Nuchal translucency beyond Down syndrome screening
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Ductus venosus pulsatility index measurement reduces the false-positive rate in first-trimester screening

E. Timmerman, K. Oude Rengerink, E. Pajkrt, B.C. Opmeer, J.A.M. van der Post and C.M. Bilardo

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ABSTRACT

Objective To investigate if ductus venosus (DV) pulsatility index for veins (PIV) and a-wave measurements can increase the accuracy of first-trimester Down syndrome screening in a high-risk population.

Methods The database of our fetal medicine unit was searched for all cases at increased first-trimester Down syndrome risk. Multivariable logistic regression was used to construct a prediction rule for chromosomal anomalies at any given maternal age, nuchal translucency multiples of the median (NT MoM) and DV-PIV MoM. The discriminative ability of the model was assessed by using receiver–operating characteristics (ROC) analysis.

Results The study population included 445 fetuses. DV-PIV was increased (≥95th percentile) in 239 (54%) and DV a-wave was abnormal in 187 fetuses (42%). In this cohort, 80% of all chromosomal anomalies were identified by an increased DV-PIV and 68% by an abnormal a-wave. The odds of chromosomal anomalies increased by a factor of 4.2 per MoM increase in DV-PIV, adjusted for NT and maternal age. The area under the ROC curve for the prediction of chromosomal anomalies was 0.79. After correction for DV-PIV, DV a-wave did not significantly add to the prediction of chromosomal anomalies.

Conclusion In a population of fetuses at increased first trimester risk for Down syndrome, the combination in a logistic regression model of NT, DV-PIV and maternal age can improve the accuracy of screening for trisomy 21 and other chromosomal anomalies. This is the first study that models the additional value of DV-PIV as a continuous variable to NT measurement alone in a high-risk first-trimester population.
INTRODUCTION

The finding of abnormal ductus venosus (DV) flow in first-trimester fetuses was originally regarded as a strong and independent marker for Down syndrome and as a potential pathophysiological explanation for the cardiac involvement of excessive nuchal fluid accumulation\(^1\). The initially reported high sensitivity of DV screening was questioned by subsequent studies and dependency on other parameters was demonstrated by the finding of a correlation between DV flow and nuchal translucency (NT) thickness\(^2,3\). In addition concerns have been raised with regard to the implementation of this rather difficult measurement in a routine setting.

Nowadays it is generally accepted that the combined finding of an enlarged NT and abnormal DV flow patterns enhances the likelihood of an abnormal karyotype. Even if the karyotype is normal, the chance of structural anomalies, in particular cardiac, or poor pregnancy outcome is increased\(^2,4-6\). To increase the sensitivity of first-trimester screening, many centres have adopted investigation of DV complementary to the combined test in first-trimester screening for aneuploidies\(^5,7,8\). Alternatively, DV measurements can be used after risk assessment has been performed, in a two-step contingent screening approach, to reduce false-positive rates in fetuses at increased risk based on NT enlargement alone or on the combined test\(^1-3,9-15\).

In our institution DV flow is assessed in all fetuses referred for increased first-trimester risk. Although DV results are not used to modify the initial risk assessment, they are taken into account in counselling parents on the need for karyotyping. The aim of this study was to investigate how DV measurements can be best used in our setting to reduce the false-positive rate of first trimester screening. A prediction model was constructed to assess sensitivity and specificity of the combination of DV pulsatility index for veins (DV-PIV) and NT to detect chromosomal anomalies in a tertiary referral center.

