Single-Step Maternal Serum Screening for Trisomy 21 in the Era of Combined or Integrated Screening

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Key Words
Maternal serum screening · Advanced maternal age · Fetal aneuploidy · Trisomy 21 · Down’s syndrome

Abstract
Single-step maternal serum screening (MSS) in the first (1MSS) or second (2MSS) trimester at maternal age ≥35 years was evaluated in the North Belgian region Flanders, where difficulties are encountered in the general introduction of combined or integrated screening algorithms. The fetal aneuploidy screening database of General Medical Laboratory AML in Antwerp was searched for 2MSS tests between 1992 and 1999 (α-fetoprotein, β-human chorionic gonadotropin (β-HCG) and unconjugated estriol, cut-off 1:300) and for 1MSS tests between 1999 and 2003 (free β-HCG and pregnancy-associated plasma protein A, cut-off 1:85). At ≥35 years, the detection rate for trisomy 21 (DR) was 93.8% (15/16) for 2MSS and the screen-positive rate (SPR) was 24.5% (504/2061). For 1MSS, these figures were 85.7% (6/7) and 17.7% (109/615) respectively. To detect one trisomy 21, missed by MSS at maternal age ≥35 years in Flanders is excellent, even without the combination with ultrasound parameters or integration of first and second trimester parameters. The simplicity of both methods allows to consider them valuable options for fetal aneuploidy screening at advanced maternal age, until high quality combined or integrated screening is accessible to all pregnant women in Belgium.

Introduction
In maternal serum screening (MSS) for fetal aneuploidy, concentrations of pregnancy-associated placent protein A (PAPP-A) and free β-human chorionic gonadotropin (HCG) are measured in the first trimester MSS (1MSS); in the second trimester MSS (2MSS), levels of HCG, α-fetoprotein (α-FP) and unconjugated estriol (E3) are used [1, 2].

As the prevalence of fetal trisomies increases with maternal age, performance of different screening methods is reported to be better at advanced maternal age compared to younger women [3–5]. For medical and economical reasons, invasive procedures for positive screening results only (‘selective invasive procedures’) are recom-
mended instead of routine invasive testing at maternal age \( \geq 35 \) years [3, 6–9]. Several reports are published on the performance of 2MSS at advanced maternal age [3, 4, 10, 11]. Few population studies are reported on 1MSS without nuchal translucency (NT) in the advanced maternal age group [12].

The integration of 1MSS and 2MSS parameters into one single algorithm and/or their combination with ultrasound parameters are recommended to improve the results of screening [9, 13–15]. However, several practical limitations are encountered when these combined or integrated methods are introduced in general screening programs for fetal aneuploidy [16]. In some European countries, i.e. Belgium and the Netherlands, these limitations currently cause a poor access for pregnant women to high-quality combined or integrated screening. In these countries, primary invasive testing for advanced maternal age is still practised without patient’s counselling on alternatives.

We investigated single-step 1MSS and 2MSS at maternal age \( \geq 35 \) years in the North Belgian region Flanders, as a cheap and simple measure on the way to combined or integrated screening.

**Materials and Methods**

Since 1992, maternal serum samples for fetal aneuploidy screening have been analysed by the General Medical Laboratory (AML) in Antwerp, Belgium. These samples were recruited from all geographic regions in Flanders.

Samples for 2MSS were obtained between 1992 and 1998, which was before the introduction of first trimester screening in Flanders. Parameters for 1MSS were sampled between 1999 and 2003. Immunoradiometric assay was used to measure concentrations of \( \alpha \)-FP (Diagnostic Products Corp., Los Angeles, Calif., USA) and (free) \( \beta \)-HCG (BioSource Europe SA, Belgium) and levels of \( E_2 \) were measured by radioimmunoassay (Diagnostic Systems Laboratories, Webster, Tex., USA) [17]. PAPP-A was measured by enzyme-linked immunosorbent assay (ELISA-DRG BioSource Belgium) [18]. The cut-off level for a positive screening result was 1:300 for 2MSS and 1:85 for the first trimester algorithm. These cut-off values were chosen at the level of 5% screen-positive rate (SPR) in the first 5,000 pregnancies screened by each algorithm.

Data on pregnancy and neonatal outcome were obtained from the referring obstetricians after delivery. Once a year a list was mailed to every clinician for completion of missing data. Non-responding obstetricians were contacted personally to report missing cases of trisomy 21. The accuracy of reported cases of trisomy 21 was evaluated by comparison of this prevalence between the AML database and the Eurocat registry for Belgium in the time periods 1992–1998 and 1999–2001 [19]. For this study, we used SPR, which refers to all pregnancies submitted to screening, instead of false-positive rate, which refers to the normal pregnancies only. The results of MSS were analysed for the total population and for pregnant women <35 and \( \geq 35 \) years of age.

