Ultrasound Obstet Gynecol 2008; **31**: 15–19
Published online 12 November 2007 in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/uog.5165

First-trimester biochemical markers of aneuploidy and the prediction of small-for-gestational age fetuses

K. SPENCER*†, N. J. COWANS*, K. AVGIDOU†, F. MOLINA† and K. H. NICOLAIDES†

**Prenatal Screening Unit, Clinical Biochemistry Department, Harold Wood Hospital, Romford, Essex and †Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK*

KEYWORDS: adverse pregnancy outcome; fetal growth restriction; free β-hCG; NT; PAPP-A; prenatal screening; trisomy

ABSTRACT

Objectives *To examine the clinical utility of the first-trimester biochemical markers of aneuploidy in their ability to predict subsequent delivery of a small-for-gestational age (SGA) infant.*

Methods *We examined singleton pregnancies with no chromosomal abnormality and with complete outcome data that had undergone screening for trisomy 21 by a combination of fetal nuchal translucency (NT) thickness and maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11 + 0 and 13 + 6 weeks' gestation. The biochemical markers were converted to multiples of the expected normal median (MoM) for a pregnancy of the same gestation. The association between free β-hCG and PAPP-A and the incidence of SGA were assessed by comparing the relative incidence at MoM cut-offs and birth-weight centile cut-offs. At various marker levels the likelihood ratios (LR) for SGA were also calculated after excluding other adverse pregnancy complications.*

Results *There were 46 262 pregnancies resulting in live births with birth weight at or above the 10th centile, and 3539 below the 10th centile for gestation (SGA). There was a significant inverse association between the risk for SGA and maternal serum PAPP-A MoM but not free β-hCG MoM. At the 5th centile of the normal outcome group for PAPP-A (0.415 MoM) the odds ratios for SGA below the 10th, 5th and 3rd centiles of normal were 2.70, 3.21 and 3.66 and the respective detection rates for SGA were 12.0%, 14.0% and 16.0%.*

Conclusions *Low levels of maternal serum PAPP-A are associated, in the absence of an abnormal karyotype, with an increased risk for subsequent delivery of an SGA infant.*

INTRODUCTION

In the first trimester of pregnancy the placentally-derived biochemical markers pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) are increasingly being used in conjunction with the ultrasound measurement of nuchal translucency thickness (NT) as part of screening programs for trisomy 21 and other aneuploidies in which approximately 90% of such anomalies can be identified[1–7] for a false positive rate of 5%. Preliminary studies[8] have shown that reduced levels of these biochemical markers – particularly PAPP-A – may be of potential value in identifying those pregnancies that may result in adverse outcome, including the delivery of a small-for-gestational age (SGA) infant.

In this study we examine the clinical utility of free β-hCG and PAPP-A measurements in their ability to predict SGA in a large cohort of women prospectively screened during the first trimester.

METHODS

All the women booked for maternity care at the following UK hospitals were offered screening for trisomy 21 by a combination of fetal NT and maternal serum free β-hCG and PAPP-A at 11 + 0 to 13 + 6 weeks' gestation: Harold Wood Hospital, Romford (between June 1998 and December 2003); King George Hospital, Goodmayes (between July 2001 and December 2003); Kent and Canterbury Hospital, Canterbury (between July 2002 and December 2003); William Harvey Hospital,

Correspondence to: Prof. K. Spencer, Prenatal Screening Unit, Clinical Biochemistry Department, King George Hospital, Barley Lane, Goodmayes, Essex, IG3 8YB, UK (e-mail: KevinSpencer1@aol.com)

Accepted: 20 August 2007

Ashford (between July 2002 and December 2003); Queen Elizabeth The Queen Mother's Hospital, Margate (between July 2002 and December 2003); King's College Hospital, London (between January 1999 and February 2000); and those attending The Fetal Medicine Centre, London (between July 1999 and December 2003). They received an information leaflet about the service and gave details about their demographic characteristics and medical history, which were entered into a computer database (PIA-Fetal Database, ViewPoint, Webling, Germany).

