Sequential triage in the first trimester may enhance advanced ultrasound scanning in population screening for trisomy 21


*Department of Obstetrics & Gynaecology, Ziekenhuis Oost Limburg, Genk and †Algemeen Medisch Laboratorium, Antwerp, Belgium and ‡Department of Obstetrics & Gynaecology, Academisch Ziekenhuis Maastricht, The Netherlands

KEYWORDS: contingent screening; first-trimester screening; population screening; trisomy 21

ABSTRACT

Objective To design a trisomy 21 screening protocol for sequential triage in the first trimester, and to evaluate whether it reduces the need for advanced ultrasound scanning to such an extent that this could be dealt with by a limited number of well-trained sonographers only.

Methods Screening results of 31 trisomy 21 affected pregnancies and 16 096 unaffected pregnancies from the first trimester screening program of Algemeen Medisch Laboratorium in Antwerp, Belgium, were used to define high-risk, intermediate-risk and low-risk groups. A serum screening result (age, pregnancy-associated plasma protein-A (PAPP-A) and free beta-human chorionic gonadotropin (β-hCG)) of ≥ 1:30 and/or a nuchal translucency thickness (NT) measurement of ≥ 3.5 mm were classified as high risk. A serum screening result of < 1:1000 together with an NT of < 3.5 mm were classified as low risk. Other results were considered intermediate risk, for which further advanced ultrasound screening would be indicated. This protocol was then evaluated prospectively in another population of 13 493 first-trimester pregnancies.

Results Of the total population, 1.9% was identified as being high risk (14 trisomy 21 pregnancies and 222 unaffected pregnancies; prevalence, 1:17), 59.6% was identified as being low risk (three trisomy 21 pregnancies and 9615 unaffected pregnancies; prevalence, 1:3206) and 38.4% was identified as being intermediate risk (10 trisomy 21 pregnancies and 6190 unaffected pregnancies; prevalence, 1:620). A similar distribution was found in the prospective arm of the study. There was no reduction of overall screening performance compared with our current first-trimester combined screening program. The number of intermediate-risk pregnancies was sufficiently low as to enable advanced ultrasound scanning by well-trained sonographers only.

Conclusion In population screening for fetal trisomy 21, sequential triage in the first trimester can be achieved using very simple methods. Pregnancies at high or at low risk can be identified easily and the number of pregnancies at intermediate risk can be reduced sufficiently to enable advanced ultrasound screening by well-trained sonographers only. A prospective study is needed to evaluate the performance of this approach and to compare its results with current combined or integrated screening algorithms. Copyright © 2006 ISUOG. Published by John Wiley & Sons, Ltd.

INTRODUCTION

First-trimester combined screening using advanced ultrasound scanning techniques (fetal nuchal translucency thickness (NT) measurement with evaluation of the fetal nasal bone, ductus venosus flow or tricuspid regurgitation) allows detection of over 90% of trisomy 21 affected pregnancies at a false-positive rate (FPR) of < 5%. In some countries, the small number of well-trained sonographers may limit the numbers of pregnant women who have access to this screening method. We recently reported that in Flanders, Belgium, while pregnant women have easy access to first-trimester combined screening, only a limited number of ultrasound examinations are performed according to the criteria defined by The Fetal Medicine Foundation (FMF). Despite efforts to increase the number of FMF-accredited sonographers in the country, many obstetricians refuse to take part in ultrasound training and audit programs. Easy and accessible integrated screening methods may offer a solution to
Sequential triage screening for fetal trisomy 21

In this problem, but these have not yet been introduced in Belgian antenatal care. Furthermore, integrated screening delays the diagnosis of an affected pregnancy until the second trimester$^{6,7}$; it has been reported that most pregnant women prefer a first-trimester over a second-trimester diagnosis$^8$.

Contingent screening was presented recently as a sequential triage screening process, using parameters from the first and/or second trimester, that is both efficient and cost-effective$^{9-11}$. In the first step of this screening process, three groups of pregnant women are identified: those with a very high risk, justifying immediate diagnostic testing, those with a very low risk, allowing interruption of the screening process, and those with an intermediate risk, who are offered further screening by ultrasound (in the first trimester) or maternal serum (in the second trimester).

In this study, we investigated whether it was possible to design a protocol for sequential triage in the first trimester, which offers easy access to screening for every pregnant woman, without decreasing the overall screening performance. We also evaluated whether the number of pregnancies eligible for second-step advanced ultrasound screening within the first trimester could be limited to such an extent that they could all be screened by the small number of FMF-accredited sonographers in the country.