In this report, we do not discuss chromosomal anomalies, other than trisomy 21 (i.e. trisomy 18), as collection and handling of detailed information on the outcome of these pregnancies is still ongoing.

**Results**

A total of 40,419 2MSS tests was performed. In this population, 60 trisomy 21 affected pregnancies were present (prevalence 1:674). In a total of 7,079 1MSS tests, 13 trisomy 21 affected pregnancies were found (prevalence 1:545). In the two time periods studied, the prevalence of trisomy 21 was 0.145 and 0.170% respectively in the AML database, which correlated well with the prevalence of 0.133 and 0.165% respectively in the Eurocat database.

Table 1 shows the performance of 2MSS for all women and for women aged <35 and \( \geq 35 \) years. The calculated risk was more than 1:300 for 5.7% of all women and the overall detection rate of trisomy 21 was 73.3%. A total of 2,061 women was aged \( \geq 35 \) years (5.1%). In this group, 15 out of 16 trisomies 21 were detected by the screening algorithm (DR 93.8%) and the SPR was 24.5% (504/2,061). Compared to primary invasive procedures in women \( \geq 35 \) years of maternal age, selective invasive testing resulted in a reduction of 75.5% (1,557/2,061) of invasive procedures for a loss of DR of only 6.2% (1/16). In order to detect one trisomy 21, which was missed by selective invasive testing following 2MSS, an additional number of 1,557 primary invasive procedures would have been necessary.

Table 2 shows the performance of 1MSS for all women and for women aged <35 and \( \geq 35 \) years. The calculated risk was 1:85 or more for 5.1% of women and the DR of trisomy 21 was 61.5%. A total of 615 women was aged \( \geq 35 \) years (8.6%). In this group the screening algorithm detected 6 out of 7 trisomies 21 (DR 85.7%) in a total of 109 positive screening results (SPR 17.7%). Compared to primary invasive procedures in women \( \geq 35 \) years of maternal age, selective invasive testing resulted in a reduction of 82.3% (506/615) of invasive procedures for a loss of DR of only 14.3% (1/7). In order to detect one trisomy 21, which was missed by selective invasive testing following 1MSS, an additional number of 506 primary invasive procedures would have been necessary.

In women aged \( \geq 35 \) years, 1MSS detected all 7 trisomy 21 affected pregnancies at a SPR of 28.6% (176/615), when a cut-off level of 1:200 was used. By changing the cut-off level from 1:85 to 1:200 at maternal age \( \geq 35 \) years, a positive screening result was found in an additional number of 67 pregnancies, which caused an 0.9% (67/7079) increase of overall SPR.
Table 1. Performance of 2MSS by α-FP, β-HCG and estriol at maternal age <35 and ≥35 years, as registered by AML in Antwerp, Belgium, between 1992 and 1998 (cut-off 1:300)

<table>
<thead>
<tr>
<th>Trisomy 21 affected pregnancies</th>
<th>2MSS</th>
<th>DR, %</th>
<th>SPR, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>60</td>
<td>44</td>
<td>16</td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>44</td>
<td>29</td>
<td>15</td>
</tr>
<tr>
<td>≥35 years</td>
<td>16</td>
<td>15</td>
<td>1</td>
</tr>
</tbody>
</table>

All pregnancies

<table>
<thead>
<tr>
<th>Overall</th>
<th>n+</th>
<th>–</th>
<th>DR, %</th>
<th>SPR, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>40,419</td>
<td>2,304</td>
<td>38,115</td>
<td>5.7</td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>38,358</td>
<td>1,800</td>
<td>36,558</td>
<td>4.9</td>
</tr>
<tr>
<td>≥35 years</td>
<td>2,061</td>
<td>504</td>
<td>1,557</td>
<td>24.5</td>
</tr>
</tbody>
</table>

+ = Positive screening result; – = negative screening result; DR = detection rate for trisomy 21; SPR = screen-positive rate.

Table 2. Performance of 1MSS by free β-HCG and PAPP-A at maternal age <35 and ≥35 years, as registered by AML in Antwerp, Belgium, between 1999 and 2002 (cut-off 1:85)

<table>
<thead>
<tr>
<th>Trisomy 21 affected pregnancies</th>
<th>1MSS</th>
<th>DR, %</th>
<th>SPR, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>13</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>≥35 years</td>
<td>7</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>

All pregnancies

<table>
<thead>
<tr>
<th>Overall</th>
<th>n+</th>
<th>–</th>
<th>DR, %</th>
<th>SPR, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>7,079</td>
<td>360</td>
<td>6,719</td>
<td>5.1</td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>6,464</td>
<td>251</td>
<td>6,213</td>
<td>3.9</td>
</tr>
<tr>
<td>≥35 years</td>
<td>615</td>
<td>109</td>
<td>506</td>
<td>17.7</td>
</tr>
</tbody>
</table>

+ = Positive screening result; – = negative screening result; DR = detection rate for trisomy 21; SPR = screen-positive rate.

