Prospective experience with contingent screening strategy for Down syndrome in Estonia

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Abstract The purpose of this study was to establish the first-trimester screening for Down syndrome (DS) in Estonia and to evaluate the potential of a contingent screening in the population of pregnant women. A prospective cohort study included non-selected pregnancies during the programme of first-trimester screening for DS in a 4-year period at a single centre. The following screening tests were evaluated: measurement of nuchal translucency (NT) and serum screening [pregnancy-associated plasma protein A and free beta subunit of human chorionic gonadotropin ($\text{f}\beta\text{-HCG}$)]; results were given as combined screening. After first-trimester screening, contingent screening protocol was used, and women were divided into three groups: high risk, low risk and an intermediate risk group. In the last group, a second-trimester triple test (AFP; total HCG and uE3) was also performed. The study group consisted of 3,194 non-selected pregnancies. In 1,387

(43.4%) women, first-trimester serum screening showed low risk ($\text{risk} \leq 1:5,000$), and no future testing was performed, in 30 (0.9%) women screening test showed high risk ($\text{risk} \geq 1:50$) and a diagnostic test was offered, and in 1,777 (55.7%) women repeated risk calculation in the second trimester was done. During the study period, there were 17 cases of trisomy 21, of which 15 (88.3%) were detected with the described screening programme. In conclusion, two-step contingent sequential screening is a better choice for Down syndrome screening in Estonia instead of previously used second-trimester screening, and it offers the advantage of earlier diagnosis.

Keywords First-trimester screening · Trisomy 21 screening · Down syndrome · PAPP-A · Free $\beta\text{-HCG}$ · Nuchal translucency

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Introduction

Down syndrome (DS) serum screening has been an integral part of routine prenatal care in recent decades. Now the most commonly used screening tests for DS screening use either second-trimester maternal serum biochemical markers or a combination of first-trimester maternal serum markers with ultrasound measurement of nuchal translucency (NT) (Wright et al. 2006). The use of ultrasound screening and placental markers for DS screening in the first trimester was first proposed by Nicolaides and colleagues in the early 1990s (Nicolaides et al. 1992; Snijders et al. 1998).

Different screening strategies are used in different countries (Boyd et al. 2008). The integrated test, in which first- and second-trimester screening markers are incorporated into a single test reporting of risk results at second

trimester only, may be disadvantageous from both a psychological and a medical point of view (Christiansen and Olesen Larsen 2002). Sequential screening policy allows offering an early diagnostic test to high-risk women and a second-trimester screening test to low-risk women. Such screening can achieve similar performances to integrated test, while only a fraction of women need second-trimester tests (Wright et al. 2004). Contingent screening uses a first-trimester test to triage the population of women screened into three groups: a high-risk group that is immediately offered a diagnostic test, a low-risk group that receives no future screening and an intermediate-risk group in which first-trimester results would be reused in the second trimester as part of a subsequent integrated test (Wald et al. 2006; Wright et al. 2006). Each screening protocol has advantages and disadvantages, and each country must determine which protocol suits it best.

Estonia is a country in Northern Europe with about 16,000 births per year. Clinical use of amniocentesis (AC) for fetal chromosome analysis was introduced in the spring of 1990. Since 1995, the AC was offered as a screening of fetal chromosomal abnormalities to selected high-risk group based on maternal age ≥ 35 years and complicated anamnesis. Non-invasive DS screening by second-trimester maternal serum screening (double/triple test) was commenced in 1999 in southern Estonia, and in 2006 the whole of Estonia was covered. Serum screening is now widely accepted in Estonia; in 2006, about 91% pregnant women under the age of 37 were monitored, with a detection rate of 66.6% and a false positive rate of 4.7% (Sitska et al. 2008a, b). In 2005, the first clinical guidelines for prenatal diagnosis in Estonia were established (revised in 2008) (Sitska et al. 2008a, b).

The aim of this study was to establish the first-trimester screening for DS in Estonia and to evaluate the potential of a contingent screening in our population of pregnant women.