METHODS

From our cohort of all fetuses with an increased risk for Down syndrome based on enlarged NT alone or at increased risk at the combined test, registered in our prenatal registry between September 1996 and March 2008, we selected those with a known DV-PIV. The Fetal Medicine Unit of the Academic Medical Centre in Amsterdam acts as a tertiary referral centre for a large geographic area. Fetuses with an increased risk for Down syndrome, owing to an enlarged NT alone or to an abnormal combined test result, are referred to our hospital for advanced first-trimester sonography, invasive testing and (genetic) counselling. First-trimester ultrasound screening is also offered routinely to all pregnant women booking at our hospital. Enlarged NT was defined as a measurement
above the 95th centile for the normal range, according to The Fetal Medicine Foundation\textsuperscript{16}. NT values were converted to multiples of the median (MoM) for gestational age. DV flow was assessed as previously described\textsuperscript{2,17}. During the first years of our study period, DV-PIV measurement was not part of the standard practice in our centre and was only carried out by one sonographer (C.M.B.). Since 2004 DV-PIV has been measured in all high-risk fetuses. We were successful in obtaining satisfactory waveforms in 98\% of cases. In order to correct for intra-fetus variation at least three different sets of waveforms were recorded and the mean PIV value of three different measurements was used for the analysis. The actual time exposure to color-flow and Doppler ultrasound was limited to a maximum of 5 min. PI values for the DV were converted to MoMs according to the reference values of Teixeira et al\textsuperscript{18}. Increased DV-PIV was defined as a measurement above the 95\textsuperscript{th} centile for the normal range. DV a-waves were recorded as positive (normal), absent or reversed (abnormal). When the a-wave was alternately normal and abnormal this was recorded as a mixed a-wave. In the analysis mixed a-waves were considered to be normal. Only when the a-wave was abnormal at all three measurements was it classified as abnormal.

Fetal karyotyping is routinely offered in our centre to all patients with an adjusted Down syndrome risk of more than 1 : 200 based on maternal age, NT and first-trimester pregnancy-associated plasma protein-A and free $\beta$-human chorionic gonadotropin. Before first-trimester serum screening was introduced as part of a national screening program in The Netherlands (2002), karyotyping was offered in cases of enlarged NT or maternal age above 36 years. Numeric chromosomal abnormalities and unbalanced translocations were classified as chromosomal anomalies.

In all cases of enlarged NT and normal karyotype a two-step ultrasound investigation at 13–16 and 20–24 weeks’ gestation was performed to exclude structural anomalies. Pregnancy outcome was obtained in all cases from standard follow-up forms filled and returned by patients, maternity wards or midwife practices and by reviewing neonatal, pathology and clinical pediatric notes. When the baby was born without structural defects or dysmorphic features, the chromosomes were assumed to be normal. In all cases of enlarged NT or antenatal suspicion of abnormal development the infant was investigated by a neonatologist, pediatric cardiologist or geneticist. Adverse pregnancy outcome was defined as chromosomal anomalies, structural anomalies, genetic disorders, intrauterine death or neonatal death and termination of pregnancy in case of severe nuchal fluid accumulation.

\textbf{Statistical analysis}

Chi-square tests were used to compare the prevalence of chromosomal anomalies and other pregnancy outcomes between the normal fetuses and those with increased DV-PIV. Mann–Whitney U-tests were used to compare continuous non-normally distributed characteristics and chi-square tests were used to compare categorical characteristics between the groups. The correlation between NT MoM and DV-PIV MoM was calculated.
with Spearman’s rho correlation coefficient, and $P < 0.05$ was considered statistically
significant. Multivariable logistic regression analysis was used to estimate the predicted
probability of chromosomal anomalies at any given maternal age, NT MoM and DV-PIV
MoM. Splines were used to determine whether the risk of chromosomal anomalies
increases at a constant rate with increasing maternal age, NT MoM and DV-PIV MoM.
Variables were transformed accordingly.

As we used logistic regression analysis, the predicted probability for chromosomal anomaly
$= 1/(1 + \exp(- (\text{constant} + (\beta \times \text{Variable 1}) + (\beta \times \text{Variable 2}) + etc.)))$, where $\beta$ is the regression coefficient for a predictor.

