Ultrasonography of the fetal nose, maxilla, mandible and forehead as markers for aneuploidy

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Trends in serial measurements of ultrasound markers in second and third trimester Down syndrome fetuses

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ABSTRACT

Objectives
To evaluate trends of nasal bone length (NBL), prenasal thickness (PT), nuchal fold (NF), prenasal thickness to nasal bone length (PT-NBL) ratio and prefrontal space ratio (PFSR), measured serially in second and third trimesters Down syndrome (DS) fetuses.

Methods
Prenatal databases were searched for cases of continuing DS pregnancies with serial measurements, taken at least two weeks apart. Trends were plotted on previously reported normal ranges.

Results
Serial measurements were available in 25 Down syndrome fetuses. Median gestational age (GA) was 25 weeks; average number of visits per case was 2.44, with a median interval of 39 days between investigations. In DS fetuses, NBL and PT showed fairly stable trends with gestation. PFSR, but especially NF, had a more unpredictable trend. The PT-NBL ratio was the most stable marker, remaining the same value in 95% of cases. NBL, PT and NF showed more deviance from the normal range with advancing gestation, but Multiple of the Median values remained stable. All but two fetuses had common markers or structural anomalies, especially heart defects.

Conclusions
The PT-NBL ratio is the most constant DS marker throughout gestation, following a predictable trend.
INTRODUCTION

Beyond the first trimester of pregnancy, prenatal ultrasound assessment of Down Syndrome (DS), has focused, among other things, on markers located in the fetal profile and neck. Short nasal bone length (NBL), increased prenasal thickness (PT) and increased nuchal fold (NF) thickness are the most frequently investigated\textsuperscript{1-12}. Recent studies have indicated that the use of ratios, such as the prenasal thickness to nasal bone length (PT-NBL) ratio and the prefrontal space ratio (PFSR), are strong and easy to use second trimester markers\textsuperscript{13-15}.

In the Netherlands, uptake of first trimester screening for DS is low\textsuperscript{16}. The combined test is free only for women of 36 years and older, whereas second trimester ultrasound screening for structural anomalies at around 20 weeks' gestation is fully covered by medical insurance\textsuperscript{17} and chosen by over 95% of women\textsuperscript{18}. This means that a considerable number of DS pregnancies remains undetected. In case of occasional detection of DS markers at the 20 weeks scan, not all women choose karyotyping and, even if they do, not all decide to terminate the pregnancy. As a result, a number of DS fetuses can be followed-up during pregnancy and trends in DS markers can be observed. In a recent study, we have demonstrated that the NBL, PT, PT-NBL ratio and PFSR are valuable and reproducible DS markers\textsuperscript{13,14,19} and assessed the detection rates, which appear to be evenly distributed throughout the second and third trimester. However, these studies were based on cross-sectional measurements in both normal and DS fetuses.

Aim of this study was to assess individual trends in a number of DS markers measured serially in the same affected fetus.

METHODS

The Fetal Medicine Units of the University Medical Center Groningen and of the Saint Antonius Hospital in Nieuwegein act as referral centers. Databases were searched (from January 2006 to October 2013) for second and third trimester ultrasound investigations in DS cases from Caucasian parents, confirmed pre- or postnatally by karyotyping. At our institutions, all ongoing pregnancies with (suspected) chromosomal abnormalities receive follow up at regular intervals. All patients consented to the serial measurement of facial markers. Cases with at least two measurements taken with a minimum interval of two weeks, were included in the study. When possible, the measurements were performed on 3D volumes after multiplanar mode correction to the exact median view in order to improve measurement accuracy\textsuperscript{20}. NF was measured on stored 2D images or, in case no images were available, the measurement was retrieved from ultrasound reports. The NBL, PT, PT-NBL ratio and PFSR were measured as described previously\textsuperscript{13}. The NF was measured on a fronto-occipital transverse view - including the cavum septum pellucidum, cerebellum and the posterior fossa - as the distance between the median point of the outer curve of the occipital bone and the outer skin edge\textsuperscript{20}.

