Ultrasound Obstet Gynecol 2006; **27**: 658–663
Published online 21 February 2006 in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/uog.2676

Prediction of pre-eclampsia by uterine artery Doppler ultrasonography and maternal serum pregnancy-associated plasma protein-A, free β-human chorionic gonadotropin, activin A and inhibin A at 22 + 0 to 24 + 6 weeks' gestation

K. SPENCER*†, C. K. H. YU†, M. SAVVIDOU†, A. T. PAPAGEORGHIOU† and K. H. NICOLAIDES†

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

KEYWORDS: activin A; inhibin A; maternal serum biochemistry; pre-eclampsia; screening; uterine artery Doppler

ABSTRACT

Objective *To investigate the potential value of combining uterine artery Doppler ultrasonography with the measurement of maternal serum pregnancy-associated plasma protein-A (PAPP-A), free β-human chorionic gonadotropin (β-hCG), activin A and inhibin A at 22 + 0 to 24 + 6 weeks' gestation, in the prediction of pregnancies that subsequently develop pre-eclampsia.*

Methods *The maternal serum PAPP-A, free β-hCG, activin A and inhibin A concentrations at 22 + 0 to 24 + 6 weeks' gestation were measured in samples obtained from women with singleton pregnancies who participated in a screening study for pre-eclampsia by transvaginal color flow Doppler measurement of the uterine artery pulsatility index (PI). A search was made of the database to identify those who subsequently developed pre-eclampsia (*n = *24) and a group of controls with normal outcome (*n = *144). Regression analysis was performed to establish any relationship between the biochemical markers themselves and between the biochemical markers and uterine artery mean PI. A multivariate Gaussian model combining various biochemical markers with uterine artery mean PI was developed using standard statistical modeling techniques and the performance of such models in discriminating cases with pre-eclampsia was evaluated by receiver–operating characteristics curve (ROC) analysis.*

Results *In the pre-eclampsia group, compared to the controls, the uterine artery mean PI and the maternal serum levels of PAPP-A, free β-hCG, activin A and inhibin A were significantly increased. The predicted detection rates of pre-eclampsia, for a false positive rate of 5%, was 50% by uterine artery mean PI, 5% by PAPP-A, 10% by free β-hCG, 35% by inhibin A and 44% by activin A. Screening by a combination of uterine artery mean PI and maternal serum activin A and inhibin A could detect 75% and 92% of patients who subsequently developed pre-eclampsia, for false positive rates of 5% and 10%, respectively.*

Conclusion *Screening for pre-eclampsia by uterine artery PI at 22 + 0 to 24 + 6 weeks' gestation can be improved by measurement of activin A and inhibin A levels.*

INTRODUCTION

Pre-eclampsia is thought to be a circulatory maladaptation disease that is characterized by defective trophoblastic invasion of the maternal spiral arteries[1]. The consequent increased resistance in the uteroplacental circulation forms the basis of screening for the condition by uterine artery Doppler ultrasonography[2]. Thus increased impedance to flow in the uterine arteries, as measured by the pulsatility index (PI) at 20–25 weeks' gestation, can identify, for a 5% false positive rate, about 45% of pregnancies that subsequently develop pre-eclampsia[2–4].

There is some evidence that the combination of certain biochemical markers, such as activin A and inhibin A, could potentially enhance the sensitivity of

Correspondence to: Dr K. Spencer, Prenatal Screening Unit, Department of Clinical Biochemistry, Harold Wood Hospital, Gubbins Lane, Romford, Essex, RM3 0BE, UK (e-mail: KevinSpencer1@aol.com)

Accepted: 1 November 2005

uterine artery Doppler velocimetry for the prediction of pre-eclampsia[5,6]. Activin A and inhibin A are dimeric glycoproteins produced by the decidua, placenta and fetal membranes. The maternal serum concentration of these proteins is increased at the time of clinical manifestation of pre-eclampsia or gestational hypertension[7–14]. Although there is some evidence that activin A and inhibin A are also elevated in the first and second trimesters[13,15,16], levels appear to decline to normal the further one gets away from the onset of clinical symptoms.