Discrimination refers to the ability of a variable (test result or model estimate) to distinguish
between patients who do and do not experience the event of interest, in this case the
presence of chromosomal anomalies\textsuperscript{19}. The discriminative ability of the model was assessed
by using receiver–operating characteristics (ROC) analysis. The area under the ROC curve
provides a quantitative summary of the discriminative ability of a predictive model and has
a range of 0.5 (no discrimination, random chance) to 1.0 (perfect discrimination).

Calibration refers to the level of correspondence between the probability of an outcome
based on the constructed prediction rule (i.e. chromosomal anomalies) and the observed
proportion of that outcome in the fetuses studied\textsuperscript{19}. Calibration was assessed by comparing
in 10 subgroups the mean predicted probability with the mean observed probability of
chromosomal anomalies. For this purpose, the cohort was split into 10 groups based
on the deciles of the calculated probabilities. The mean predicted probability and the
mean observed fraction per group were calculated. The predicted and observed means
are shown in a calibration plot. SPSS 16.0 (SPSS Inc., Chicago, IL, USA) was used for all
analyses, except for the splines, which were analyzed in SAS 9.2 (SAS Institute Inc., Cary,
NC, USA).

RESULTS

Four hundred and seventy-nine fetuses with an increased first-trimester risk for Down
syndrome ($\geq 1 : 200$ based on the combined test or based on NT, gestational age and
maternal age) and a known DV-PIV were included. Karyotype and outcome were known
in 445 fetuses (93%). Of these, 239 (54%) had an increased DV-PIV ($\geq 95$th centile). The
a-wave was abnormal (zero or reversed) in 187 fetuses (42%). Characteristics of fetuses
with normal and increased DV-PIV are shown in Table 1.

Overall adverse outcome rate was 46% (206 of 445 fetuses). Adverse outcome sources
according to DV-PIV and a-wave normality or abnormality are reported in Table 2.
Chromosomal anomalies were present in 14% of fetuses with a normal DV-PIV and in 46%
of fetuses with an abnormal DV-PIV (relative risk (RR) 3.4, $P < 0.01$). The figure was 17.4%
Table 1. Characteristics of fetuses with normal and increased DV-PIV (mean or median and range)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total</th>
<th>DV-PIV &lt; P95</th>
<th>DV-PIV ≥ P95</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRL (mean)</td>
<td>61 (40-86)</td>
<td>62 (40-85)</td>
<td>60 (40-86) *</td>
</tr>
<tr>
<td>NT MoM</td>
<td>2.3 (0.7-8.6)</td>
<td>2.0 (0.7-8.2)</td>
<td>2.7 (0.8-8.6) *</td>
</tr>
<tr>
<td>Maternal age</td>
<td>34.5 (19-45)</td>
<td>34.7 (19-45)</td>
<td>34.3 (21-45)</td>
</tr>
<tr>
<td>Male fetuses</td>
<td>60%</td>
<td>63%</td>
<td>58%</td>
</tr>
</tbody>
</table>

Data are given as mean (range), median (range) or percent.
* Significant difference between normal and abnormal DV-PIV (Mann-Whitney-U resp. Chi squared test, p< 0.05)
NT MoM, nuchal translucency multiples of the median.

Table 2. Outcome according to ductus venosus pulsatility index for veins (DV-PIV) and normality of a-wave

