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Second-trimester soft markers: relation to first-trimester nuchal translucency in unaffected pregnancies

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KEYWORDS: Down syndrome; nuchal translucency; prenatal; sequential screening; soft-markers; sonography

ABSTRACT

Objective *Genetic sonography following first-trimester combined screening appears to increase substantially detection rates for Down syndrome but it relies on the unproved assumption of independence between these tests. In this study we aimed to investigate the relationship between first-trimester nuchal translucency (NT) and a series of second-trimester soft markers and structural defects in unaffected pregnancies.*

Methods *NT measurement in the first trimester was followed by second-trimester scan (18 to 23 + 6 weeks) including examination for three categorical markers (intracardiac echogenic foci, hyperechogenic bowel and structural defects) and measurement of nasal bone length, nuchal-fold thickness, femur length, humerus length, renal pelvis diameter and prenasal thickness. All continuous variables were expressed in multiples of the median (MoM) for gestation and correlation coefficients between log-transformed NT and second-trimester variables were calculated. In addition, frequencies of soft markers and structural defects in cases with increased NT were compared to those with normal NT, using MoM cut-offs.*

Results *In a dataset of 1970 cases, NT was significantly correlated ($P < 0.05$) with all second-trimester continuous variables, the correlation being strongest for nuchal-fold thickness ($r = 0.10$). There was a higher frequency of cases with second-trimester nuchal-fold thickness above the 97.5th centile (10.7 vs. 2.2%) and hyperechogenic bowel (2.4 vs. 0.1%) in cases with increased NT.*

Conclusions *Straightforward reassessment of risk using likelihood ratios derived from the second-trimester genetic sonogram might lead to inaccurate estimates. Multivariate models using continuous second-trimester variables might be preferable in sequential screening strategies. Copyright © 2012 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Down syndrome screening has shifted from the second to the first trimester in recent years. First-trimester combined screening offers both better performance and the advantages of providing earlier reassurance or a safer termination of pregnancy, if required[1–3]. Nevertheless, first-trimester sonography has not obviated the need for a second-trimester scan to exclude major structural defects and other recognizable complications of pregnancy[4].

During second-trimester sonography, a series of 'soft markers' or major structural defects are reported in 70–75% of Down syndrome cases[4–6]. The presence or absence of such findings can be used to modify the background risk by applying positive or negative likelihood ratios[4–6]. The use of this approach in sequential screening strategies, both in models[7] and in prospective interventional studies[8–10], has resulted in identification of about 50% of Down syndrome cases that had remained undetected following first-trimester combined screening, with minimal impact on false-positive rates[7–10].

The use of second-trimester likelihood ratios relies on the assumption of independence between first- and second-trimester markers, an assumption that has not been fully investigated[4]. In fact, it would be reasonable to expect some kind of relationship between them. Cardiac defects and echogenic foci, for instance, are known to be associated with increased nuchal translucency (NT)[11,12]. On the other hand, NT and second-trimester nuchal-fold thickness, despite presumably sharing a similar pathophysiology, were not found to be related in three independent studies[13–15].

More recently, theoretical models incorporating highly discriminatory soft markers (nuchal-fold thickness, nasal bone length and prenasal thickness) have predicted even higher detection rates (81% for a 5% false-positive rate)[16–19]. However, correlations between these second-

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and first-trimester markers have not been described, precluding their incorporation into sequential screening protocols.

In this study we investigated the relationship between first-trimester NT and a series of second-trimester soft markers and structural defects in unaffected pregnancies, in order to obtain reliable parameters for planning sequential screening strategies.

METHODS

This was a prospective cohort study including viable singleton pregnancies, scanned from 11 to 13 + 6 weeks' gestation (crown–rump length (CRL) 45–84 mm) from August 2007 to December 2009 at the University of São Paulo Obstetrics and Gynecology Department. Informed consent was obtained in all cases and the study was approved by the local ethics committee.

NT measurement was performed by Fetal Medicine Foundation-accredited examiners, using previously described guidelines[20]. Three different ultrasound machines were used: Voluson 730 Expert (GE Healthcare, Milwaukee, WI, USA), Voluson 730 Pro (GE Healthcare) and Envisor (Philips Healthcare, Eindhoven, The Netherlands). Gestation was dated based on CRL[21], and all patients were offered a second appointment for an anomaly scan between 18 and 23 + 6 weeks. Follow-up was obtained by letter, phone contact or search in the local cytogenetic database or hospital medical records. Exclusion criteria were: cases showing major structural defects during the first-trimester scan; prenatal or postnatal diagnosis of chromosomal abnormalities; failure to return at 18 to 23 + 6 weeks; pregnancy loss at any stage; lack of follow-up; and stillbirth.