Other potentially useful biochemical markers of impaired placentation are pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG), which are now increasingly being used, in combination with fetal nuchal translucency thickness, in first-trimester screening for chromosomal defects[17]. Recently, PAPP-A has been shown to be an insulin-like growth factor (IGF) binding protein (IGFBP) protease[18]. Since IGFBPs have a key role in modulating IGF activity, PAPP-A could be important in regulating fetal growth and development[19] and in trophoblastic invasion of the decidua[20]. The maternal serum concentration of PAPP-A is increased soon after the onset of pre-eclampsia[21], but at 11–14 weeks' gestation the levels are decreased in those pregnancies that subsequently develop pre-eclampsia[15,22].

The aim of this study was to investigate the potential value of combining uterine artery Doppler ultrasonography with the measurement of maternal serum PAPP-A, free β-hCG, activin A and inhibin A at 22 + 0 to 24 + 6 weeks' gestation in the prediction of pregnancies that subsequently develop pre-eclampsia.

PATIENTS AND METHODS

This was a case-control study in which uterine artery PI and maternal serum PAPP-A, free β-hCG, activin A and inhibin A concentrations were measured at 22 + 0 to 24 + 6 weeks' gestation in 24 singleton pregnancies that subsequently developed pre-eclampsia and 144 normal controls, matched for gestation and duration of storage of samples. The patients were recruited from a screening study for pre-eclampsia by transvaginal color flow Doppler measurement of the uterine artery PI[3]. Blood samples were also collected and the serum stored at −20°C. A search was made of the database to identify all singleton pregnancies that had maternal serum collected and that had also had 22 + 0 to 24 + 6 week Doppler investigations. The hospital notes and delivery suite records were then searched for each one of these patients to identify any pregnancy complications and obtain delivery details. Specifically pregnancies with pre-eclampsia were identified in which pregnancy-induced hypertension was defined by a diastolic blood pressure of ≥ 110 mmHg on any one occasion or a diastolic blood pressure of ≥ 90 mmHg on two consecutive occasions 4 h apart in women with no pre-existing hypertensive or renal disease, and the presence of either more than 300 mg of total protein in a 24-hour urine collection or a 1 + albumin on reagent strip[23].

Free β-hCG and PAPP-A were measured by the Kryptor Immunoassay system (Brahms AG, Berlin, Germany), the performance of which has been described previously[17]. Activin A and inhibin A were measured in duplicate by commercial quantitative enzyme linked immunosorbent assay (ELISA) techniques (Serotec, Kidlington, UK), the performance of both of which has also been previously described[15,24,25].

Statistical analysis

Biochemical marker results were expressed as a multiple of the median (MoM) value in normal pregnancies using the values obtained in this study. Comparison of the biochemical data in the pre-eclampsia group with the normal group was by means of unpaired *t*-test of unequal variance after $\log_{10}$ transformation of the MoM values to achieve a Gaussian distribution of the data. Regression analysis was performed to establish any relationship between the biochemical markers themselves and between the biochemical markers and uterine artery mean PI. The detection rate for pre-eclampsia and false positive rate for different cut-offs in marker levels were calculated and receiver–operating characteristics (ROC) curves were constructed to establish clinical utility. A multivariate Gaussian model combining various biochemical markers with uterine artery mean PI (after $\log_{10}$ transformation to achieve Gaussianarity) was developed using standard statistical modeling techniques, and the performance of such models in discriminating cases with pre-eclampsia was assessed by ROC analysis. All statistical analysis was performed using Analyse-It (Smart Software, Leeds) and Microsoft Excel or with SPSS 12 (SPSS, Woking).

RESULTS

The uterine artery mean PI was significantly higher in the pre-eclampsia group than in the controls [1.70 vs. 0.908, $P < 0.001$; $\log_{10}$ mean PI (SD) 0.1982 (0.1195) vs. −0.0342 (0.1681)]. Similarly, the maternal serum levels of PAPP-A, free β-hCG, activin A and inhibin A were significantly higher in the pre-eclampsia group than in the controls (Table 1, Figure 1). In the pre-eclampsia group there was no significant correlation between uterine artery mean PI and any of the biochemical markers, and in the controls there was only a small but significant correlation with activin levels (Table 2). A significant correlation was seen between free β-hCG and PAPP-A and inhibin A in the pre-eclampsia group and between inhibin A and PAPP-A and free β-hCG in the control group.