Maternal serum free β-hCG and PAPP-A were measured using the Kryptor analyzer (Brahms AG, Berlin, Germany) as previously described[1] and an ultrasound examination was carried out to measure fetal NT and crown–rump length (CRL) and to diagnose any major fetal abnormalities. All scans were carried out by sonographers who had obtained The Fetal Medicine Foundation Certificate of Competence in the 11 + 0 to 13 + 6-week scan (www.fetalmedicine.com). Patient-specific risks were calculated by a multivariate approach using biochemical population parameters as outlined in a previous study[1], likelihood ratios based on delta NT – the difference from the normal median NT at the measured CRL – as outlined in a previous study[9] and the gestational age-related risk of trisomy 21 at the time of screening[10]. Data on pregnancy outcomes were obtained from the cytogenetics laboratories, the National Chromosomal Anomaly Register, the patients themselves, their general practitioners, or the maternity units in which they delivered.

The measured free β-hCG and PAPP-A were converted to multiples of the expected normal median (MoM) for a pregnancy of the same gestational age using values established in a previous study[8] corrected for maternal weight[11,12], self-recorded smoking status[13,14] and ethnicity[11,15].

The fetal database was searched to identify singleton pregnancies resulting in live births with no features of a chromosomal abnormality and with complete data on maternal ethnic origin, maternal weight, cigarette smoking status, gestational age at delivery and birth weight. The women were assigned to two groups – the normal pregnancy group was defined as those pregnancies in which a live baby was delivered after 37 complete weeks of gestation with a birth weight at or above the 10th centile for gestational age[16]. The SGA group was classified into three subgroups according to birth weight below the 10th, 5th and 3rd centile for gestational age[16]. Cases included in this study were not part of our previous study[8] but were part of those included in a recent study of PAPP-A and ADAM12[17].

The association between serum metabolites and the incidence of SGA was assessed by comparing the relative incidence at a number of MoM cut-offs and at various birth-weight centile cut-offs. Likelihood ratios (LR) for SGA were also calculated.

Regression analysis was used to determine the significant independent contributors in the prediction of SGA from the following variables: maternal serum PAPP-A and free β-hCG as MoMs, maternal weight as kg, cigarette smoking (yes or no) and maternal ethnic origin (Caucasian, Afro-Caribbean, Oriental (Chinese or Japanese) and Asian (Indian or Pakistani)). Statistical analysis was performed with Analyse-It (Analyse-It Software Ltd, Leeds, UK), SPSS for Windows (v.13, Chicago, IL, USA) and Excel for Windows 2003 (Microsoft Corp., Redmond, WA, USA). A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

The normal group comprised 46 262 pregnancies resulting in live births at term with no features of chromosomal abnormality. The SGA group comprised 3539 pregnancies resulting in live births at any gestation with no features of chromosomal abnormality. In 1605 cases the birth weight was below the 5th centile and in 979 cases it was below the 3rd centile. The characteristics of the normal pregnancy group and the SGA group are summarized in Table 1.

Multiple regression analysis demonstrated significant independent contributions in predicting SGA from PAPP-A, as well as maternal weight, smoking and ethnic origin (Table 2).

In SGA pregnancies the median PAPP-A MoM was significantly lower than that in the normal group, but the median free β-hCG was not statistically significantly different from normal (Table 3). At the 5th centile of the normal outcome group for PAPP-A (0.415 MoM) the odds ratios for SGA below the 10th, 5th and 3rd centiles were 2.70, 3.21 and 3.6574 (Table 3) and the respective detection rates for SGA were 12.0%, 14.0% and 16.0%. For free β-hCG at the 5th centile of normal (0.41 MoM) the odds were only marginally increased.

