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ORIGINAL RESEARCH

PAPP-A2 and Inhibin A as Novel Predictors for Pregnancy Complications in Women With Suspected or Confirmed Preeclampsia

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BACKGROUND: We aimed to evaluate the value of inhibin A and PAPP-A2 (pregnancy-associated plasma protein-A2) as novel biomarkers in the prediction of preeclampsia-related complications and how they compare with angiogenic biomarkers.

METHODS AND RESULTS: Making use of a secondary analysis of a prospective, multicenter, observational study, intended to evaluate the usefulness of sFlt-1 (soluble Fms-like tyrosine kinase-1)/PlGF (placental growth factor) ratio, we measured inhibin A and PAPP-A2 levels in 524 women with suspected/confirmed preeclampsia. Women had a median gestational age of 35 weeks (range, 20–41 weeks) while preeclampsia occurred in 170 (32%) women. Levels of inhibin A and PAPP-A2 were significantly increased in women with preeclampsia and in maternal perfusate of preeclamptic placentas. Inhibin A and PAPP-A2 (C-index = 0.73 and 0.75) significantly improved the prediction of maternal complications when added on top of the traditional criteria; gestational age, parity, proteinuria, and diastolic blood pressure (C-index = 0.60). PAPP-A2 was able to improve the C-index from 0.75 to 0.77 when added on top of the sFlt-1/PlGF ratio for the prediction of maternal complications. To discriminate fetal/neonatal complications on top of traditional criteria, inhibin A and PAPP-A2 showed additive value (C-index = 0.79 to 0.80 and 0.82, respectively) but their discriminative ability remained inferior to that of sFlt-1/PlGF ratio or PlGF. Interestingly, the PAPP-A2/PlGF ratio alone showed remarkable value to predict pregnancy complications, being superior to sFlt-1/PlGF ratio in the case of maternal complications.

CONCLUSIONS: Inhibin A and PAPP-A2 show significant potential to predict preeclampsia-related pregnancy complications and might prove beneficial on top of the angiogenic markers.

Key Words: inhibin A ■ sFlt-1 ■ PlGF ■ preeclampsia ■ PAPP-A2

Preeclampsia is a multisystem disorder unique to pregnancy, characterized by the new onset of hypertension and proteinuria, intrauterine growth restriction, or evidence of other end-organ damage occurring after 20 weeks of gestation.[1,2] Affecting 5% to 7% of all pregnant women, preeclampsia poses a great threat to maternal and fetal well-being worldwide.[3] Because the clinical presentation and course of preeclampsia can vary considerably, it remains important to identify those women at risk for developing severe complications such as eclampsia, pulmonary edema, hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome, liver and kidney damage, along with iatrogenic preterm birth, perinatal morbidity, and mortality.[4] Since hypertension and proteinuria, the classical hallmarks of the disorder, have shown poor value to predict adverse outcomes,[5] several biochemical markers are emerging to improve diagnostic tools applied to women with a clinical suspicion or diagnosis of preeclampsia.

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CLINICAL PERSPECTIVE

What is New?

- This is the first study to investigate inhibin A and PAPP-A2 (pregnancy-associated plasma protein-A2) as potential biomarkers for adverse pregnancy outcome in women with suspected or confirmed preeclampsia.

What Are the Clinical Implications?

- PAPP-A2 showed the highest value to predict preeclampsia-related pregnancy complications when added on top of traditional criteria and shows comparable value to that of the angiogenic markers sFlt-1 (soluble Fms-like tyrosine kinase-1), placental growth factor (PlGF), and sFlt-1/PlGF ratio.
- PAPP-A2 shows incremental predictive value when added on top of the sFlt-1/PlGF ratio, while the PAPP-A2/PlGF ratio performed better than sFlt-1/PlGF ratio to predict maternal complications.
- Future studies are necessary to validate our findings and to evaluate whether PAPP-A2 and inhibin A can be used in clinical practice.