The predicted detection rates of pre-eclampsia, for different false positive rates, by uterine artery mean PI and maternal serum biochemical markers are shown in Table 3 and Figure 2. Screening by a combination of uterine artery mean PI and maternal serum activin A and inhibin A could identify 75% of patients who subsequently developed pre-eclampsia for a false positive rate of 5%. Alternatively,

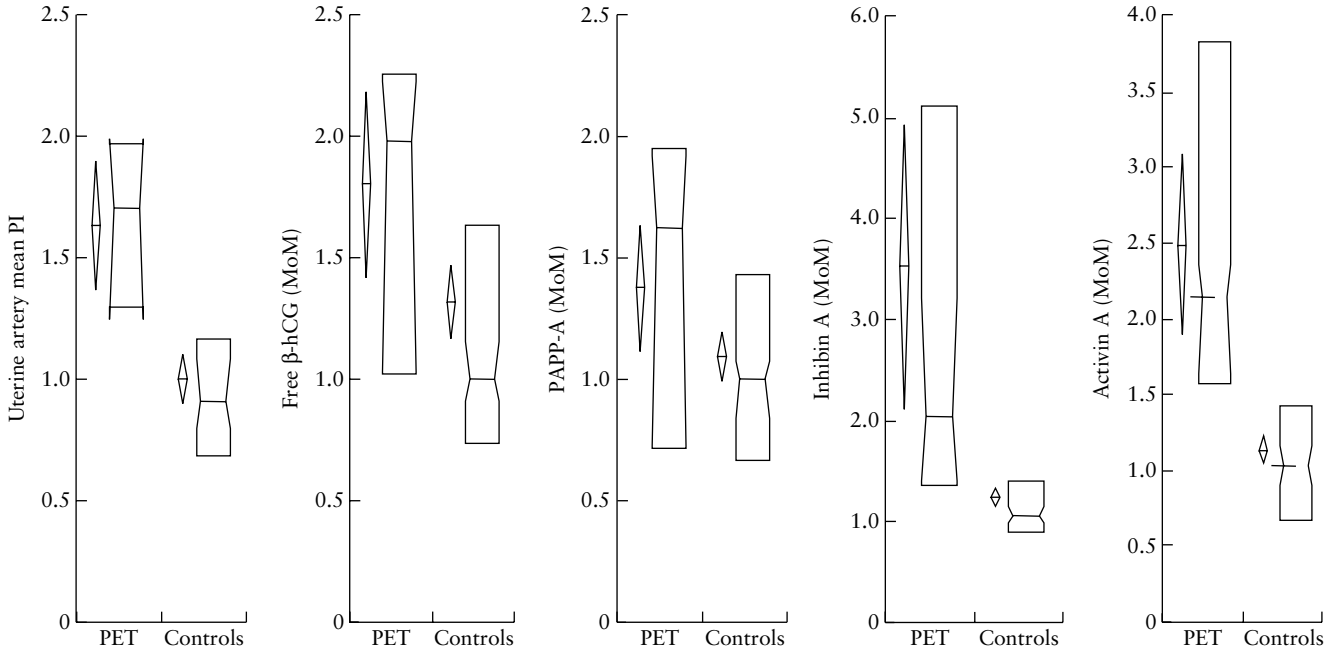

Figure 1 Box–whisker plots for various markers in controls compared with those in cases with pre-eclampsia (PET). The diamonds indicate means and their 95% confidence intervals. The notched boxes show the medians and their 95% confidence intervals and the upper/lower interquartile ranges. hCG, human chorionic gonadotropin; MoM, multiple of the median; PAPP-A, pregnancy-associated plasma protein-A; PI, pulsatility index.

