $P < 0.05$ was considered statistically significant.

**Figure 3**

a. Doppler recording of ductus venosus flow (upper trace) and hepatic artery flow (lower trace) in a 12-week fetus with trisomy 21. The sample gate includes the ductus venosus and the hepatic artery, the waveforms of which are very clearly recorded in spite of no clear color Doppler visualization of the vessel. Note the high velocities.

b. Doppler recording with the sample gate including the ductus venosus only. The hepatic artery waveform can still be seen faintly in the upper channel.

c. Doppler recording with the sample gate moved slightly toward the fetal aorta to include only the hepatic artery waveform.
RESULTS

Crown–rump length, NT, DV and HA measurements and fetal outcome were collected in 59 fetuses. Cases in which measurements were not complete or were not satisfactory were excluded. The success rate in obtaining clear HA, DV and both DV and HA waveforms was 81, 73 and 65%, respectively of the fetuses referred to C.M.B. after a finding of increased risk at first-trimester assessment. Maternal age, CRL, NT measurement, a priori and calculated risk at first-trimester screening are shown according to fetal and neonatal outcome in Table 1.

<table>
<thead>
<tr>
<th>Maternal age (years)</th>
<th>CRL (mm)</th>
<th>NT (mm)</th>
<th>A priori risk</th>
<th>Risk after combined test</th>
<th>Risk ≥ 1:200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal NT + favourable outcome</td>
<td>37 (28 - 42)</td>
<td>61 (46 - 80)</td>
<td>1.7 (1.0 - 2.5)</td>
<td>1:189 (54 - 792)</td>
<td>1:924 (9 - 6224)</td>
</tr>
<tr>
<td>Enlarged NT + favourable outcome</td>
<td>34 (28 - 42)</td>
<td>71 (52 - 84)</td>
<td>3.0 (2.6 - 5.3)</td>
<td>1:325 (53 - 852)</td>
<td>1:50 (8 - 677)</td>
</tr>
<tr>
<td>Trisomy 21</td>
<td>36 (28 - 43)</td>
<td>64 (53 - 90)</td>
<td>4.2 (3.1 – 12)</td>
<td>1:156 (33 – 624)</td>
<td>1:2 (2 - 37)</td>
</tr>
<tr>
<td>Other chromosomal anomaly</td>
<td>39 (32 - 42)</td>
<td>52 (45 – 58)</td>
<td>7.0 (2.8 – 8)</td>
<td>1:89 (36 - 448)</td>
<td>1:2 (2 - 18)</td>
</tr>
<tr>
<td>Adverse outcome (euploid)</td>
<td>31 (24 - 41)</td>
<td>48 (47 – 54)</td>
<td>5.0 (3.1 – 7)</td>
<td>1:512 (56 - 1003)</td>
<td>1:12 (3 – 14)</td>
</tr>
<tr>
<td>All</td>
<td>35 (24 - 43)</td>
<td>61 (45 – 90)</td>
<td>2.9 (1 – 12)</td>
<td>1:204 (33 - 1003)</td>
<td>1:50 (2 - 6224)</td>
</tr>
</tbody>
</table>

Figure 4 Mean hepatic artery pulsatility index (PI) and 95% CI’s in relation to nuchal translucency thickness (NT) and fetal outcome.
The NT was increased (≥95th centile) in 34 fetuses (58%). The karyotype was abnormal in 16 of these cases (47%) (trisomy 21, n = 12; trisomy 18, n = 3; 47XXY, n = 1). The pregnancy was terminated in 14 of the 16 chromosomally abnormal pregnancies, and two trisomy 21 fetuses died in utero. Two pregnancies were terminated due to abnormal fetal development. The remaining 16 fetuses with an enlarged NT were live born. Anomalies were confirmed or diagnosed after birth in three cases (one Noonan syndrome, one unspecified genetic syndrome with corpus callosum agenesis and one ventricular septal defect and pulmonary atresia). The other 13 fetuses with increased NT and the 25 with normal NT were phenotypically normal at birth and had an uneventful neonatal period.