Nonstandard Abbreviations and Acronyms

DBP	diastolic blood pressure
HELLP	hemolysis, elevated liver enzymes, low platelet count
PAPP-A2	pregnancy associated plasma protein-A2
PlGF	placental growth factor
sFlt-1	soluble Fms-like tyrosine kinase-1

The pathogenesis underlying preeclampsia remains uncertain, although it is well-recognized that placental ischemia triggers the release of placental factors into the maternal circulation, leading to the clinical syndrome of preeclampsia.[3] While certain placental factors, including the sFlt-1 (soluble Fms-like tyrosine kinase-1), placental growth factor (PlGF), or their ratio have been established as good candidate biomarkers for the prediction of preeclampsia or adverse outcomes,[6,7] other biomarkers are still being uncovered. In recent years, the circulating factors inhibin A and PAPP-A2 (pregnancy-associated plasma protein-A2) have emerged as novel biomarkers for preeclampsia.[8–10] Inhibin A is a glycoprotein hormone belonging to the transforming growth factor family,[11] while PAPP-A2 is an insulin growth factor (IGF) binding protein protease thought to be involved in the regulation of IGF bioavailability[8]. Both inhibin A and PAPP-A2 are abundantly expressed in the placenta, and have been reported to be significantly elevated in the maternal circulation as well as placentas of pregnancies complicated by preeclampsia.[8,12–15] Yet, current evidence on the role of these factors as biomarkers, particularly for the development of preeclampsia-related adverse outcomes remains scarce.

We hypothesized that inhibin A and PAPP-A2 could be better discriminating markers for the prediction of adverse outcome in women with suspected or confirmed preeclampsia than the angiogenic markers. In this secondary analysis, we examined the value of inhibin A and PAPP-A2 as predictors and compared their value to that of sFlt-1, PlGF, and sFlt-1/PlGF ratio. In addition, we explored their levels in placental perfusate of healthy pregnant women versus women with an established diagnosis of preeclampsia.

METHODS

All supporting data are available within the article and its online supplemental information.

Study Design and Participants

This was a secondary analysis of a prospective cohort study involving women with suspected or confirmed preeclampsia enrolled between December 2013 through April 2016 at 3 Dutch hospitals (Erasmus Medical Center, Maasstad Hospital in Rotterdam and Reinier de Graaf Hospital in Delft) with the aim of evaluating the sFlt-1/PlGF ratio for the prediction of preeclampsia-related complications. All subjects provided written informed consent to participate in the study, which was approved by the local research ethics committee (MEC-2013-202). Inclusion and exclusion criteria were described previously by Saleh et al.[6] Women with singleton pregnancies who had a confirmed clinical diagnosis of preeclampsia, or had symptoms such as hypertension, proteinuria, right upper quadrant abdominal pain, severe headache, visual disturbances, elevated liver enzymes, or decreased platelet count were included in the study. Preeclampsia was defined according to the definition of the International Society for the Study of Hypertension in Pregnancy (ISSHP) of 2001 which was de novo hypertension (systolic blood pressure of ≥140 and diastolic blood pressure [DBP] of ≥90 mm Hg) and proteinuria (protein-to-creatinine ratio ≥30 mg/mmol or ≥300 mg/24 hours or 2+ dipstick) at or after 20 weeks of pregnancy or superimposed preeclampsia defined as chronic

hypertension with the new onset of proteinuria or sudden increase of blood pressure or appearance of thrombocytopenia and increased liver enzymes or a sudden increase of proteinuria in patients with a preexisting proteinuria. HELLP syndrome was defined as hemolysis, elevated liver enzymes and low platelet count, in the presence or absence of hypertension. Women with only suspicion of preeclampsia but without gestational hypertension were defined as suspected preeclampsia.

Data Collection

For the analysis of sFlt-1 and PlGF, blood was taken at study entry only, and after centrifugation, serum was stored at −80°C until analysis. All samples were measured postpartum to avoid influence on decision making of the treating physicians.

