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# Evaluation of first trimester maternal serum and ultrasound screening for Down's syndrome in Eastern and Northern Finland

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The current trend in prenatal diagnosis is that trisomy screening is being moved to the first trimester and ultrasonographic nuchal translucency measurement is included in risk calculation. It is likely that biochemical screening in the second trimester will gradually be given up. In Eastern and Northern Finland, during the year 1999 we offered first-trimester ultrasonographic and serum screening for trisomy 21, with measurements of maternal serum PAPP-A and  $\beta$ -hCG. A total of 2515 pregnant women participated in the screening, yielding the detection of eight fetuses with Down's syndrome. Six affected fetuses (75%) were detected by means of first-trimester serum screening. Since we were in the phase of collecting data for the Finnish medians for PAPP-A and  $\beta$ -hCG, the women were not given the estimates of risk for trisomy 21. Only 1602 of the 2515 enrolled women had the combination of first-trimester ultrasonographic and serum screening performed, and in that group there were five fetuses with Down's syndrome. The combination ultrasonographic and serum approach yielded a Down's syndrome detection rate of 80% (four out of five) with a 5% false positive rate, whereas in nuchal translucency based-screening the detection rate was 60%, with a 5% false positive rate. *European Journal of Human Genetics* (2001) 9, 404–408.

**Keywords:** prenatal diagnosis; Down's syndrome; first trimester trisomy screening; maternal serum screening

## Introduction

Prenatal diagnosis has become an increasingly important part of the management of pregnancies in Western countries. This is due to both the delay in childbearing following longer education among women, and to a growing awareness of the frequency and importance of congenital disorders. Furthermore, the current social trend is toward smaller families. Down's syndrome is still the primary reason for families to seek prenatal counselling. The current standard of care has

been to offer women the maternal serum test in the 15th–16th weeks of pregnancy. However, there is a growing demand to have a screening test earlier, in the first trimester. Nicolaides<sup>1</sup> in the UK and Ville<sup>2</sup> in France introduced the first trimester ultrasound screening for trisomy in 1992. These early findings were later confirmed by larger studies, which demonstrated that foetal nuchal translucency is an efficient method of screening for Down's syndrome in the first trimester<sup>3,4</sup>. In 1992, Wald *et al.*<sup>5</sup> proposed the use of pregnancy associated plasma protein A (PAPP-A) and placental protein 14 in trisomy 21 screening. Published findings report that combining free  $\beta$ -hCG and PAPP-A with maternal age and crown-rump length by using mathematical models allows the detection of trisomy 21 at a rate of 53–68% with a 5% screen-positive rate<sup>6–8</sup>. Scott *et al.*<sup>9</sup> assessed nuchal translucency, free  $\beta$ -hCG and maternal age; multi-

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variant discriminant analysis enabled an 87.5% detection rate in high-risk population with a 14% false positive rate. The efficacy of biochemical screening increases if the measurement of nuchal translucency thickness is included in calculating the risk figure. The sensitivity of Down's syndrome detection is thus increased to 85–89% at a fixed false positive rate of 5%.<sup>10–19</sup> The results of the published screening tests are shown in Table 1.

In our study we have studied the efficacy of combining the first trimester maternal serum test and foetal nuchal translucency measurement in screening for Down's syndrome in Finland.

### Subjects and methods

Since the granting of the maternity allowance provided by the Finnish state is dependent on the completion of a registration procedure, most pregnant women sign themselves into antenatal clinics at health centres, usually between their 6th and 13th weeks of pregnancy. Health care providers, mostly midwives, gave information on the research project to all pregnant women in Eastern and Northern Finland during their first visit, emphasising that the participation in the screening was completely voluntary. All subjects gave informed written consent before being enrolled in the study. The Research-Ethics Committee of the participating hospitals approved the study.

The screening test, PAPP-A and  $\beta$ -hCG and ultrasound were offered free of charge. Blood samples were drawn in primary care centres and in maternity clinics of the participating university and central hospitals. Gestational ages ranged from 10 weeks 0 days to 13 weeks 6 days and were based on ultrasound (most cases, 82%) or last menstrual period if ultrasound was not done. Blood samples were sent to Wallac OY, Turku, Finland where the maternal serum PAPP-A and  $\beta$ -hCG concentrations were analysed. The within and between assay variation for  $\beta$ -hCG were both <3.4% and for PAPP-A <1.4% and

**Table 1** Estimated detection rate for trisomy 21 by a combination of maternal age, foetal nuchal translucency and first-trimester maternal serum PAPP-A and free  $\beta$ -hCG at a 1–14% screen-positive rate

Study	Trisomy 21	Gestation (weeks)	Screen-positive (%)	Detection (%)
Brizot <i>et al.</i> <sup>10</sup>	80	10–14	5.0	89
Scott <i>et al.</i> <sup>9</sup>		10–13	14.0	87.5
Zimmerman <i>et al.</i> <sup>11</sup>	4	10–13	2*	39*
Wald <i>et al.</i> <sup>13</sup>	86+77	10–14	5.0	80
Orlandi <i>et al.</i> <sup>12</sup>	11	9–14	5.0	87
de Graaf <i>et al.</i> <sup>16</sup>	37	10–14	5.0	85
de Biasio <i>et al.</i> <sup>15</sup>	13	10–14	3.3	85
Spencer <i>et al.</i> <sup>17</sup>	210	10–14	5.0	89
Krantz <i>et al.</i> <sup>18</sup>	50	10–14	4.5	87.5
Spencer <i>et al.</i> <sup>19</sup>	7	10–14	6.7	86

\*For an increased nuchal translucency and decreased PAPP-A.

