First trimester risk assessment for trisomy 21 in twin pregnancies combining nuchal translucency and first trimester biochemical markers

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ABSTRACT

Objective The aim is to describe the performance of first-trimester combined risk assessment in twin pregnancies.

Methods Maternal serum free beta-human chorionic gonadotrophin and pregnancy-associated plasma protein A (PAPP-A) were determined at 8 to 12 weeks and fetal nuchal translucency (NT) was measured at 11 to 13+6 weeks. The individual risk was estimated for each fetus using the combined test in dichorionic twins. In monochorionic twins, the mean risk assessment of the two fetuses was used. An invasive diagnostic procedure was offered when the risk was $\geq 1 : 270$ in either one of the fetuses.

Results From February 2007 to June 2011, 447 twin pregnancies were enrolled in this study. There were 402 (89.9%) dichorionic and 45 (10.1%) monochorionic twins. In dichorionic twins, mean crown–rump length (CRL) was 63.9 mm; median NT multiples of the median (MoM) was 0.97; median B-hCG was MoM 1.74; median PAPP-A was 1.72. In monochorionic twins, mean CRL was 61.9 mm; median NT MoM was 0.98; median B-hCG MoM was 1.44; and median PAPP-A was 1.51. Two pregnancies with Down syndrome were detected by first trimester screening, both in dichorionic twins. The false positive rate was 5.7% (95% confidence interval 4.1–7.3) and 4.4% (95% confidence interval 0.1–8.8%) in dichorionic and monochorionic twins, respectively.

Conclusions The combined test in twins appears to be a good method for Down syndrome screening with a high detection rate and an acceptable false-positive rate. © 2012 John Wiley & Sons, Ltd.

INTRODUCTION

The incidence of twin pregnancies is increasing as a result of the widespread use of assisted reproduction technologies and the increasing maternal age. Because the rate of autosomal trisomy increases with maternal age, multiple pregnancies are at a higher risk than the general population.\textsuperscript{1} Although amniocentesis or chorionic villus sampling (CVS) are recommended in cases at high risk for fetal aneuploidy, in multiple pregnancies invasive procedures are more challenging and women who conceive after assisted reproduction technologies (ART) are usually more reluctant to accept invasive testing. For all these reasons, the aneuploidy-screening test should be as accurate as possible in twin pregnancies.

In the past, the counselling of patients regarding the risk of fetal aneuploidy was based on maternal age alone. In singleton pregnancies, this counselling has been refined because of the addition of first and second trimester biochemistry and aneuploidy ultrasound markers. In patients with multiples, counselling regarding the risk of fetal chromosomal abnormalities is far more complex because of several issues. Defining ‘advanced maternal age’ in these women is complicated not only because there is more than one fetus, but also because zygosity has an impact on risk.\textsuperscript{2,3} A noninvasive method of determining zygosity in dichorionic twin pregnancies does not currently exist, making accurate counselling of these patients impossible. The addition of maternal serum markers to risk assessment is difficult because it is not possible to determine the contribution of each individual fetus to the analyses values.\textsuperscript{4} Risk assessment in twin pregnancies with nuchal translucency (NT) does provide fetus-specific risk. The detection rate with the use of maternal age and NT has been described as similar to that in singleton pregnancies, although the false-positive rate is higher.\textsuperscript{5}

Not many reports have been published about the benefit of the combination of maternal age, maternal serum biochemistry and nuchal translucency for the screening for Down syndrome
in twin pregnancies.6–10 Our aim is to describe the performance of first-trimester combined risk assessment in twin pregnancies and compare these results to the performance of the test in singletons in our population.

MATERIALS AND METHODS
From February 2007 to June 2011, 894 twin fetuses and 9868 singleton pregnancies attending our unit for first trimester screening were enrolled in this study. Exclusion criteria were pregnancies with missing data or an uncompleted follow up.

Both in singletons and in twins, biochemical risk assessment for Down syndrome (maternal serum free beta-human chorionic gonadotrophin (free-βHCG) and pregnancy-associated plasma protein A (PAPP-A)) was performed between 8 and 13+6 weeks’ gestation and NT scan was performed between 11 and 13+6 weeks (crown–rump length (CRL) 45–84 mm), following the Fetal Medicine Foundation Guidelines.11 The ultrasound examination was transvaginal, transabdominal or combined, when necessary. The risk assessment was calculated using the Combined Test in two steps (maternal age, maternal serum levels of free-βHCG and PAPP-A at 8 to 13+6 weeks’ gestation and NT measurement at 11 to 13+6 weeks’ gestation). When pregnancy was achieved with egg donors, the age of the egg donor was used.

