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Combined ultrasound biometry, serum markers and age for Down syndrome risk estimation

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ABSTRACT

Objective *To compare Down syndrome screening efficiency of the standard serum triple analyte screen to that of a four-component screen consisting of ultrasound biometry and serum markers in the second trimester.*

Methods *The Down syndrome screening efficiency of the triple screen, i.e. alpha-fetoprotein (AFP), unconjugated estriol (E3), hCG and maternal age, was compared with the four-marker algorithm, i.e. humerus length, nuchal thickness, AFP and hCG plus maternal age. A quadrivariate Gaussian algorithm was used to calculate individual Down syndrome odds. Receiver operating characteristic (ROC) curves plotting sensitivity against false-positive rate were constructed for each algorithm and the areas under the curves were compared to determine which was superior. Sensitivity and false-positive rates at different Down syndrome risk thresholds were also compared.*

Results *There were 46 cases of Down syndrome (1.9%) with 2391 normal singleton pregnancies in a referral population in which triple screen, fetal biometry and karyotype had been done. The gestational age range for the study was 14–24 completed weeks. The median maternal age for the study group was 35.0 years (14.0–46.0 years). The areas (SE) under the ROC curves were 0.75(0.04) and 0.93(0.02) for the standard triple and the four-marker screen, respectively ($P < 0.001$). At a 10% false-positive rate, detection was 45.7% for the triple and 80.4% for the four-marker screen.*

Condusions *A new algorithm combining humerus length and nuchal thickness measurement with serum AFP, hCG and maternal age substantially improved Down syndrome screening efficiency compared with the traditional triple screen. The model appears promising and should be evaluated in an independent data set.*

INTRODUCTION

Several studies have reported using ultrasound biometry markers for adjusting Down syndrome risk[1–3]. A large recent multicenter trial of 241 Down syndrome second-trimester fetuses found that 85% of the affected group had ultrasound abnormalities[4]. Among Down syndrome cases without gross structural defects, 75% had subtle ultrasound markers or abnormal biometry. Humerus length and nuchal thickness measurements were the most important biometry markers for Down syndrome. The latter finding agreed with previously reported observations[5,6].

Maternal serum screening (including triple screen) is now widely available[7] and, apart from maternal age, is the only other officially sanctioned Down syndrome screening method[8]. By combining maternal serology and ultrasound biometry, Down syndrome screening efficiency can be improved further. Normal nuchal thickness measurements reduced Down syndrome risk in second-trimester pregnancies with an abnormal triple screen[9]. That study had limited application because only crude group risk estimates and not individual Down syndrome odds could be derived using that information. In a more recent study[10], the screening performance was improved when humerus length measurement replaced unconjugated estriol (E3) in the triple screen.

The purpose of this study was to determine the screening efficiency of a new algorithm that includes humerus length and nuchal thickness data with multiple marker serum screening because evidence points to the superiority of nuchal thickness over other biometry markers. We also compared the new algorithm with the traditional triple-marker screen.

METHODS

From January l992 to November 1997, biparietal diameter (BPD), humerus length and nuchal thickness were measured

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sonographically as described previously[1,5] in second-trimester (14–24 completed weeks) singleton pregnancies before genetic amniocentesis. Nuchal thickness was measured from the outer edge of the occipital bone to the skin surface. Serum biochemical screening was done before ultrasound in all instances. Only fetuses with Down syndrome or a normal karyotype were considered. In most cases, triple-marker serum screening tested alpha-fetoprotein (AFP), hCG and unconjugated E3. In 500 normal and four Down syndrome cases, only serum for AFP was collected at amniocentesis. The hCG and unconjugated E3 were subsequently measured from those stored (up to 2 years), masked serum specimens. The serum analytes were standardized based on menstrual dates as is the current clinical practice. AR study cases had triple-screen data. A small number of cases in which amniocentesis was declined but neonatal karyotyping was done were also included. Failure to record BPD, nuchal thickness or humerus length data, detection of a cystic hygroma or lack of serum screening were exclusionary criteria. Some of the cases in this study were used in previous reports from the database[3,5]. Twenty-five additional Down syndrome cases were added to the current report, compared with our previous study that integrated humerus length with serum screening[10]. Thirty-one of the affected fetuses are reported in the latter report[10], while 18 of the Down syndrome cases from this study were used in the multicenter trial[5]. A total of 35 of the Down syndrome cases have been reported in previous publications.