<4.8%, respectively. The analytical sensitivities of  $\beta$ -hCG and PAPP-A were 0.2 ng/ml and 5 mU/l, respectively. The results are given as multiples of medians (MoM). Quality was assessed regularly, using in-house controls. The risk figures for biochemical tests, nuchal translucency and for combined test were calculated using the software program of Wallac 1T risk calculation program (research version),<sup>20</sup> which is based on the principles previously introduced. Wallac's program has been extended to include pregnancies before the 11th week of pregnancy.

The assay laboratory was provided with the following patient information: date of birth, weight, first day of last menstrual period, gestational age by ultrasound and the nuchal translucency thickness (if available), diabetic status, use of insulin, and the number of fetuses. Nuchal translucency was estimated in university or central hospitals or in health-care centres by personnel trained in tertiary university centres. The results of the serum test were not given to the patients.

In the ultrasound screening we used a cut-off limit of  $\geq 3$  mm for the nuchal translucency thickness, and the risk figure was also calculated by the Wallac program. The risk figure program takes into account the maternal age, crown-rump length and foetal nuchal translucency thickness. The estimate of risk for Down's syndrome for a specific maternal age remained investigational. If the nuchal translucency thickness was  $\geq 3$  mm, foetal chromosome investigation was offered.

In the biochemical screening we used the risk-calculation program of Wallac. Women with a risk greater than that of a 35-year-old at the same gestational age, ie 1:250, were considered to be at increased risk for Down's syndrome. We also calculated the cut-off limit for a fixed false positive rate of 5%.

In the combined biochemical and nuchal translucency screening we used the Wallac program for calculating the risk figure. Corrections were made for maternal weight but not for diabetic status or smoking. The cut-off limit for increased risk was set at 1:250.

In order to identify Down's syndrome infants born to screen-negative women enrolled in the study, we contacted all maternity clinics and the Finnish Register of Congenital Malformation, and the National Research and Development Centre for Welfare and Health, to get information on newborns with Down's syndrome.

### Results

The participants comprised 2515 volunteer pregnant women in Eastern and Northern Finland during the 10th–13th weeks of pregnancy in 1999. First the Finnish medians for maternal serum concentration of PAPP-A and  $\beta$ -hCG were calculated. The expected number of Down's syndrome in our study group according to the Finnish Register of Congenital Malformation was 7.8 (1:324). Among the screenees there

were eight cases of trisomy 21 (1:314) and two cases of trisomy 18 (1:1257). Of the pregnant women, 17.5% were older than 35 years, a figure that conforms exactly to the average Finnish pregnancy statistics.<sup>21</sup> Nuchal translucency of the foetus was measured in 1602 of the pregnancies. Five cases of trisomy 21 and both cases of trisomy 18 were found in that group.

#### The biochemical test only

In the population screened by the biochemical test only, a total of 249 women (9.9%=initially positive) had the adjusted estimated risk of at least 1 in 250. Six foetuses of the eight (75%) affected by Down's syndrome (Tables 2 and 3) and the foetuses with trisomy 18 were detected on the basis of serum screening. At a fixed 5% false positive rate, the cut-off limit was 1:140 and the detection rate remained the same, 75%. In the age group of 16–20 years the false positive rate was 14% ( $n=63$ ), and in the age group of women  $\geq 35$  years the false positive rate was 18% ( $n=460$ ).

#### Nuchal translucency only

In the population screened by nuchal translucency only, a total of 189 women (7.5%=initially positive) had the adjusted estimated risk of at least 1 in 250 for Down's syndrome calculated by the risk figure program. Among the 1602 screenees there were five pregnancies affected by Down's syndrome (the frequency of Down's was 1:320), and two by trisomy 18. Three foetuses affected by Down's syndrome, 60% (Tables 2 and 3), and the foetuses with trisomy 18 were detected by nuchal translucency screening.

#### Biochemical and nuchal translucency combined

In the screened population, a total of 136 women (5.4%=initially positive) constituted the risk group for Down's syndrome. Among the screenees there were five pregnancies affected by Down's syndrome, and two by trisomy 18. Four foetuses affected by Down's syndrome, 80% (Tables 2 and 3), and the foetuses with trisomy 18 were detected on the basis of the combined screening.