In fetuses with normal NT and normal outcome the mean HA-PI was 2.03 ± 0.46. HA-PI was significantly lower (independent samples t-test, $P < 0.05$) in fetuses with enlarged NT (1.76 ± 0.37), enlarged NT and chromosomal anomalies (all, 1.60 ± 0.28; trisomy 21, 1.57 ± 0.26) and in chromosomally normal fetuses with enlarged NT and adverse outcome (1.66 ± 0.27) (Table 2, Figure 4). Fetuses with chromosomal anomalies had low mean HA-PI, irrespective of the degree of NT enlargement (Figure 5). The relationships between HA-PI and NT and DV-PIV are shown in Figure 6. In fetuses with enlarged NT there was a significant correlation between NT and DV-PIV (Spearman rho, 0.30) and a significant negative correlation between HA-PI and NT (Spearman rho, 0.34) and between HA-PI and DV-PIV (Spearman rho, 0.58). Figure 7 shows the relationship between HA-PI and CRL for the whole group.

### Table 2

Hepatic artery pulsatility index in relation to nuchal translucency thickness (NT) and fetal outcome. SEM, standard error of the mean.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>N</th>
<th>mean</th>
<th>SD</th>
<th>SEMean</th>
<th>95%CI mean</th>
<th>min</th>
<th>max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal NT + favourable outcome</td>
<td>25</td>
<td>2.03</td>
<td>0.46</td>
<td>0.09</td>
<td>1.85 – 2.21</td>
<td>1.52</td>
<td>3.00</td>
</tr>
<tr>
<td>Enlarged NT + favourable outcome</td>
<td>13</td>
<td>1.99</td>
<td>0.40</td>
<td>0.11</td>
<td>1.77 – 2.21</td>
<td>1.44</td>
<td>2.90</td>
</tr>
<tr>
<td>Trisomy 21</td>
<td>12</td>
<td>1.57</td>
<td>0.26</td>
<td>0.07</td>
<td>1.43 – 1.71</td>
<td>1.32</td>
<td>2.12</td>
</tr>
<tr>
<td>Other chromosomal anomaly</td>
<td>4</td>
<td>1.69</td>
<td>0.36</td>
<td>0.18</td>
<td>1.33 – 2.05</td>
<td>1.25</td>
<td>2.00</td>
</tr>
<tr>
<td>Adverse outcome (euploid)</td>
<td>5</td>
<td>1.66</td>
<td>0.27</td>
<td>0.12</td>
<td>1.42 – 1.90</td>
<td>1.40</td>
<td>2.03</td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td>1.87</td>
<td>0.43</td>
<td>0.06</td>
<td>1.75 – 1.99</td>
<td>1.25</td>
<td>3.00</td>
</tr>
</tbody>
</table>
Figure 5. Mean hepatic artery pulsatility index (PI) in fetuses with normal outcome and chromosomally abnormal fetuses in relation to nuchal translucency thickness. P95, 95th percentile.

Figure 6. Scatterplots of hepatic artery pulsatility index (PI) with regard to NT and fetal outcome

a. plotted against nuchal translucency thickness (NT)
b. plotted against ductus venosus pulsatility index for veins (DV-PIV)
DISCUSSION

This is the first prospective study on HA flow in first trimester fetuses. The finding of low-resistance HA flow in fetuses with enlarged NT is an unfavourable prognostic factor, as it is highly associated with chromosomal anomalies (trisomy 21 and other aneuploidies), genetic syndromes and fetal anomalies. DV-PIV and HA-PI show opposite trends and are inversely related. This suggests that low-resistance HA flow, detected along with a high pulsatility DV waveform (increased DV-PIV), may be indicative of hemodynamic changes triggered by the same pathological event. This study confirms our preliminary observation that when low-resistance HA flow is observed in fetuses with very large NTs or with hydrops of varying severity it is associated with a high incidence of adverse outcome7.

The present study involved a cohort of prospectively investigated predominantly high-risk fetuses, reflecting the tertiary referral centre nature of our Fetal Medicine Unit, and calls therefore for larger studies of unselected first-trimester fetuses. However, Doppler studies must be performed cautiously early in pregnancy. Besides the ALARA recommendation the Doppler examination should be limited in time and only carried out in the late first trimester.

