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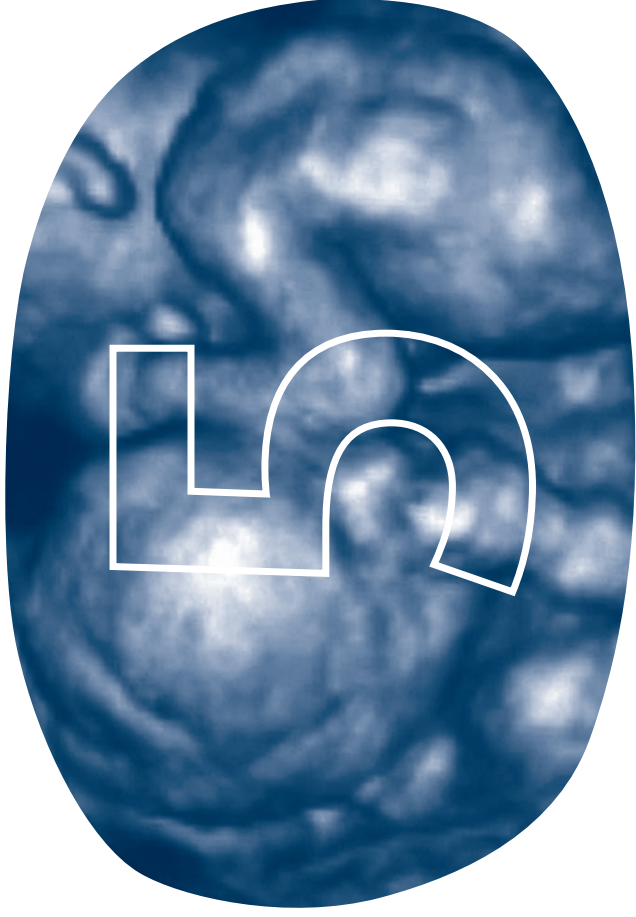
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**ADAM12s and PP13 as first trimester screening
markers for adverse pregnancy outcome**

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Submitted

Abstract

• • • Objective

To assess the screening performance of first trimester serum A-Disintegrin-And-Metalloprotease 12s (ADAM12s) and Placental Protein 13 (PP13) for preeclampsia (PE), gestational hypertension (GH) and small-for-gestational-age (SGA) fetuses.

• • • Method

In this retrospective case-control study cases were matched for gestational and maternal age at sampling. Results were expressed as multiples of the median (MoM) and compared using Mann-Whitney U test. Screening performance was assessed by Receiver-Operator-Characteristics (ROC) curves.

• • • Results

Seventeen cases of preeclampsia, 30 cases of gestational hypertension and 8 cases of small-for-gestational-age fetuses were matched with 165 controls. Decreased first trimester ADAM12s and PP13 levels were found in GH, PE and SGA cases, however, only significantly lower for ADAM12s and GH. ROC-analysis yielded area-under-the-curve (AUC) for ADAM12s and PP13 of 0.63 and 0.59 for PE, 0.68 and 0.57 for GH and 0.59 and 0.62 for SGA, respectively. Combined ADAM12s and PP13 did not improve AUC. When specificity set at 80%, corresponding detection rate of ADAM12s was 52% for GH.

• • • Conclusion

Combined ADAM12s and PP13 measurements in this study do not predict adverse pregnancy outcome, but decreased first trimester ADAM12s and PP13 levels are associated with PE and SGA although only significantly for ADAM12s and gestational hypertension.

Introduction

Preeclampsia, gestational hypertension and intrauterine growth restriction are major causes of maternal and fetal mortality and morbidity¹. Although the pathogenesis is only partly understood, it is assumed that impaired trophoblastic invasion and subsequent placental insufficiency play an important role^{2,3}.

Early monitoring of trophoblastic invasion is generally believed to enable us in identifying women at high risk for developing pregnancy complications that require more intensive surveillance during pregnancy. In addition, early identification of high risk pregnancies can be useful when designing clinical trials regarding early treatment or intervention to prevent fetal or maternal complications.