Table 1 Biochemical marker levels in cases with pre-eclampsia and controls

	Pre-eclampsia (n = *24*)	*Controls* (n = *144*)	P
Free β-hCG			
Median MoM	1.98	1.00	0.003
Mean $\log_{10}$ MoM	0.1976	0.0270	
SD $\log_{10}$ MoM	0.2385	0.2879	
PAPP-A			
Median MoM	1.62	1.00	
Mean $\log_{10}$ MoM	0.0864	−0.0373	0.02
SD $\log_{10}$ MoM	0.2275	0.2691	
Inhibin A			
Median MoM	2.03	1.05	<0.001
Mean $\log_{10}$ MoM	0.3957	0.0530	
SD $\log_{10}$ MoM	0.3507	0.1707	
Activin A			
Median MoM	2.14	1.02	<0.001
Mean $\log_{10}$ MoM	0.3351	0.0017	
SD $\log_{10}$ MoM	0.2307	0.2181	

hCG, human chorionic gonadotropin; MoM, multiple of the median; PAPP-A, pregnancy-associated plasma protein-A; SD, standard deviation.

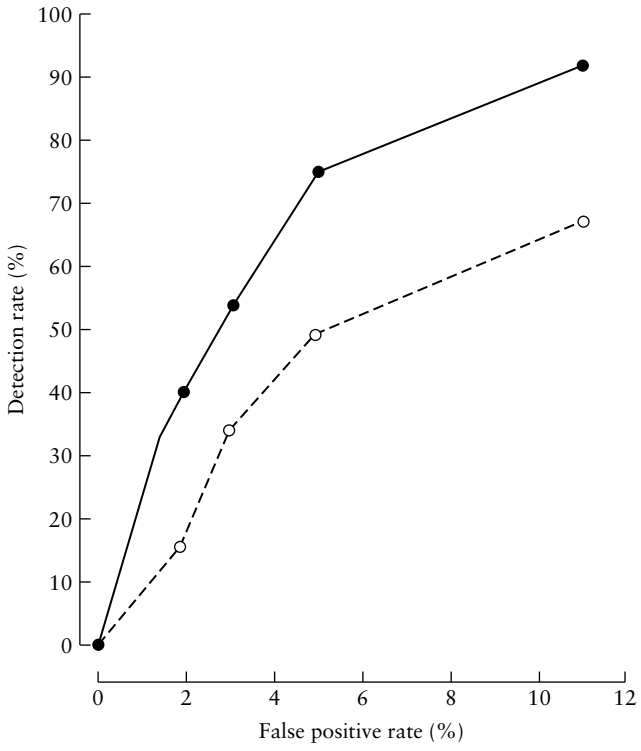

Figure 2 Receiver–operating characteristics plot for screening for pre-eclampsia by uterine artery mean pulsatility index (PI) (------) and uterine artery mean PI combined with maternal serum activin A and inhibin A (———).

the detection rate was 92% at a false positive rate of 10%.

In women in which the pre-eclampsia was severe enough to warrant delivery prior to 35 weeks' gestation ($n = 10$) compared with those who delivered at or after 35 weeks ($n = 14$), the median marker MoMs were 1.92 vs. 1.11 ($P = 0.28$) for PAPP-A, 2.07 vs. 1.16 ($P = 0.10$) for free β-hCG, 3.20 vs. 1.78 ($P = 0.23$) for inhibin A and 2.09 vs. 2.20 ($P = 0.10$) for activin A. The difference in mean PI was 1.99 vs. 1.31 ($P < 0.001$). The number of cases that delivered before 35 weeks' gestation was insufficient to allow a full ROC analysis.

Table 2 Marker correlations in pre-eclampsia cases and controls

	Pre-eclampsia				
Controls	*Mean PI*	*Free β-hCG*	*PAPP-A*	*Inhibin A*	*Activin A*
Mean PI	—	0.22 ($P = 0.499$)	0.35 ($P = 0.298$)	0.24 ($P = 0.457$)	−0.13 ($P = 0.697$)
Free β-hCG	0.02 ($P = 0.896$)	—	0.11 ($P = 0.198$)	0.26 ($P = 0.002$)	0.03 ($P = 0.678$)
PAPP-A	0.02 ($P = 0.860$)	0.52 ($P = 0.009$)	—	0.18 ($P = 0.034$)	−0.03 ($P = 0.752$)
Inhibin A	0.18 ($P = 0.129$)	0.48 ($P = 0.017$)	0.23 ($P = 0.278$)	—	0.03 ($P = 0.688$)
Activin A	0.23 ($P = 0.046$)	−0.17 ($P = 0.433$)	0.10 ($P = 0.626$)	−0.32 ($P = 0.125$)	—

hCG, human chorionic gonadotropin; PAPP-A, pregnancy-associated plasma protein-A; PI, pulsatility index.