The second-trimester ultrasound examiners were blinded to the first-trimester results. Second-trimester fetal anatomical survey was followed by a search for a number of soft markers, including categorical and continuous variables, evaluated using previously described standard techniques[4–6,9,19,22]. Categorical variables were: presence or absence of intracardiac echogenic foci (in the apical four-chamber view), hyperechogenic bowel (as bright as the iliac bone) and structural defects. Continuous variables were: nasal bone length, nuchal-fold thickness, femur and humerus length, largest renal pelvis anteroposterior diameter and prenasal thickness.

All continuous variable measurements, including first-trimester NT, were converted into multiples of the gestation-specific normal median (MoMs) using previously described techniques[23,24]. In short, fetuses were divided into 12 roughly equal gestational-age (GA) groups and the median in each group was regressed against the median GA, weighted by the number of women in that group. NT measurements were converted into MoMs using regression of the medians for the nearest integer CRL value. The best-fit polynomial regression model for each variable was chosen and medians were log-transformed if appropriate. Standard deviations (SD) for log-transformed MoM variables were estimated robustly, by the 90^{th}–10^{th} centile range divided by 2.563[23].

Log-transformed NT-MoMs were then plotted against log-transformed continuous variable MoMs. Whenever a statistically significant correlation was found ($P \leq 0.05$), the Pearson correlation coefficient was calculated. Measurements less than or greater than 3 SD from the median were excluded to avoid interference of extreme values (outliers) on the correlation coefficient.

Statistical analysis was performed using 'Analyse-it standard-edition' (Analyse-it Software, Ltd, Leeds, UK), an add-in software for Microsoft Excel (Microsoft, Redmond, USA). The minimum sample size necessary for finding a significant correlation coefficient was calculated, assuming a small effect size ($r = 0.10$) for an 80% power and a 0.05 alpha risk, as being 783 cases[25].

Subsequently, the frequency of soft markers (increased nuchal-fold thickness, renal pyelectasis, short femur, short humerus, hyperechogenic bowel, echogenic focus) and structural defects in the group with increased NT (above the 95^{th} centile) was compared with the group with normal NT (Fisher's or chi-square test), using MoM cut-offs: 2.5^{th} (short femur or humerus) or 97.5^{th} centiles (nuchal fold and renal pelvis).

RESULTS

From the initial population of 2257 pregnancies there were 287 exclusions, resulting in a final dataset of 1970 cases. Reasons for exclusions were: failure to return for the 18 to 23 + 6-week scan ($n = 131$), pregnancy loss/stillbirth ($n = 63$), no postnatal follow-up ($n = 55$), first-trimester major abnormalities ($n = 20$), chromosomal abnormalities ($n = 17$) and bilateral renal agenesis ($n = 1$).

First-trimester scan was performed from 11 to 11 + 6 weeks in 19.4%, from 12 to 12 + 6 weeks in 52.8% and from 13 to 13 + 6 weeks in 27.8%. Median CRL was 61.8 mm and median NT 1.65 mm, which was 0.04 mm below the expected median according to the Fetal Medicine Foundation algorithm[26]. NT measurement medians fitted a quadratic regression model well ($NT = -1.641 + (0.08325 \times GA) - (0.0004927 \times GA^2)$). After MoM conversion, NT median was 0.9957 and $\log_{10}$SD was 0.0904.

Second-trimester scan was performed from 18 to 19 + 6 weeks in 20.9%, from 20 to 21 + 6 weeks in 71.2% and from 22 to 22 + 6 weeks in 7.9% of cases. All MoM-converted continuous-variable medians (nasal bone length, femur length, humerus length, largest renal pelvis, prenasal thickness and nuchal-fold thickness) were close to 1.0 and fitted a log-normal distribution as assessed by histograms and QQ plots. Table 1 summarizes their distribution parameters. A statistically significant correlation between log NT-MoMs and all second-trimester continuous variables was found (Table 1). The Pearson correlation coefficient was then calculated for each variable. This was low in all cases (Table 1), the highest being that of nuchal-fold thickness ($r = 0.10$; Figure 1).