Discussion

This study provides information on single-step MSS in Flanders, Belgium, both in the first and in the second trimester, without their combination with ultrasound parameters or the use of integrated algorithms.

The trisomy 21 affected pregnancies were reported adequately by the Flemish obstetricians, as is shown by the correlation between the AML database and the Eurocat Register of Birth Defects in Belgium for the prevalence of trisomy 21 between 1992 and 2001 [19].

The performances of both the second and first trimester algorithms we used in this study were very similar to other reports. Wald et al. [2] reviewed 2MSS in 1997 and found detection rates for trisomy 21 varying between 42 and 89% at SPR between 3 and 13.3%. We found a DR of 73.3% at a SPR of 5.7% of all pregnancies. At maternal age ≥35 years, these rates were respectively 93.8 and 24.5% in our study. This is also similar to other reports [3, 4, 11]. 1MSS was reported to achieve a DR for trisomy 21 varying between 60 and 85% at SPR of 5–25% [18, 20–22]. In our study, trisomy 21 DR was 61.5% and the overall SPR was 5.1% at a cut-off of 1:85. For 1MSS combined with NT, an increase of DR for chromosomal anomalies was reported with advancing maternal age [5, 21, 23]. Few data are reported on this association within the algorithm 1MSS without NT [12]. In our study, at a cut-off of 1:85, 1MSS detected 61.5% of all trisomy 21 affected pregnancies and 85.7% at ≥35 years. The overall SPR was 5.1 and 17.7% for women aged ≥35 years. At a cut-off level of 1:200, our algorithm detected all cases of trisomy 21 at maternal age ≥35 years, and the SPR differed less than 1% from the SPR at cut-off of 1:85.

We evaluated the rate of invasive procedures by primary invasive testing at advanced maternal age versus selective invasive procedures only for positive MSS results. At maternal age ≥35 years, our data indicate that selective invasive testing only for positive 1MSS or 2MSS achieves a reduction of over 75% of invasive procedures compared to routine invasive testing. For first trimester chorionic villus sampling and second trimester amniocenteses, a procedure-related complication rate of 0.5–1% is reported [24]. We would need to perform an additional number of 506 primary invasive procedures to detect one trisomy 21 missed by 1MSS, which as a result would cause an expected number of 2–5 lost pregnancies. In the population screened by 2MSS, we would even need to perform an additional number of 1,557 invasive procedures to detect one trisomy 21 missed by the algorithm, which would then lead to an expected number of 8–15 lost pregnancies. Our data confirm the findings of others [3, 6–8]. According to several reviews, the performance of combined or integrated screening algorithms is much better than for single-step MSS [6, 9, 13–15]. In our study population, we also expect an improvement of the screening performance from the combination or integration of ultrasound parameters, obtained in the first (NT, nasal bone) or in the second trimester (genetic scan), into the maternal serum algorithms. However, several practical limitations are reported in introducing combined or integrated screening for general obstetric populations; these include scheduling of testing within relatively narrow ges-
ational age intervals, availability of appropriately trained ultrasonographers and costs [16]. Due to these limitations, high-quality combined or integrated screening is not yet available or easily accessible for all pregnant women in many developed countries, i.e. Belgium and the Netherlands. Therefore many obstetricians currently still prefer to recommend primary invasive testing by amniocentesis or chorionic villus sampling to women ≥ 35 years of age, meanwhile offering non-invasive screening methods to younger women. In Belgium, this practice is likely to continue until training and audit of a sufficient number of ultrasonographers and National Health Insurance funding of multiple parameter screening is accomplished. As our data show, a strong reduction of primary invasive procedures and subsequent complications in Flanders may already be achieved by single-step MSS for women at advanced maternal age. Selective invasive testing following 1MSS or 2MSS is easy to introduce and accessible to all pregnant women. This approach may also be the first step towards the general introduction of combined or integrated screening strategies, which are far more superior to the single-step approach.

We conclude that single-step MSS in the first or second trimester in Flanders achieves excellent trisomy 21 detection rates and reduces substantially the number of primary invasive procedures for women aged ≥ 35 years. The simplicity of both methods allows to consider them valuable options for fetal aneuploidy screening at advanced maternal age, until combined or integrated screening is accessible to all pregnant women in Belgium.

Acknowledgements

We wish to thank all Flemish obstetricians contributing to the continuing expansion of the database of trisomy screening in Flanders.

References