<table>
<thead>
<tr>
<th>DV-PIV</th>
<th>a-wave</th>
<th>Trisomy 21</th>
<th>Other chromosomal anomalies</th>
<th>Structural anomalies</th>
<th>Miscarriage / IUFD</th>
<th>Other adverse outcome</th>
<th>Overall adverse outcome</th>
<th>Favourable outcome</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; P95</td>
<td>&lt; P95</td>
<td>15 (7.3%)</td>
<td>13 (6.3%)</td>
<td>14 (6.8%)</td>
<td>3 (1.5%)</td>
<td>9 (4.4%)</td>
<td>54 (26.2%)</td>
<td>152 (73.8%)</td>
<td>206 (100%)</td>
</tr>
<tr>
<td>≥ P95</td>
<td>≥ P95</td>
<td>57 (23.8%)</td>
<td>54 (22.6%)</td>
<td>20 (8.4%)</td>
<td>11 (4.6%)</td>
<td>10 (4.1%)</td>
<td>152 (63.6%)</td>
<td>87 (36.4%)</td>
<td>239 (100%)</td>
</tr>
<tr>
<td>normal</td>
<td>normal</td>
<td>24 (9.3%)</td>
<td>21 (8.1%)</td>
<td>17 (6.6%)</td>
<td>4 (1.6%)</td>
<td>10 (3.9%)</td>
<td>76 (29.5%)</td>
<td>182 (70.5%)</td>
<td>258 (100%)</td>
</tr>
<tr>
<td>abnormal</td>
<td>abnormal</td>
<td>48 (25.7%)</td>
<td>46 (24.6%)</td>
<td>17 (9.1%)</td>
<td>10 (5.3%)</td>
<td>9 (4.8%)</td>
<td>130 (69.5%)</td>
<td>57 (30.5%)</td>
<td>187 (100%)</td>
</tr>
</tbody>
</table>

Data are given as n (%).
* Significant difference between normal and abnormal DV-PIV or a-wave (chi-square test, P < 0.05).
IUFD, intrauterine fetal death. P 95, 95th percentile

Table 3. Outcome parameters for ductus venosus pulsatility index for veins (DV-PIV) ≥95th percentile, a-wave abnormality and the combination of both, for all chromosomal anomalies and for trisomy 21.

<table>
<thead>
<tr>
<th>All chromosomal anomalies</th>
<th>DV-PIV ≥ P95</th>
<th>a-wave abnormal</th>
<th>Both abnormal</th>
<th>DV-PIV ≥ P95</th>
<th>a-wave abnormal</th>
<th>Both abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 111/139)</td>
<td>(n = 94/139)</td>
<td>(n = 94/139)</td>
<td></td>
<td>(n = 57/72)</td>
<td>(n = 48/72)</td>
<td></td>
</tr>
<tr>
<td>Sn</td>
<td>0.80</td>
<td>0.68</td>
<td>0.68</td>
<td>0.79</td>
<td>0.67</td>
<td>0.67</td>
</tr>
<tr>
<td>Sp</td>
<td>0.58</td>
<td>0.70</td>
<td>0.70</td>
<td>0.51</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>RR</td>
<td>3.4</td>
<td>2.9</td>
<td>2.9</td>
<td>3.3</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>(2.4 - 5.0)</td>
<td>(2.1 - 3.9)</td>
<td>(2.2 - 4.0)</td>
<td>(1.9 - 5.6)</td>
<td>(1.8 - 4.3)</td>
<td>(1.8 - 4.4)</td>
<td></td>
</tr>
<tr>
<td>OR</td>
<td>5.5</td>
<td>4.8</td>
<td>4.9</td>
<td>4.0</td>
<td>3.4</td>
<td>3.5</td>
</tr>
<tr>
<td>(3.4 - 8.8)</td>
<td>(3.1 - 7.4)</td>
<td>(3.2 - 7.6)</td>
<td>(2.2 - 7.3)</td>
<td>(2.0 - 5.7)</td>
<td>(2.0 - 5.9)</td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>0.46</td>
<td>0.50</td>
<td>0.51</td>
<td>0.24</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td>NPV</td>
<td>0.86</td>
<td>0.83</td>
<td>0.83</td>
<td>0.93</td>
<td>0.91</td>
<td>0.91</td>
</tr>
</tbody>
</table>

P95, 95th percentile; Sn, sensitivity; Sp, specificity; RR, relative risk; OR, odds ratio; NPV, negative predictive value; PPV, positive predictive value.
and 50.3% in cases of normal and abnormal a-wave, respectively (RR 2.9, \( P < 0.01 \)) (Tables 2 and 3). Trisomy 21 accounted for 52% of the chromosomal anomalies (Table 4). Of the 306 chromosomally normal fetuses, a favourable outcome was recorded in 85% when the DV-PIV was normal and in 68% when the DV-PIV was increased (\( P < 0.01 \)). According to normal or abnormal a-wave this was 85% and 61%, respectively (\( P < 0.01 \)). The sensitivity, specificity and RRs of an increased DV-PIV and abnormal a-wave for chromosomal anomalies are reported in Table 3.