Table 1 Distribution parameters of continuous variables, correlation coefficients between log-transformed second-trimester markers (expressed as multiples of the median (MoM)) and log-transformed first-trimester nuchal translucency thickness (MoMs) and their respective level of significance

Marker	n	*Mean (mm)*	*Median (MoM)*	*log_{10}SD (MoM)*	*Correlation coefficient (95% CI)*	P
Nasal bone length (NB)	1957	5.82	0.9956	0.0537	−0.05 (−0.09 to −0.002)	0.043
Femur length (FL)	1970	33.4	0.9986	0.0225	0.05 (0.01 to 0.10)	0.017
Humerus length (HL)	1888	32.0	0.9965	0.0242	0.05 (0.002 to 0.09)	0.041
Largest renal pelvis (RP)	1889	2.4	1.0018	0.1857	0.07 (0.03 to 0.12)	0.002
Prenasal thickness (PT)	1062	3.4	1.0048	0.0687	0.09 (0.03 to 0.15)	0.005
Nuchal fold thickness (NF)	1970	4.1	1.0039	0.0796	0.10 (0.06 to 0.15)	< 0.001

MoMs were computed from regressed medians for gestational age (GA) (weeks): NB = $10^{\wedge}(0.9639 - (0.0468 \times GA) + (0.001792 \times GA^2))$; FL = $-76.41 + (7.971 \times GA) - (0.1279 \times GA^2)$; HL = $-43.57 + (4.964 \times GA) - (0.06246 \times GA^2)$; RP = $-15.73 + (1.654 \times GA) - (0.03795 \times GA^2)$; PT = $-2.543 + (0.2888 \times GA)$; NF = $0.578 + (0.06403 \times GA) + (0.005201 \times GA^2)$.

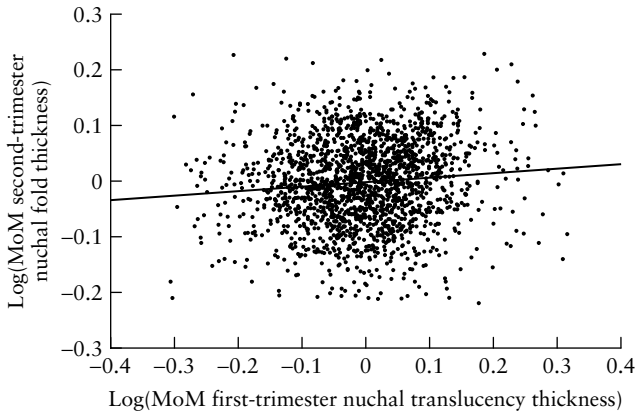

Figure 1 Scatterplot of first-trimester nuchal translucency thickness vs. second-trimester nuchal-fold thickness (given as log-transformed multiples of the median (MoM) for gestation), showing a small ($r = 0.10$) although significant ($P < 0.001$) positive correlation.

The relationship between NT in the first trimester and nuchal-fold thickness in the second was further demonstrated by the higher frequency of cases ($P < 0.01$) with second-trimester nuchal edema (> 97.5th centile) in the group of fetuses with increased NT (Table 2): 10.7% (9/84) vs. 2.2% (41/1886). The same was true ($P < 0.01$) when we used a 6-mm nuchal-fold cut-off: 7.1% (6/84) vs. 0.9% (17/1886).

Hyperechogenic bowel was also found to be more frequent ($P = 0.02$) in cases with increased NT (Table 2). The frequency of other classical soft markers did not diverge significantly among cases with normal or increased NT. However, the presence of at least one marker was significantly more prevalent (30.8 vs. 15.5%) in cases with increased NT (Table 2).

DISCUSSION

The present study has demonstrated a relationship between first-trimester NT and a series of second-trimester soft markers, the most significant of which was nuchal-fold thickness. Despite the correlation coefficient being only 0.10, this resulted in a 4–5-fold increase in the number of cases with increased nuchal-fold thickness in the group with NT above the 95th centile.

These findings have major implications for sequential screening strategies involving second-trimester sonography. Previous studies have speculated that second-trimester nuchal-fold edema and first-trimester NT may represent a different underlying etiology, allowing a straightforward reassessment of risk using likelihood ratios derived from previously unscreened populations[13–15]. These studies showed no significant correlation between NT and nuchal fold but they lacked the power to exclude a small correlation coefficient[13–15].