Table 3 Area under the receiver–operating characteristics curve (AUC) and detection rates at 5% and 10% false positive rates (FPR) when screening for pre-eclampsia by uterine artery Doppler velocimetry and maternal serum levels of free β-hCG, PAPP-A, inhibin A and activin A

		Detection rate (%)	
Method of screening	*AUC*	*FPR 5%*	*FPR 10%*
Uterine artery mean PI	0.872	50	66
Maternal serum free β-hCG	0.688	10	15
Maternal serum PAPP-A	0.630	5	10
Maternal serum inhibin A	0.819	35	53
Maternal serum activin A	0.850	44	60
Maternal serum inhibin A and activin A	0.949	67	83
Uterine artery mean PI and maternal serum inhibin A	0.913	67	75
Uterine artery mean PI and maternal serum activin A	0.935	57	75
Uterine artery mean PI, and maternal serum activin A and inhibin A	0.970	75	92

hCG, human chorionic gonadotropin; PAPP-A, pregnancy-associated plasma protein-A; PI, pulsatility index.

DISCUSSION

The findings of this study, that measurement of the uterine artery mean PI at 22 + 0 to 24 + 6 weeks' gestation can, for a 5% false positive rate, identify about 50% of pregnancies that subsequently develop pre-eclampsia, are compatible with the results of previous large-scale prospective screening studies[3]. In addition, our findings suggest that the performance of screening can be improved by combining uterine artery Doppler scan with maternal serum biochemical analysis. Thus, a combination of uterine artery mean PI and maternal serum activin A and inhibin A could detect 75 and 92%, respectively, of patients who subsequently develop pre-eclampsia, with false positive rates of 5 and 10%.

Two previous studies have also reported that the detection rate of pre-eclampsia was higher by combining uterine artery Doppler sonography and maternal serum biochemistry than with either method alone. Thus, Aquilina *et al.*[5], in a study that included 35 patients who subsequently developed pre-eclampsia, reported that for a false positive rate of about 7% the detection rate with uterine artery Doppler at 18–22 weeks' gestation was 60% and this increased to 71% by combining Doppler scan with maternal serum inhibin A levels at 15–19 weeks. Florio *et al.*[6] measured activin A and inhibin A levels in 58 asymptomatic pregnant women at 24 weeks' gestation with Doppler evidence of increased impedance to flow in the uterine arteries. The probability of developing pre-eclampsia was calculated for different combinations of results. Activin A achieved a detection rate of 60% at a false positive rate of 10%, while inhibin A had a detection rate of 41% with a false positive rate of 10%. This compares well with our results of 60% for activin A and 53% for inhibin A in an unselected population at a 10% false positive rate.

The findings of maternal serum biochemical markers at 22 + 0 to 24 + 6 weeks, together with the results of previous studies, suggest that the pattern of serum concentration changes across pregnancy in women developing pre-eclamsia.

The maternal serum PAPP-A levels in pregnancies that subsequently develop pre-eclampsia are reduced at 11–14 weeks' gestation[15,22,26–31] and mildly increased at 22 + 0 to 24 + 6 weeks. In women with pre-eclampsia in the third trimester, the levels are increased[21,31,32]. Similarly, tissue PAPP-A levels in placental extracts of pre-eclamptic patients at term are higher than normal[33].

The maternal serum free β-hCG levels in pregnancies that subsequently develop pre-eclampsia may be mildly reduced at 11–14 weeks[15,16,22]. At 15–20 weeks' gestation the median free β-hCG in patients who developed pre-eclampsia was 1.41 MoM for intact or total hCG[14,34–36] and 1.17 MoM for free β-hCG[9,37,38]. At

30–40 weeks the level in those who developed severe pre-eclampsia was 4.26 MoM[39]. In established pre-eclampsia the levels of hCG are increased[40–42].