**METHODS**

The principles of sequential triage screening, as evaluated in this paper, are presented in Figure 1. This protocol was designed to operate according to the specific organization of antenatal care in Belgium. All pregnancies are submitted to first-trimester maternal serum screening at around 10 weeks or at the time of the first-trimester ultrasound examination between 11 and 14 weeks. Maternal age, pregnancy-associated plasma protein-A (PAPP-A) and free beta-human chorionic gonadotropin (β-hCG) are used to calculate an individual’s risk for fetal trisomy 21. These results are categorized into three groups: high risk, intermediate risk and low risk. Pregnancies with a high risk are referred for immediate invasive testing. All other pregnancies are submitted to first-trimester ultrasound screening with measurement of fetal NT. The pregnancies with very high NT measurements are also referred for invasive testing, irrespective of the prior serum screening risk. The pregnancies with a low serum screening risk and normal NT values are withdrawn from further screening. The pregnancies in the serum screening intermediate risk group, and with an NT within the normal range, undergo further screening with advanced first-trimester ultrasound screening methods by trained sonographers.

We used the data of the first-trimester combined screening program (maternal age, PAPP-A, free β-hCG and NT), as recorded by Algemeen Medisch Laboratorium in Antwerp, Belgium, to develop and evaluate this sequential triage screening model. The methods of parameter handling, risk calculation and follow-up of pregnancy outcome are reported elsewhere$^{4,12}$. Since 1$\text{st}$

![Figure 1 Protocol for sequential triage in the first trimester.](image-url)

February 2005, biochemical analysis of first-trimester serum parameters has been performed using the Kryptor analyser (Brahms Diagnostica GmbH, Berlin, Germany). Six of 17 FMF-trained sonographers in the country participate in the Algemeen Medisch Laboratorium screening program.

To develop the model, we used data from screened pregnancies that delivered before 30$\text{th}$ November 2004. For this group, we established a receiver–operating characteristics (ROC) curve for the serum screening algorithm currently in use (maternal age + PAPP-A + free β-hCG) to evaluate the balance between detection rate and FPR. This curve was used to define the optimum cut-off point, above which invasive testing would be justified. The cut-off point was chosen at the level where FPR approximated 1%; this was at a risk of 1 : 30. We also used this curve to define the cut-off point below which no further screening was needed, at a risk of 1 : 1000. Calculated risks between 1 : 30 and 1 : 1000 were classified as being intermediate. The cut-off for NT measurement, justifying immediate invasive testing, was set at 3.5 mm, which is the 99$\text{th}$ percentile of The FMF’s reference range$^{13}$. We evaluated the overall detection rate and FPR of this sequential triage screening strategy and compared their performance with that of the first-trimester combined algorithm that is currently used.
We evaluated our new sequential triage screening protocol prospectively in pregnancies screened in the Algemeen Medisch Laboratorium first-trimester screening program that were delivered after 1st December 2004. The screen-positive rate (SPR) was evaluated for the sequential triage screening protocol and compared with that of our current first-trimester combined algorithm.

RESULTS

To develop the model, we used a total of 16,127 screening tests, of which 31 were from trisomy 21 affected pregnancies (prevalence = 1:520) and 16,096 were from unaffected pregnancies. The mean maternal age in this population was 29.2 years.

The data are presented in Figure 2. With the algorithm maternal age + PAPP-A + free β-hCG, 45.2% (14/31) of trisomy 21 affected pregnancies had a calculated risk of ≥1:30, compared with only 1.4% (222/16,096) of unaffected pregnancies. These pregnancies would be submitted to immediate invasive testing. Similarly, an NT measurement of ≥3.5 mm was found in 4/17 of the remaining trisomy 21 affected pregnancies (12.9% (4/31) of all trisomy 21 affected pregnancies), compared with only 0.4% (69/15,874) of the remaining unaffected pregnancies. These pregnancies would also be submitted to invasive testing. Thus a serum screening risk of ≥1:30 and/or an NT measurement of ≥3.5 mm detected 58% (18/31) of trisomy 21 affected pregnancies at an FPR of 1.8% (222 + 69 = 291/16,096).

A total of 9,618 pregnancies (59.6%) had a serum screening risk of <1:1000 and an NT measurement of <3.5 mm. These pregnancies would be withdrawn from further screening. This group included 9.7% (3/31) of trisomy 21 affected pregnancies. These data indicate that, at the given cut-off values, a population may be identified in which the probability of a false-negative screening result is only 1:3206 (3/9618).

