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# Comparison and integration of first trimester fetal nuchal translucency and second trimester maternal serum screening for fetal Down syndrome

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**Background** It is uncertain whether first trimester nuchal translucency (NT) is more effective than the well-established second trimester serum screening for fetal Down syndrome or whether their combination works best. We report data from a large multicentre non-interventional trial in which all subjects underwent both first and second trimester screening.

**Methods** All women who attended the obstetric clinic before 15 weeks' gestation were recruited. An ultrasound examination was performed at 10 to 14 weeks to measure the NT. The nuchal measurements were not acted upon unless the fetus showed gross features of hydrops fetalis. All women had serum alpha-fetoprotein (AFP) and human chorionic gonadotrophin (hCG) assay at 15 to 20 weeks. The Down syndrome risk assigned by serum screening was disclosed and amniocentesis was offered if this assigned risk was $\geqslant 1:250$ or if the women were 35 years and older. The efficacy of different combinations of screening markers was compared.

**Results** Between January 1997 and August 2000, 17 590 women were recruited (19% $\geqslant$35 years old). After excluding subjects who miscarried, defaulted the serum test and other reasons, 16 237 pregnancies were analysed. Of these, 35 pregnancies were affected by Down syndrome (2.2 cases per 1000 pregnancies). At a false-positive rate of 5%, the detection rate of Down syndrome by NT alone, NT and age, serum hCG, AFP and age, and NT, hCG, AFP and age were 61%, 69%, 73% and 86%, respectively.

**Conclusion** Integration of NT and second trimester serum AFP and hCG assay yielded the best screening efficacy for Down syndrome. 

KEY WORDS: Down syndrome; screening; ultrasound; serum screening; nuchal translucency

## INTRODUCTION

Prenatal diagnosis of Down syndrome can be accurately made by cytogenetic studies on samples obtained from invasive procedures such as amniocentesis or chorionic villus sampling (CVS). Because these procedures are associated with a 0.5% to 1% risk of miscarriage, the common approach is to perform non-invasive screening tests to define an individual woman's risk of having a Down syndrome pregnancy before subjecting her to a diagnostic test. The most commonly employed screening test is based on the combination of maternal age and maternal serum assay of alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG) and other analytes in the second trimester (Palomaki *et al*., 1997). This yields a detection rate of 60–70% for a 5% false-positive rate (Cuckle, 2000; Lam *et al*., 1998). Recently, efforts were made to increase the screening efficacy by adding new markers such as inhibin-A (Wald *et al*., 1996; Lam and Tang, 1999) and to move screening forward into the first trimester (Malone *et al*., 2000). The most commonly used marker for Down syndrome in the first trimester is sonographic measurement of nuchal translucency (NT), though studies of its efficacy have yielded widely conflicting results with detection rates ranging from 29% to 91% (Malone *et al*., 2000). Because of the spontaneous loss of Down syndrome fetuses between the first and second trimesters, direct comparison of the screening efficacy derived from first trimester intervention studies with that derived from second trimester studies will be invalid (Malone *et al*., 2000). Recently, Wald *et al*. (1999) proposed a new concept of screening by integrating first and second trimester markers that may improve the screening efficacy dramatically. In the present study, we report our data on a large multicentre non-interventional trial in which all subjects underwent both first and second

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trimester methods of screening to assess the relative efficacy of different methods of screening.

## METHODS

### Subjects

This was a multicentre study conducted between the years 1997 and 2000 in Hong Kong. Pregnant women who attended at or before 14 completed weeks of gestation and who agreed to be screened for fetal Down syndrome were recruited. A written informed consent was obtained from all subjects and they were told that the NT was to be recorded for research purpose and they would not be informed of the results.

### Ultrasound examination

All subjects underwent an abdominal and/or vaginal ultrasound examination at 10–14 weeks of gestation. The ultrasound examinations were performed by either one of the authors who were experienced obstetricians and who had undergone training in NT measurements. The gestational age was ascertained by the measurement of fetal crown–rump length at or before 13 weeks, and by the measurement of biparietal diameter and head circumference between 13 and 14 weeks of gestation. A sagittal section of the fetus was obtained for the measurement of NT to the nearest 0.1 mm (Nicolaides *et al*., 1992). NT was always measured when the fetal neck was in a neutral position (Whitlow *et al*., 1998) and the image was adequately magnified according to the guidelines of the Fetal Medicine Foundation of London and all the ultrasound images were continuously audited by the chief investigator (Y.H.L.) (Herman *et al*., 1998). For each fetus, the two best NT measurements were averaged. To adjust for the effect of gestational age, all the NT measurements were converted to multiples of the median (MoM) for the gestational day (Yagel *et al*., 1998; Lam *et al*., 1999). The NT were not disclosed to the subjects or their obstetricians or acted upon unless the fetus showed ultrasound features of generalised hydropic changes.