Chorionicity was determined and was considered monochorionic in the presence of a single placenta and the absence of the lambda sign and was considered dichorionic when the placentas were not adjacent or the lambda sign was present.12 In twin pregnancies, the larger of the two CRL measurements was used to estimate the overall gestational age of the pregnancy. Biochemistry values were determined using the Kryptor analyzer (BRAHMS®). These values were expressed as multiples of the median (MoM) adjusted for number of fetuses, maternal weight, history of chromosome anomalies, smoking habit and ethnicity. In case of multiple pregnancies the conversion to MoM was carried out after the gestation. When pregnancy was achieved with egg donors, the age of the egg donor was used.

Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Dichorionic twins</th>
<th>Monochorionic twins</th>
<th>Singletons</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean maternal age (years ± SD)</td>
<td>35.4 ± 4.5</td>
<td>34.2 ± 3.2</td>
<td>33.5 ± 4</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean gestational age maternal serum sampling (days ± SD)</td>
<td>67.2 ± 8.8</td>
<td>70.7 ± 7.9</td>
<td>66.9 ± 9</td>
<td>0.300</td>
</tr>
<tr>
<td>ART conception (%)</td>
<td>30.3</td>
<td>2.2</td>
<td>4.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean maternal weight (Kg ± SD)</td>
<td>62.7 ± 10.9</td>
<td>61 ± 9.5</td>
<td>62.1 ± 10.3</td>
<td>0.344</td>
</tr>
<tr>
<td>Cigarette smoking (%)</td>
<td>10.9</td>
<td>13.3</td>
<td>10.7</td>
<td>0.289</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>99.2</td>
<td>100</td>
<td>98.9</td>
<td>0.978</td>
</tr>
<tr>
<td>Non-Caucasian</td>
<td>0.8</td>
<td>0</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Mean CRL (mm ± SD)</td>
<td>63.9 ± 8.7</td>
<td>61.9 ± 7.9</td>
<td>63.4 ± 8.41</td>
<td>0.063</td>
</tr>
<tr>
<td>Median NT MoM (mm)</td>
<td>0.97</td>
<td>0.98</td>
<td>0.97</td>
<td>0.460</td>
</tr>
</tbody>
</table>

RESULTS
During the study period (February 2007–June 2011), 447 twin pregnancies and 9816 singletons fulfilled the inclusion criteria. Fifty-four cases were excluded from the study.

Among the multiple pregnancies, 402 (89.9%) were dichorionic and 45 (10.1%) were monochorionic. The mean maternal age was 35 years (range 20–50) for dichorionic twins and 33 for monochorionic twins (range 27–40), with 44% and 20% over 36 years in dichorionic and monochorionic twin gestations, respectively. The characteristics of the study population can be seen in Table 1.

Among twin gestations undergoing the combined test, 5.8% (52/894) had results indicating high risk for Down syndrome (cut off: 1/270): 48 cases were in dichorionic and 4 in monochorionic twin pregnancies. Of them, 39 fetuses (75%) were in women older than 36 years. After counselling, 12 women declined invasive diagnostic test. Amniocentesis
was performed in 38 women undergoing invasive diagnostic testing and CVS in 2. There was one fetal loss because of a chorioamnionitis post amniocentesis. In the remaining cases, the pregnancy continued for at least 28 days after the procedure.

Down syndrome was identified in two fetuses of two different pregnancies; both of them were dichorionic twins and in patients older than 36 years. In case 1, maternal age was 38 years old, the affected fetus had NT of 2.6 mm, CRL of 51 mm, free β-hCG was 126, 1.4 MoM and PAPP-A was 0.93, with a calculated Down syndrome risk of 1/9. In case 2, maternal age was 37 years old, the fetus had NT of 3.2 mm, CRL of 72 mm, free-β-hCG of 1.52 MoM, PAPP-A of 0.79 MoM, with a calculated Down syndrome risk of 1/34. In case 1, the patient declined the offer of an invasive diagnostic test and Down syndrome was diagnosed at birth. In case 2, the mother elected to undergo embryo reduction of the affected fetus. The surviving twin progressed to a normal healthy delivery.