Patients and methods

All women seeking antenatal care at Tartu University Hospital during the first trimester were enrolled in the study group. The screening was completely voluntary; all subjects gave written consent before being enrolled in screening. The study period lasted from 1st February 2005 to 31st December 2008.

NT thickness was measured at the gestational age between 11⁺⁰ and 13⁺⁶ weeks of pregnancy (CRL 45–84 mm), using standard procedures by a Fetal Medicine Foundation (FMF) certified sonographer or by a well-trained sonographer working under the supervision of a certified one. If NT was either not measured properly or

CRL was less than 45 mm at the time of NT measurement, results were ignored by laboratory.

Serum screening in first trimester was performed in the 8⁺⁰–13⁺⁶ weeks of pregnancy. All serum samples were assayed for pregnancy-associated plasma protein-A (PAPP-A) and free β -chorionic gonadotropin (f β -HCG) using Immulite DPC 2000 (distributed by Siemens Medical Solutions Diagnostics). For individual risk calculation (combined and/or serum-based risk), we used Prisca 4.0 software (distributed by Siemens Medical Solutions Diagnostics), which has an algorithm for trisomy 21 and trisomy 18. Results were given in MoMs (multiple of medians) and corrected for maternal weight, smoking, ethnicity, maternal diabetes and in vitro fertilisation.

At the beginning of screening programme (in 2005), there was a pilot study of 500 women in whom both first- and second-trimester serum screening was performed. Second-trimester serum was analysed for alphafetoprotein (AFP), total human chorionic gonadotropin (HCG) and unconjugated oestriol (uE3); serum-based risk and/or integrated risk were calculated with the software Prisca 4.0 (distributed by Siemens Medical Solutions Diagnostics), which has an algorithm for trisomy 21 and trisomy 18. Results were given in MoMs (multiple of medians) and corrected for maternal weight, smoking, ethnicity, maternal diabetes and in vitro fertilisation. After analysis of 500 tests, screening cut-off was worked out and applied.

From the beginning of 2006, two-step contingent sequential screening was introduced. After first-trimester combined screening, we triaged the population of women screened into three groups (Fig. 1):

The high-risk group consisted of women with an increased risk after first-trimester combined screening. Their individual risk for trisomy 21 or trisomy 18 was higher than 1:50 or NT >3 mm, and chorionic villous sampling (CVS) or AC for fetal karyotyping was offered after the first-trimester combined screening result. Provisional results from FISH analysis were available within 48 h and a final diagnosis by conventional karyotyping within 2 weeks.

The low-risk group consisted of women whose individual risk calculation after the first-trimester combined test was less than 1:5,000 and who received no future screening.

The intermediate-risk group included women with a medium-risk calculation: a first-trimester combined risk for DS from 1:51 to 1:5,000 or to whom we could not offer the combined screening method because of a lack of ultrasound (US) scan data. To these women, the second-trimester serum screening test (AFP; HCG and uE3) was offered. AC for fetal chromosome investigation was performed to the high-risk group—the risk of trisomy 21 was $\geq 1:270$ and for trisomy 18 $\geq 1:100$. To women with individual integrated

Fig. 1 Used screening protocol in 2006–2008 in Estonia

risk calculation $>1:1,000$ for trisomy 21 (intermediate risk), the second-trimester genetic sonograms were performed looking for additional sonographic markers like increased nuchal skinfold, short humerus, short femur, echogenic bowel, pyelectasis, echogenic intracardiac focus, absence or hypoplasia of nasal bone and choroid plexus cysts. AC for fetal chromosome investigation was offered if there were two or more sonographic markers.

To identify Down syndrome infants born to screening-negative women who participated in our screening, we contacted all cytogenetic laboratories in Estonia.