A weighted-regression equation for expected humerus length based on measured BPD was developed. Standardization of humerus length based on BPD rather than menstrual dates permits the inclusion of women with uncertain dates. The observed humerus length was divided by the expected median humerus length based on BPD to express humerus length values as multiples of the median (MoM). Down syndrome likelihood ratios based on humerus length MoM values were determined as previously reported[3]. Humerus length distributions used in the current report were recalculated based on the larger numbers of Down syndrome cases accumulated prospectively in our biometry database, i.e. 106 cases.

Using a method similar to that described for humerus length, a formula for expected nuchal thickness based on BPD was determined, and the measured nuchal thickness value was expressed as MoM[11]. In that report, log nuchial thickness MoM values for unaffected and Down syndrome cases were normally distributed. Those data were based on 66 Down syndrome cases collected prospectively and entered into our biometry database between 1990 and 1997. The same nuchal thickness distributions were used for risk calculation in the current study. The log transformed MoM values for the means and standard deviations, and correlation coefficients for the serum markers were those used in a recent publication by Wald *et al.*[12]. Humerus length, nuchal thickness, AFP and hCG MoM data were log transformed to achieve a Gaussian distribution. The median was used to approximate the mean, and to minimize the influence of outliers the standard deviation was calculated by subtracting the tenth from the 90th percentile and dividing by 2.563[12]. Stepwise logistic regression analysis was performed to see which of the biometry and serum markers were significant independent predictors of Down syndrome.

A quadrivariate Gaussian algorithm correcting for pairwise correlations[12,13] was developed using the Statistical Package for Social Sciences (SPSS, Chicago, Illinois, USA) computer program. The quadrivariate algorithm integrated the Down syndrome risk associated with the level of each of the ultrasound and serum analytes after taking into consideration the correlation between analyte pairs. An overall likelihood ratio or Down syndrome odds based on the combination of the four markers was derived. Multiplying the age-related risk by a summary likelihood ratio gave the individual Down syndrome risk based on the four markers plus maternal age. Detection and false-positive rates were calculated at different Down syndrome risk thresholds[12], which allowed us to calculate individual adjusted Down syndrome risks, based on combined multiple ultrasound and serology markers plus maternal age. The method of calculating the adjusted likelihood ratio and then multiplying the value by the age-related risk is the current and prevailing standard by which Down syndrome odds are calculated[12,13].

Receiver operating characteristic (ROC) curves plotting Down syndrome detection rate against the false-positive rate at different screening thresholds (above one in 10 to above one in 500) were generated for the traditional triple-marker screen and the combined biometry and serum marker algorithms. The areas under the ROC curves were calculated and compared to determine statistically significant differences in Down syndrome screening efficiency between the two algorithms. The Graph ROC for Windows computer package (Graph ROC for Windows, version 2.0; Department Clinical Chemistry, University of Turku, Turku, Finland) was used for the areas under the ROC curves[14]. The statistical significance of the differences of the areas under the curves was determined by calculating the Z score. The latter was determined from the area and standard error of each curve together with the estimated correlation between the areas of each curve. Using statistical tables of normal distribution the corresponding P value (two-tailed test) based on the Z score was determined[15]. A significantly larger area under either screening curve (triple screen or serum plus biometry markers) would provide statistical proof of the superiority of that algorithm. $P < 0.05$ was considered statistically significant.

RESULTS

There were 2391 study cases, 46 (1.9%) of which had Down syndrome. For normal cases the median (range) maternal age was 35.0 (14.0–46.0) years while the median (range) gestational age was 17.1 (14.0–24.0) weeks. Corresponding values for Down syndrome cases were 38.0 (17.0–44.4) years and 16.6 (15.0–21.6) weeks, respectively. Among normal cases 85.5% were white

Table 1 Ultrasound biometry and maternal serum analytes in Down syndrome prediction

Characteristic	*β-coefficient*	*SE*	P
Log_{10} nuchal thickness	11.9881	1.6885	< 0.001
Log_{10} hCG	4.1747	0.8222	< 0.001
Log $_{10}$ humerus length	− 36.989	5.8432	< 0.001
Log_{10} AFP	− 5.1813	1.0377	< 0.001
Maternal age	0.32	0.0456	< 0.001

All characteristics except age are expressed as multiples of the median. Analysis was based on stepwise logistic regression. hCG = ????; AFP = alpha-fetoprotein.