Table 2 First trimester B-hCG, PAPP-A and NT in unaffected fetuses

<table>
<thead>
<tr>
<th></th>
<th>Dichorionic twins</th>
<th>Monochorionic twins</th>
<th>Singletons</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median B-hCG MoM</td>
<td>1.74</td>
<td>1.44</td>
<td>0.97</td>
<td>0.01</td>
</tr>
<tr>
<td>Median PAPP-A MoM</td>
<td>1.72</td>
<td>1.51</td>
<td>0.96</td>
<td>0.03</td>
</tr>
<tr>
<td>Median NT MoM</td>
<td>0.97</td>
<td>0.98</td>
<td>0.97</td>
<td>0.460</td>
</tr>
<tr>
<td>Mean log_{10} B-hCG</td>
<td>0.26</td>
<td>0.20</td>
<td>-0.01</td>
<td></td>
</tr>
<tr>
<td>Mean log_{10} PAPP-A</td>
<td>0.24</td>
<td>0.17</td>
<td>-0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Mean log_{10} NT</td>
<td>-0.02</td>
<td>-0.02</td>
<td>-0.01</td>
<td>0.460</td>
</tr>
<tr>
<td>log_{10} standard deviation B-hCG</td>
<td>0.23</td>
<td>0.29</td>
<td>0.25</td>
<td>0.389</td>
</tr>
<tr>
<td>log_{10} standard deviation PAPP-A</td>
<td>0.24</td>
<td>0.24</td>
<td>0.25</td>
<td>0.88</td>
</tr>
</tbody>
</table>

NT, nuchal translucency; MoM, multiples of the median; B-hCG, free beta-human chorionic gonadotrophin; PAPP-A, pregnancy-associated plasma protein A.

Table 3 Median markers values observed in spontaneous versus assisted conceived chromosomally normal twin pregnancies

<table>
<thead>
<tr>
<th></th>
<th>Spontaneous conception (n = 649)</th>
<th>Assisted conception (n = 245)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median B-hCG MoM</td>
<td>1.96</td>
<td>2.00</td>
<td>0.32</td>
</tr>
<tr>
<td>Median PAPP-A MoM</td>
<td>1.72</td>
<td>1.84</td>
<td>0.25</td>
</tr>
<tr>
<td>Median NT MoM</td>
<td>0.98</td>
<td>0.97</td>
<td>0.96</td>
</tr>
</tbody>
</table>

n, number of cases; MoM, multiples of the median; NT, nuchal translucency; B-hCG, free beta-human chorionic gonadotrophin; PAPP-A, pregnancy-associated plasma protein A.

Table 4 Combined Test results in dichorionic, monochorionic and singleton fetuses

<table>
<thead>
<tr>
<th></th>
<th>DS risk ≥ 270</th>
<th>DR</th>
<th>FPR</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichorionic</td>
<td>48/804</td>
<td>100%</td>
<td>5.7%</td>
<td>4.2%</td>
<td>99.9%</td>
</tr>
<tr>
<td>Monochorionic</td>
<td>4/90</td>
<td>—</td>
<td>4.4%</td>
<td>—</td>
<td>99.9%</td>
</tr>
<tr>
<td>Singletons</td>
<td>632/9868</td>
<td>94.1%</td>
<td>5.9%</td>
<td>8.1%</td>
<td>99.9%</td>
</tr>
</tbody>
</table>

DS, Down syndrome; DR, detection rate; FPR, false positive rate; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

In singleton pregnancies, Down syndrome was identified in 52 fetuses.

If we compare biochemical markers and NT between unaffected fetuses, in dichorionic, monochorionic and singletons, we observe significant differences in β-hCG and PAPP-A values, but not in NT measurement (Table 2). No significant differences were found in either serum analyses or NT measurements between the spontaneous and the assisted conceptions for the chromosomally normal twin pregnancies (Table 3).

In our global population, combined test for the detection of Down syndrome had a DR of 98.1%, FPR of 5.9%, PPV of 7.7% and NPV of 99.9%. In Table 4 we show the results of the combined test in dichorionic, monochorionic and singleton fetuses.

In twin pregnancies, the FPR of the combined test per fetus was 5.7% (95% CI 4.1–7.3) in dichorionic and 4.4% (95% CI 0.1–8.8%) in monochorionic twins. The FPR was higher in singletons: 5.9 (95% CI 5.5–6.4). If we distinguish, between patients older/younger than 36 years old, results are worse in the older patient group (Table 5).