The individual likelihood ratios for SGA at each specific MoM level of PAPP-A are shown in Figure 1. The best-fit equations for PAPP-A, using a power regression data fit,

Table 1 Demographic and pregnancy characteristics of the study groups

Parameter	*Appropriate for gestation*	*Small for gestation (< 10th centile)*
n	46 262	3539
Maternal age (years)	31.5 (5.5)	30.4 (5.9)
Maternal weight (kg)	66.5 (13.2)	62.7 (13.0)
Cigarette smoker (%)	10.8	21.2
Ethnicity (%)		
Caucasian	85.9	74.4
Afro-Caribbean	3.5	4.6
Indian/Pakistani	6.1	14.9
Other	4.5	6.0
Crown–rump length (mm)	63.2 (8.6)	61.7 (8.8)
Gestational age at delivery (weeks)	39.6 (1.2)	37.5 (3.1)
Birth weight (kg)	3.5 (0.4)	2.3 (0.4)

Values are either mean (SD) or percentage.

Table 2 Multiple regression analysis in the prediction of small-for-gestational age (SGA) fetuses ($n = 49\,801$)

	SGA < 3rd centile		*SGA < 5th centile*		*SGA < 10th centile*	
Variable	*OR (95% CI)*	P	*OR (95% CI)*	P	*OR (95% CI)*	P
PAPP-A MoM	0.427 (0.373–0.489)	< 0.0001	0.485 (0.438–0.537)	< 0.0001	0.535 (0.500–0.573)	< 0.0001
Maternal weight (kg)	0.984 (0.978–0.989)	< 0.0001	0.976 (0.972–0.981)	< 0.0001	0.975 (0.972–0.978)	< 0.0001
Smoker	2.791 (2.394–3.255)	< 0.0001	2.640 (2.332–2.988)	< 0.0001	2.596 (2.376–2.836)	< 0.0001
Caucasian	1		1		1	
Afro-Caribbean	2.460 (1.800–3.362)	< 0.0001	2.305 (1.791–2.968)	< 0.0001	2.500 (2.106–2.969)	< 0.0001
Indian/Pakistani	2.970 (2.457–3.590)	< 0.0001	2.866 (2.468–3.329)	< 0.0001	3.013 (2.712–3.346)	< 0.0001
Other ethnic group	1.535 (1.186–1.986)	< 0.001	1.490 (1.212–1.832)	< 0.0001	1.415 (1.218–1.643)	< 0.0001

OR, odds ratio; PAPP-A MoM, pregnancy-associated plasma protein-A multiples of the median.

Table 3 Median multiples of the median (MoM) for free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) in pregnancies with small-for-gestational age (SGA) infants, probability of difference from the appropriately grown group and the odds ratio (OR) for SGA at the 5th centile of normal (corresponding to 0.41 MoM free β-hCG and 0.415 MoM PAPP-A)

	Free β-hCG			*PAPP-A*		
Small for gestation	*Median MoM*	P	*OR*	*Median MoM*	P	*OR*
< 10th centile	0.979	0.159	1.43	0.823	< 0.001	2.70
< 5th centile	0.992	0.665	1.60	0.790	< 0.001	3.21
< 3rd centile	0.978	0.410	1.71	0.748	< 0.001	3.66

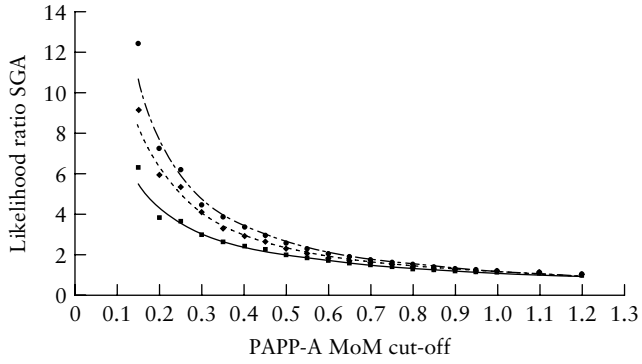

Figure 1 Likelihood ratio for small-for-gestational age (SGA) fetuses based on the pregnancy-associated plasma protein-A multiples of the median (PAPP-A MoM). Symbols represent individual data points, while the smoothed lines represent the best fit to the data. –■–, SGA < 10th centile; -◆-, SGA < 5th centile; –·●·–, SGA < 3rd centile.