Outcome Measures

Patients diagnosed with (partial) HELLP at initial inclusion (n=37) were excluded from the calculation of maternal complications. Maternal complications were defined as the development of 1 of the following after inclusion into the study; (partial) HELLP syndrome, eclampsia, pulmonary edema, subcapsular liver hematoma, cerebral hemorrhage/edema or infarction, visual disturbances, placental abruption, postpartum hemorrhage (blood loss ≥1000 mL after delivery), and acute renal failure (absolute increase in the serum creatinine concentration of ≥0.3 mg/dL [26.4 µmol/L] from baseline; ≥50% increase in serum creatinine; or oliguria with <0.5 mL/kg per hour for a period of 6 hours).

Fetal/neonatal complications were defined as admittance to the neonatal intensive care unit; neonatal birth weight <10th percentile according to Perinatal Registration, The Netherlands; endotracheal intubation; intraventricular or intracranial hemorrhage; other intracerebral abnormalities; development of sepsis; respiratory distress syndrome; bronchopulmonary dysplasia defined as chronic lung disease developing in preterm neonates treated with oxygen and positive-pressure ventilation, with radiographic signs of inflammation and scarring, in need of artificial ventilation 4 weeks post-partum and at 36 weeks postmenstrual age; post-hemorrhagic ventricular dilatation; periventricular leukomalacia, necrotizing enterocolitis, and fetal or neonatal death. All patients (n=524) were used for the calculation of fetal/neonatal complications.

Patient demographics, physical examination, laboratory test results, maternal and fetal/neonatal complications (diagnosed by treating physicians) were obtained from patient's electronic medical records and ascertained by 2 independent researchers.

Perfusion Studies

Placental perfusate samples were obtained from previously conducted placental perfusion experiments in which transplacental drug transfer was evaluated. These perfusion experiments were previously described by Hitzerd et al.[16,17] Perfusion experiments were conducted in healthy placentas and preeclamptic placentas. In brief, maternal and fetal perfusion media consisted of Krebs-Henseleit buffer at 37°C, supplemented with heparin (final concentration; 2500 IU/L) and aerated with 95% O_2 - 5% CO_2. The fetal circulation (closed circuit; flow rate, 6 mL/minute) was established by cannulating the chorionic artery and corresponding vein of an intact cotyledon. Maternal circulation (closed circuit; flow rate 12 mL/minute) was created by placing 4 blunt cannulas in the intervillous space. At t = 0, at a concentration of ~10×C_{max}, either endothelin receptor antagonists, PDE-5 (Phosphodiesterase-5) inhibitor sildenafil, or no drug as a control were added to the maternal circulation to verify transfer to the fetal circulation.[16,17] These high concentrations were chosen to prevent underestimation of transfer. Samples of the maternal and fetal circulations were taken every 30 minutes until the end of the experiment (180 minutes) for the determination of biomarker concentrations, and stored immediately at −80°C.

Biochemical Measurements

Serum levels of sFlt-1 and PlGF were measured in 524 samples, using an automated analyzer (Cobas 6000, e-module; Roche Diagnostics, Mannheim, Germany), as described previously.[6] Analysis of sFlt-1 and PlGF in placental perfusate was also performed by an automated analyzer (Cobas 6000, e module; Roche Diagnostics, Rotterdam, The Netherlands). ELISAs (AL-123 and AL-109) from Ansh Labs, Texas were used to determine the levels of inhibin A and PAPP-A2. Maternal perfusate was diluted 1:100 for PAPP-A2 and 1:5 for inhibin A, while fetal perfusate was run undiluted. Serum was diluted 1:80 for PAPP-A2 and 1:4 for inhibin A. Greater dilutions were applied in case levels were above the highest standard. The analytical characteristics of the ELISAs have been published elsewhere.[18–20] The coefficients of variation for inhibin A (determined at concentrations of 101 and 345 pg/mL) and PAPP-A2 (determined at concentrations of 1.03 and 3.13 ng/mL) were 4.7% and 3.4% and 4.3% and 3.7%, respectively. The samples were masked to the personnel running the assays.

