Maternal plasma inhibin A at 11–13 weeks of gestation in hypertensive disorders of pregnancy

Ranjit Akolekar, Ryoko Minekawa, Alina Veduta, Ximena C. Romero and Kypros H. Nicolaides*

Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK

Objective   To investigate the potential value of maternal plasma inhibin A in first-trimester screening for preeclampsia (PE).

Method   The concentration of inhibin A at 11–13 weeks was measured in samples from 121 pregnancies that developed PE, 87 cases of gestational hypertension (GH) and 208 normal controls. The distributions of inhibin A multiple of median (MoM) in the control and hypertensive groups were compared. Logistic regression analysis was used to derive algorithms for the prediction of hypertensive disorders.

Results   The maternal plasma inhibin A MoM was significantly higher in the early and late PE groups (1.55 MoM and 1.24 MoM, respectively; $p < 0.0083$), compared to the controls (0.98 MoM), but not in GH. Significant contributions for the prediction of PE were provided by maternal factors, plasma inhibin A and uterine artery pulsatility index (PI) and with combined screening the detection rates for early and late PE were 88% and 42%, respectively, for a false positive rate of 10%.

Conclusion   The proposed combined screening test could be used to identify women at high risk for PE and intensive monitoring in such patients would lead to earlier identification of the disease which could potentially improve pregnancy outcome. Copyright © 2009 John Wiley & Sons, Ltd.

KEY WORDS: inhibin A; first trimester screening; preeclampsia; uterine artery Doppler

INTRODUCTION

Inhibin A is a dimeric glycoprotein hormone produced by many tissues but in normal pregnancy the main source of circulating inhibin A is the placenta (Muttukrishna *et al*., 1997a; Petraglia *et al*., 1997; Florio *et al*., 2001). Several studies have reported that in patients with established preeclampsia (PE) there is a 1.5- to 8.5-fold increase in the maternal plasma inhibin A concentration (Table 1). There is also evidence that increased levels of inhibin A precede the clinical onset of PE and may be evident from the first trimester of pregnancy (Table 1).

The underlying mechanism for PE is thought to be impaired placentation due to inadequate trophoblastic invasion of maternal spiral arteries, documented by the findings of both histological and Doppler ultrasound studies of uterine arteries (Khong *et al*., 1986; Pijnenborg *et al*., 1991; Yu *et al*., 2005; Plasencia *et al*., 2007). The resulting placental hypoxia leads to the release of inflammatory factors which cause endothelial cell activation and damage (Redman 1991; Roberts *et al*., 1993; Granger *et al*., 2001). The exact function of inhibin A in pregnancy and its role in the pathogenesis of PE are uncertain but there is evidence that inhibin A has autocrine and paracrine roles in the placenta thereby affecting trophoblastic function (Muttukrishna *et al*., 1997b; Petraglia *et al*., 1997).

Previous studies reported that at 11–13 weeks of gestation in pregnancies that subsequently develop PE the uterine artery pulsatility index (PI), assessed by Doppler ultrasound, is increased (Plasencia *et al*., 2007) and the maternal serum concentration of pregnancy-associated plasma protein A (PAPP-A), which is thought to be involved in placental growth and development, is reduced (Poon *et al*., 2009). The differences between pregnancies developing PE from controls in uterine artery PI and serum PAPP-A are particularly marked in severe early onset disease requiring delivery before 34 weeks (early PE) (Plasencia *et al*., 2007; Poon *et al*., 2009). The individual risk for the development of early and late PE can be calculated from a combination of maternal demographic characteristics and obstetric history, the uterine artery PI and maternal serum PAPP-A (Poon *et al*., 2009).

The aim of this study was to first investigate further the maternal plasma concentration of inhibin A in the first trimester of pregnancy in cases that subsequently developed hypertensive disorders and secondly to estimate the potential performance of screening for PE by a combination of maternal factors, uterine artery PI and maternal serum PAPP-A and inhibin A.

METHODS

Study population

This was a prospective screening study for hypertensive complications of pregnancy in women attending for

---

*Correspondence to: Kypros H. Nicolaides, Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, Denmark Hill, London SE5 9RS, UK.
E-mail: kypros@fetalmedicine.com

Table 1—Studies reporting on the association between maternal serum inhibin A concentration and preeclampsia