Over the last decade, various authors examined the potential of first trimester maternal marker screening of impaired placental development to identify high risk pregnancies, such as spiral artery Doppler measurements, uterine artery Doppler measurements or maternal serum markers, such as soluble Flt, Placenta Growth Factor (PlGF), pregnancy associated plasma protein-A (PAPP-A) or free beta human Chorionic Gonadotrophin (free β -hCG)⁴⁻⁹. However, large and even controversial differences in screening performance for detecting complicated pregnancies are reported.

A Disintegrin And Metalloprotease 12s (ADAM12s) is a placenta-derived glycoprotein produced by trophoblasts, which is involved in growth and differentiation¹⁰. Literature has reported low serum levels of ADAM12s preceding pregnancies complicated by preeclampsia or intrauterine growth restriction¹¹⁻¹⁵. Although one study showed no association between ADAM12s and preeclampsia or fetal growth restriction in singletons¹⁶ and also in twin pregnancies this relationship was not confirmed¹⁷.

Placental Protein 13 (PP13) is a small dimer protein involved in implantation and spiral artery modification¹⁸. Previous studies have shown a relationship of low serum PP13 levels and subsequent development of preeclampsia^{19,20}. Some authors have used additional markers to increase screening performance, such as uterine artery Doppler or uterine artery Doppler combined with serum levels of PAPP-A^{21,22}. However, one report by Cowans et al. (2008) did not show a significant relationship between low levels of PP13 and fetal growth restriction²³.

To our knowledge, there are no reports published regarding the combined predictive value of ADAM12s and PP13. Therefore, we performed a retrospective matched case-control study to investigate the potential value of combined first trimester maternal serum ADAM12s and PP13 measurements for prediction of preeclampsia (PE), gestational hypertension (GH) or small-for-gestational-age (SGA) fetuses.

Methods

A retrospective matched case-control study was performed at the VU university medical center, Amsterdam. Maternal serum samples were collected between 9+0 till 13+6 weeks of gestation as part of the routine first trimester screening program for Down syndrome from

2004 till 2007 and unused material was stored at -20°C for research purposes. Before starting our case-control study, we evaluated an in house protocol and assessed the stability of PP13 and ADAM12s. We found stable concentrations for the entire study period. Stability of PP13 and ADAM12s at room and refrigerator temperature have been described previously^{24,25}. For ADAM12s the results of our stability protocol, including results on stability at -20°C have been published elsewhere²⁶.

Gestational age at sample date was based on both dating scan and first day of last menstrual period. When a difference of less than 7 days existed, gestational age was based on the first day of the last period. If a difference of at least 7 days existed, a second dating scan was performed 2 weeks later. Gestational age was then averaged from both dating scans. Pregnancy course and outcome were obtained by filled in questionnaires and delivery room records as well as examination of the medical records and scanning of medical databases. All participating women in this study gave informed consent. The study was approved by our local Medical Ethic Committee.

According to the criteria of the International Society for the Study of Hypertension in Pregnancy cases of gestational hypertension were identified. GH was defined as two recordings of diastolic blood pressure above 90 mmHg at least 4 hours apart in a previously normotensive woman. The same criteria were used to identify cases of PE, defined as GH combined with proteinuria exceeding 300 mg/24 h or two readings of at least 2+ by dipstick on urine analysis after 20 weeks of gestation²⁷. SGA was defined as birth weight below the tenth percentile²⁸. Each case was matched for gestational age at sampling and maternal age with three control cases. In addition, medical records were studied for data concerning known confounders such as smoking status, ethnicity, primigravida and maternal weight. Pregnancy outcome data was evaluated for peak diastolic pressure, gestational age at delivery, fetal gender and neonatal weight at birth. We excluded multiple pregnancies, serum samples with unknown gestational age and pregnancies with fetal abnormalities. Control cases delivered at term a healthy baby.

Serum ADAM12s was measured 'blinded' for clinical outcome, using a semi-automatically performed time-resolved immunofluorometric assay (AutoDELFIA, PerkinElmer, Turku, Finland). Interassay CV for the ADAM12s assay was below 5% at all levels. Serum PP13 was measured blinded for clinical outcome, using a solid phase enzyme linked immunosorbent assay (ELISA), (AutoDELFIA, PerkinElmer, Turku, Finland). Interassay CV for the PP13 assay was below 5% at all levels.