We found that at 22 + 0 to 24 + 6 weeks' gestation the maternal serum levels of activin A and inhibin A in women who subsequently develop pre-eclampsia are on average twice as high as in normal pregnancies. Previous studies reported that at 11–14 weeks the levels in those who subsequently develop pre-eclampsia are about 1.5 MoM for both activin A and inhibin A[16,43], whereas in women with pre-eclampsia, at 25–41 weeks, the median levels of activin A and inhibin A ranged from 2.59 to 9.64 MoM and 1.92 to 8.47 MoM, respectively[7,8,10,32,44]. A longitudinal study of 71 women who subsequently developed pre-eclampsia reported that the median MoM levels of both inhibin A and activin A increased with gestation from 8 to 30 weeks[13]. Studies of women at 15–20 weeks' gestation reported that in those who subsequently developed pre-eclampsia the median values of inhibin A were increased and ranged from 1.09 to 2.01 MoM[9,36,38,45], whereas the levels of activin A were not significantly different from normal[34].

CONCLUSIONS

In this study we used standard statistical analysis, as used in screening for trisomy 21, to establish a method of screening for pre-eclampsia by a combination of uterine artery Doppler ultrasonography and maternal serum biochemical testing at 22 + 0 to 24 + 6 weeks' gestation. Such an approach can improve the detection rate, for a false positive rate of 10%, from 66% with uterine artery Doppler alone to more than 90% by combining Doppler velocimetry with maternal serum activin A and inhibin A levels. The results also suggest that for PAPP-A, free β-hCG and inhibin, levels of these markers are even further elevated in cases of severe pre-eclampsia.