compared with 77.3% of the Down syndrome cases. Corresponding percentages for blacks were 8.8% and for others 15.9% vs. 6.8%, respectively. The racial breakdown was not statistically different between Down syndrome and normal cases ($\chi^2 = 2.849$, 2 d.f., $P = 0.241$). There were no significant differences in maternal or fetal ages between races. The equation used for median humerus length was[10]:

$$\text{Humerus length} = 6.30134 + \text{BPD} \times 0.77612.$$

Calculation of the median nuchal thickness value MoM was based on the previously published equation[11]:

$$\text{Nuchal thickness} = 0.6684 + 0.0628 \times \text{BPD}.$$

Based on stepwise logistic regression analysis the significant characteristics for detecting Down syndrome were humerus length, nuchal thickness, AFP, hCG and maternal age (Table 1). The value of the β-coefficient shows the relative contribution of each screening marker to Down syndrome risk prediction in the data set. The negative value of the β-coefficient indicates that the risk of Down syndrome increased as the particular characteristic decreased in value. The discriminating efficiency of the four-marker combination (plus age) was 98.3%. The unconjugated E3 MoM level did not contribute significantly to Down syndrome risk predictions. The same result was obtained with log transformation of the unconjugated E3 MoM value. The mean(SD) of the analytes in normal and Down syndrome cases is shown in Table 2. The pairwise correlation coefficients between analytes are shown in Table 3. The truncation limits used were: log AFP (0.3–3.3 MoM), log hCG (0.2–5.0 MoM)[12], log humerus length (0.8–1.25 MoM) and log nuchal thickness (0.8–2.0 MoM).

Table 4 compares the sensitivity and false-positive rates

Table 2 Screening values for Down syndrome markers

Parameter	*Down syndrome*	*Normal subject*
Log_{10} AFP	− 0.1427(0.1965)	0.00(0.1936)
Log_{10} hCG	0.3023(0.2606)	0.00(0.2336)
Log_{10} humerus length	− 0.024(0.028)	0.00(0.0304)
Log $_{10}$ nuchal thickness	0.099(0.140)	0.00(0.100)

Values are mean(SD). Values of analytes expressed as multiples of the median log alpha-fetoprotein (AFP) and ??? (hCG) values[12].

Table 3 Screening parameters*

Parameters	*Down syndrome (n = 46)*	*Normal (n = 2345)*
Log_{10} AFP–Log_{10} hCG†	0.1282	− 0.0199
Log_{10} AFP–log_{10} humerus	− 0.1912	− 0.0359
Log_{10} AFP–log_{10} nuchal thickness	0.2281	0.0620
Log_{10} hCG–log_{10} nuchal thickness	0.1047	− 0.0237
Log_{10} nuchal thickness–log_{10} humerus	− 0.0779	0.0475

*Correlation coefficient between pairs of markers (Down syndrome and normal groups). hCG = ????; AFP = alpha-fetoprotein; humerus = humerus length. †Reference 12.

of the triple-marker and four-marker screens at identical Down syndrome risk thresholds. The detection rate was consistently higher, while maintaining lower false-positive rates, with the four-marker algorithm. Table 5 compares the sensitivity of the two algorithms at fixed false-positive rates, showing the superiority of the four-marker algorithm.

The ROC curves plotting detection rates against the false-positive rate for the traditional triple-marker and four-marker screens are shown in Figure 1. The area under the curve (SE) for the four-marker algorithm was 0.93(0.02) and 0.75(0.04) for the traditional triple screen. The difference in the areas was highly statistically significant ($P < 0.001$), proving the superiority of four-marker screening. In a previous study we found that a combined triple-marker test that replaced unconjugated E3 with humerus length significantly improved screening performance[10]. Superior screening was also shown when the four-marker screen was compared with AFP, hCG plus humerus length (area 0.93 vs. 0.89; $P = 0.4$), confirming that the further addition of nuchal thickness data improved performance over addition of humerus length alone to the serum screen. Of 38 fetuses with gross structural anomalies on ultrasound, 26 had normal karyotypes (seven cardiac defects and 19 other anomalies, including intracranial and abdominal wall defects). Twelve had Down syndrome (eight cardiac anomalies and four others: clubbed feet, omphalocele, duodenal atresia and Dandy–Walker malformation).