Author	Gestation (w)	Kit used for analysis	Preeclampsia		Controls		p value
			n	Inhibin A	n	Inhibin A	
During preeclampsia							
Muttukrishna *et al.*, 1997b	25–33	Not stated	20	3.05 ng/mL[a]	20	0.36 ng/mL	<0.001
Silver *et al.*, 1999	25–42	Serotec	60	2.6 MoM	60	1.00 MoM	<0.0001
Gratacos *et al.*, 2000	30–37	Serotec	20	2.18 ng/mL[a]	60	0.50 ng/mL	<0.001
Keelan *et al.*, 2002	30–38	Not stated	22	15.1 ng/mL[b]	22	5.3 ng/mL	<0.001
Zeeman *et al.*, 2002	34–40	Serotec	77	1806.3 pg/mL[b]	83	936.0 pg/mL	<0.001
Florio *et al.*, 2002	25–38	Serotec	21	2.46 MoM	42	1.00 MoM	<0.001
Bersinger *et al.*, 2003	25–39	Not stated	19	3080.0 pg/mL[a]	19	1510.0 pg/mL	<0.01
Hanisch *et al.*, 2004	29–35	Serotec	21	1325.5 pg/mL[a]	11	346.1 pg/mL	<0.05
Hamar *et al.*, 2006	26–38	DSL	31	1863.7 pg/mL[a]	16	572.6 pg/mL	<0.05
Paiwattananupant *et al.*, 2008	33–39	DSL	30	1229.7 pg/mL[a]	30	839.1 pg/mL	<0.01
Before preeclampsia							
Cuckle *et al.*, 1998	13–18	Serotec	28	2.01 MoM	701	1.00 MoM	<0.001
Räty *et al.*, 1999	12–20	Biosource	22	1.09 U/mL[a]	7	0.85 U/mL	NS
Sebire *et al.*, 2000	10–14	Not stated	9	233 pg/mL[a]	759	167 pg/mL	<0.05
Grobman *et al.*, 2000	14–28	Serotec	12	700.0 pg/mL[a]	24	613.0 pg/mL	NS
D'Anna *et al.*, 2002	15–18	Serotec	20	1.43 MoM	40	1.03 MoM	NS
Davidson *et al.*, 2003	15–20	Not stated	39	206 pg/mL[b]	155	188 pg/mL	NS
Florio *et al.*, 2003	24	Serotec	18	131.2 pg/mL[a]	40	91.9 pg/mL	<0.05
Ay *et al.*, 2005	16–18	Serotec	14	3.36 MoM	164	0.99 MoM	<0.001
Wald *et al.*, 2006	15–20	DSL	96	1.39 MoM	480	1.00 MoM	<0.05
Spencer *et al.*, 2006	22–24	Serotec	24	2.03 MoM	144	1.05 MoM	<0.001
Kim *et al.*, 2006	14–23	DSL	40	414 pg/mL[b]	80	280 pg/mL	<0.001
Zwahlen *et al.*, 2007	11–13	Serotec	52	0.46 mg/mL[b]	104	0.28 mg/mL	<0.05
Kang *et al.*, 2008	10–21	Not stated	32	1.73 MoM	3044	1.00 MoM	<0.001
Spencer *et al.*, 2008a	11–14	DSL	64	1.24 MoM	240	1.00 MoM	<0.001

[a] mean values;
[b] median values;
MoM, multiple of the median.

their routine first hospital visit in pregnancy at King's College Hospital, London, UK. In this visit, which is held at $11^{+0}-13^{+6}$ weeks of gestation, first, all women have an ultrasound scan to confirm gestational age from the measurement of the fetal crown-rump length (CRL), secondly, diagnose any major fetal abnormalities and thirdly, measure fetal nuchal translucency (NT) thickness as part of screening for chromosomal abnormalities. In addition, the maternal serum PAPP-A and free beta-human chorionic gonadotropin (ß-hCG) are determined and the results are combined with the fetal NT to calculate the patient-specific risk for trisomy 21 (Snijders *et al.*, 1998; Kagan *et al.*, 2008a). We recorded maternal characteristics and medical history, measured the uterine artery PI by transabdominal color Doppler (Plasencia *et al.*, 2007) and stored serum and plasma at $-80\,°C$ for subsequent biochemical analysis. A written informed consent was obtained from the women agreeing to participate in the study, which was approved by King's College Hospital Ethics Committee.

We prospectively examined 8234 singleton pregnancies between March 2006 and March 2007. In 147 (1.8%) cases there was subsequent development of PE, 135 (1.6%) cases developed gestational hypertension (GH) and 7922 cases were unaffected by PE or GH. In this study we measured maternal plasma inhibin A in a case-control population of 121 PE, 87 GH and 208 controls. The selection of the specific samples from each

group of hypertensive disorders was simply based on availability. The cases and controls were matched for length of storage of their blood samples and none of the samples were previously thawed and refrozen.

This study is part of a research program on the early prediction of pregnancy complications and the data from these patients on serum PAPP-A were included in a previous publication (Poon *et al.*, 2009).

Maternal factors

Patients were asked to complete a questionnaire on maternal age, racial origin, cigarette smoking during pregnancy, method of conception, medical history, medication, parity, obstetric history and family history of PE in the mother. The questionnaire was then reviewed by a doctor together with the patient. The maternal weight and height were measured and the body mass index (BMI) was calculated in kg/m^2.

Outcome measures

The definitions of PE and GH were those of the International Society for the Study of Hypertension in Pregnancy (Davey *et al.*, 1988). In GH the diastolic blood pressure should be 90 mmHg or more on at

least two occasions 4 h apart developing after 20 weeks of gestation in previously normotensive women in the absence of significant proteinuria. In PE there should be GH with proteinuria of 300 mg or more in 24 h or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens, if no 24-h collection is available.

Sample analysis

Duplicate plasma samples of 50 μL were used to measure inhibin A concentration by a quantitative enzyme-linked immunoassay (ELISA) technique using DSL-10-28 100 inhibin A immunoassay (Diagnostic systems laboratories, Inc. Webster, Texas, USA). The assays were performed on an automated ELISA processor (Dade-Behring BEP 2000, Liederbach, Germany). Absorbance readings were taken on a VICTOR[3] plate reader (PerkinElmer Life and Analytical Sciences, Turku, Finland) and inhibin A concentrations were determined using MultiCalc software (PerkinElmer Life and Analytical Sciences, Turku, Finland). The lower limit of detection of the assay was 1.0 pg/mL and the between-batch imprecision was 15.4% at an inhibin A concentration of 94.5 pg/mL and 8.2% at 361.0 pg/mL. All samples were analyzed in duplicate and those with a coefficient of variation exceeding 10% were reanalyzed.