Patient characteristics of both cases and controls are presented as medians (range) and percentages (%) and tested for significance with Mann-Whitney U and chi-square tests. ADAM12s and PP13 results were expressed in multiples of the median (MoM) for control cases. Differences in ADAM12s and PP13s MoM's between cases and controls were evaluated for significance with non-parametric Mann-Whitney U tests on the nontransformed MoM's, since log-transforming the ADAM12s and PP13 MoM's did not improve the fitting curve and were not normally distributed.

Receiver-operator-characteristics (ROC) curves were used to assess screening performance for ADAM12s, PP13 and both combined. Values of $p < 0.05$ were considered to be significant.

Results

We identified 30 cases of GH, 17 PE and 8 cases of SGA fetuses and matched 165 control cases. Patient and pregnancy outcome characteristics are shown in Table 1 and 2, and are consistent with the matching criteria described in the Methods section. Because of the matching procedure no significant differences in gestational age and maternal age at sample collection were found between controls and cases. In addition, no differences between cases and controls for smoking, ethnicity, primigravida and maternal weight were found.

Table 1 Maternal characteristics in the four outcome groups

Characteristic	Cases				
	Controls	PE	GH	SGA	p-value
Number	165	17	30	8	
Median GA at sampling in days (range)	82.1 (63-97)	80.1 (64-94)	80.0 (63-96)	84.9 (65-95)	0.55
Median maternal age in years at sampling (range)	34.4 (19.9-41.2)	35.7 (30.3-41.6)	34.7 (27.5-43.4)	34.7 (30.4-37.8)	0.91
Median maternal weight (kg) (range)	66.1 (50-110)	69.8 (58-77)	72.3 (56-110)	64.0 (58-78)	0.16
Ethnicity (% Caucasian)	95.6	93.8	100	100	0.65
Primigravida (%)	59.5	53.8	68.0	100	0.19
Non-smoking (%)	95.2	90.9	100	85.7	0.47

PE - preeclampsia; GH - gestational hypertension; SGA - small for gestational age; GA - gestational age
* p-value < 0.05 by post hoc testing

Logistic regression analysis demonstrated no significant confounding from maternal age, ethnicity, smoking, primigravida, fetal gender and neonatal weight on ADAM12s and PP13 levels.

Outcome characteristics are described in Table 2. As expected, we found significantly increased peak diastolic pressure in pregnancies complicated by GH/PE compared with the controls. Median neonatal weight at birth in pregnancies complicated by SGA-fetuses was significantly reduced compared with controls. In addition, we found significantly decreased gestational age at delivery in pregnancies complicated by PE or SGA-fetuses. In controls, median ADAM12s concentration was 405 $\mu\text{g/L}$ (range 101 - 923 $\mu\text{g/L}$) compared with 324 $\mu\text{g/L}$ (range 85 - 761 $\mu\text{g/L}$) in cases. In controls, median PP13 concentration was 57.7 pg/mL (range 15.6 - 147 pg/mL) compared with 54.6 pg/mL (range 22.2 - 110 pg/mL) in cases. ADAM12s and PP13 are independent analytes, log regression analysis showed only a weak correlation (maximum $r = 0.2$) between both markers in the four outcome groups.

Table 2 Pregnancy outcome characteristics in the four outcome groups

Characteristic	Cases				p-value
	Controls	PE	GH	SGA	
Number of cases	165	17	30	8	
Median peak diastolic tension in mm Hg (range)	75.6 (60-90)	101.9* (90-120)	94.3* (90-110)	89.5 (80-115)	<0.05
Median GA at delivery in days	288	266*	275	261*	< 0.05
Fetal gender (% female)	47.8	14.3	56.3	28.7	0.07
Median neonatal weight at birth (gram)	3546	3015	3383	2196*	0.05

PE – preeclampsia; GH – gestational hypertension; SGA – small for gestational age fetuses; GA – gestational age
* P-value < 0.05 by post hoc testing

Table 3 shows median MoM values for ADAM12s and PP13 for controls and cases, all reporting reduced MoM values in the GH/PE/SGA cases. However, only ADAM12s MoM values are significantly reduced in GH cases.