Table 4 Comparison of Down syndrome screening performance: three-and four-marker screens

Down syndrome risk	*Three marker*		*Four marker*	
	Sensitivity (%)	*FPR (%)*	*Sensitivity (%)*	*FPR (%)*
≤ 1/20	37.1	4.7	51.4	1.8
≤ 1/40	51.4	9.3	65.7	3.7
≤ 1/70	57.1	10.8	71.4	4.9
≤ 1/80	62.9	18.3	77.1	5.7
≤ 1/100	71.7	30.0	78.3	7.9
≤ 1/190	84.8	54.3	87.0	14.0
≤ 1/250	84.8	54.3	93.5	20.4

FPR = false-positive rate (1 – specificity); three-marker screen = alpha-fetoprotein (AFP), hCG and unconjugated estriol plus age; four-marker screen = AFP, hCG, humerus length and nuchal thickness plus age.

Table 5 Down syndrome detection rate at fixed false-positive rates: three- and four-marker screens

	Detection rate (%)	
FPR	*Three marker*	*Four marker*
1.0	4.4	39.1
3.0	30.4	54.4
5.0	37.0	67.4
7.0	41.3	71.7
9.0	45.7	78.3
10.0	45.7	80.4
15.0	50.0	87.0
25.0	60.9	95.6

FPR = false positive rate; three-marker screen = alpha-fetoprotein (AFP), hCG and unconjugated estriol plus age; four-marker screen = AFP, hCG, humerus length and nuchal thickness plus.

After elimination of these 38 cases, the area under the ROC curve was still significantly larger for the four-marker vs. the traditional triple screen (0.94 vs. 0.80; $P = 0.001$). When cases in which only AFP was measured initially were eliminated and the comparisons redone, the four-marker screen was still superior to the triple screen.

Finally, the addition of serum data significantly improved the screening performance over that of the individual biometry markers. The area under the curve for age plus nuchal thickness vs. the four-marker screen was 0.80 vs. 0.93 ($P = 0.003$), while values for humerus length plus age vs. the four-marker screen were 0.82 vs. 0.93 ($P = 0.019$).

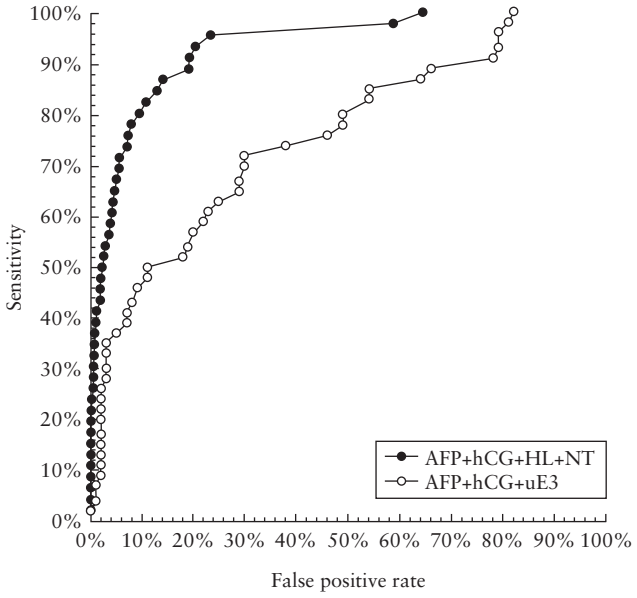

Figure 1 Receiver operating characteristics curves of the traditional triple screen and the four-marker screen (AFP = alpha-fetoprotein, hCG, NT = nuchal thickness, HL = humerus length, uE3 = unconjugated serum estriol, and maternal age) for Down syndrome screening in the second trimester. On the *x*-axis false-positive rate (100 − specificity in percentage) is given and on the *y*-axis the sensitivity is represented.

DISCUSSION

Triple analyte serum screening, the current gold standard, is widely used[7] and apart from maternal age is the only officially sanctioned prenatal screening test for Down syndrome. A recent collaborative trial[4] found that second-trimester ultrasound markers were sensitive for detecting Down syndrome. The number of Down syndrome cases reported in that study, 241, exceeded that of almost all previous studies related to biochemical or ultrasound screening for that aneuploidy. In an earlier report we observed that substituting humerus length data for unconjugated E3 improved risk prediction over triple-marker serum screen[9]. It has also been shown that normal nuchal thickness (under 6 mm), normal humerus length (observed divided by expected value of at least 0.92) and normal fetal structural survey significantly reduced the risk of Down syndrome and clinically significant chromosome defects in pregnancies with triple-screen risks of at least one in 270, or increased risk of trisomy 18[16]. In the present study, we hypothesized that by combining information from the two most important biometry markers, humerus length and nuchal thickness, with serum markers to determine individual pregnancy risk, we could improve screening efficiency. This hypothesis was confirmed. Comparison of the areas under the screening curves confirmed a highly statistically significant improvement in performance of the four- over the triple-marker screening. Unconjugated E3 lacked significance when all screening indices were considered. We found that adding nuchal thickness data to the serum markers further improved efficiency over adding humerus length data alone.