The HA arises in most fetuses from the celiac artery (CA), and less frequently (18% of cases) from the superior mesenteric artery10. The common HA branches off into the right, left and middle HA. In first-trimester fetuses the vessel is seen in continuity with the CA, branching off the aorta and running in a supero-anterior direction towards the DV, with which it comes into close contact (Figure 2). Due to the small fetal size it is impossible to exactly map CA branching in the first trimester. However, sampling the vessel close to the DV ensures that the recorded arterial waveforms originate from the HA.
Waveforms from the HA were easy to record in the first trimester, particularly in cases with low-resistance flow. At variance with DV flow patterns, HA flow appears constantly abnormal with less fluctuation. This may represent an advantage over DV investigations. The first report on low-resistance HA flow in the fetus dates back to 1999. Low-resistance HA flow had been documented in second-trimester growth-restricted fetuses. The authors suggested that in cases of shunting of oxygenated umbilical venous blood through the DV, the total blood supply to the liver is maintained by a concomitant, significant increase in arterial blood flow through the HA. The liver can be seen as a fourth preferential organ besides heart, brain and adrenal glands, where a preferential perfusion occurs in cases of poorly oxygenated umbilical venous blood to maintain liver function. Doppler umbilical venous flow quantification in similar circumstances provides evidence of a significant increase in venous blood shunting through the dilated isthmic part of the DV, at the expense of fetal hepatic perfusion. Other clinical reports have documented the presence of an arterial redistribution to the right liver lobe at early stages of hemodynamic adaptation in growth restricted fetuses. In a recent study that provided reference ranges for HA flow in the second and third trimesters, an activated HABR was documented in three cases – two anemic fetuses and one that was growth restricted. In all cases the PI was low and in the two anemic fetuses it returned to within normal ranges after transfusion. The vascular architecture of the human liver is embryologically very complex. Vasculogenesis starts very early in pregnancy and only small changes in vascular architecture occur after 25 weeks. Hepatic perfusion is maintained constant by an adenosine-mediated regulation of blood supply to the organ. HA blood flow is therefore not controlled by the metabolic status of the hepatocytes, but rather by changes in portal flow. When portal flow decreases, less adenosine is washed away and the local concentration rises, resulting in arterial dilation and increased hepatic artery flow – i.e. the HABR.

In this study evidence of low-resistance flow in the HA suggests endothelial response to vasoactive substances from as early as the late first trimester of pregnancy. Although velocities were not measured systematically, these tended to be high in fetuses with low-resistance HA flow. It is not clear which mechanism – the hematopoietic, as in cases of anemia, the hepatopoietic, as in cases of hypoxia-induced impaired liver function or some other mechanism – enhances liver perfusion in these challenged first-trimester fetuses.

In first-trimester fetuses hypoxia is postulated as a common denominator for abnormal DV flow, altered right ventricular diastolic function and discordant ventricular afterload, possibly to ensure cerebral perfusion. The concomitant reduction in portal flow may cause adenosine accumulation, resulting in HA dilatation to maintain adequate liver perfusion.

The fetal liver has a prominent role in regulating fetal growth. It differentiates from being an essentially hematopoietic organ in the first trimester to a hepatopoietic organ during
the second trimester. Signals from the extracellular matrix, soluble factors secreted by neighbouring cells, or direct cell–cell interactions are crucial for the differentiation of the hepatocytes. That liver function is impaired in second-trimester chromosomally abnormal fetuses can be inferred from association with low levels of alpha-fetoprotein, hematopoietic disorders and hepatomegaly. Arterial liver perfusion may be increased to preserve hematopoietic activity in these pregnancies from early in gestation.

The association of HABR with genetic syndromes such as Noonan is also intriguing. Interestingly, the Noonan infant in the present study was hydropic at birth, had hepatomegaly and was subsequently diagnosed with juvenile myelomonocytic leukemia. PTPN11 encodes the protein tyrosine phosphatase SHP-2, relaying signals from growth factor receptors to Ras and other effectors. Mutant SHP-2 proteins induce aberrant growth in multiple hematopoietic compartments. It is not clear if in this case the excessive hematopoiesis is the cause or the effect, as in fetal anemia, of the increased HA flow to the liver.

In conclusion, the finding of low-resistance HA flow in first-trimester fetuses can be regarded as an unfavourable prognostic sign irrespective of the pathophysiological mechanism, which remains unclear. Further studies involving larger cohorts of first-trimester fetuses are needed to confirm this preliminary observation and to elucidate its clinical value and the pathophysiological background.
REFERENCES