NT MoM and DV-PIV MoM were significantly correlated, both in chromosomally abnormal (\( r = 0.24, P = 0.005 \)) as well as in chromosomally normal fetuses (\( r = 0.35, P < 0.001 \)). Logistic regression analysis showed that in this high-risk population maternal age, NT MoM and DV-PIV MoM were independent predictors for chromosomal anomalies. Addition of information on the normality or abnormality of the DV a-wave to a model including maternal age, NT MoM and DV-PIV MoM did not significantly influence the chance of chromosomal anomalies. Results of the univariable and multivariable analysis for the prediction of chromosomal anomalies are presented in Table 5.

### Table 4. Chromosomal anomalies in all fetuses and subdivided according to normal or increased ductus venosus pulsatility index of veins (DV-PIV)

<table>
<thead>
<tr>
<th>Overall</th>
<th>DV-PIV &lt; P95</th>
<th>DV-PIV &gt; P95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal karyotype</td>
<td>306 (68,7%)</td>
<td>178 (86,4%)</td>
</tr>
<tr>
<td>Trisomy 21</td>
<td>72 (16,2%)</td>
<td>15 (7,3%)</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>34 (7,6%)</td>
<td>4 (1,9%)</td>
</tr>
<tr>
<td>Trisomy 13</td>
<td>7 (1,6%)</td>
<td>3 (1,5%)</td>
</tr>
<tr>
<td>45X</td>
<td>8 (1,8%)</td>
<td>2 (1,0%)</td>
</tr>
<tr>
<td>Other</td>
<td>18 (4,0%)</td>
<td>4 (2,0%)</td>
</tr>
<tr>
<td>Total</td>
<td>445 (100%)</td>
<td>206 (100%)</td>
</tr>
</tbody>
</table>

Data are given as n (%).
P95 = 95th percentile
* Significant difference between normal and abnormal DV-PIV (Chi squared test, \( p < 0.05 \))

### Table 5. Results of univariable and multivariable analyses for the prediction of chromosomal anomalies.

<table>
<thead>
<tr>
<th></th>
<th>Univariable analyses</th>
<th>Multivariable analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio (95% CI)</td>
<td>( P )</td>
</tr>
<tr>
<td></td>
<td>Odds ratio (95% CI)</td>
<td>( P )</td>
</tr>
<tr>
<td>NT MoM</td>
<td>2.7 (2.0-3.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>2.3 (1.6-3.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DV-PIV MoM</td>
<td>5.9 (3.8-9.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>4.2 (2.6-6.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DV a-wave (ab)normality</td>
<td>4.8 (3.1 - 7.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>1.7 (0.8 - 3.5)</td>
<td>0.15</td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>1.1 (1.0-1.2)</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>1.2 (1.1-1.3)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

DV, ductus venosus; MoM, multiples of the median; NT, nuchal translucency; PIV, pulsatility index for veins.
Based on the logistic regression analysis a prediction model for chromosomal anomalies at any given maternal age, NT MoM and DV-PIV MoM was constructed:

\[
\text{Predicted probability of chromosomal anomaly} = \frac{1}{1 + \exp\left(-7.26 + (0.44 \times \text{NT MoM}) + (1.21 \times \text{DV-PIV MoM}) + (0.10 \times \text{maternal age})\right)}.
\]

Splines showed that for NT the risk for chromosomal anomalies increased at a constant rate up to 3.5 MoM (linear association). An additional increase in NT MoM above 3.5 did not increase the risk for chromosomal anomalies, therefore all NT MoM values above 3.5 MoM were set in the model to 3.5. Furthermore splines showed a linear association between maternal age and chromosomal anomalies between the ages of 20 and 30 years. DV-PIV was linearly associated with chromosomal anomalies up to 2.0 MoM. The ROC curve derived from this analysis is shown in Figure 1a, while the ROC curve for a combination of maternal age, NT MoM and DV-PIV MoM is shown in Figure 1b.