The results from this study suggest that modifying first-trimester Down-syndrome risk by using fixed likelihood ratios derived from the second-trimester genetic sonogram findings may lead to inaccurate estimates. This calculation depends critically on the assumption that the first-trimester markers and the anomaly scan results are independent predictors of risk. The fact that the presence of soft markers was twice as common in cases with increased NT implies that this assumption is incorrect.

In fact, none of the classical soft markers seemed to be independent of the first-trimester scan: nuchal edema and echogenic bowel were found in this study to be more prevalent in fetuses with increased NT. Other continuous soft markers (femur and humerus length and renal pelvis diameter) were found to be positively correlated with first-trimester NT. The remaining two markers (structural defects and echogenic focus) have previously been linked to increased NT. Cardiac defects are known to be associated with markedly enlarged NT[11]. Similarly, echogenic foci were found, in a large series involving 259 fetuses with NT above the 95th centile, to be statistically more prevalent in such fetuses (8.1%) than in those with normal NT (2.9%)[12]. The fact that our study failed to demonstrate such associations could be because of an insufficient number of cases with an increased NT ($n = 84$).

Concerns about the validity of using genetic sonogram likelihood ratios to modify first-trimester results are further exacerbated by their lack of standardization. In fact, there are wide variations in the definition of individual markers and in the reported likelihood ratios

Table 2 Comparison of second-trimester soft-marker frequencies in cases with normal nuchal translucency (NT) and in those with increased NT (> 95th centile), stratified according to whether NT was < or ≥ 3 mm

Marker	*Frequency in cases with normal NT (≤ 95th centile)*	*Frequency in cases with NT > 95th centile*			P*
		NT > 95th centile	*NT from 95th centile to 2.9 mm*	*NT ≥ 3.0 mm*	
Nuchal edema (> 97.5th percentile)	41/1886 (2.2)	9/84 (10.7)	3/61 (4.9)	6/23 (26.1)	< 0.01†
Short humerus (< 2.5th percentile)	46/1886 (2.4)	2/82 (2.4)	1/59 (1.7)	1/23 (4.3)	1.00
Short femur (< 2.5th percentile)	46/1886 (2.4)	4/84 (4.8)	4/61 (6.6)	—	0.32
Renal pyelectasis (> 97.5th percentile)	44/1810 (2.4)	4/79 (5.1)	3/58 (5.2)	1/21 (4.8)	0.28
Intracardiac echogenic focus	180/1885 (9.5)	9/84 (10.7)	8/61 (13.1)	1/23 (4.3)	0.72†
Hyperechogenic bowel	2/1886 (0.1)	2/84 (2.4)	2/61 (3.3)	—	0.02
Structural defect	16/1886 (0.8)	1/84 (1.2)	—	1/23 (4.3)	1.00
Any finding	272/1750 (15.5)	24/78 (30.8)	17/57 (29.8)	7/21 (33.3)	< 0.01†

Data given as *n* (%). *Comparisons between cases with normal NT and cases with NT > 95th centile only; Fisher's test used except where indicated. †Chi-square test.

associated with each of these markers, individually or in combination[27]. The examination is largely subjective, therefore it is difficult to ensure quality.

Lau and Evans[27] argued that second-trimester sonographic screening programs should focus on the evaluation of a few strong markers. Most soft markers are too weak or subjective to be useful in the post first-trimester screening era. Some more recently described second-trimester soft markers – nasal bone length and prenasal thickness – along with nuchal-fold thickness seem to be a better choice[16]. These are all objective, simple measurements that can be interpreted as continuous variables, and are, therefore, amenable to external audit and quality assurance. It has been shown that these markers, like NT, are best expressed in MoMs and likelihood ratios calculated continuously from a log-Gaussian distribution[17,28,29]. Statistical modeling using nasal bone length, prenasal thickness and nuchal-fold thickness has predicted large detection rates and, if confirmed in prospective studies, this would have important implications for health planners[16].