A total of 6,200 pregnancies (38.4%), including 10 trisomy 21 affected pregnancies, had an NT measurement of <3.5 mm and a serum screening risk of between 1:30 and 1:1000. These were the pregnancies that would be submitted to advanced first-trimester ultrasound screening. Of the 10 trisomy 21 affected pregnancies, three were missed by our current combined algorithm. These pregnancies were scanned by non-FMF-trained sonographers and had NT values of 1.0 mm (0.77 MoM), 1.2 mm (0.80 MoM) and 1.6 mm (0.93 MoM), respectively. Modeled calculations in our current combined screening algorithm showed that an artificial increase of 0.5 mm in the measured NT value would reverse the screening result from negative (<1:300) to positive (≥1:300) for two of these three pregnancies.

Figure 3 presents the results of our prospective evaluation of the sequential triage protocol for the screened population who delivered after 1st December 2004. As many of these pregnancies were still ongoing at the time of writing and data on pregnancy outcome were not yet available, we could not evaluate detection rate or FPR in this population, and so we used the SPR instead: the number of positive screening results divided by the total number of screened pregnancies. The results were very similar to those of Figure 2. Of all screened pregnancies, 1.5% would be submitted to immediate invasive testing for a serum screening risk of ≥1:30 and/or NT measurement of ≥3.5 mm, 60.8% would be withdrawn from further screening and 37.8% would be submitted to further advanced ultrasound screening.

In Table 1, detection rate and FPR are compared between sequential triage screening and combined First-trimester maternal serum screening (age, PAPP-A, free β-hCG).

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First trimester maternal serum screening
(age, PAPP-A, free $\beta$-hCG)
13,493 (100%)

- $< 1 : 1000$
  - 8,224 (61.0%)
  - $\geq 1 : 30$
  - 151 (1.1%)
  - Between $1 : 30$ and $1 : 1000$
  - 5,118 (37.9%)

- NT $< 3.5$ mm
  - 8,198 (60.8%)
  - NT $\geq 3.5$ mm
  - 26 (0.2%)

- NT $\geq 3.5$ mm
  - 22 (0.2%)
  - NT $< 3.5$ mm
  - 5,096 (37.8%)

Invasive procedure (1.5%)
Stop
Further screening

Figure 3 Prospective evaluation of the protocol for sequential triage: decision tree in the Algemeen Medisch Laboratorium population with delivery after 1st December 2004. free $\beta$-hCG, beta-human chorionic gonadotropin; NT, nuchal translucency thickness; PAPP-A, pregnancy-associated plasma protein-A.

Table 1 Comparison of the overall performance of sequential triage screening and first-trimester combined screening in the Algemeen Medisch Laboratorium populations with delivery before 30th November and after 1st December 2004: number of cases of trisomy 21 (T21) apparently detected

<table>
<thead>
<tr>
<th>Screening protocol</th>
<th>Actual T21 pregnancies ($n = 31$): detection ($n$ (%))</th>
<th>Unaffected pregnancies ($n = 16,096$): false positives ($n$ (%FPR))</th>
<th>All pregnancies: screen positives ($n$ (%SPR))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequential triage</td>
<td>25 (80.6)</td>
<td>1118 (6.9)</td>
<td>750 (5.6)</td>
</tr>
<tr>
<td>Combined ($\geq 1 : 300$)</td>
<td>25 (80.6)</td>
<td>1161 (7.2)</td>
<td>775 (5.7)</td>
</tr>
</tbody>
</table>

For the second step in the sequential triage screening process, nuchal translucency (NT) thickness measurements were used with a documented underestimation according to The FMF’s reference range. It is expected that the use of advanced ultrasound scanning methods, according to The FMF’s criteria, would achieve higher detection rates at lower false-positive rates. *Delivery before 30th November 2004. †Delivery after 1st December 2004.

DISCUSSION

There are approximately 600 consultants and residents in obstetrics and gynecology active in Flanders today. Amongst them, only 17 participate in the program for training and audit of first-trimester ultrasound screening, organized by The FMF. This number is too low to offer ultrasound screening that meets The FMF’s criteria to all pregnant women requesting screening. Contingent screening has been reported as a sequential triage protocol, which offers a practical solution to the difficulties encountered with introducing combined or integrated screening strategies into population screening for fetal aneuploidy. Its main goal is to offer screening to as many pregnant women as possible but to present advanced screening methods selectively to a limited number of pregnancies. Here we report the development and evaluation of a first-trimester sequential triage screening model, useful for the specific organization of antenatal care in Belgium.