Results of the calibration analysis of the model are given in Figure 2, which shows the association between the mean calculated probability for chromosomal anomalies and the mean observed fraction of chromosomal anomalies for each of the 10 decile groups. There were 81 fetuses (18% of the population) with a predicted risk of <10%. In this group the observed chromosomal anomaly rate was 5%. In the 10% of fetuses (i.e. 45 fetuses) with the lowest predicted probability, there were no chromosomal anomalies, and 93% of the fetuses in this group had a favourable outcome.

**Figure 1.** Receiver–operating characteristics curves for the prediction of chromosomal anomalies by maternal age, nuchal translucency multiples of the median (NT MoM) and ductus venosus pulsatility index for veins (DV-PIV) MoM (a), and their combination (predicted probability) (b). Area under the curve (AUC) for maternal age = 0.58 (95% CI, 0.52–0.64); AUC for NT MoM = 0.72 (95% CI, 0.67–0.76); AUC for DV-PIV MoM = 0.73 (95% CI, 0.68–0.78); AUC for combination of maternal age, NT MoM and DV-PIV MoM = 0.79 (95% CI, 0.74–0.83).
DISCUSSION

This study demonstrates that in fetuses with high first-trimester Down syndrome risk (≥1 : 200) the risk of chromosomal anomalies significantly increases with increased DV-PIV (RR 3.4) or abnormal DV a-wave (RR 2.9). In this cohort, 80% of the chromosomal anomalies were identified by an increased DV-PIV (≥95th percentile) and 68% by an abnormal a-wave. When DV-PIV is analyzed as a continuous variable, the odds of chromosomal anomalies increase by a factor of 4.2 per MoM increase in DV-PIV, adjusted for NT and maternal age.

This is the first published model including DV-PIV as a continuous variable for further refinement of risk assessment in first-trimester fetuses referred in view of a high risk for chromosomal anomalies.

The combination in a prediction model of DV-PIVMoM, NT MoM and maternal age, used as continuous variables, has a better predictive ability for chromosomal anomalies than does NT MoM or DV-PIV MoM alone. After correction for maternal age, NT MoM and DV-PIVMoM in a prediction model, normality or abnormality of the DV a-wave did not significantly influence the chance of chromosomal anomalies. The odds of chromosomal anomalies increase by a factor 4.2 per MoM increase in DV-PIV. The area under the ROC curve, providing a quantitative summary of the discriminative ability of the model19, is 0.79. This confirms that DV-PIV provides good discrimination between fetuses with and without chromosomal anomalies and can refine risk assessment at any cut-off point. The calibration – the level of correspondence between model-based probability and observed number of chromosomal anomalies19 – is especially good in the lower range, thereby
correctly identifying fetuses with the lowest risk of chromosomal anomalies. As the model performance is only internally validated in the same population it was derived from, we cannot exclude an overestimation of its predictive ability. A prospective validation of the model is therefore needed. If prospectively validated, the model can potentially reduce the number of needed karyotyping procedures by 10%. This estimation is based on the fact that in our population of fetuses selected by a risk cut-off of 1:200, in the 10% with the lowest predicted probability there were no chromosomal anomalies. The impact may be even greater when a lower cut-off is used, as confirmed by Borrell et al. in a general population, where addition of DV-PIV to risk assessment in the combined test reduced the false-positive rate (FPR) at all cut-off values.