Maternal serum PAPP-A was measured using the DELFIA XPRESS analyzer (PerkinElmer Life and Analytical Sciences, Waltham, USA). The variation of the DELFIA XPRESS PAPP-A assay was determined in 20 runs with two replicates using this system. The calibration curve of the first run was used as a reference curve during the 14-day-period. The intra- and interassay variations were 1.2% and 2.1%, respectively, at a PAPP-A concentration of 462 mU/L, 1.4% and 2.3% at 2124 mU/L and 1.3% and 2.5% at 5543 mU/L.

Statistical analysis

The following steps were taken. First, the distributions of uterine artery PI, PAPP-A and inhibin A were made Gaussian after logarithmic transformation. Distributions were confirmed to be Gaussian using the probability plots and Kolmogorov–Smirnov test. Second, multiple regression analysis was used to determine which of the factors amongst the maternal characteristics and gestation were significant predictors of log inhibin A in the unaffected group. Then the distribution of log inhibin A expressed as multiples of the median (MoM) of the unaffected group, were determined in the PE and GH groups of the case-control population. Third, the measured uterine artery PI was converted into MoM after adjustment for gestation, maternal age, BMI and racial origin, as described earlier (Plasencia et al., 2007). Fourth, the measured PAPP-A was converted into MoM after adjustment for gestation, maternal age, racial origin, weight, parity, cigarette smoking status and method of conception as described earlier (Kagan et al., 2008b). Fifth, Kruskall–Wallis test with Dunn's procedure and Bonferroni correction was used to compare median MoM

of inhibin A, uterine artery PI and PAPP-A between the outcome groups. Sixth, regression analysis was used to determine the significance of association between log inhibin A MoM and log uterine artery PI MoM in the different outcome groups. Seventh, logistic regression analysis was used to determine which of the factors amongst the maternal characteristics, log uterine artery PI MoM, log inhibin A MoM and log PAPP-A MoM had a significant contribution in predicting early and late PE. Eighth, the detection and false positive rates (FPR) were calculated as the respective proportions of PE (detection rate) and unaffected pregnancies (FPR) with MoM values above given cut-offs. Ninth, the performance of screening was determined by receiver operating characteristic (ROC) curves analysis.

The statistical software package SPSS 15.0 (SPSS Inc.,Chicago, IL), MedCalc for windows, version 9.6.2.0 (MedCalc Software, Mariakerke, Belgium) and XLSTAT-Pro 2008 (Addinsoft, USA) were used for data analyses.

RESULTS

The maternal characteristics of each of the outcome groups are compared in Table 2.

Unaffected group

Multiple regression analysis in the unaffected group of the case-control population demonstrated that for log inhibin A and significant independent contributions were provided by maternal weight and racial origin:
log expected inhibin A = 2.596−0.003 × maternal weight in kg + (0.127 if Black, 0 if other racial origins); $R^2 = 0.078$, $p < 0.0001$.

In each patient we used this formula to derive the expected log inhibin A and then expressed the observed value as a MoM of the expected (Table 3). Similarly, we used previously derived formulae for uterine artery PI and PAPP-A to calculate the respective MoM values (Plasencia et al., 2007; Kagan et al., 2008b).

Hypertensive disorders

Plasma inhibin A and uterine artery PI were significantly higher in early and late PE than in controls, but not in GH than in controls (Table 3, Figure 1). Serum PAPP-A was significantly lower in early PE than in controls but not in late PE or GH than in controls (Table 3). There were no significant associations between plasma inhibin A and uterine artery PI in either of the controls ($p = 0.120$) or the hypertensive groups (early PE $p = 0.568$, late PE $p = 0.492$, GH $p = 0.671$).

Screening for early PE

Logistic regression analysis demonstrated that in the prediction of early PE there were significant contributions from log MoM uterine artery PI (OR 4.442E[5],