#### Discussion

It is generally believed that screening for trisomies using maternal age alone has a low detection rate and a high false positive rate.<sup>10</sup> This may be true for countries where the proportion of older women (>35 years of age) is 10% or less. But in Finland, where 17.5% of pregnant women are 35 years of age or older, the 'age screening' would detect more than half of all cases of Down's syndrome. In the whole country of Finland, 58% of cases of trisomy 21 in 1999 were in the group of older women and 39% of all detected Down's syndromes were found in that group (the uptake of prenatal diagnostics is not 100%). The proportion of older women in the screened population has an

**Table 2** Results of screening for trisomy 21 by assay of maternal serum (PAPP-A and  $\beta$ -hCG), by foetal nuchal translucency and by combining maternal serum biochemistry and foetal nuchal translucency at 10–13 weeks of gestation using 1:250 as a cut-off limit. There were eight cases of Down's syndrome in the maternal serum group and five cases in the other groups

	Total	Screen positives n (%)	No of Down's detected	f
Maternal serum	2515	249 (9.9)	6/8	1:35
Nuchal translucency	1602	189 (7.5)	3/5	1:63
Combined	1602	136 (5.4)	4/5	1:34

f=frequency of Down's in screen positive cases.

**Table 3** Cases of trisomy 21 identified in the screened population during the period January 1, 1999 to December 31, 1999. NT=foetal nuchal translucency thickness

Case	Age	Gestation	NT mm	NT only risk	Biochem risk	Combined risk
1	27	12+4	1.7	1:1584	1:35	1:64
2	30	12+5	0.6	1:2111	1:1875	1:2142
3	21	10+3	–	–	1:37	–
4	35	12+6	–	–	1:37	–
5	31	13+0	5.0	1:10	1:294	1:10
6	38	12+4	3.7	1:10	1:83	1:10
7	44	13+0	6.2	1:10	1:10	1:10
8	43	11+5	–	–	1:30	–

influence on the prevalence of Down's syndrome. The number of Down's cases will increase with the increasing proportion of older women,  $\geq 35$  years.

The proportion of older women also has an influence on the false positive rate, since the risk programs emphasise the maternal age: the greater the proportion of older women, the higher the false positive rate. On the other hand, the greater the proportion of older women, the better the detection rate. The rising false positive rate is an important issue in populations with a high proportion of older women since it increases the number of invasive procedures. Therefore, in the future, it would be important to find a risk program independent of maternal age.

At a fixed 5% false positive rate Krantz *et al.*<sup>18</sup> reported a 91% detection rate and a cut-off figure of 1:270. Spencer *et al.*<sup>19</sup> reported an 86% detection rate and a cut-off figure of 1:300. In our study, a fixed 5% false positive rate, the cut-off figure was 1:200 with an 80% detection rate. The differences in cut-off figures may reflect the difference in the proportion of older women: in Krantz's study 34.6% of the women were  $\geq 35$  years old and the frequency of Down's was 1:173, in Spencer's study 12.7% of the screenees were aged  $\geq 35$  years, and the frequency of Down's was 1:537. In the study by Zimmerman *et al.*<sup>11</sup> the

frequency of Down's was 1:287, but 63% of the screenees were in the group of older women ( $\geq 35$  years). The expected and detected numbers of Down's syndrome in our study match closely.

A potential bias in our study could result from the loss of foetuses with Down's syndrome. We have not studied the foetal chromosomes in pregnancy losses. Studies by Snijders *et al.*<sup>22</sup> and Morris *et al.*<sup>23</sup> found that the foetal loss rate between late first trimester and term was *ca.* 31%. This means that the detection rate of the screening test is overestimated, since undetected cases of Down's syndrome were aborted before term. A significant proportion of foetal losses occur between first and second trimester; therefore in order for a first trimester test to be considered superior to one performed in the second trimester, the detection rate should be at least 8.3% higher.<sup>24</sup>

Using first trimester biochemical or ultrasound screening alone it is possible to detect 63% and 74% respectively of trisomy 21 cases at a fixed 5% screen-positive rate.<sup>1,2,5,6</sup> Each screening method has a Down's syndrome detection rate similar or better than mid trimester double or triple serum screening. Furthermore, the first trimester screening offers the advantage of having the screening results earlier, allowing more time for counselling and consideration of the result.

Modern trisomy screening combines first trimester serum biochemical test with the thickness of foetal nuchal translucency. Years of practical experience in this line of screening have already been gained, as shown in Table 1. Our findings are in keeping with the studies of combined screening published earlier.<sup>10–14</sup> The studies referred to show clearly that biochemical screening is more efficient if the nuchal translucency is included in calculating the risk figure: the efficacy increases by 15%. This is true the other way round too: the efficacy of nuchal translucency screening can be improved by adding biochemical data to the risk figure software program. In our study the additional effect of ultrasound was not so obvious, possibly due to the rather limited number of trisomy cases. Despite limited numbers of Down's cases in nuchal translucency screening, we endorse the view that the application of the risk figure program is superior to the use of a simple cut-off limit of  $\geq 3$  mm in estimation of the risk for Down's syndrome in nuchal translucency screening.

We also draw attention to the important issue of the audit and quality assurance in the ultrasound screening context. Since January 2000 in one tertiary University centre (Oulu), ultrasound audit has been carried out by an independent agency (Fetal Medicine Foundation).

This study suggests that by combining the biochemical and ultrasound screening tests, we have a test which can be performed already in the first trimester and which promises a better sensitivity than the current testing procedures used in Down's syndrome screening.

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