were described by:

$$\text{LR SGA} < 10^{\text{th}}\ \text{centile} = 1.091 \times (\text{PAPP-A MoM} - 0.8543)$$

$$\text{LR SGA} < 5^{\text{th}}\ \text{centile} = 1.139 \times (\text{PAPP-A MoM} - 1.053)$$

$$\text{LR SGA} < 3^{\text{rd}}\ \text{centile} = 1.170 \times (\text{PAPP-A MoM} - 1.161)$$

The corresponding regression coefficients for goodness of fit were 0.995, 0.994 and 0.996.

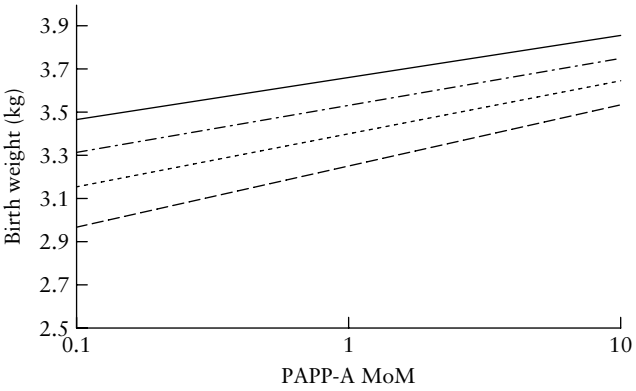

Figure 2 Correlation of maternal serum pregnancy-associated plasma protein-A multiples of the median (PAPP-A MoM) with birth weight for pregnancies delivering at 38 (– – – –), 39 (·····), 40 (–·–·) and 41 (——) weeks' gestation.

Table 4 Correlation of pregnancy-associated plasma protein-A (PAPP-A MoM) (as $\log_{10}$) with birth weight at various gestational ages (GA)

GA at delivery (weeks)	*Correlation coefficient*	P	n
38	0.1457	< 0.001	8030
39	0.1288	< 0.001	11 740
40	0.1144	< 0.001	16 042
41	0.0937	< 0.001	10 570

Smith *et al.*[18] showed a significant positive correlation between first-trimester levels of PAPP-A and eventual birth weight at 38 to 41 weeks' gestation. Analysis of our data confirms this positive correlation with first-trimester levels of PAPP-A (Figure 2 and Table 4).

DISCUSSION

The data from this study confirm that the incidence of SGA is higher in women of Afro-Caribbean and Indian or Pakistani origin than in Caucasians, in cigarette smokers than in non-smokers and is inversely associated with maternal weight. Additionally, there is an association between low maternal serum PAPP-A at 11 + 0 to 13 + 6 weeks and subsequent delivery of an SGA infant.

Table 5 Studies reporting on the association between first-trimester maternal serum pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG), and delivery of small-for-gestational age (SGA) infants

				Serum PAPP-A				*Serum free β-hCG*			
		SGA		*Median*	*< 5th centile MoM*		*OR or RR*	*Median*	*< 5th centile MoM*		*OR or RR*
Reference	*Total* n	*Centile*	n	*MoM*	*OR or RR*	*DR*	*< 0.5 MoM*	*MoM*	*OR or RR*	*DR*	*< 0.2 MoM*
Ong *et al.*[8]	4692	< 10th	395	0.967	1.47	7.8		1.033	1.36	6.1	
		< 5th	171	0.900	2.43	12.9		1.017	1.56	7.0	
		< 3rd	103	0.819	2.75	14.6		1.038	1.73	7.8	
Smith *et al.*[22]	8469	< 5th	370	0.830	2.90	12.4		0.900	1.30	8.1	
Yaron *et al.*[28]	1622	< 5th	49				3.30				NS
De Leon *et al.*[29]	145	—	10	0.760				1.700			
Krantz *et al.*[24]	8012	< 5th	384		2.70	9.7			1.30	5.1	
Dugoff *et al.*[23]	33 395	< 10th	2994		2.47	10.5			1.55		
		≤ 5th	1300		2.81	12.2			NS		
Morssink *et al.*[30]	800	< 5th	73	0.830				0.950			
Tul *et al.*[31]	1136	< 10th	51	0.760			2.36				
This study	49 801	< 10th	3539	0.823	2.70	12.0		0.979	1.43	6.5	
		< 5th	1605	0.790	3.21	14.0		0.992	1.60	7.4	
		< 3rd	979	0.748	3.66	16.0		0.978	1.71	7.9	