Data were compared to the reference values derived from our previous reports on euploid fetuses\textsuperscript{14,19} or compared to reference values derived from the literature\textsuperscript{8,9,21,22,23}; the NBL and PT
increased with gestation from 3.3 mm at 15 weeks’ gestation to 9.6 mm at 33 weeks (NBL = -6.927 + (0.83*GA)-(0.01*GA²)) and from 2.3 mm at 15 weeks to 6.1 mm at 33 weeks (PT = 0.212 × GA – 0.873), respectively. The PT-NBL ratio and PFSR were stable throughout gestation, with a mean of 0.61 (95th percentile = 0.80) and 0.97 (5th percentile = 0.55), respectively.

Measurements below the 5th percentile (for NBL and PFSR) or above the 95th percentile (for NF, PT and PT-NBL ratio) of the reference ranges, were considered abnormal. Measurements below the 5th percentile (for the NBL and PFSR) or above the 95th percentile (for PT, NF, and the PT-NBL ratio) of the reference ranges were considered abnormal. Multiple of the Median (MoM) values were created for the NBL, PT and NF in order to correct for gestational age (GA). In a previous study of both euploid and DS fetuses13,19 we have investigated intra- and interobserver variability. Additional ultrasound findings at the examination in the participating referral centers were documented when available and classified as structural and non-structural anomalies (not including the profile markers NBL, PT, NF, PT-NBL ratio and PFSR).

Images were obtained by a General Electric Voluson 730 Expert ultrasound or E8 system equipped with a RAB4-8L probe (GE Medical Systems, Kretz Ultrasound, Zipf, Austria). Images and volumes were stored and examined either offline on 4D View software version 7.0 (GE Medical Systems, Kretz Ultrasound, Zipf, Austria) or on stored images in the General Electric ultrasound system. Markers were measured by two examiners (F.I.V. and E.J.P.), who were blinded to gestational age and to previous measurements, but not to karyotype.

Correlation coefficients were calculated by Pearson’s correlation test. A p-value of less than 0.05 was considered statistically significant. Averaged trendlines for serial measurements in individual fetuses were calculated by the mixed models analysis in SPSS. This analysis models the covariance structure of data and is the best model to create a trendline from repeated measurements. It expresses the relationship with time, corrects for random effects, deals with missing data and is especially suitable for small samples. The data were analyzed using the statistical software SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA) and Excel for windows 2000.

RESULTS

A total of 25 Down syndrome fetuses were included in the analysis. The median GA was 25‘0 weeks; 20‘4 weeks (range 14 – 26 weeks) at initial measurement and 29‘2 weeks (range 22 – 36 weeks) at final measurement. Median interval between measurements was 39 days (range 14 – 98 days) with an average number of 2.44 visits per case; in 14 fetuses measurements were performed twice, in 10 three times and in one fetus four times. Of all the measurements (except for the NF measurements), 54% was performed on 2D images and 46% on 3D volumes. The percentage of DS fetuses with an abnormal first measurement, last measurement or the same outcome (both normal or abnormal) at both measurements is displayed in table 1.

Overall NBL, PT and NF measurements increased significantly with GA (p < 0.01). However in 41.7% of cases, the NF did not increase in at least one consecutive measurement. NBL and PT did not increase in at least one consecutive measurement in 4.8% and 13.6%, respectively.

Longitudinal trends in individual markers measured in DS fetuses, are presented in figure 1, together with the mean measurement in normal fetuses8,14,19,21.
Trends in serial measurements of profile markers in DS fetuses | 95

Figure 1 | Serial measurements of ultrasound markers in individual DS cases, compared to the mean of euploid fetuses. DS, down syndrome; NBL, nasal bone length; PT, prenasal thickness; NF, nuchal fold; PFSR, prefrontal space ratio.
The overall trend in serial measurements in DS fetuses was calculated by mixed model analysis and compared to the corresponding normal range for each marker\textsuperscript{8,14,19,23} (figure 2).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.pdf}
\caption{Mixed model analysis showing the trend line of serial measurements in 25 DS foetuses (longitudinal), compared to the corresponding mean in euploid fetuses. DS, down syndrome; NBL, nasal bone length; PT, prenasal thickness; NF, nuchal fold; PFSR, prefrontal space ratio.}
\end{figure}
Trends in serial measurements of profile markers in DS fetuses

Table 1 | Percentage of DS fetuses with an abnormal first and last measurements or with the same outcome at first and last measurement (both normal and abnormal). NBL, nasal bone length; PT, prenasal thickness; NF, nuchal fold; PFSR, prefrontal space ratio.