This study also found some correlation between first-trimester NT and both prenasal thickness and nasal bone length. Although independency between the first and second trimesters is necessary for interpreting these variables dichotomously (with an increase defined against a fixed cut-off in millimeters), multivariate Gaussian techniques have long been used to deal with correlations between variables in multimarker serum screening, and this does not preclude their use in sequential screening[17]. In fact, it is widely thought that correlations between screening markers will tend to degrade screening performance. However, recent evidence has demonstrated that these correlations can either decrease or increase screening performance, provided a multivariate model is used[30]. In practice, these effects are usually modest, because most screening markers are not highly correlated with each other[30].

In conclusion, the findings of this study have two important practical implications. First, we should be cautious about re-evaluation of the risk assessed in the first trimester based on the currently published second-trimester sonographic-marker likelihood ratios, as this could result in inaccurate estimates. Second, reliable sequential screening strategies involving sonographic markers would almost certainly require multivariate risk analysis.

The present study has provided all the necessary parameters in unaffected pregnancies (median, SDs and correlation coefficients) to construct a multivariate risk model involving first-trimester NT, second-trimester nasal bone length, prenasal thickness and nuchal-fold thickness. However, more information is needed on correlations between these variables and other first-trimester markers in both normal and Down syndrome fetuses, to evaluate how they might be incorporated into contingent screening protocols.

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REFERENCES

1. Bindra R, Heath V, Liao A, Spencer K, Nicolaides KH. One-stop clinic for assessment of risk for trisomy 21 at 11–14 weeks: a prospective study of 15 030 pregnancies. *Ultrasound Obstet Gynecol* 2002; **20**: 219–225.
2. Malone FD, Canick JA, Ball RH, Nyberg DA, Comstock CH, Bukowski R, Berkowitz RL, Dar P, Dugoff L, Craigo SD, Timor IE, Carr SR, Wolfe HM, Dukes K, Canick JA, Malone FD. First-trimester or second-trimester screening, or both, for Down's syndrome. *N Engl J Med* 2005; **353**: 2001–2011.
3. ACOG Committee on Practice Bulletins. ACOG Practice Bulletin No. 77: screening for fetal chromosomal abnormalities. *Obstet Gynecol* 2007; **109**: 217–227.
4. Nicolaides KH. Screening for chromosomal defects. *Ultrasound Obstet Gynecol* 2003; **21**: 313–321.
5. Nyberg DA, Souter VL, El-Bastawissi A, Young S, Luthhardt F, Luthy DA. Isolated sonographic markers for detection of