### Serum screening

All subjects underwent serum screening in the second trimester by the assay of human chorionic gonadotrophin (hCG) and alpha-fetoprotein (AFP) between 15 and 20 weeks of gestation. The Down syndrome risk assigned by serum screening was disclosed and women with a risk at or above 1 : 250 were offered amniocentesis (Lam *et al*., 1998). The risk cut-off was chosen to achieve a detection rate of around 60% and a false-positive rate (i.e. amniocentesis rate) of around 5% (Lam *et al*., 1998). Women aged 35 years and older or those who had other risk factors for fetal chromosomal disorders were given the options of undergoing chorionic villus sampling (CVS) at 10 to 12 weeks or amniocentesis at 15 to 20 weeks (Lam *et al*., 2000) but a serum sample would still be taken a few weeks after CVS or just prior to amniocentesis for hCG and AFP assay.

### Outcome ascertainment

Pregnancy outcome was retrieved from the hospital record. If this was not available, the woman was contacted by phone. In order to ensure complete ascertainment of the Down syndrome pregnancies, records of the cytogenetic laboratories responsible for performing karyotyping for the five participating hospitals were reviewed up to July 2001.

### Statistical analysis

All markers were expressed as MoMs of the unaffected pregnancies at a given gestational age. AFP and hCG MOMs were adjusted for maternal weight as described by Neveux *et al*. (1996) using commercially available software (Robert Macial, USA). The detection rates and false-positive rates of the screening methods [first trimester NT, second trimester hCG and AFP assay and an integration of the first and second trimester markers (the integrated test)] were obtained by following the model-based likelihood ratio approach described by Royston and Thompson (1992). Specifically, all the markers were first logarithmic (base 10) transformed and a multivariate Gaussian distribution was fitted for each of the affected and unaffected pregnancies. Adequacy of each of the two multivariate Gaussian distributions was examined by using Shapiro-Wilks and $\Omega$ tests in each and all subsets of the three markers and by comparing the observed and predicted likelihood ratios (Royston and Thompson, 1992). Using the age-specific risk of having a Down syndrome pregnancy and the maternal age distribution in Hong Kong in 1994 (Hong Kong College of Obstetricians and Gynaecologists, 1996), the detection rate and false-positive rate for a particular risk cut-off level were obtained numerically for different screening methods. Thus, the receiver-operating characteristic curves (ROC, i.e. plot of detection rate against false-positive rate over different risk cut-off levels) could be constructed.

Screening accuracy of the different methods was then compared by their detection rates at a 5% false-positive rate, the total area under the ROC curve, and the partial area under the ROC curve (defined as the part of the curve where the false-positive rate was between 0% and 10%). Estimates of the three measures were computed as above while their 95% confidence intervals (CI) were obtained and comparisons were examined by parametric bootstrap of size 300 since no standard methods were available (Efron and Tibshirani, 1993). Specifically, 300 sets of samples were re-sampled from the two fitted multivariate Gaussian distributions for the affected and unaffected pregnancies. The analysis was repeated on these samples and thus 300 sets of bootstrapped measure

estimates were obtained for each screening method. The 95% CI of a measure for a screening method was then taken as the 2.5th and 97.5th percentiles of the corresponding bootstrapped measure estimates. With the same 300 sets of bootstrapped estimates, a non-parametric Friedman's test was used to examine the overall difference and pair-wise differences of a measure among the different screening methods. All the statistical analysis was performed using the Statistical Analysis System (SAS) version 8.

## RESULTS

Between January 1997 and August 2000, 17 590 women were recruited. After excluding those pregnancies in which NT were unsuccessful ($n=39$), those affected by chromosomal abnormalities other than trisomy 21 ($n=48$) or other major non-chromosomal abnormalities ($n=160$), subjects who defaulted the second trimester serum test ($n=1015$) and those who miscarried after NT measurements and before the second trimester serum test ($n=91$), 16,237 pregnancies which completed both first and second trimester screening were analysed. The mean maternal age of unaffected pregnancies was 30.5 years (19% were 35 years and older). The mean gestational age at ultrasound scan and gestational age at second trimester serum screening were 87 days and 16 weeks, respectively. Of these 16,237 pregnancies, 117 women (0.7%) underwent CVS (none were affected by Down syndrome) and 1913 women (11.8%) underwent amniocentesis. A total of 35 pregnancies were affected by Down syndrome (2.2 cases per 1000 pregnancies) and all the diagnosis were ascertained at or beyond 15 weeks of gestation or after birth. Pregnancy outcome was ascertained in 15,253 pregnancies (93.9%).