Results

During the study period (2005–2008), 3,194 women agreed to participate in the routine first-trimester serum screening. Their average age at giving birth was 28.5 years, and 355 (11.1%) of women were older than 35 years of age. We were able to give 2,718 women (85.1% of all those who tested) a combined risk calculation (NT with first-trimester serum screening). In 14.9% of tests, NT was either not

measured properly or CRL was less than 45 mm at the time of NT measurement. The median CRL was 61.5 mm (range 38–83 mm), and the median NT was 1.63 mm (range 0.2–8.8 mm). In 32 cases (1.2%), NT was greater than 3 mm. The median gestational age at the time of blood sampling was 12^{+2} weeks (with a range of 8–13 weeks).

High-risk group

Of the 3,194 women screened in the first trimester, 30 (0.93%) had a combined risk higher than 1:50. Twelve women accepted an invasive procedure on the basis of a first-trimester risk calculation after counselling from a geneticist. CVS was the procedure chosen by seven and AC by five women. Eighteen of the high-risk women decided to repeat serum screening in the second trimester. In ten of these women, the risk remained high ($>1:50$), and nine of the women accepted an invasive procedure. Low risk in second trimester was reported in eight women, and a second-trimester genetic sonogram was the first choice of these women. Among the high-risk group, 18 (60%) of women were carrying a baby with a chromosomal

abnormality (Table 1): Down syndrome was diagnosed in 13 cases, Edwards syndrome in two cases, triploidy (69, XXX) in one case and Turner syndrome or its variations in two cases.

NT was greater than 3 mm in 32 cases (1.2%). Chromosomal abnormality or congenital defect was diagnosed in ten of them (32%): Down syndrome in six cases, and Edwards syndrome, Turner syndrome, diaphragmal hernia or congenital heart defect in one case each.

Low-risk group

Of the women screened in the first trimester, 1,387 (43.4%) had a combined risk lower than 1:5,000. No false-negative cases were registered.

Intermediate-risk group

In 1,777 (55.7%) women, we found intermediate risk in the first trimester. Among them, 65 (3.7%) women did not pass second trimester due to their choice. In 1,712 (96.3%), second-trimester serum screening was performed. An invasive procedure was offered to 79 (2.5%) women after second-trimester screening due to a high-risk test result. In the intermediate-risk group, six (0.3%) aneuploidies were ascertained (Table 1): Down syndrome in two cases, Turner syndrome variation in one case and *de novo* translocation in one case. There were two cases in intermediate-risk group

that resulted in the birth of a child with Down syndrome and accounted as false-negative test results (Table 1). In the first case, the first-trimester screening result was intermediate (1:3,611) and she had negative result at second trimester (1:2,492). In the second case, the first-trimester result was 1:3,534 and she had also intermediate risk at second trimester (1:607). The genetic sonogram was offered to her and as it was normal according to our screening policy, invasive procedure was not offered to the family.

Detection rate

The detection rate (DR) in the study period with the developed screening programme was 88.3%, the false-positive rate (FPR) 3.4% (0.9% after first-trimester screening and 2.5% after second-trimester screening).

Discussion

We performed a prospective cohort study including non-selected pregnancies during the programme of first-trimester screening for DS in a 4-year period at a single centre, with the main purpose of evaluating the potential of a contingent screening in the population of pregnant women.

We chose the two-step contingent sequential screening because the protocol we used has the potential to

Table 1 Ascertained chromosomal aberrations

First trimester results	High risk ≥1:50		Intermediate risk 1:51 – 1:5,000		Low risk ≤1:5,000
Number of women	30 (0.93%)		1777 (55.64%)		1387 (43.44%)
Second trimester screening	0		1712 (96.3%)*		0
Second trimester results	High risk ≥1:270		Intermediate risk 1:270 – 1:1,000		Low risk ≤1:1,000
Number of women	79		167		1466
Karyotype normal	12		166		1465
Karyotype abnormal	18		1 **		1 **
	DS 13 cases (76.5% of DS)		DS 1 case (5.9% of DS)		DS 1 case (5.9% of DS)
	ES 2 cases TS 2 cases		<i>de novo</i> translocation 1 case		
	Triploidy 1 case				0