Table 2—Maternal characteristics in the four outcome groups

Maternal characteristics	Control (n = 208)	Early preeclampsia (n = 26)	Late preeclampsia (n = 95)	Gestational hypertension (n = 87)
Maternal age in years, median (IQR)	31.9 (28.7–35.3)	32.7 (27.4–38.7)	31.6 (26.7–36.3)	33.4 (30.1–35.9)
BMI in kg/m^2, median (IQR)	25.2 (23.0–29.1)	27.3 (23.7–32.0)	27.0 (23.7–33.3)*	26.6 (24.1–31.1)
CRL in mm, median (IQR)	64.1 (59.7–71.0)	68.8 (58.3–74.5)	62.0 (58.0–68.9)	62.2 (57.6–69.1)
GA at sampling (weeks), median (IQR)	12.4 (12.2–13.0)	12.4 (12.2–13.3)	12.3 (12.1–12.5)	12.3 (12.1–13.0)
Racial origin				
White, *n* (%)	146 (70.2)	11 (42.3)	41 (43.2)	66 (75.9)
Black, *n* (%)	41 (19.7)	11 (42.3)**	40 (42.1)***	16 (18.4)
Indian or Pakistani, *n* (%)	14 (6.7)	2 (7.7)	7 (7.4)	0**
Chinese or Japanese, *n* (%)	2 (1.0)	0	2 (2.1)	1 (1.1)
Mixed, *n* (%)	5 (2.4)	2 (7.7)	5 (5.3)	4 (4.6)
Parity				
Nulliparous, *n* (%)	80 (38.5)	13 (50.0)	61 (64.2)	48 (55.2)
Parous—no previous PE, *n* (%)	122 (58.7)	6 (23.1)**	22 (23.2)	29 (33.3)
Parous—previous PE, *n* (%)	6 (2.9)	7 (26.9)	12 (12.6)**	10 (11.5)*
Family history of PE—Mother (*n*, %)	7 (3.4)	3 (11.5)	11 (11.6)**	9 (10.3)*
Cigarette smoker, *n* (%)	16 (7.7)	0	6 (6.3)	7 (8.0)
Conception				
Spontaneous, *n* (%)	201 (96.6)	23 (88.5)	91 (95.8)	84 (96.6)
Assisted, *n* (%)	7 (3.4)	3 (11.5)	4 (4.2)	3 (3.4)
Medical history				
None, *n* (%)	201 (96.6)	21 (80.8)	89 (93.7)	84 (96.6)
Chronic hypertension, *n* (%)	1 (0.5)	4 (15.4)	4 (4.2)*	0
Diabetes mellitus, *n* (%)	2 (1.0)	0	1 (1.1)	2 (2.3)
Thrombophilia, *n* (%)	3 (1.4)	1 (3.8)	1 (1.1)	1 (1.1)
Others, *n* (%)	1 (0.5)	0	0	0
Medication during pregnancy				
None, *n* (%)	188 (90.4)	22 (84.6)	87 (91.6)	75 (86.2)
Antihypertensives, *n* (%)	0	2 (7.7)*	2 (2.1)	0
Insulin, *n* (%)	2 (1.0)	0	1 (1.1)	2 (2.3)
Antiasthmatics, *n* (%)	5 (2.4)	0	3 (3.2)	4 (4.5)
Thyroxin, *n* (%)	3 (1.4)	1 (3.8)	1 (1.1)	2 (2.3)
Aspirin, *n* (%)	3 (1.5)	1 (3.8)	0	2 (2.3)
Antidepressant, *n* (%)	2 (1.0)	0	1 (1.1)	1 (1.1)
Antiepileptic, *n* (%)	4 (1.9)	0	0	1 (1.1)
Others, *n* (%)	1 (0.5)	0	0	0

BMI, body mass index; CRL, crown-rump length; GA, gestational age; IQR, interquartile range. Note: Comparisons between each outcome group with controls (Chi-square test and Fisher's exact test for categorical variables and Kruskall–Wallis test and Dunn's procedure with Bonferroni correction for continuous variables): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

95% CI 413.0–4.8E^8; $p < 0.0001$), log MoM PAPP-A (OR 0.099, 95% CI 0.011–0.929; $p = 0.043$) log MoM inhibin A (OR 249.6, 95% CI 9.3–6.688E^3; $p = 0.001$), history of chronic hypertension (OR 143.3, 95% CI 8.3–2.463E^3; $p = 0.001$), Black ethnic origin (OR 5.5, 95% CI 1.4–21.8; $p = 0.016$), mixed ethnic origin (OR 16.3, 95% CI 1.3–198.1; $p = 0.029$) and parous with no previous PE (OR 0.159, 95% CI 0.039–0.651; $p = 0.011$) but not BMI ($p = 0.190$) or family history of PE ($p = 0.068$); $R^2 = 0.608$, $p < 0.0001$.

The estimated detection rates of early PE at fixed FPR of 5% and 10% in screening by maternal obstetric history and characteristics, inhibin A, PAPP-A, uterine artery PI and by their combinations are shown in Table 4 and the areas under the ROC curves are shown in Table 5. The estimated detection rate of screening for early PE by inhibin A was 23.1% and 30.8% at respective FPR of 5% and 10% and the values increased to 84.6% and 88.5% in screening by a combination of maternal obstetric history and characteristics, inhibin A and uterine artery PI.

Screening for late PE

Logistic regression analysis demonstrated that in the detection of late PE there were significant contributions from log MoM uterine artery PI (OR 32.0, 95% CI 3.4–302.5; $p = 0.002$), log MoM inhibin A (OR 21.0, 95% CI 4.6–96.1; $p < 0.0001$), BMI (OR 1.1, 95%CI 1.1–1.2; $p < 0.001$), Black ethnic origin (OR 3.8, 95% CI 2.0–7.3; $p < 0.0001$) and parous with no previous PE (OR 0.123, 95% CI 0.062–0.243; $p < 0.0001$) but not from log MoM PAPP-A ($p = 0.283$), history of chronic hypertension ($p = 0.266$) and family history of PE ($p = 0.063$) $R^2 = 0.377$, $p < 0.0001$.