REFERENCES

1. Lyall F. The human placental bed revisited. *Placenta* 2002; **23**: 555–562.
2. Papageorghiou AT, Yu CK, Nicolaides KH. The role of uterine artery Doppler in predicting adverse pregnancy outcome. *Best Pract Res Clin Obstet Gynaecol* 2004; **18**: 383–396.
3. Papageorghiou AT, Yu CKH, Bindra R, Pandis G, Nicolaides KH. Multicenter screening for pre-eclampsia and fetal growth restriction by transvaginal uterine artery Doppler at 23 weeks of gestation. *Ultrasound Obstet Gynecol* 2001; **18**: 441–449.
4. Yu CKH, Smith GCS, Papageorghiou AT, Cacho AM, Nicolaides KH. An integrated model for the prediction of pre-eclampsia using maternal factors and uterine artery Doppler velocimetry in unselected low-risk women. *Am J Obstet Gynecol* 2005; **193**: 429–436.
5. Aquilina J, Thompson O, Thiliganathan B, Harrington K. Improved early prediction of pre-eclampsia by combining second-trimester maternal serum inhibin A and uterine artery Doppler. *Ultrasound Obstet Gynecol* 2001; **17**: 477–484.
6. Florio P, Reis FM, Pezzani I, Luisi S, Severi FM, Petraglia F. The addition of activin A and inhibin A measurement to uterine artery Doppler velocimetry to improve the early prediction of pre-eclampsia. *Ultrasound Obstet Gynecol* 2003; **21**: 165–169.
7. Petraglia F, De Vita D, Gallinelli A, Aguzzoli L, Genazzani AR, Romero R, Woodruff TK. Abnormal concentration of maternal serum activin-A in gestational diseases. *J Clin Endocrinol Metab* 1995; **80**: 558–561.
8. Muttukrishna S, Knight PG, Groome NP, Redman CW, Ledger WL. Activin A and inhibin A as possible endocrine markers for pre-eclampsia. *Lancet* 1997; **349**: 1285–1288.
9. Cuckle H, Sehmi I, Jones R. Maternal serum inhibin A can predict pre-eclampsia. *Br J Obstet Gynaecol* 1998; **105**: 1101–1103.
10. Silver HM, Lambert-Messerlian GM, Star JA, Hogan J, Canick JA. Comparison of maternal serum total activin A and inhibin A in normal, pre-eclamptic and non proteinuric gestational hypertensive pregnancies. *Am J Obstet Gynecol* 1999; **180**: 1131–1137.
11. Aquilina J, Barnett A, Thompson O, Harrington K. Second-trimester maternal serum inhibin A concentrations as an early marker for pre-eclampsia. *Am J Obstet Gynecol* 1999; **181**: 131–136.
12. D'Antona D, Reis FM, Benedetto C, Evans LW, Groome NP, de Kretser DM, Wallace EM, Petraglia F. Increased maternal serum activin A but not follistatin levels in pregnant women with hypertensive disorders. *J Endocrinol* 2000; **165**: 157–162.
13. Muttukrishna S, North RA, Morris J, Schellenberg JC, Taylor RS, Asselin J, Ledger W, Groome N, Redman CW. Serum inhibin A and activin A are elevated prior to the onset of pre-eclampsia. *Hum Reprod* 2000; **15**: 1640–1645. Erratum in *Hum Reprod* 2001; **16**: 2477.
14. Lambert-Messerlian GM, Silver HM, Petraglia F, Luisi S, Pezzani I, Maybruck WM, Hogge WA, Hanley-Yanez K, Roberts JM, Neveux LM, Canick JA. Second-trimester levels of maternal serum human chorionic gonadotropin and inhibin A as predictors of pre-eclampsia in the third trimester of pregnancy. *J Soc Gynecol Invest* 2000; **7**: 170–174.
15. Ong CT, Liao AW, Spencer K, Munim S, Nicolaides KH. First-trimester maternal serum free beta-human chorionic gonadotropin and pregnancy associated plasma protein-A as predictors of pregnancy complications. *BJOG* 2000; **107**: 1265–1270.
16. Sebire NJ, Roberts L, Noble P, Wallace E, Nicolaides KH. Raised maternal serum inhibin A concentration at 10 to 14 weeks of gestation is associated with pre-eclampsia. *BJOG* 2000; **107**: 795–797.
17. 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.
18. Lawrence JB, Oxvig C, Overgaard MT, Sottrup-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; **96**: 3149–3153.
19. Conover CA, Bale LK, Overgaard MT, Johnstone EW, Laursen UH, Fuchtbauer EM, Oxvig C, van Deursen J. Metalloproteinase pregnancy-associated plasma protein A is a critical growth regulatory factor during fetal development. *Development* 2004; **131**: 1187–1194.
20. van Kleffens M, Groffen C, Lindenbergh-Kortleve DJ, van Neck JW, Gonzalez-Parra S, Dits N, Zwarthoff EC, Drop SL. The IGF system during fetal-placental development of the mouse. *Mol Cell Endocrinol* 1998; **140**: 129–135.
21. Toop K, Klopper A. Concentration of pregnancy-associated plasma protein-A (PAPP-A) in patients with pre-eclamptic toxaemia. *Placenta* 1981; **4**: 167–173.
22. Spencer K, Yu CKH, Cowans NJ, Otigbah C, Nicolaides KH. Prediction of pregnancy complication by first-trimester maternal serum PAPP-A and free β-hCG and with second-trimester uterine artery Doppler. *Prenat Diagn* 2005; **25**: 949–953.