Table 3 Median MoM values for ADAM12s and PP13 in controls and cases

	n	Median ADAM12s MoM	Median PP13 MoM
Controls	165	1.00	1.00
Gestational Hypertension	30	0.77*	0.95
Preeclampsia	17	0.90	0.77**
Small for gestational age fetus	8	0.88	0.89

MoM – Multiple of Median; ADAM12s – A Disintegrin And Metalloprotease 12s; PP13 – Placental Protein 13
* P-value < 0.05; ** P-value = 0.07

Table 4 shows ROC curve analysis for ADAM12s, PP13 and combined ADAM12s and PP13. Results are expressed by area under the curve (AUC). Detection rate was assessed when specificity was set at 80%. Figure 1 shows the ROC curves for combined ADAM12s and PP13 measurements for prediction of PE, GH and SGA fetuses, as well as the ROC curve for ADAM12s measurement for prediction of GH.

Table 4 Overview of Receiver-operating Characteristics curve analysis of ADAM12s, PP13 and combined ADAM12s & PP13 for prediction of PE, GH and SGA fetuses expressed in AUC. Detection rate was assessed when specificity was set at 80%

Case	Serum Marker	AUC	p-value	Detection Rate
PE	ADAM12s	0.63	0.08	41%
	PP13	0.57	0.21	24%
	ADAM12s & PP13	0.63	0.08	31%
GH	ADAM12s	0.68	<0.01	52%
	PP13	0.57	0.22	32%
	ADAM12s & PP13	0.66	0.06	48%
SGA	ADAM12s	0.59	0.41	26%
	PP13	0.62	0.26	38%
	ADAM12s & PP13	0.60	0.34	31%

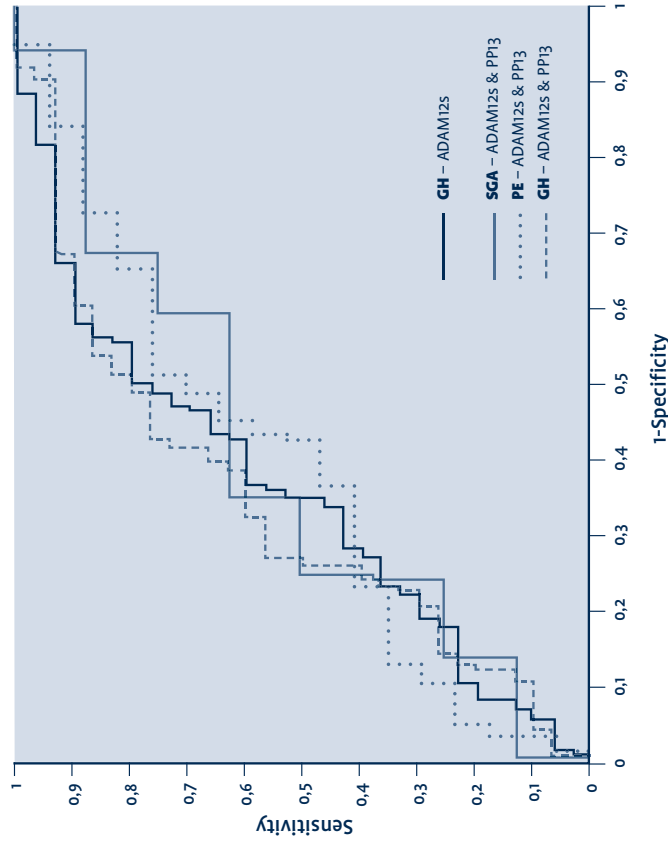
AUC – Area under the Curve; PE – preeclampsia; ADAM12s – A Disintegrin And Metalloprotease 12s; PP13 – Placental Protein 13; GH – gestational hypertension; SGA – small for gestational age

Discussion

To our knowledge this is the first study that assesses the predictive values of combined ADAM12s and PP13 measurements for GH/PE complicated pregnancies as well as pregnancies complicated by SGA-fetuses. In this retrospective matched case-control study, the main findings are that ADAM12s and PP13 were decreased in GH, PE and SGA cases, however, decreased ADAM12s was only significantly associated with GH. Detection rate is 52% when specificity is set at 80%, showing a fair association between GH and ADAM12s. The strength of the study is that all samples were conducted from the same sample base of the national screening program for Down syndrome and therefore included women are likely to be comparable between the controls and cases. Controls and cases were matched for relevant confounding factors such as maternal age and gestational age at sampling. Our study is limited by the small number of cases; it would be most interesting in creating subgroups of early versus late onset preeclampsia and intrauterine growth restricted fetuses with fetal weight below 5th instead of 10th percentile. However, no sufficient data were present.