Table 5 False positive rate per fetuses in monochorionic twins, dichorionic twins and singletons, depending on the age of the mother

<table>
<thead>
<tr>
<th>FPR per fetus (95% CI)</th>
<th>Dichorionic twins</th>
<th>Monochorionic twins</th>
<th>Singletons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global</td>
<td>5.7 (4.1–7.3)</td>
<td>4.4 (0.1–8.8%)</td>
<td>5.9 (5.5–6.4)</td>
</tr>
<tr>
<td>≤ 35 years old</td>
<td>2.2 (0.9–3.6)</td>
<td>4.2 (0.8–9)</td>
<td>3.5 (3–3.9)</td>
</tr>
<tr>
<td>&gt; 36 years old</td>
<td>10.2 (7–13.3)</td>
<td>5.6 (0–17)</td>
<td>11.6 (10.5–12.8)</td>
</tr>
</tbody>
</table>

FPR, false positive rate; CI, confidence interval.
Combined test appeared to be an effective screening strategy, both in dichorionic twins and singletons (Table 6). Because we had no cases of Down syndrome in monochorionic twins, we could not establish the ROC curve in this group.

**DISCUSSION**

In patients with monochorionic twins, the designation of 'advanced maternal age' is the same as for women with a singleton. However, defining 'advanced maternal age' in women with dizygotic twins, triplets and high-order multiples is far more complex. Advanced age is a very poor screening tool in twin pregnancies, because of the difficulty of determining the age-specific risk in twins and its high false positive rate. Since 2004, the International Down Syndrome Screening Group has not considered advanced maternal age an indication for invasive procedures. This is especially important in twin pregnancies, where the presence of advanced maternal age is frequent. In our study, 44.5% and 20% in dichorionic and monochorionic pregnancies, respectively, were older than 36 years old. Moreover, 30.3% of dichorionic twin pregnancies were conceived after ART, signalling increased maternal concern for risk of pregnancy loss because of genetic diagnostic procedures.

The addition of maternal serum markers (first or second trimester maternal serum markers) to risk assessment is difficult because they cannot identify the fetus at risk. For instance, the detection rate achieved by second-trimester biochemical marker is lower than in singletons because the unaffected co-twin can mask the abnormal serum values of the affected twin. First trimester ultrasound NT measurement associated with maternal age has the additional advantage of allowing calculation of the specific risk for each twin. In a previous series reported by our group, the sensitivity of NT screening in multiple pregnancies was 85.7% and 79.6% in singletons, with a false positive rate of 3.5% in twins and 4.8% in singletons.

In this present series of 447 twin sets, the use of the combined test for the screening of trisomy 21 enabled the detection of all cases of Down syndrome with a false positive rate of 5.7% in dichorionic fetuses and 4.4% in monochorionic fetuses (cut off ≥1/270). Wide CIs were observed around the FPR because of the small number of affected fetuses. The performance of this screening method appears to be as good as in singletons, or even better in the case of dichorionic twin pregnancies, as shown by the ROC curves. Our series is the first one describing a false positive rate lower in twins than in singleton pregnancies. There are a few series in the literature about the performance of the combined test in twin pregnancies (Table 7). Our results are comparable to those reported previously. Note the decrease in the FPR in the latest studies. The increase in specificity in the publications of Goncé et al. and our present series could be due to the performance of the combined test in two steps. We believe that the sooner maternal serum sampling is performed, the more accurate these markers seem to be for the screening of Down syndrome. The decrease in the FPR is important because it leads to a decreased need for invasive procedures. Invasive techniques are more difficult in twin pregnancies and are associated with a higher risk of fetal loss.

Regarding the biochemical markers, β-hCG and PAPP-A values in our series followed a slightly different distribution compared with those published by Spencer. We recommend that every centre establishes its own biochemical marker distributions to apply a correcting factor depending on the chorionicity for the screening of Down syndrome in twin pregnancies. We previously reported the distribution of the serum markers in monochorionic and dichorionic twin pregnancies in our population. In our series, PAPP-A and β-hCG MoM do not rise to twice the level of unaffected

### Table 6 ROC curve in dichorionic twins and singletons

<table>
<thead>
<tr>
<th>ROC Curve</th>
<th>AUC</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichorionic twins</td>
<td>0.998</td>
<td>0.995–1</td>
</tr>
<tr>
<td>Singletons</td>
<td>0.991</td>
<td>0.987–0.995</td>
</tr>
</tbody>
</table>

ROC, receiver operating characteristic curve; AUC, area under the curve; CI, confidence interval.

