Prenatal characteristics of false negative cases from first-trimester screening of Down syndrome (trisomy 21)

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INTRODUCTION

Since 2004, all pregnant women in Denmark have been offered a combined risk assessment in the first trimester for Down Syndrome (DS), on the basis of maternal age, nuchal translucency (NT) measurement and biochemical tests for plasma free β-human chorionic gonadotropin (hCGβ), and pregnancy-associated plasma protein-A (PAPP-A). This risk assessment is free of charge and performed by sonographers and doctors certified by the Fetal Medicine Foundation in London, UK, at fetal medicine units at local departments of obstetrics and gynecology. Blood samples are taken by medical laboratory technicians at the hospital or by general practitioners, and hCGβ and PAPP-A measured by the departments of clinical biochemistry.

If the risk for DS is higher than 1:300 at the time of testing, it is considered as increased, and the women are offered a chorionic villus sample or an amniocentesis to determine the fetal karyotype. Besides patient concerns and ethical considerations, chorionic villus sample and amniocentesis have a 0.5–1% risk of a procedure-related pregnancy loss.1

In all departments of obstetrics and gynecology in Denmark, the screening results are registered in the software program Astraia, and the final risk is automatically calculated. Results of all prenatal and postnatal chromosome analyses are forwarded to the Danish Cytogenetic Central Registry in Aarhus. Since 2008, most results from the prenatal screening program have been automatically uploaded from the local Astraia servers to the Danish Fetal Medicine Database (DFMD).

According to a previous study, the detection rate of the combined test in Denmark is 90% for a 3.5% false positive rate.2 In 2006, 84.4% of all pregnant women in Denmark underwent first-trimester risk assessment,2 and today, the figure is more than 90%. Since the introduction of the new screening program in Denmark, the number of postnatally diagnosed children with DS is more than halved.2 The number of postnatally diagnosed DS children in Denmark was 63 in 2004 (of 64,690 births corresponding to 0.98%), 29 in 2008 (0.45%) and 26 in 2009 (0.41%).

The aim of our study was to investigate prenatal characteristics of false negative DS cases and identify possible ways to improve the detection rate of first-trimester screening for DS.

According to the Danish Cytogenetic Central Registry, the total number of postnatally diagnosed children with DS born in Denmark between 1.1.2005 and 31.12.2009 was 148. For each of the 148 cases, we collected all relevant data on the prenatal screening from case records, Astraia and the DFMD. Parts of the data on 22 cases from the period of 2005 to 2007 were available from a previous study concerning the detection rate of the combined screening in Denmark.2

Flowchart on the study material and criteria of inclusion are shown in Figure 1. In addition to the 51 postnatally diagnosed cases, we included four cases of prenatally diagnosed false negative cases. These cases had a final risk lower than 1:300 but still had an invasive test, either because they insisted or because of a previous DS pregnancy.

The total number of prenatally detected (true positive) DS cases between 2005 and 2009 was 643. Of these, 420 were diagnosed with DS before week 14. Screening parameters in the false negative cases were compared with true positive cases, matched on department of screening, screening year and maternal age. From each department, two true positive cases were matched for each false negative case. In total, the
group of matched controls consisted of 110 true positive cases from the period of 2005 to 2009. The match criteria were as follows: (1) NT scan and risk calculation performed at the same screening department, (2) screened within the same ±2 years and (3) same maternal age or as close to the age of the false negative mother as possible. The median maternal age in false negative cases was compared with the median maternal age for the total number of true positive cases screened before week 14 in Denmark in 2005–2009 (n=420).

Results on screening parameters are shown in Table 1. Of the 55 false negative cases, 25 were girls and 30 were boys, which is not a significant difference (p=0.5). Twenty-four cases (44%) had a risk between 1:300 and 1:1000. Of these, ten (18%) had a risk assessment between 1:300 and 1:500. The lowest risk among the false negative cases was 1:67760.

We also calculated the percentages of false negative cases among all DS cases in different age groups. The percentages of false negative cases declined from 29% in the group of women between 20 and 25 years, 28% in the group between 26 and 30 years, 12% in the group between 31 and 35 years to 5% in the group of women aged 36 years or older.

To the best of our knowledge, this is one of the first studies focusing on false negative DS cases in the first-trimester combined screening program. We are aware of only one previous