DR, detection rate; NS, not significant; OR, odds ratio; RR, relative risk.

Low serum PAPP-A presumably indicates impaired placentation. Certainly a plausible hypothesis to explain how low PAPP-A can reflect poor placental function and lead to potential SGA is the role of PAPP-A as an insulin-like growth factor binding protein (IGFBP)-4 and -5 protease[19,20]. Lowered levels of PAPP-A would have less of a protease effect on IGFBPs leading to higher levels of bound (biologically inactive) IGF-I and -II and thus reduced fetal growth. One recent study has shown a significant correlation of PAPP-A levels with second-trimester ultrasound measures of fetal growth such as femur length and abdominal circumference[21].

The data on biochemical markers in relation to SGA essentially confirm the initial observations we made in our earlier smaller study[8] and the data presented recently from other studies[22–24]. Since the introduction of first-trimester screening for aneuploidy using maternal serum PAPP-A and free β-hCG in conjunction with fetal NT thickness, more data in limited studies have become available, but the clinical usefulness of the data provided is often obscured by the wide range of presentation of the data. Table 5 summarizes the published data with respect to the various biochemical marker levels, odds ratios and detection rates. The presentation of our data in terms of likelihood ratios for SGA at any marker level for PAPP-A should enable clinicians to better assess an individual's risk and to use this information when counseling the patient about invasive testing, or when the results of invasive testing have identified a normal karyotype.

There is accumulating evidence that increased surveillance of high-risk fetuses reduces perinatal morbidity and mortality, with a systematic review showing that the use of umbilical artery Doppler is advantageous in monitoring such fetuses[25] but not in unselected populations[26]. Lindqvist and Molin[27] found that an awareness of SGA before delivery, in combination with a structured program of surveillance for those identified as SGA, was related to a four-fold lowered risk of adverse fetal outcome. We believe that there is now sufficient evidence to use lowered PAPP-A as part of this structured approach to identifying SGA-affected pregnancies.

ACKNOWLEDGMENTS

This study was supported by a grant from The Fetal Medicine Foundation (Charity No: 1037116) and by a grant from NHS R&D (RF4:Risk Assessment in Pregnancy) to KS.

REFERENCES

1. Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free β-human chorionic gonadotropin and pregnancy associated plasma protein-A. *Ultrasound Obstet Gynecol* 1999; **13**: 231–237.
2. Tul N, Spencer K, Noble P, Chan C, Nicolaides KH. Screening for trisomy 18 by fetal nuchal translucency thickness and maternal serum free β-hCG and pregnancy associated plasma protein-A at 10–14 weeks of gestation. *Prenat Diagn* 1999; **19**: 1035–1042.
3. Spencer K, Ong C, Skentou H, Liao AW, Nicolaides KH. Screening for trisomy 13 by fetal nuchal translucency and maternal serum free beta-hCG and pregnancy associated plasma protein A at 10–14 weeks of gestation. *Prenat Diagn* 2000; **20**: 411–416.
4. Spencer K, Liao AW, Skentou H, Cicero S, Nicolaides KH. Screening for triploidy by fetal nuchal translucency and maternal serum free β-human chorionic gonadotropin and pregnancy associated plasma protein-A at 10–14 weeks of gestation. *Prenat Diagn* 2000; **20**: 495–499.
5. Spencer K, Spencer CE, Power M, Dawson C, Nicolaides KH. Screening for chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one-stop clinic: a review of three years prospective experience. *BJOG* 2003; **110**: 281–286.