* - 3.7% of women missed second trimester screening by their choice

** - false negative cases, as there were no indication to offer prenatal diagnosis.

DS – Down syndrome; ES – Edwards syndrome, TS – Turner syndrome

considerably reduce second-trimester sampling with little impact on overall screening performance when compared to a policy of nondisclosure screening where all markers are measured on all women (Wright et al. 2006). Contingent screening is not a single test but the application of a sequence of tests; there are many cut-off combinations in which the different tests can be used (Wald et al. 2006). Some authors have chosen the three-stage screening protocol which requires the selection of four risk cut-offs, each of which will affect the overall performance of screening (Wright et al. 2006). We choose to implement the two-step screening protocol by presenting the results as high risk (cut-off 1:50), intermediate risk and low risk (cut-off 1:5,000) after first-trimester combined screening. The DR for the contingent screening during the study period was 88.3% for Down syndrome-affected pregnancies with FPR of 3.4%. The DR and FPR are comparable to some reported results from screening programmes (Crossley et al. 2002; Rozenberg et al. 2007; Okun et al. 2008; Wortelboer et al. 2009), but performance was nevertheless lower than the published results of large prospective studies (Spencer and Nicolaides 2003; Nicolaides et al. 2005). The lower performance rate may be due to a small study group where every case played a major role on DR calculation, and also median maternal age in our study group was 3 years younger than in the large studies.

The contingent sequential screening protocol with a two-step screening protocol offers advantages and disadvantages. The contingent screening protocol offers first-trimester low-risk group women earlier reassurance that they are not at risk of fetal chromosomal abnormalities. The cut-off for the first trimester ($\text{risk} \leq 1:5,000$) we used was much lower than that presented in the literature (Christiansen and Olesen Larsen 2002; Benn et al. 2005; Wald et al. 2006; Vadiveloo et al. 2009; Cocciolone et al. 2008), and this made it possible to give closure screening to about 40% of women. We choose to implement a very low screening-negative cut-off because we needed to calculate our own multiple of medians (MoM), but our laboratory throughput was low. When there will be enough tests in our database, we will perform new statistical analysis to evaluate different cut-offs and their value to screening strategy.

Using very low cut-off, more women had to wait for second-trimester screening results, which may create more anxiety. Therefore, we realized the importance of the fact that in counselling patients with intermediate risks, it is important to indicate that these are not 'high-risk' results but simply a sub-group for whom a second round of testing could possibly be beneficial (Benn et al. 2005). Infrequently, two independent test results may give a discrepant individual risk calculation that is difficult to interpret. A multi-step screening protocol generates additional subsets of patients who have high-risk results; potentially, all such

women could request invasive testing (Huttlly et al. 2006; Cocciolone et al. 2008). Benn et al. found little evidence that two-step screening resulted in additional invasive testing (Benn et al. 2007). In our study, the women who had high risk in the first trimester but low risk in the second trimester chose US scan for additional investigation and not invasive testing. This suggests that women did find the revised risk to be reassuring (Benn et al. 2007). In that group, we did not find any Down syndrome.

At the same time, contingent screening has the advantage of achieving an earlier diagnosis in a varying proportion of women. In the first trimester, high-risk results (individual $\text{risk} \geq 1:50$) represented 0.9% of all tests, and with only first-trimester screening we were able to detect 76.5% (13 of 17) of DS prenatally during the study period. Contingent screening strategy added two more cases to prenatally diagnosed DS and raised DR to 88.3% with FPR of 3.4%. It is a better result comparing the second-trimester screening with a detection rate of 66.6% and a false-positive rate of 4.7% which was used earlier in Estonia (Sitska et al. 2008a, b).

Earlier diagnosis allows women greater privacy and safer termination of affected pregnancies (Mennuti and Driscoll 2003). With appropriate patient counselling, contingent sequential screening can potentially provide most patients with early reassurance or diagnosis while engaging the power of additional testing for those patients who would reap the most benefit (Benn et al. 2005).

In conclusion, our study has demonstrated that two-step contingent sequential screening is effective and feasible in clinical practice. It is a better choice for Down syndrome screening in Estonia instead of previously used second-trimester screening and offers the advantage of earlier diagnosis.

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