NT measurements were successful in 99.8% of cases and this was performed by abdominal ultrasound examination in 98.6%. NT increased with gestational age and a log-linear model fitted the data well (NT median $=10^{0.005342\times\text{gestational days}-0.259}$). The log means ($\pm$standard deviations) of NT MOM for affected and unaffected pregnancies were 0.25 ($\pm$0.17) and 0.00 ($\pm$0.13), respectively. NT was not correlated with maternal age, hCG and AFP, except a small significant correlation between NT MOM and hCG MOM in the Down syndrome pregnancies ($r=$ 0.363, $p=0.03$; Pearson correlation test). Figure 1 shows the ROC curves of the different methods of screening for Down syndrome. The areas under the ROC curves by different methods of screening are shown in Table 1. The differences in the areas between different methods of screening were statistically significant ($p<0.001$). The integrated test yielded the best result. Second trimester hCG and AFP screening performed slightly better than NT and the difference was statistically significant (Table 1). At a 5% false-positive rate (a risk cut-off of 1 : 320), the detection rate of Down syndrome pregnancies by the integrated test was 85.7% (95% CI, 76.2–92.1%). This was greater than the detection rate achieved with second trimester hCG, AFP and age (73.2%; 95% CI 63.4–82.9%), NT and age (69.3%; 95% CI 56–76.1%) or NT alone (60.8%; 95% CI 41.7–69.4%). Pair-wise comparisons of NT and age with hCG, AFP and age, NT and age with the integrated test,

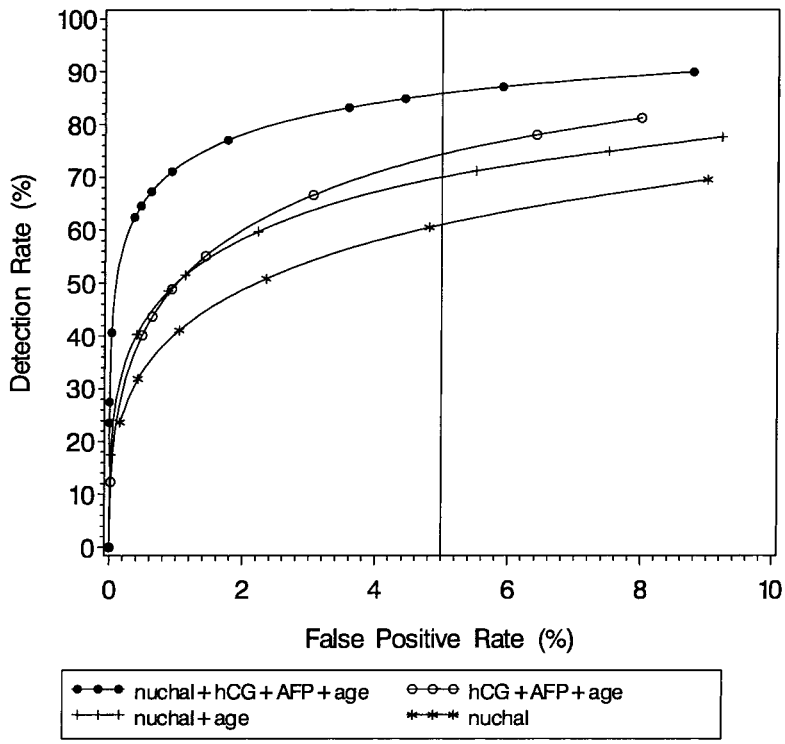


Figure 1—Receiver-operating characteristic (ROC) curves for different combinations of screening test for Down syndrome

 

Table 1—Area under the receiver-operating characteristic (ROC) curves for different methods of screening

| Screening test | Total area (95% CI) | Partial area[a] (95% CI) |
|---|---|---|
| NT and age[b] | 0.881 (0.840–0.943) | 0.065 (0.053–0.074) |
| hCG, AFP and age[b] | 0.924 (0.904–0.968) | 0.069 (0.059–0.079) |
| NT, hCG, AFP and age[b] | 0.947 (0.919–0.977) | 0.083 (0.073–0.089) |

[a]Area of the curves restricted to the zone where false-positive rates were between 0% and 10%.
[b]$p < 0.001$, non-parametric Friedman test.
AFP, α-Fetoprotein; CI, confidence intervals; hCG, human chorionic gonadotrophin; NT, nuchal translucency.

hCG, AFP and age with the integrated test showed statistically significant differences in the detection rates (pair-wise $p$ values were all $< 0.001$). Conversely, the integrated test resulted in a much lower false-positive rate than the other combinations to achieve a 70% detection of Down syndrome pregnancies (Table 2).