6. Avgidou K, Papageorghiou A, Bindra R, Spencer K, Nicolaides KH. Prospective first-trimester screening for trisomy 21 in 30,564 pregnancies. *Am J Obstet Gynecol* 2005; **192**: 1761–1767.
7. Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. Mutlitcenter study of first-trimester screening for trisomy 21 in 75821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. *Ultrasound Obstet Gynecol* 2005; **25**: 221–226.
8. Ong CYT, Liao AW, Spencer K, Munim S, Nicolaides KH. First trimester maternal serum free β human chorionic gonadotrophin and pregnancy associated plasma protein A as predictors of pregnancy complications. *BJOG* 2000; **107**: 1265–1270.
9. Spencer K, Bindra R, Nix ABJ, Heath V, Nicolaides KH. Delta-NT or NT MoM: which is the most appropriate method for calculating accurate patient-specific risks for trisomy 21 in the first trimester? *Ultrasound Obstet Gynecol* 2003; **22**: 142–148.
10. Snijders RJM, Sundberg K, Holzgreve W, Henry G, Nicolaides KH. Maternal age- and gestation-specific risk for trisomy 21. *Ultrasound Obstet Gynecol* 1999; **13**: 167–170.
11. Spencer K, Ong CYT, Liao AWK, Nicolaides KH. The influence of ethnic origin on first trimester biochemical markers of chromosomal abnormalities. *Prenat Diagn* 2000; **20**: 491–494.
12. Spencer K, Bindra R, Nicolaides KH. Maternal weight correction of maternal serum PAPP-A and free β-hCG when screening for trisomy 21 in the first trimester of pregnancy. *Prenat Diagn* 2003; **23**: 851–855.
13. Spencer K, Ong CYT, Liao AWJ, Papademetriou D, Nicolaides KH. First trimester markers of trisomy 21 and the influence of maternal cigarette smoking status. *Prenat Diagn* 2000; **20**: 852–853.
14. Spencer K, Bindra R, Cacho AM, Nicolaides KH. The impact of smoking status when screening for chromosomal anomalies using maternal serum biochemistry and fetal nuchal translucency thickness in the first trimester of pregnancy. *Prenat Diagn* 2004; **24**: 169–173.
15. Spencer K, Heath V, El-Sheikhah A, Ong CYT, Nicolaides KH. Ethnicity and the need for correction of biochemical and ultrasound markers of chromosomal anomalies in the first trimester: a study of Oriental, Asian and Afro-Caribbean populations. *Prenat Diagn* 2005; **25**: 365–369.
16. Yudkin PL, Aboualfa M, Eyre JA, Redman CW, Wilkinson AR. New birth weight and head circumference centiles for gestational ages 24 to 42 weeks. *Early Hum Dev* 1987; **15**: 45–52.
17. Cowans NJ, Spencer K. First trimester ADAM12 and PAPP-A as markers for intrauterine fetal growth restriction through their roles in the insulin-like growth factor system. *Prenat Diagn* 2007; **27**: 264–271.
18. Smith GCS, Stenhouse EJ, Crossley JA, Aitken DA, Cameron AD, Connor JM. Early pregnancy origins of low birth weight. *Nature* 2002; **417**: 916.
19. Lawrence JB, Oxvig C, Overgaard MT, Sotrup-Jensen L, Gleich GJ, Hays LG, Yates JR, Conover CA. The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. *Proc Natl Acad Sci USA* 1999; **95**: 3149–3153.
20. Laursen LS, Overgaard MT, Soe R, Boldt HB, Sottrup-Jensen L, Giudice LC, Conover CA, Oxvig C. Pregnancy-associated plasma protein-A (PAPP-A) cleaves insulin-like growth factor binding protein (IGFBP)-5 independent of IGF: implications for the mechanism of IGFBP-4 proteolysis by PAPP-A. *FEBS Lett* 2001; **504**: 36–40.