The estimated detection rates of late PE at fixed FPR of 5% and 10% in screening by maternal obstetric

Table 3—Median (IQR) for inhibin A, uterine artery pulsatility index (PI) and PAPP-A in the four outcome groups

	Serum inhibin A (median, IQR)		Uterine artery PI (median, IQR)		Serum PAPP-A (median, IQR)	
	MoM	pg/mL	MoM	Unit	MoM	mU/L
Unaffected	0.98 (0.72–1.43)	244.0 (171.9–341.0)	1.05 (0.83–1.30)	1.68 (1.32–2.04)	1.00 (0.69–1.45)	2.80 (1.81–4.60)
Early preeclampsia	1.55 (0.95–2.05)*	378.8 (243.6–529.8)	1.56 (1.20–1.69)*	2.49 (1.96–2.66)	0.62 (0.42–1.11)*	2.63 (0.95–3.36)
Late preeclampsia	1.24 (0.89–1.65)*	322.3 (210.3–444.5)	1.26 (0.92–1.44)*	2.00 (1.54–2.36)	0.95 (0.58–1.31)	2.71 (1.55–4.32)
Gestational hypertension	1.07 (0.80–1.42)	253.1 (196.0–333.2)	1.12 (0.89–1.33)	1.77 (1.42–2.08)	0.87 (0.62–1.44)	2.01 (1.54–3.38)

Note: Comparisons between outcome groups by Kruskall–Wallis test and Dunn's procedure with Bonferroni correction,
* $p < 0.0083$.

history and characteristics, inhibin A, PAPP-A, uterine artery PI and by their combinations are shown in Table 4 and the areas under the ROC curves are shown in Table 5. The estimated detection rate of screening for late PE by inhibin A was 13.7% and 16.8% at respective FPR of 5% and 10% and the values increased to 36.8% and 55.8% in screening by a combination of maternal obstetric history and characteristics and plasma inhibin A.

DISCUSSION

The findings of this study that at 11–13 weeks of gestation, women who subsequently develop PE have increased maternal plasma levels of inhibin A, reduced serum PAPP-A and increased uterine artery PI are consistent with previous reports (Sebire *et al.*, 2000; Plasencia *et al.*, 2007; Zwahlen *et al.*, 2007; Spencer *et al.*, 2008a; Spencer *et al.*, 2008b). The maternal plasma inhibin A and uterine artery PI are significantly higher in early and late PE but not in GH whereas maternal serum PAPP-A is significantly lower in early PE but not in late PE and GH.

In the unaffected controls, the measured concentration of maternal plasma inhibin A decreased with maternal weight and was higher in Black than in White women. Consequently, as in the case of uterine artery PI and serum PAPP-A the measured concentration of inhibin A must be adjusted for these variables before comparing with pathological pregnancies (Plasencia *et al.*, 2007; Kagan *et al.*, 2008b). Most of the previous studies did not make any adjustments for these variables, except a few that adjusted only for gestational age (Florio *et al.*, 2002; Ay *et al.*, 2005; Kim *et al.*, 2006;
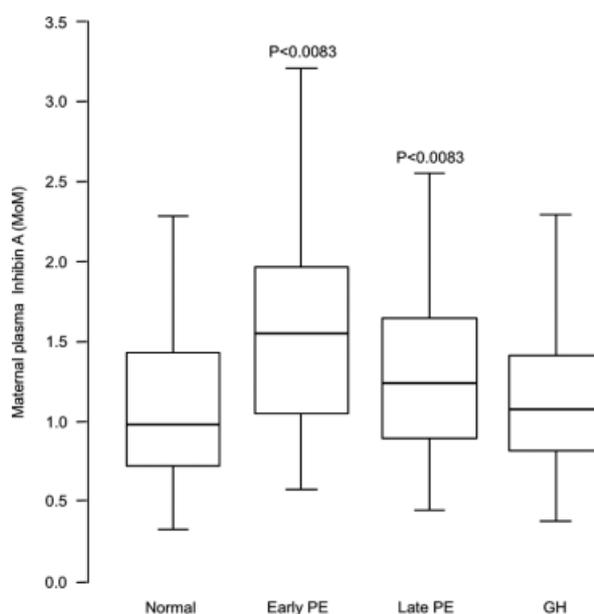

Figure 1—Box-and-whisker plot of (median, interquartile range and range) of inhibin A multiple of median (MoM) in pregnancy outcome groups: controls, early preeclampsia (PE), late PE and gestational hypertension (GH)

Table 4—Detection rates of early and late preeclampsia at fixed false positive rates (FPR) of 5% and 10% in screening by maternal obstetric history and characteristics, inhibin A, pregnancy-associated plasma protein A (PAPP-A), uterine artery PI and by their combinations

	Detection rate (%) (95% CI)			
	Early preeclampsia		Late preeclampsia	
Screening test	FPR 5%	FPR 10%	FPR 5%	FPR 10%
History/characteristics	47.5 (27.6–68.6)	57.7 (36.9–76.6)	31.6 (22.4–41.9)	43.2 (33.0–53.7)
Inhibin A	23.1 (9.0–43.7)	30.8 (14.4–51.8)	13.7 (7.5–22.3)	16.8 (9.9–25.9)
PAPP-A	23.1 (9.0–43.7)	42.3 (23.4–63.1)	—	—
Uterine artery PI	50.0 (29.9–70.1)	73.1 (52.2–88.4)	19.0 (11.6–28.3)	22.1 (14.2–31.8)
History with				
Inhibin A	46.2 (26.6–66.6)	61.5 (40.6–79.7)	36.8 (27.2–47.4)	55.8 (45.2–66.0)
PAPP-A	69.2 (48.2–85.6)	69.2 (48.2–85.6)	—	—
Inhibin A and PAPP-A	57.7 (36.9–76.6)	73.1 (52.2–88.4)	—	—
Uterine artery PI	65.4 (44.3–82.8)	84.6 (65.1–95.5)	31.6 (22.4–41.9)	46.3 (36.0–56.8)
Uterine artery PI and PAPP-A	61.5 (40.6–79.7)	80.8 (60.6–93.4)	—	—
Inhibin A and uterine artery PI	84.6 (65.1–95.5)	88.5 (69.8–97.4)	33.7 (24.3–44.1)	42.1 (32.0–52.7)
Inhibin A, PAPP-A and uterine artery PI	80.8 (60.6–93.4)	88.5 (69.8–97.4)	—	—