In the US and other places, screening for Down syndrome is primarily offered to women under 35 years of age (ACOG Committee, 1994). In the present study, 13,120 women were less than 35 years old and 12 of these pregnancies were affected by Down syndrome (0.9 cases per 1000 pregnancies). Re-analysis of the data showed that using a risk cut-off of 1 : 320 (that would result in an overall false-positive rate of 5%), the integrated test would detect 75% of the Down syndrome pregnancies at a false-positive rate of 2.6% in women less than 35 years old. Using this risk cut-off, 352 young subjects would be screen-positive of which nine were true-positives and 343 were false-positives. The odds of being affected given a positive screening result in the young subjects were 1 : 38. The corresponding detection rate, false-positive rate and odds of being affected given a positive screening result for women 35 years and older were 93.3%, 15.1% and 1 : 22, respectively. Figure 2 shows the ROC curves of the different methods of screening for Down syndrome in women less than 35 years old. The integrated test again yielded the best result.

Table 2—Efficacy of different screening methods when the false-positive rate is fixed at 5% or the detection rate is fixed at 70%

| Screening test | Detection rate (%) | False-positive rate (%) | Risk cut-off[a] |
|---|---|---|---|
| NT | 60.8 | 5 | – |
| | 70.0 | 9.4 | – |
| NT | 69.3 | 5 | 1 : 186[a] |
| | 70.0 | 5.2 | 1 : 198[b] |
| HCG, AFP and age | 73.2 | 5 | 1 : 147[a] |
| | 70.0 | 4.1 | 1 : 120[b] |
| NT, hCG, AFP and age | 85.7 | 5 | 1 : 320[a] |
| | 70.0 | 0.8 | 1 : 44[b] |

[a]Risk cut-off used when the false-positive rate is fixed at 5%.
[b]Risk cut-off used when the detection rate is fixed at 70%.
AFP, α-Fetoprotein; hCG, human chorionic gonadotrophin; NT, nuchal translucency.

## DISCUSSION

This is the first report of data on pregnancies that have undergone both first and second trimester screening without action being taken on the first trimester NT measurements. This allows a valid comparison of the screening performance of NT versus second trimester serum screening. Despite the use of a double test (hCG and AFP) that may be inferior to a test that incorporates unconjugated estriol ($uE_3$) and inhibin-A (Wald *et al*., 1999), we demonstrated that NT detects 69% of Down syndrome fetuses which is inferior to the 73% detection rate of the double test. Examination of the ROC curves confirms that the screening efficacy of NT is inferior to the double test (Figure 1). We realise the vital importance of sonographer training and auditing to the performance of NT-based screening. The tight distribution (SD = 1.3 MOM) of NT in the controls provides some evidence to this quality control. Despite this effort, the present data on NT is slightly inferior than that reported by the Fetal Medicine Foundation (Snijders *et al*., 1998). In that study 96,127 patients at both high and low risk had nuchal screening at 22 centres in the UK. At a 5% false-positive rate, 77% of the Down syndrome pregnancies were detected (Snijders *et al*., 1998). Because that was an interventional study, the majority of the Down syndrome cases were ascertained in the first trimester. It is well known that some of the Down syndrome pregnancies are spontaneously miscarried between the first and second trimesters (Morris *et al*., 1999). Detection rate will be exaggerated if NT preferentially detects cases that are more likely to miscarry. We believe that the present study provides a better estimation of the screening performance of NT because of its non-interventional design and the fact that all the Down syndrome cases were ascertained at or beyond the second trimester.

The present data show that the integrated test provides the highest screening efficacy. The detection rate can be increased from 73% to 86% at a 5% false-positive rate. However, extra resources will be needed for ultrasound examination. In the present study, it meant an extra cost of 16 237 NT measurements to detect five additional Down syndrome fetuses that would have been missed by the serum double test. Whether this is cost-effective depends largely on the cost of an ultrasound examination in the locality versus the cost of, and suffering associated with, raising a child with Down syndrome. Conversely, the integrated test reduces the false-positive rate from 5% to 0.8% to achieve the current 70% detection rate of Down syndrome pregnancies. This strategy will reduce substantially the number of unaffected pregnancies subjected to the risk of amniocentesis and hence the resultant unnecessary fetal loss. It will reduce the cost spent on unnecessary amniocentesis. Because fewer women will be labelled screen-positive, this may reduce