fetal Down syndrome in the second trimester of pregnancy. *J Ultrasound Med* 2001; **20**: 1053–1063.
6. Bromley B, Lieberman E, Shipp TD, Benacerraf BR. The genetic sonogram: a method of risk assessment for Down syndrome in the second trimester. *J Ultrasound Med* 2002; **21**: 1087–1096; quiz 1097–1098.
7. Krantz DA, Hallahan TW, Macri VJ, Macri JN. Genetic sonography after first-trimester Down syndrome screening. *Ultrasound Obstet Gynecol* 2007; **29**: 666–670.
8. Salomon LJ, Chevret S, Bussieres L, Ville Y, Rozenberg P. Down syndrome screening using first-trimester combined tests and contingent use of femur length at routine anomaly scan. *Prenat Diagn* 2010; **30**: 783–789.
9. Aagaard-Tillery KM, Malone FD, Nyberg DA, Porter TF, Cuckle HS, Fuchs K, Sullivan L, Comstock CH, Saade GR, Eddleman K, Gross S, Dugoff L, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, Bianchi DW, D'Alton ME; First and Second Trimester Evaluation of Risk Research Consortium. Role of second-trimester genetic sonography after Down syndrome screening. *Obstet Gynecol* 2009; **114**: 1189–1196.
10. Rozenberg P, Bussieres L, Chevret S, Bernard JP, Malagrida L, Cuckle H, Chabry C, Durand-Zaleski I, Bidat L, Lacroix I, Moulis M, Roger M, Jacquemot MC, Bault JP, Boukobza P, Boccara P, Vialat F, Giudicelli Y, Ville Y. Screening for Down syndrome using first-trimester combined screening followed by second-trimester ultrasound examination in an unselected population. *Am J Obstet Gynecol* 2006; **195**: 1379–1387.
11. Atzei A, Gajewska K, Huggon IC, Allan L, Nicolaides KH. Relationship between nuchal translucency thickness and prevalence of major cardiac defects in fetuses with normal karyotype. *Ultrasound Obstet Gynecol* 2005; **26**: 154–157.
12. Prefumo F, Presti F, Thilaganathan B, Carvalho JS. Association between increased nuchal translucency and second trimester cardiac echogenic foci. *Obstet Gynecol* 2003; **101**: 899–904.
13. Salomon LJ, Bernard JP, Taupin P, Benard C, Ville Y. Relationship between nuchal translucency at 11–14 weeks and nuchal fold at 20–24 weeks of gestation. *Ultrasound Obstet Gynecol* 2001; **18**: 636–637.
14. Pandya PP, Snijders RJ, Johnson S, Nicolaides KH. Natural history of trisomy 21 fetuses with increased nuchal translucency thickness. *Ultrasound Obstet Gynecol* 1995; **5**: 381–383.
15. Maymon R, Zimerman AL, Weinraub Z, Herman A, Cuckle H. Correlation between nuchal translucency and nuchal skinfold measurements in Down syndrome and unaffected fetuses. *Ultrasound Obstet Gynecol* 2008; **32**: 501–505.
16. Miguelez J, Moskovitch M, Cuckle H, Zugaib M, Bunduki V, Maymon R. Model-predicted performance of second-trimester Down syndrome screening with sonographic prenasal thickness. *J Ultrasound Med* 2010; **29**: 1741–1747.
17. Cuckle HS, Benn PA. Multi-Marker Maternal Serum Screening for Chromosomal Abnormalities. In *Genetic Disorders and the Fetus: Diagnosis, Prevention and Treatment* (6th edn), Milunsky A, Milunsky JM (eds). Wiley–Blackwell: Oxford, 2010; 771–818.
18. Maymon R, Levinsohn-Tavor O, Cuckle H, Tovbin Y, Dreazen E, Wiener Y, Herman A. Second trimester ultrasound prenasal thickness combined with nasal bone length: a new method of Down syndrome screening. *Prenat Diagn* 2005; **25**: 906–911.
19. Persico N, Borenstein M, Molina F, Azumendi G, Nicolaides KH. Prenasal thickness in trisomy-21 fetuses at 16–24 weeks of gestation. *Ultrasound Obstet Gynecol* 2008; **32**: 751–754.
20. Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. *Am J Obstet Gynecol* 2004; **191**: 45–67.
21. Hadlock FP, Shah YP, Kanon DJ, Lindsey JV. Fetal crown–rump length: reevaluation of relation to menstrual age (5–18 weeks) with high-resolution real-time US. *Radiology* 1992; **182**: 501–505.
22. Bunduki V, Ruano R, Miguelez J, Yoshizaki CT, Kahhale S, Zugaib M. Fetal nasal bone length: reference range and clinical application in ultrasound screening for trisomy 21. *Ultrasound Obstet Gynecol* 2003; **21**: 156–160.
23. Reynolds T. Screening by test combination: a statistical overview. In *Screening for Down's Syndrome*, Grudzinskas JG, Chard T, Chapman M, Cuckle H (eds). Cambridge University Press: Cambridge, 1994; 47–71.
24. Reynolds TM, Penney MD. The mathematical basis of multivariate risk screening: with special reference to screening for Down's syndrome associated pregnancy. *Ann Clin Biochem* 1990; **27**: 452–458.
25. Cohen J. A power primer. *Psychol Bull* 1992; **112**: 155–159.
26. Wright D, Kagan KO, Molina FS, Gazzoni A, Nicolaides KH. A mixture model of nuchal translucency thickness in screening for chromosomal defects. *Ultrasound Obstet Gynecol* 2008; **31**: 376–383.
27. Lau TK, Evans MI. Second-trimester sonographic soft markers: what can we learn from the experience of first-trimester nuchal translucency screening? *Ultrasound Obstet Gynecol* 2008; **32**: 121–125.
28. Benn PA, Kaminsky LM, Ying J, Borgida AF, Egan JF. Combined second-trimester biochemical and ultrasound screening for Down syndrome. *Obstet Gynecol* 2002; **100**: 1168–1176.
29. Borrell A, Mercade I, Casals E, Borobio V, Seres A, Soler A, Fortuny A, Cuckle H. Combining fetal nuchal fold thickness with second-trimester biochemistry to screen for trisomy 21. *Ultrasound Obstet Gynecol* 2007; **30**: 941–945.
30. Morris JK, Wald NJ. The effect of correlations between screening markers on screening performance. *J Med Screen* 2007; **14**: 151–157.