Table 5—Comparison of the performance of screening for preeclampsia by maternal obstetric history and characteristics, inhibin A, PAPP-A, uterine artery PI and by their combinations by receiver-operating characteristics curve analysis

	Area under receiver-operating characteristics curve analysis, mean (95% CI)	
Screening test	Early preeclampsia	Late preeclampsia
History/characteristics	0.806 (0.749–0.854)	0.783 (0.732–0.828)
Inhibin A	0.679 (0.615–0.738)	0.625 (0.568–0.680)
PAPP-A	0.705 (0.643–0.763)	—
Uterine artery PI	0.829 (0.774–0.875)	0.623 (0.566–0.678)
History with		
Inhibin A	0.876 (0.827–0.916)	0.815 (0.767–0.857)
PAPP-A	0.866 (0.815–0.907)	—
Inhibin A and PAPP-A	0.896 (0.849–0.932)	—
Uterine artery PI	0.899 (0.853–0.934)	0.799 (0.750–0.843)
Uterine artery PI and PAPP-A	0.919 (0.877–0.951)	—
Inhibin A and uterine artery PI	0.938 (0.899–0.965)	0.823 (0.775–0.864)
Inhibin A, PAPP-A and uterine artery PI	0.938 (0.899–0.965)	—

Spencer *et al.*, 2006; Wald *et al.*, 2006; Kang *et al.*, 2008; Spencer *et al.*, 2008a) or gestational age and maternal weight (Cuckle *et al.*, 1998; Silver *et al.*, 1999; D'Anna *et al.*, 2002). In our study there was no change with fetal CRL within the narrow gestational range of 11–13 weeks.

In women with established PE the level of inhibin A has been reported to be substantially increased (Table 1). However, there is conflicting evidence in the literature regarding inhibin A levels before the clinical onset of the disease. Possible explanations for this discrepancy include failure to correct for maternal characteristics (as discussed in the previous paragraph), small sample sizes, variations in the severity of PE and differences in the methods of analysis. It is also likely that the further back in pregnancy one gets from the clinical manifestation of PE the deviation from normal in serum metabolites is less (Kang *et al.*, 2008; Spencer *et al.*, 2008a).

The mechanism of increased maternal plasma levels of inhibin A in pregnancies destined to develop PE is uncertain. Immunohistochemical studies have localized inhibin A to the syncytiotrophoblast layer (McCluggage *et al.*, 1998) and it was postulated that the increased plasma levels in PE may be the consequence of reactive hyperplasia of the cytotrophoblast cells leading to increased production or due to functional alterations in syncytiotrophoblast giving rise to increased leakage of placental proteins into the maternal circulation (Aquilina *et al.*, 1999). There is evidence that in established PE the increased plasma levels may be secondary to increased placental m-RNA production (Silver *et al.*, 2002). In contrast, *in vitro* studies demonstrated that in placental explants low oxygen tension and hypoxia down regulate the expression of the inhibin A gene (Manuelpillai *et al.*, 2003). Our finding of lack of a significant association between uterine artery PI and plasma inhibin A in either the PE or the unaffected group contradicts the hypothesis of impaired placentation and placental hypoxia as the cause of increased inhibin A in PE. A previous study in the second trimester has also reported a lack of significant association between inhibin A and uterine artery PI (Spencer *et al.*, 2006).

Maternal plasma inhibin A in combination with factors from the maternal history and uterine artery PI could provide effective first-trimester screening for subsequent development of PE. We estimated that at 10% false positive rate such combined screening could identify 88% of early PE and 42% of late PE. This is particularly important because it is early rather than late disease which is associated with increased risk of perinatal mortality and morbidity and both short- and long-term maternal complications (Witlin *et al.*, 1999; Irgens *et al.*, 2001; von Dadelszen *et al.*, 2003). Previous studies have also reported that inhibin A can be combined with uterine artery PI to achieve high detection of PE, but they did not distinguish between early and late PE. Aquilina *et al.* (2001) reported that serum inhibin A at 15–19 weeks together with uterine artery Doppler at 18–22 weeks could detect 71% of cases developing PE at an FPR of 7%. Spencer *et al.* (2006) reported that the detection rate of PE with inhibin A and uterine artery PI at 22–24 weeks was 75% at an FPR of 10%. In another study by Spencer *et al.* (2008a), inhibin A at 11–13 weeks together with uterine artery PI at 22–24 weeks had a detection rate of 68% for an FPR of 5%.

Identification of women at high risk for PE could potentially improve pregnancy outcome because intensive maternal and fetal monitoring in such patients would lead to an earlier diagnosis of the clinical signs of the disease and the associated fetal growth restriction and avoid the development of serious complications through such interventions as the administration of antihypertensive medication and early delivery. The proposed combined screening test could also be used for effective identification of the high-risk group for future studies investigating the potential role of pharmacological interventions starting from the first trimester to improve placentation and reduce the prevalence of the disease.

REFERENCES

Aquilina J, Barnett A, Thompson O, Harrington K. 1999. Second-trimester maternal serum inhibin A concentration as an early marker for preeclampsia. *Am J Obstet Gynecol* **181**: 131–136.