Conversion of humerus length and nuchal thickness data to MoM values based on BPD has two important advantages, allowing us to handle biometry data in a mathematically identical fashion to the serum markers. This makes possible adjusted likelihood ratio calculations based on a multivariate Gaussian algorithm. That algorithm currently forms the basis of triple-marker serum screening. Individual risk estimation is more desirable than grouping subjects into broad categories such as low and high risk[17]. Errors in fetal dating, usually an important factor in Down syndrome screening, were eliminated with reliance on measured BPD rather than menstrual dates.

The potential problems raised by inability to take appropriate biometry measurements in individual subjects need to be addressed further. In almost all instances, such difficulties are a result of fetal position. They can be surmounted by employing a number of strategies such as repeating the attempt at measurement after the woman has ambulated for a brief period or rescheduling the examination for another day. Transvaginal imaging usually overcomes the difficulty in measuring nuchal thickness or BPD due to low fetal head position. The inconvenience of such strategies might not be justified for a study, but they would be justified if combined biometry plus biochemical screening became accepted clinical practice.

The Down syndrome screening performance of combined

biochemical and ultrasound markers has been reported for the first trimester[18]. In that report, Noble *et al.*, using nuchal translucency measurement, reported an 80% detection rate and a 5% false-positive rate. When maternal serum-free β-hCG data were added, the detection rate increased to 85%. In addition to the multicenter trial[9], other studies from individual centers reported high sensitivity values (87% and 92.8%) using multiple sonographic markers for detecting Down syndrome in the second trimester[19,20]. DeVore and Alfi[19] used sophisticated color Doppler techniques to aid diagnosis of cardiac defects in Down syndrome, whereas Vintzileos *et al.*[20] used several other sonographic markers with humerus length and nuchal thickness for detecting affected fetuses. As we limited our study to relatively simple and more objective measurements it underestimates the full capabilities of ultrasound diagnosis.

We compared screening efficiency of two protocols in the same women, a high-risk population referred for genetic amniocentesis. Consistently higher sensitivities and lower false-positive rates were achieved with the four-marker screen. Even if higher than the currently reported triple-test screening performance (higher sensitivity and lower false-positive rates) were achieved in our study population, the added advantage of biometry is still likely to ensure superiority of the four-marker screen. The superior performance of the four-marker screen should hold in a low-risk group, although the absolute values of the screening characteristics (e.g. sensitivity, false-positive rate and positive predictive values) are likely to change. Concerns have been raised about whether variability associated with ultrasound biometry would prevent its application as a screening method. The measurements chosen, humerus length and nuchal thickness, are commonly measured in tertiary centers and are not difficult to perform. The results of a large collaborative study involving eight institutions confirmed that, despite the potential for variability in measurements, high Down syndrome screening efficiency of ultrasound markers can be maintained[4]. Use of screening ultrasound on a large scale appears to be feasible. The biometry measurements could also be made in non-referral centers after appropriate training of ultrasound personnel. The current cost of an ultrasound study hinders realization of a combined screening protocol as we described in a low-risk population. Repeating this study in a population in which the risk level is similar to that of the entire population of women who currently have triple-marker screening would be desirable. In a high-risk population, ultrasound evaluation is almost always performed before amniocentesis. There would be no extra costs associated with our algorithm in a high-risk population to further improve Down syndrome risk estimations.

We found that the new algorithm for estimating individual Down syndrome odds significantly improved Down syndrome screening efficiency over traditional triple-marker screening. Our paradigm permits the integration of any group of markers. We have previously shown that ultrasound can be combined with urine analytes[21] for Down syndrome screening. Preliminary data suggest that individual urine markers have superior Down syndrome screening performance than traditionally reported for serum markers[22]. Our results at this time must be regarded as preliminary. Prospective studies in which subjects and sonographers are masked to the results of the serum screens, and in which posterior combined risk is determined before amniocentesis, are desirable to confirm the clinical value of the new algorithm.

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