23. Davey DA, MacGillivray I. The classification and definition of the hypertensive disorders of pregnancy. *Am J Obstet Gynecol* 1988; **158**: 892–898.
24. Spencer K, Liao AW, Ong CY, Geerts L, Nicolaides KH. Maternal serum levels of dimeric inhibin A in pregnancies affected by trisomy 21 in the first trimester. *Prenat Diagn* 2001; **21**: 441–444.
25. Spencer K, Liao AW, Skentou H, Ong CY, Nicolaides KH. Maternal serum levels of total activin A in first-trimester trisomy 21 pregnancies. *Prenat Diag* 2001; **21**: 270–273.
26. 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, pre-eclampsia and stillbirth. *J Clin Endocrinol Metab* 2002; **87**: 1762–1767.
27. 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.
28. 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.
29. Krantz D, Goetzl L, Simpson JL, Thom E, Zachary J, Hallahan TW, Silver R, Pergament E, Platt LD, Filkins K, Johnson A, Mahoney M, Hogge WA, Wilson RD, Mohide P, Hershey D, Wapner R; 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.
30. 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.
31. Bersinger NA, Smarason AK, Muttukrishna S, Groome NP, Redman CW. Women with pre-eclampsia have increased serum levels of pregnancy-associated plasma protein-A (PAPP-A), inhibin A, acitivin A and soluble E-selectin. *Hypertens Pregnancy* 2003; **23**: 45–55.
32. Hughes G, Bischoff P, Wilson G, Smith R, Klopper A. Assay of placental proteins to determine risk. *Br Med J* 1980; **280**: 671–673.
33. Bersinger NA, Groome N, Muttukrishna S. Pregnancy-associated and placental proteins in the placental tissue of normal pregnant women and patients with pre-eclampsia. *Eur J Endocrinol* 2002; **147**: 1785–1793.
34. Davidson EJ, Riley SC, Roberts SA, Shearing CH, Groome NP, Martin CW. Maternal serum activin, inhibin, human chorionic gonadotropin and α-fetoprotein as second-trimester predictors of pre-eclampsia. *BJOG* 2003; **110**: 46–52.
35. Muller F, Savey L, Le Fiblec B, Bussieres L, Ndayizamba G, Colan JC, Giraudet P. Maternal serum human chorionic gonadotropin level at fifteen weeks is a predictor for pre-eclampsia. *Am J Obstet Gynecol* 1996; **175**: 37–40.
36. Lee L-C, Sheu B-C, Shau W-Y, Liu D-M, Lai T-J, Lee Y-H, Hunag S-C. Mid trimester β-hCG levels incorporated in a mutlifactorial model for the prediction of severe pre-eclampsia. *Prenat Diagn* 2000; **20**: 738–743.
37. Luckas M, Hawe K, Meekins J, Neilson J, Walkinshaw S. Second-trimester serum free human chorionic gonadotropin levels as a predictor of pre-eclampsia. *Acta Obstet Gynecol Scand* 1998; **77**: 381–384.
38. Raty R, Koskinen P, Alanen A, Irjala K, Matinlauri I, Ekblad U. Prediction of pre-eclampsia with maternal mid-trimester total renin, inhibin A, AFP and free β-hCG levels. *Prenat Diagn* 1999; **19**: 122–127.
39. Gurbuz A, Karateke A, Mengulluoglu M, Gedikbasi A, Ozturkmen M, Kabaca C, Sahinoglu Z. Can serum HCG values be used in the differential diagnosis of pregnancy complicated by hypertension? *Hypertens Pregnancy* 2004; **23**: 1–12.
40. Smith GV, Smith OW. Anterior pituitary-like hormone in late pregnancy toxemia: summary of results since 1932. *Am J Obstet Gynecol* 1939; **36**: 618–624.
41. Crosignani PG, Trojsi L, Attanasio AE, Lombroso Finzi GC. Value of hCG and hCS measurements in clinical practice. *Obstet Gynecol* 1974; **44**: 673–681.
42. Hsu CD, Chan DW, Iriye B, Johnson TRB, Hong SF, Repke JT. Elevated serum human chorionic gonadotropin as evidence of a secretory response in severe pre-eclampsia. *Am J Obstet Gynecol* 1994; **170**: 1135–1138.
43. Ong CYT, Liao AW, Munim S, Spencer K, Nicolaides KH. First-trimester maternal serum activin A in pre-eclampsia and fetal growth restriction. *J Matern Fetal Neonatal Med* 2004; **15**: 176–180.
44. Laivuori H, Kaaja R,Turpeinen U, Stenman U-H, Ylikorkala O. Serum activin A and inhibin A elevated in pre-eclampsia: no relation to insulin sensitivity. *Br J Obstet Gynaecol* 1999; **106**: 1298–1303.
45. Grobman WA, Wang EY. Serum levels of activin A and inhibin A and the subsequent development of pre-eclampsia. *Obstet Gynecol* 2000; **96**: 390–394.