Aquilina J, Thompson O, Thilaganathan B, Harriangton K. 2001. Improved early prediction of pre-eclampsia by combining second trimester maternal serum inhibin A and uterine artery Doppler. *Ultrasound Obstet Gynecol* **17**: 477–484.

Ay E, Kavak ZN, Elter K, Gokaslan H, Pekin T. 2005. Screening for pre-eclampsia by using maternal serum inhibin A, activin A, human chorionic gonadotropin, unconjugated estriol, and alpha-fetoprotein levels and uterine artery Doppler in the second trimester of pregnancy. *Aust N Z J Obstet Gynaecol* **45**: 283–828.

Bersinger NA, Smárason AK, Muttukrishna S, Groome NP, Redman CW. 2003. Women with preeclampsia have increased serum levels of pregnancy-associated plasma protein A (PAPP-A), inhibin A, activin A and soluble E-selectin. *Hypertens Pregnancy* **22**: 45–55.

Cuckle H, Sehmi I, Jones R. 1998. Maternal serum inhibin A can predict pre-eclampsia. *Br J Obstet Gynecol* **105**: 1101–1103.

D'Anna R, Baviera G, Corrado F, Leonardi I, Buemi M, Jasonni VM. 2002. Is mid-trimester maternal serum inhibin A a marker of preeclampsia or intrauterine growth restriction? *Acta Obstet Gynecol Scand* **81**: 540–543.

Davey DA, MacGillivray I. 1988. The classification and definition of the hypertensive disorders of pregnancy. *Am J Obstet Gynecol* **158**: 892–898.

Davidson EJ, Riley SC, Roberts SA, Shearing CH, Groome NP, Martin CW. 2003. Maternal serum activin, inhibin, human chorionic gonadotropin and alpha-fetoprotein as second trimester predictors of pre-eclampsia. *Br J Obstet Gynecol* **110**: 46–52.

Florio P, Cobellis L, Luisi S, *et al.* 2001. Changes in inhibins and activin secretion in healthy and pathological pregnancies. *Mol Cell Endocrinol* **180**: 123–130.

Florio P, Ciarmela P, Luisi S, *et al.* 2002. Pre-eclampsia with fetal growth restriction: placental and serum activin A and inhibin A levels. *Gynecol Endocrinol* **16**: 365–372.

Florio P, Reis FM, Pezzani I, Luisi S, Severi FM, Petraglia F. 2003. 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* **21**: 165–169.

Granger JP, Alexander BT, Llinas MT, Bennett WA, Khalil RA. 2001. Pathophysiology of hypertension during preeclampsia linking placental ischemia with endothelial dysfunction. *Hypertension* **38**: 718–722.

Gratacos E, Casals E, Gomez O, *et al.* 2000. Inhibin A serum levels in proteinuric and nonproteinuric pregnancy-induced hypertension: evidence for placental involvement in gestational hypertension? *Hypertens pregnancy* **19**: 315–321.

Grobman WA, Wang EY. 2000. Serum levels of activin A and inhibin A and the subsequent development of preeclampsia. *Obstet Gynecol* **96**: 390–394.

Hanisch CG, Pfeiffer KA, Schlebusch H, Schmolling J. 2004. Adhesion molecules, activin and inhibin—candidates for the biochemical prediction of hypertensive diseases in pregnancy? *Arch Gynecol Obstet* **270**: 110–115.

Hamar BD, Buhimschi IA, Sfakianaki AK, *et al.* 2006. Serum and urine inhibin A but not free activin A are endocrine markers of severe pre-eclampsia. *Am J Obstet Gynecol* **195**: 1636–1645.

Irgens HU, Reisaeter L, Irgens LM, Lie RT. 2001. Long term mortality of mothers and fathers after pre-eclampsia: population based cohort study. *BMJ* **323**: 1213–1217.

Kagan KO, Wright D, Baker A, Sahota D, Nicolaides KH. 2008a. Screening for trisomy 21 by maternal age, fetal nuchal translucency thickness, free beta-human chorionic gonadotropin, and pregnancy associated plasma protein-A. *Ultrasound Obstet Gynecol* **31**: 618–624.

Kagan KO, Wright D, Spencer K, Molina FS, Nicolaides KH. 2008b. First trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy associated plasma protein-A: impact of maternal and pregnancy characteristics. *Ultrasound Obstet Gynecol* **31**: 493–502.

Kang JH, Farina A, Park JH, *et al.* 2008. Down syndrome biochemical markers and screening for preeclampsia at first and second trimester: correlation with the week of onset and the severity. *Prenat Diagn* **28**: 704–709.

Keelan JA, Taylor R, Schellenberg JC, Groome NP, Mitchell MD, North RA. 2002. Serum activin A, inhibin A, and follistatin concentrations in preeclampsia or small for gestational age pregnancies. *Obstet Gynecol* **99**: 267–274.

Khong TY, De Wolf F, Robertson WB, Brosens I. 1986. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. *Br J Obstet Gynaecol* **93**: 1049–1059.

Kim SY, Ryu HM, Yang JH, *et al.* 2006. Maternal serum and amniotic fluid inhibin A levels in women who subsequently develop severe preeclampsia. *J Korean Med Sci* **21**: 452–456.

Manuelpillai U, Schneider-Kolsky M, Thirunavukarasu P, Dole A, Waldron K, Wallace EM. 2003. Effect of hypoxia on placental activin A, inhibin A and follistatin synthesis. *Placenta* **24**: 77–83.