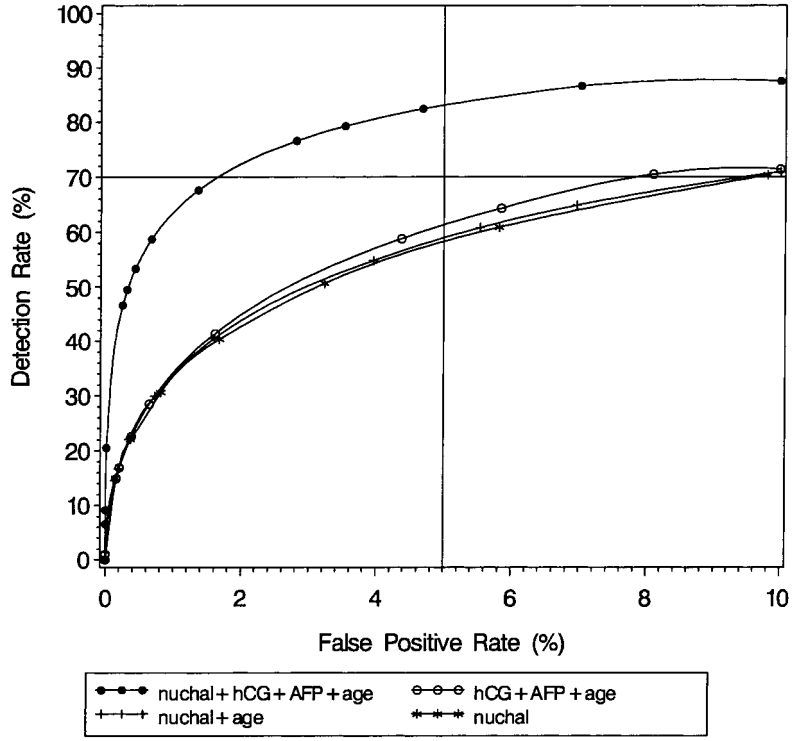


Figure 2—Receiver-operating characteristic (ROC) curves for different combination of screening for Down syndrome for women under 35 years of age

the unnecessary anxiety associated with receiving an abnormal result that may persist into the postpartum period (Marteau *et al.*, 1992). Nevertheless, cost-effectiveness was not the research question of interest to the present study and hence this issue should be addressed by further studies.

Besides the better screening efficacy, the integrated test has several other potential advantages. First, it will avoid the problem of sequential screening and the confusion created by giving first and second trimester results independently that may be contradictory. Second, the diagnostic test will be amniocentesis rather than CVS. A meta-analysis of randomised trials comparing these two invasive procedures showed that first trimester CVS was associated with more sampling and technical failures, more false-positive and false-negative results and more pregnancy losses (Alfirevic *et al.*, 2000). CVS is also considered to be a more difficult procedure to learn (Wijnberger *et al.*, 2000). Thus, it is probably easier and safer to implement the integrated test in centres with an existing second trimester screening protocol and established expertise in amniocentesis rather than moving screening into the first trimester. The strategy also allows continuation of second trimester AFP screening for neural tube defect and provides Down syndrome screening for the occasional late attendees. Third, the integrated test will avoid detecting Down syndrome cases that are destined to miscarry between the first and second trimesters.

The main disadvantage of the integrated test is the time lag between the first screening by NT and reporting of the screening results which may take up to 5 weeks and so delay the diagnosis of an affected pregnancy beyond 15 weeks of gestation. Some women may find this waiting and delay unacceptable. Conversely, some women may not return for second-trimester serum screening thus making the integration impossible. Because of these potential advantages and disadvantages of the integrated test, we believe that the women should be given the information and be allowed to choose between different methods of screening.

It is well known that both NT and the second trimester serum test can detect aneuploidies other than Down syndrome (Chitty, 1998). In the present study we have excluded pregnancies affected by other chromosomal abnormalities from analysis. Because the number of fetuses affected by each individual aneuploidy was few, we could not assess whether the integrated test was useful in detecting these cases. This issue should be addressed by further studies.

An important limitation of the present study is that we cannot ascertain pregnancy outcome in 6% of the subjects. In Hong Kong there is no Down syndrome registry, however each of the participating hospitals have a registry of birth malformations. To ensure complete ascertainment of the Down syndrome cases we have tried to contact the women individually and have reviewed the records of the cytogenetic laboratories. According to the maternal age distribution of the subjects studied and the second trimester incidence of Down syndrome (Cuckle *et al.*, 1987; Snijders *et al.*, 1999), the estimated number of Down syndrome pregnancies in the present series should be around 38, i.e. a similar figure to the ascertained cases. Hence

 

comparison of efficacy of different methods of screening in the present series should be valid.

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