21. Leung TY, Chan LW, Leung TN, Fung TY, Sahota DS, Lau TK. First-trimester maternal serum levels of placental hormones are independent predictors of second-trimester fetal growth parameters. *Ultrasound Obstet Gynecol* 2006; **27**: 156–161.
22. Smith GCS, Stenhouse EJ, Crossley JA, Aitken DA, Cameron AD, Connor JM. Early pregnancy levels of pregnancy-associated plasma protein-A and the risk of intrauterine growth restriction, premature birth, preeclampsia and stillbirth. *J Clin Endocrinol Metab* 2002; **87**: 1762–1767.
23. Dugoff L, Hobbins JC, Malone FD, Porter TF, Luthy D, Comstock CH, Hankins G, Berkowitz RL, Merkatz I, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, Vidaver J, D'Alton ME. First-trimester maternal serum PAPP-A and free-beta subunit human chorionic gonadotropin concentrations and nuchal translucency are associated with obstetric complications: a population-based screening study (the FASTER Trial). *Am J Obstet Gynecol* 2004; **191**: 1446–1451.
24. Krantz D, Goetzl L, Simpson JL, Thom E, Zachary JM, Hallahan TW, Silver R, Pergament E, Platt LD, Filkins K, Johnson A, Mahoney M, Hogge WA, Wilson RD, Mohide P, Hershey D, Wapner RJ. First Trimester Maternal Serum Biochemistry and Fetal Nuchal Translucency Screening (BUN) Study Group. Association of extreme first-trimester free human chorionic gonadotropin-β, pregnancy-associated plasma protein A, and nuchal translucency with intrauterine growth restriction and other adverse pregnancy outcomes. *Am J Obstet Gynecol* 2004; **191**: 1452–1458.
25. Alfirevic Z, Neilson JP. Doppler ultrasonography in high-risk pregnancies: systematic review with meta-analysis. *Am J Obstet Gynecol* 1995; **172**: 1379–1387.
26. Goffinet F, Paris-Llado J, Nisand I, Breart G. Umbilical artery Doppler velocimetry in unselected and low risk pregnancies: a review of randomised controlled trials. *Br J Obstet Gynaecol* 1997; **104**: 425–430.
27. Lindqvist PG, Molin J. Does antenatal identification of small-for-gestational age fetuses significantly improve their outcome? *Ultrasound Obstet Gynecol* 2005; **25**: 258–264.
28. Yaron Y, Heifetz S, Ochshorn Y, Lehavi O, Orr-Urtreger A. Decreased first trimester PAPP-A is a predictor of adverse pregnancy outcome. *Prenat Diagn* 2002; **22**: 778–782.
29. De Leon J, Sifuentes G, Hopkins C, Noble V, Gimpel T, Myles T, Santolaya-Forgas J. Maternal serum free β-hCG levels in uncomplicated pregnancies at the 10^{th}–15^{th} week of gestation and the development of obstetric complications. *J Reprod Med* 2004; **48**: 89–92.
30. Morssink LP, Kornman LH, Hallahan TW, Kloosterman MD, Beekhuis JR, de Wolf BTHM, Mantingh A. Maternal serum levels of free β-hCG and PAPP-A in the first trimester of pregnancy are not associated with subsequent fetal growth retardation or preterm delivery. *Prenat Diagn* 1998; **18**: 147–152.
31. Tul N, Pusenjak S, Osredkar J, Spencer K, Novak-Antolic Z. Predicting complications of pregnancy with first-trimester maternal serum free β-hCG, PAPP-A and inhibin-A. *Prenat Diagn* 2003; **23**: 990–996.