McCluggage WG, Ashe P, McBride H, Maxwell P, Sloan JM. 1998. Localization of the cellular expression of inhibin in trophoblastic tissue. *Histopathology* **32**: 252–256.

Muttukrishna S, Child TJ, Groome NP, Ledger WL. 1997a. Source of circulating levels of inhibin A, pro alpha C-containing inhibins and activin A in early pregnancy. *Hum Reprod.* **12**: 1089–1093.

Muttukrishna S, Knight PG, Groome NP, Redman CW, Ledger WL. 1997b. Activin A and inhibin A as possible endocrine markers for preeclampsia. *Lancet* **349**: 1285–1288.

Paiwattananupant K, Phupong V. 2008. Serum inhibin A level in preeclampsia and normotensive pregnancy. *Hypertens pregnancy* **27**: 337–343.

Petraglia F. 1997. Inhibin, activin and follistatin in the human placenta—a new family of regulatory proteins. *Placenta* **18**: 3–8.

Pijnenborg R, Anthony J, Davey DA, *et al.* 1991. Placental bed spiral arteries in the hypertensive disorders of pregnancy. *Br J Obstet Gynaecol* **98**: 648–655.

Plasencia W, Maiz N, Bonino S, Kaihura C, Nicolaides KH. 2007. Uterine artery Doppler at 11^{+0} to 13^{+6} weeks in the prediction of pre-eclampsia. *Ultrasound Obstet Gynecol* **30**: 742–749.

Poon LCY, Maiz N, Valencia C, Plasencia W, Nicolaides KH. 2009. First-trimester maternal serum pregnancy-associated plasma protein-A and pre-eclampsia. *Ultrasound Obstet Gynecol* **33**: 23–33.

Räty R, Koskinen P, Alanen A, Irjala K, Matinlauri I, Ekblad U. 1999. Prediction of pre-eclampsia with maternal mid-trimester total rennin, inhibin A, AFP and free β-hCG levels. *Prenat Diagn* **19**: 122–127.

Redman CWG. 1991. Pre-eclampsia and the placenta. *Placenta* **12**: 301–308.

Roberts JM, Redman CW. 1993. Pre-eclampsia: more than pregnancy-induced hypertension. *Lancet* **341**: 1447–1451.

Sebire NJ, Roberts L, Noble P, Wallace E, Nicolaides KH. 2000. Raised maternal serum inhibin A concentration at 10–14 weeks of gestation is associated with pre-eclampsia. *Br J Obstet Gynecol* **107**: 795–797.

Silver HM, Lambert-Messerlian GM, Star JA, Hogan J, Canick JA. 1999. Comparison of maternal serum total activin A and inhibin A in normal, preeclamptic, and nonproteinuric gestationally hypertensive pregnancies. *Am J Obstet Gynecol* **180**: 1131–1137.

Silver HM, Lambert-Messerlian GM, Reis FM, Diblasio AM, Petraglia F, Canick JA. 2002. Mechanism of increased maternal serum total activin A and inhibin A in preeclampsia. *J Soc Gynecol Investig* **9**: 308–312.

Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH. 1998. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. *Lancet* **352**: 343–346.

Spencer K, Cowans NJ, Nicolaides KH. 2008a. Maternal serum inhibin-A and activin-A levels in the first trimester of pregnancies developing pre-eclampsia. *Ultrasound Obstet Gynecol* **32**: 622–626.

Spencer K, Cowans NJ, Nicolaides KH. 2008b. Low levels of maternal serum PAPP-A in the first trimester and the risk of pre-eclampsia. *Prenat Diagn* **28**: 7–10.

Spencer K, Yu CK, Savvidou M, Papageorghiou AT, Nicolaides KH. 2006. Prediction of pre-eclampsia by uterine artery Doppler ultrasonography and maternal serum pregnancy-associated plasma protein-A, free beta-human chorionic gonadotropin, activin A and inhibin A at 22 + 0 to 24 + 6 weeks' gestation. *Ultrasound Obstet Gynecol* **27**: 658–663.

von Dadelszen P, Magee LA, Roberts JM. 2003. Subclassification of pre-eclampsia. *Hypertens Pregnancy* **22**: 143–148.

Wald NJ, Morris JK, Ibison J, Wu T, George LM. 2006. Screening in early pregnancy for pre-eclampsia using Down syndrome quadruple test markers. *Prenat Diagn* **26**: 559–564.

Witlin GA, Saade GR, Mattar FM, Sibai BM. 1999. Predictors of neonatal outcome in women with severe pre-eclampsia or eclampsia between 24 and 33 weeks' gestation. *Am J Obstet Gynecol* **1**: S19.

Yu CK, Smith GC, Papageorghiou AT, Cacho AM, Nicolaides KH. 2005. An integrated model for the prediction of preeclampsia using maternal factors and uterine artery Doppler velocimetry in unselected low-risk women. *Am J Obstet Gynecol* **193**: 429–436.

Zeeman GG, Alexander JM, McIntire DD, Byrd W, Leveno KJ. 2002. Inhibin A levels and severity of hypertensive disorders due to pregnancy. *Obstet Gynecol* **100**: 140–144.

Zwahlen M, Gerber S, Bersinger NA. 2007. First trimester markers for pre-eclampsia: placental vs non-placental protein levels. *Gynecol Obstet Invest* **63**: 15–21.