**Table 1** Medians for maternal and fetal parameters in false negative and true positive screened DS cases

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FN median (n=55)</th>
<th>Range</th>
<th>TP median (n=110)</th>
<th>Range</th>
<th>p</th>
<th>FN+TP Median (n=165)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT (mm)</td>
<td>1.9</td>
<td>1.0–3.0</td>
<td>3.0</td>
<td>0.9–11.0</td>
<td>&lt;0.0001</td>
<td>2.3</td>
</tr>
<tr>
<td>CRL (mm)</td>
<td>66.6</td>
<td>45.0–84.0</td>
<td>62.0</td>
<td>45.0–82.0</td>
<td>0.048</td>
<td>63.0</td>
</tr>
<tr>
<td>hCGβ (MoM)</td>
<td>1.40</td>
<td>0.37–6.40</td>
<td>1.73</td>
<td>0.36–6.0</td>
<td>0.13</td>
<td>1.63</td>
</tr>
<tr>
<td>PAPP-A (MoM)</td>
<td>0.63</td>
<td>0.29–2.54</td>
<td>0.33</td>
<td>0.12–2.20</td>
<td>&lt;0.0001</td>
<td>0.45</td>
</tr>
<tr>
<td>GA (days)</td>
<td>75.5</td>
<td>56.0–96.0</td>
<td>74.0</td>
<td>56.0–95.0</td>
<td>0.31</td>
<td>74.0</td>
</tr>
<tr>
<td>Maternal weight (kg)</td>
<td>67.0</td>
<td>49.0–116.0</td>
<td>66.3</td>
<td>49.0–122.0</td>
<td>0.51</td>
<td>67.0</td>
</tr>
<tr>
<td>Maternal BMI (kg/m²)</td>
<td>23.5</td>
<td>18.6–41.6</td>
<td>23.0</td>
<td>18.0–40.6</td>
<td>0.21</td>
<td>23.2</td>
</tr>
</tbody>
</table>

DS, Down Syndrome; FN, false negatives; TP, true positives; GA, gestational age at the time of biochemical testing; MoM, multiples of median; BMI, body mass index.
paper concerning false negative cases\textsuperscript{3} but with a smaller group of false negative cases than this study. Furthermore, our study represents a nationwide non-selected population.

We found in false negative DS cases that especially the maternal age is lower, NT smaller and PAPP-A level higher compared with true positive cases. In addition, we found a remarkable high percentage (29\%) of false negative screened cases among all DS cases in the group of women between 20 and 25 years.

Because maternal age constitutes the basis of the final risk calculation, it is essential that the age-related risk is consistent with the actual risk of carrying an affected fetus. The current algorithm used for risk calculation in the combined screening includes an adjusted age-related risk based on the natural risk of giving birth to a child with DS. The adjustment is based on an estimation that 30\% of affected cases are miscarried after week 12.\textsuperscript{4} As a result of the advancement in screening for DS and as a consequence of well-established screening programs in many countries, it might now be possible to calculate the risk of carrying an affected fetus in the first trimester on the basis of prevalence data from first trimester. With the optimisation of the age-related risk algorithm used for risk calculation, it might be possible to increase the detection rate among younger women.

It is remarkable that we found a significant difference in CRL at the time of NT examination in the group of false negative and true positive cases. Because the median CRL was significantly larger in the group of false negative cases compared with true positive cases, it is apparent to consider if the false negative cases were screened later than the true positive cases, which could also explain the normal nuchal translucencies among false negatives. Yet, this hypothesis is unlikely because the “normalization” of a previously enlarged CRL would not happen in 2 days, which is the difference in time of screening between the groups. Another way to explain the larger CRL among false negative cases could be that DS cases with many co-morbidities are already growth-restricted in first trimester. If severe DS-affected fetuses are smaller than unaffected fetuses, the gestational age is underestimated leading to wrong multiples of medians for the biochemical markers. This might explain why cases with nearly normal screening parameters and no growth restriction end up with a false negative screening result. But no previous investigations have shown a first-trimester growth restriction among DS-affected fetuses.\textsuperscript{5}

In the first trimester of pregnancy, the level of PAPP-A is doubled every third to fourth day. We therefore investigated the gestational age at the time of biochemical testing but found no difference in gestational age between the groups. Furthermore, we did not find a significant difference in the level of hCG\textsubscript{b} between false negative and true positive cases.

The levels of PAPP-A and hCG\textsubscript{b} have been weight-adjusted when calculating the final risk in Astraia. In this study, we investigated if obese women are more likely to have a false negative result in the first-trimester combined screening. We did not find a significant difference in maternal weight or body mass index between false negative and true positive cases. Our results indicate that the maternal weight correction used in Astraia is sufficient.

In conclusion, the age-related risk algorithm used for risk calculation could possibly be optimized. Additionally, investigations on growth restrictions in the first trimester among affected fetuses as well as the potential of an earlier NT scan would be interesting. Because 44\% of all false negative cases have a risk between 1:300 and 1:1000, contingent screening could be another way to increase the detection rate. However, we still need an additional fetal or placental marker to significantly improve the detection rate of the first-trimester combined screening. Significant improvement may come from the use of free fetal DNA in the maternal circulation for aneuploidy screening.

**ACKNOWLEDGEMENT**

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**WHAT’S ALREADY KNOWN ABOUT THIS TOPIC?**

- In DS cases, maternal age, NT and hCG\textsubscript{b} (multiples of median) are high, whereas PAPP-A (multiples of median) is low.

**WHAT DOES THIS STUDY ADD?**

- This study describes in details the distribution of maternal age, NT, crown rump length and biochemical markers of false negative cases from the first trimester combined screening for DS.

**REFERENCES**