Our model offers easy access to screening, as both first-trimester maternal serum and ultrasound screening are available in every antenatal clinic in Belgium. Maternal serum screening, as the most important component in our
algorithm\(^4\), is offered as the very first step in the sequential triage screening protocol. It is very easy for every (FMF-trained or non-trained) obstetrician performing first-trimester ultrasound examinations to discriminate between an NT measurement above or one below 3.5 mm. In our audit of NT measurements in Flanders, we demonstrated that NT measurements around the 95\(^{th}\) percentile, performed by non-FMF-trained sonographers, were close to The FMF’s reference values\(^1\). However, in the present study, less than 0.5\% of NT values were above The FMF’s reference 99\(^{th}\) percentile (Figures 2 and 3). Despite this, the first step of our sequential triage screening protocol successfully identified: nearly 60\% of trisomy 21 affected pregnancies at a FPR of <2\%, and a 60\% proportion of pregnancies in which the prevalence of fetal trisomy 21 was less than 1/3000. As a result, the second step of the sequential triage screening process could be limited to >40\% of the population. In the prospective arm of the study, shown in Figure 3, sequential triage screening indeed efficiently identified a 1.5\% high-risk group and a 60.8\% low-risk group, leaving only 37.8\% of the population who required further screening.

The results of this sequential triage screening approach, using some NT measurements performed by non-FMF-trained sonographers in the second step (Figures 2 and 3), are summarized in Table 1. This preliminary evaluation illustrates that the first step of this sequential triage does not decrease the overall screening performance, compared with our current first-trimester combined screening program. We are now conducting a prospective evaluation of this sequential triage sequential screening protocol, to evaluate its feasibility, efficiency and cost with respect to prenatal population screening for trisomy 21.

In contrast to combined screening, the sequential triage approach required a further screening of 38\% of the total population with a second ultrasound examination. For this, advanced ultrasound scanning techniques, according to The FMF’s criteria, could be used. These techniques include accurate measurement of NT, and evaluation of the fetal nasal bone, ductus venosus flow and tricuspid regurgitation\(^1,2\). It is expected that this advanced ultrasound screening protocol may increase the overall detection of trisomy 21 in the population. Compared with the non-FMF-trained sonographers, The FMF trainees would be more likely to allocate higher NT values to the affected pregnancies that were missed by our current program, and they would also be able to add more information through the use of other ultrasound parameters (nasal bone, ductus venosus flow or tricuspid regurgitation). We estimate that advanced ultrasound screening would detect 9/10 trisomy 21 affected pregnancies in the intermediate risk group. While this would bring the overall detection rate in our total population to 87\% (27/31). Similarly, selective advanced ultrasound screening, following sequential triage screening, is expected to reduce the FPR to a lower level than that of our current first-trimester combined screening program.

An alternative for selective advanced ultrasound scanning may be the selective offer of second-trimester maternal serum screening to women in the intermediate risk group, and integration of first- and second-trimester parameters\(^10,11\). This screening method might also improve the overall screening result compared with our current results.

We are aware that sequential triage screening requires a rigid protocol and strict organization to be sure that every participant (doctor, laboratory and patient) adheres to its guidelines. This would have to be taken into account should this screening method be introduced into daily clinical practice. We also acknowledge that the second step in our sequential triage screening model will increase the cost of screening in approximately 40\% of our pregnant population, compared with combined first-trimester screening. These costs, however, may be justified by the fact that a higher number of trisomy 21 detections will be achieved than are with the current screening program. The rise of costs can be reduced by a decreased number of invasive procedures in unaffected pregnancies. In any case, the extra costs of the sequential triage screening model would be less compared with the uniform application of integrated first- and second-trimester screening, in which 100\% of the population is submitted to the second step of the screening process.

We have designed a model for sequential triage screening, suitable for the Belgian organization of antenatal care. Using very simple techniques, this model identifies efficiently high-risk and low-risk pregnancies, and reduces the need for advanced ultrasound screening selectively to a limited number of pregnancies. A small number of FMF-trained sonographers can deal with these advanced ultrasound screening examinations. The expectation that this protocol should offer a higher detection rate for trisomy 21 at a lower FPR than do the current screening methods is being investigated in an ongoing prospective study.

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