<table>
<thead>
<tr>
<th></th>
<th>Abnormal at first measurement</th>
<th>Abnormal at last measurement</th>
<th>Same trend at first and last measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBL</td>
<td>66%</td>
<td>76%</td>
<td>81%</td>
</tr>
<tr>
<td>PT</td>
<td>82%</td>
<td>86%</td>
<td>86%</td>
</tr>
<tr>
<td>NF</td>
<td>83%</td>
<td>66%</td>
<td>50%</td>
</tr>
<tr>
<td>PT-NBL ratio</td>
<td>95%</td>
<td>95%</td>
<td>95%</td>
</tr>
<tr>
<td>PFSR</td>
<td>94%</td>
<td>75%</td>
<td>69%</td>
</tr>
</tbody>
</table>

Median PT-NBL ratio and PFSR were 1.30 and 0.32, respectively. MoM values for the NF, NBL, and PT, were 1.60, 0.71 and 1.50, respectively. There were no significant correlations between GA and NF MoM, NBL MoM, PT MoM, PT-NBL and PFSR. NBL was the only marker which became increasingly more abnormal with GA (p = 0.035).

An overview of the soft markers (besides profile markers) and structural anomalies observed in 22/25 fetuses is presented in table 2. No structural anomalies were observed in 3 (14%) fetuses and no soft markers were observed in 3 (14%) of fetuses. Two (9%) fetuses did not have any soft marker or structural anomaly. When the NBL, PT, NF, PT-NBL ratio and PFSR were added as markers, all fetuses were identified. All 3 fetuses which underwent first trimester combined testing had an increased risk for DS. In one case, non-invasive prenatal testing (NIPT) was performed.

Table 2 | Additional ultrasound findings in 22 DS fetuses at the ultrasound exam. In 3 cases it was not possible to retrieve information from the database. *Soft markers and abnormal findings: ventriculomegaly, aberrant right subclavian artery, mild hydronephrosis, echogenic intracardiac focus, brachycephaly, echogenic bowel, mild pyelectasis, sandal gap, short humerus and femur. ** Profile markers: NBL, PT, NF, PT-NBL ratio and PFSR.

<table>
<thead>
<tr>
<th>DS fetuses (n = 22)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft markers and abnormal findings* (besides profile markers**)</td>
<td>86%</td>
</tr>
<tr>
<td>Structural anomalies</td>
<td>86%</td>
</tr>
<tr>
<td>Congenital heart defects</td>
<td>55%</td>
</tr>
<tr>
<td>Both soft markers and structural anomalies present</td>
<td>91%</td>
</tr>
<tr>
<td>One or more profile markers</td>
<td>100%</td>
</tr>
<tr>
<td>Median number of soft markers observed per fetus</td>
<td>1 (average 1.8, range 0 – 5)</td>
</tr>
<tr>
<td>Median number of structural anomalies observed per fetus</td>
<td>1 (average 1.4, range 0 – 3)</td>
</tr>
<tr>
<td>Previous first trimester combined screening</td>
<td>14%</td>
</tr>
</tbody>
</table>
DISCUSSION

In this study we report longitudinal trends in 5 ultrasound markers measured serially in 25 DS fetuses. NBL, PT, and PT-NBL ratio seem to follow a constant trend with proportional increase of the first two and stability of the third with gestation, whereas NF and PFSR show a great variability and no clear trend. The constant trends observed in NBL, PT and PT-NBL ratio confirm the robustness of these ultrasound markers as also inferred by their high reproducibility in affected fetuses. Conversely, no longitudinal trends were observed in NF measurements, where, in spite of a large number of abnormal first measurements (83%), only 50% of fetuses followed the same trend at subsequent measurements and in 42% no increase in measurements was observed with gestation.