NT MoM and DV-PIV MoM were significantly correlated in this study. Logistic regression analysis showed a significant increase of Nagelkerke R² when DV-PIV MoM was added to NT MoM (R² = 0.13–0.20), indicating that the second test still adds predictive value. The reported high detection rates of DV-PIV (≥95th percentile) for trisomy 21 and other chromosomal anomalies (79% and 80%, respectively) are difficult to compare with previous studies reporting on detection rates of NT and DV, based on populations of fetuses with both enlarged and normal NT1–3, 7, 9, 13, 14, 21, 22. Moreover, some workers only analyzed the a-wave1, 3, 7, 21, and others both DV-PIV and a-wave2, 9, 22, 23.

Prefumo et al.8 reported, in fetuses with a Down syndrome risk of ≥1:300, a likelihood ratio for abnormal DV flow of 9.4. Maiz et al.6 found in a general population that an abnormal DV a-wave had an odds ratio for chromosomal anomalies of 19. In our high-risk population the RRs for chromosomal anomalies in cases of increased DV-PIV or abnormal a-wave were 3.4 and 2.9, respectively.

In our population an increased DV-PIV was associated with a higher detection rate for chromosomal anomalies than abnormal DV a-wave (detection rate of 80% and 68%, respectively), but specificity showed opposite trends (specificity 58% and 70%, respectively). The lower specificity of DV-PIV can, at least partly, be overcome by using DV-PIV as a continuous variable, as shown by the predictive model where DV-PIV MoM has a significant influence on the risk of chromosomal anomalies at any cut-off point (odds ratio 4.2). This is in agreement with the findings of Borrell et al.20 in a low-risk population, where adding DV-PIV to the combined test reduced FPR at all tested cut-off values.

When considering implementing DV assessment in screening policies, aspects such as the learning curve and repeatability of the waveforms should be considered2, 24. Intra- and inter-observer reproducibility of DV studies show variable results25–27. In this study all DV measurements were performed by two experienced operators. A mixed a-wave – the situation where one of three repeat measurements was abnormal – was recorded in 15% of the fetuses and these cases were classified as normal for purposes of analysis. Such variation in DV waveforms is also reported in second- and third trimester fetuses28, 29. Whether it is the result of a true biological variation or purely a methodological problem...
(contamination of nearby vessel), ultrasonographers should be aware of it and repeat the investigation to confirm results, especially in an otherwise normal fetus.

DV measurements could directly be integrated into the first-trimester screening algorithm\textsuperscript{4,6,7,18}, or, alternatively, be used in a two-step contingent screening approach, reserving them for fetuses at increased or intermediate risk, to reduce FPR\textsuperscript{1–3,9–15}. Nicolaides et al.\textsuperscript{13} advocate individual risk-oriented two-stage screening in fetuses with a risk of between 1 : 101 and 1 : 1000.

In this study DV was measured prospectively on all referred patients. Although DV results were not used to modify the initial risk assessment, they were taken into account in counselling the parents on the need for karyotyping. In view of the fact that DV-PIV can reduce the FPR of the combined test we also envisage a role for DV-PIV in a two-step approach in fetuses at intermediate or high risk after the combined test.

Technical and interpretative difficulties also call for restricting DV screening to a two-step approach, where the investigation is carried out in units with sufficient experience and awareness of the pitfalls. In a routine setting, the possible negative effects (anxiety) produced by the finding of an abnormal DV flow in an otherwise normal fetus probably will not outweigh the positive effect on detection rates.

Another positive effect of using DV assessment in a contingent screening policy is that the investigation will take place late in the first trimester, when the potential bio-effects of color and pulsed Doppler\textsuperscript{30} and the chance of finding an abnormal flow pattern are reduced\textsuperscript{6}.

In a population of fetuses at increased first-trimester risk for Down syndrome the combination of NT, DV-PIV and maternal age can improve the accuracy of screening for trisomy 21 and other numeric chromosomal anomalies. This is the first study that models the additional value of DV-PIV as a continuous variable to NT measurement alone in a high-risk first-trimester population.
REFERENCES