It is rather remarkable that a combination of ADAM12s and PP13 hardly improves predictive values for PE and SGA compared with single ADAM12s or PP13 measurements, since several studies have reported their individual relationship with PE and fetal growth restriction. Only three studies showed no relationship between PP13 and (late) PE or ADAM12s with PE and fetal growth restriction^{16;23;29}.

Figure 1 Receiver-operating characteristics curve for the prediction of preeclampsia (PE), gestational hypertension (GH) and small-for-gestational-age (SGA) fetuses using a combination of median MoM value of combined ADAM12s and PPI3 measurements (area under the curve: 0.63 for PE, 0.66 for GH and 0.60 for SGA) and ADAM12s (area under the curve: 0.68 for GH)



For PPI3 Chafetz et al. found good predictive values for PE with detection rates of 91% and specificity of 90% in a case-control study among 425 pregnant women¹⁹. These values were also stated by Conen et al. in a prospective study, in which detection rate was 80% with an FPR of 20%. Differences with our findings can be explained by the difference in gestational age (GA) at sampling: Chafetz et al. performed sampling at a median GA of 10+6 weeks and Conen et al. performed sampling in week 6+0 till 10+0 of gestation. It has been reported that early first trimester PPI3 measurements might have better predictive values due to its role in early placental development³⁰. The most recent study by Khalil et al. showed predictive values of detection rates of approximately 90% with a false positive rate (FPR) of 20%. They included only women with a high a priori risk of PE, such as chronic hypertension, pre-gestational diabetes or renal diseases which can explain the differences with our study group which consisted mostly of women with a low a priori risk. Akolekar found only a significant relationship between PPI3 and early PE and not for late PE. Cases in this study were not differentiated into early or late PE²⁹.

ADAM12s has been previously described as a potential predictive marker for adverse pregnancy outcome: Cowans and Spencer (2007) describe in a case-control study among 1705 cases versus 414 control cases significant lower levels of ADAM12s in SGA neonates (birthweight below 10th percentile) and significant elevated levels in large for gestational age neonates (birthweight above 90th percentile)¹¹. The significant relationship between decreased ADAM12s levels and PE has firstly been reported by Laigaard et al. (2005) in a case control study of 324 controls versus 160 cases of PE¹². We cannot fully explain the differences with our results, however, matching criteria could be more stringent in this study and the small number of cases in our study may have influenced the results. Moreover, Laigaard reports the use of a different assay to measure ADAM12s; the use of different antibodies to detect ADAM12s might explain the differences in results found between these two studies. This was also stated in a recent study by Wortelboer and coworkers³¹.

Combined screening performance of ADAM12 and PPI3 has not been described in literature before. Other studies have shown combinations of PPI3 with first trimester or second trimester uterine artery Doppler velocimetry and PAPP-A^{21,22}. Especially early onset preeclampsia is found to be highly detectable with these combinations. ADAM12s and uterine artery Doppler showed a detection rate of 66% for PE. Pihl et al.(2008) combined ADAM12s with free β -hCG and PAPP-A for prediction of SGA neonates; the authors stated that this combination is reaching clinical relevance with a detection rate of 39% with a false positive rate of 10%.¹⁴ Poon et al. (2008) did not find improvement in predictive value of adverse pregnancy outcome such as PE, GH or preterm delivery when adding ADAM12s measurements to PAPP-A, uterine artery Doppler velocimetry and high a priori risk based on obstetric history¹⁶.

In conclusion, we believe that decreased first trimester levels of ADAM12s may be useful in early prediction of GH. Decreased levels of PPI3 were found in cases with adverse pregnancy outcome such as PE, GH or SGA, however, not significantly. Combining ADAM12s and PPI3 does not appear to improve screening performance. Further studies should evaluate the predictive role of ADAM12s in complicated pregnancies, possible in combination with other promising first trimester markers, such as PAPP-A or PIGF or second trimester uterine artery Doppler measurements. One can argue to select pregnancies with a high a priori risk to improve screening performance.

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