### Table 7 Performance of the combined test in twin pregnancies. Review of the literature

<table>
<thead>
<tr>
<th>N</th>
<th>&gt;35 years (%)</th>
<th>Mean GA at maternal serum sampling (days)</th>
<th>N Down syndrome</th>
<th>Median B-hCG MoM (unaffected twins)</th>
<th>Median PAPP-A MoM (unaffected twins)</th>
<th>Median NT MoM (unaffected twins)</th>
<th>FPR (%)(per fetus)</th>
<th>DR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goncé et al.</td>
<td>161</td>
<td>15 (&gt;37)</td>
<td>77</td>
<td>4</td>
<td>1.72</td>
<td>2.01</td>
<td>1.05</td>
<td>3.5</td>
</tr>
<tr>
<td>Chasen et al.</td>
<td>519</td>
<td>46.5</td>
<td>–</td>
<td>7</td>
<td>0.97</td>
<td>1.12</td>
<td>–</td>
<td>7</td>
</tr>
<tr>
<td>Goncé et al.</td>
<td>100</td>
<td>36 (&gt;34)</td>
<td>77</td>
<td>3</td>
<td>1.57</td>
<td>1.96</td>
<td>1.02</td>
<td>3.6</td>
</tr>
<tr>
<td>Spencer et al.</td>
<td>206</td>
<td>–</td>
<td>85</td>
<td>4</td>
<td>2.15</td>
<td>1.93</td>
<td>–</td>
<td>6.9</td>
</tr>
<tr>
<td>Orlandi et al.</td>
<td>30</td>
<td>–</td>
<td>84</td>
<td>7</td>
<td>1.72</td>
<td>1.61</td>
<td>0.9</td>
<td>10.6</td>
</tr>
<tr>
<td>Current study</td>
<td>447</td>
<td>30.6%</td>
<td>67.2 DC/70.7 MC</td>
<td>2</td>
<td>1.74 DC/1.44 MC</td>
<td>1.72 DC/1.51 MC</td>
<td>0.97 DC/0.98 MC</td>
<td>5.7 DC/4.4MC</td>
</tr>
</tbody>
</table>

DC, dichorionic; MC, monochorionic; FPR, false positive rate; DR, detection rate; GA, gestational age; MoM, multiples of the median; N, number of cases; NT, nuchal translucency; B-hCG, free beta-human chorionic gonadotrophin; PAPP-A, pregnancy-associated plasma protein A.

*Twins pregnancies conceived with assisted reproduction.*

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singletons. This deviation could be due to an earlier sampling for biochemical markers in our two-step screening approach.

In our twins’ series, we did not find an increase of NT measurement in the group of monochorionic twins, nor any differences in NT measurements in spontaneous gestations and ART conceptions, unlike previously reported. Moreover, in twins no significant differences were found in serum analyses between the spontaneous and the ART conceptions for the chromosomally normal pregnancies. On the contrary, as our group previously reported, we did find significant differences in maternal biochemical markers in singletons (Table 8). We believe these different results are due to the smaller number of cases in twin pregnancies.

To improve the performance of the screening, our group is studying the possibility of adding another marker as (ductus venosus pulsatility index) either in a combined or a contingent test.

Acknowledgements

Under the auspices of the Cátedra d’ Investigació en Obstetricia i Ginecologia de la Universitat Autònoma de Barcelona.

Table 8: Biochemical serum markers and nuchal translucency in spontaneous gestations and assisted conceptions in singletons

<table>
<thead>
<tr>
<th></th>
<th>Assisted conceptions (n=638)</th>
<th>Spontaneous gestations (n=9756)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean maternal age</td>
<td>35.02 ± 3.6</td>
<td>33.2 ± 3.7</td>
</tr>
<tr>
<td>(years ± SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;35 years</td>
<td>50.9%</td>
<td>31.3%</td>
</tr>
<tr>
<td>Blank chorionicity MoM</td>
<td>0.83±(0.03–5.13)</td>
<td>1.05(0.04–11.37)</td>
</tr>
<tr>
<td>PAPP-A MoM (median and range)</td>
<td>0.95±(0.17–5.26)</td>
<td>0.92(0.01–7.38)</td>
</tr>
<tr>
<td>NT MoM (median and range)</td>
<td>0.97(0.31–4.74)</td>
<td>0.97(0.25–6.02)</td>
</tr>
</tbody>
</table>

SD, standard derivation; NT, nuchal translucency; β-hCG, free beta-human chorionic gonadotrophin; PAPP-A, pregnancy-associated plasma protein A; MoM, multiples of the median; N, number of cases.

*p < 0.05.

References