Unfortunately, large studies investigating the reproducibility of NF measurements in both normal and DS fetuses are lacking. We speculate that the reason for the great variation in NF and the apparent lack of trend in the measurement with gestation is probably the consequence of the difficulty in standardizing the scanning plane where the measurement is taken. A slight change in the angulation of the probe used to obtain the view where the NF is measured, can in fact produce a great variation in the measurement. Furthermore, the position of the fetus in utero can also influence the NF measurement. Especially at later gestational ages, when the fetal head is more often flexed, it can be particularly challenging to visualize the NF and impossible to measure it with the neck in a neutral position.

A limitation of this study and possible cause for the variation in NF, is the fact that some of the NF values were not measured on stored pictures, but derived from the data stored in the database, whereas all other facial markers were (re) measured off-line by the examiners in the same stored picture of a fetal profile. Albeit this limitation, we decided to include the NF in the study, as this is a widely used DS marker.

Three underlying pathological mechanisms have been advocated for the presence of nuchal skin edema in DS fetuses: changes in the extracellular matrix, abnormalities of lymphatic vessels and cardiac dysfunction. In this cohort, a cardiac anomaly was present in 55% of the fetuses with known additional ultrasound findings. This was mostly an atrio-ventricular septal defect that is normally not associated with cardiac failure or other kinds of edema or fluid retention. In DS fetuses, an altered venous-lymphatic differentiation of the endothelial cells of the jugular lymphatic sacs has been proposed to occur in the late first or beginning second trimester, causing nuchal edema. Fewer studies on pathological examinations of NF in the late second trimester are available. Its pathophysiological background should probably be sought in the altered hydrophilic property of the skin, in combination with the evolution in the second trimester of an enlarged nuchal translucency (NT) in the first trimester. However, the exact relationship between the neck edema present in the first trimester as enlarged NT and in the second trimester as thickened NF, remains controversial. Unfortunately, the majority (85%) of the fetuses in this study had no combined first trimester screening (including NT measurement).

Also the PFSR showed a considerable variation in longitudinal trends, with an abnormal first measurement in 94%, abnormal last measurement in 75%, but constant in only 69% of the cases. Also for the PSFR, this “instability” may be attributable to the vulnerability of a ratio combining...
measurements influenced by the assessment of an angle dependent on good visualization of bony landmarks\textsuperscript{11}. Thus, slight variations in one of the components may be heavily reflected in the accuracy of the “combined” marker. However, despite the considerable variation in consecutive measurements, the marker was below the 5\textsuperscript{th} percentile in the majority of cases.

Of all investigated markers the PT-NBL ratio confirms its superiority. Ninety-five percent of DS fetuses had an abnormal ratio at the first and last measurement and remained constant throughout gestation.

The mixed model analysis expresses how one would expect a marker to evolve within time after being measured at a certain point during gestation. The trendlines in figure 2 show that the PT-NBL ratio and PFSR diverge from their corresponding normal ranges, but follow exactly the same trend. In the other markers (NBL, PT, NF) more divergence from the normal range with advancing gestation is observed, suggesting that the degree of abnormality of the marker increases with advancing age. However, their relative deviation from the normal range remains unchanged, as confirmed by the fact that MoM values for these markers remained stable throughout gestation. These findings are confirmed by Maymon et al\textsuperscript{6} and Cusick et al\textsuperscript{10}, who found constant MoM values for NBL and PT-NBL ratio during gestation, whereas Persico et al\textsuperscript{7} and Miguelez et al\textsuperscript{34} reported an increase in delta PT and MoM PT during gestation.

In terms of discriminative power for DS, the only marker in this study showing a potentially statistically significant increase in detection rate with advancing gestation is the NBL. This is at variance with the findings of our previous study on a cross-sectional cohort of 159 DS cases, where the detection rate of all markers, including NBL, did not change with gestation\textsuperscript{13}. Another limitation of this study is that part of the measurements was performed on 2D images and part on 3D volumes. However, in another study we found that measurements of the NBL, PT, PT-NBL ratio and PFSR were not significantly influenced by the acquisition method\textsuperscript{35}.

Furthermore, GA at time of measurement and number of measurements vary per case, making comparison among cases more challenging.

In conclusion, this study offers insight in the natural history of 5 ultrasound markers in DS fetuses and confirms the strength of the PT-NBL ratio. The PT-NBL ratio follows a stable trend during gestation when measured in the same fetus, with little deviation between measurements in the second and third trimester of pregnancy.
REFERENCES