Statistical Analysis

Data are reported as median with interquartile range for continuous variables and as number with percentage for categorical variables. The normality of continuous

Table 1. Patient Characteristics According to Clinical Diagnosis

Parameter	Suspected Preeclampsia (n=249)	GH (n=105)	Preeclampsia/HELLP (n=170)
Age, y	31 (27–35)	30 (27–34)	32 (28–36)†
Gestational age, wks	35 (31–38)	36 (34–38)	33 (29–36)*,†
Nulliparous, n (%)	136 (55)	70 (67)	101 (59)
Current smoker, n (%)	16 (7)	6 (6)	7 (4)
Race, n (%)			
White	175 (70)	82 (78)	105 (62)†
Black	36 (15)	12 (11)	31 (18)
Other	38 (15)	11(11)	34 (20)
Antihypertensives use, n (%)	51 (21)	29 (28)	100 (59)*,†
History of preeclampsia, n (%)	36 (15)	9 (9)	27 (16)
Preexisting hypertension, n (%)	58 (23)	0 (0)*	39 (23)†
Preexisting proteinuria, n (%)	12 (5)	0 (0)	7 (4)
Clinical findings at time of admission			
SBP, mm Hg	130 (120–138)	145 (140–150)*	143 (130–154)*
DBP, mm Hg	82 (75–89)	91 (85–97)*	90 (85–98)*
uPCR, mg/mmol	17 (11–27)	16 (11–21)	57 (36–219)*,†
LD, U/L	178 (159–205)	189 (166–210)	216 (183–279)*,†
ALT, U/L	14 (10–19)	14 (11–18)	19 (12–46)*,†
Creatinine, µmol/L	55 (50–62)	59 (54–66)*	61 (54–72)*
Uric acid, mmol/L	0.27 (0.23–0.32)	0.29 (0.24–0.34)	0.33 (0.27–0.39)*,†
Platelet count, 10^9/L	238 (188–279)	227 (180–276)	211 (160–254)*,†
sFlt-1, pg/mL	3140 (1834–5207)	4902 (2394–7226)*	5641 (1870–10382)*
PlGF, pg/mL	189 (112–361)	110 (71–211)*	73 (33–132)*,†
sFlt-1/PlGF ratio	18 (6–40)	41 (15–87)*	71 (22–272)*,†
Inhibin A, pg/mL	1165 (595–1965)	1484 (862–2606)*	2248 (1374–4071)*,†
PAPP-A2, ng/mL	151 (75–300)	281 (145–471)*	380 (171–555)[a]
Pregnancy outcomes			
Sex (Male/Female), n (%)	129/120 (52/48)	49/56 (47/53)	91/79 (54/46)
Gestational age at birth, wks	38 (37–40)	38 (37–39)	36 (30–37)*,†
Birth weight, g	3275 (2832–3658)	3140 (2646–3541)	2218 (1158–3173)*,†
Birth weight percentile < 10, n (%)	23 (9)	17 (16)	36 (21)*
Time until delivery, d	19 (9–41)	9 (3–21)*	3 (1–13)*,†

Values are median (interquartile range) or number (%).

ALT indicates alanine aminotransferase; DBP, diastolic blood pressure; GH, gestational hypertension; HELLP, hemolysis, elevated liver enzymes, low platelet count; LD, lactate dehydrogenase; PAPP-A2, pregnancy-associated plasma protein-A2; PlGF, placental growth factor; SBP, systolic blood pressure; sFlt-1, soluble Fms-like tyrosine kinase-1; and uPCR, urinary protein-to-creatinine ratio.

*indicates comparison with suspected preeclampsia at a significance level of $P < 0.05$;

†indicates comparison with gestational hypertension at a significance level of $P < 0.05$.

variables was assessed using the Shapiro-Wilk W test. For the comparison of continuous variables between >2 groups, 1-way ANOVA or Kruskal-Wallis test in the case of non-normal distribution was applied with a Dunnett or Bonferroni correction for multiple testing. For the comparison of categorical variables between ≥2 groups, Fisher exact and X^2 (Chi-square) were applied. Logistic regression analysis was used to study the association between the dichotomous outcomes (maternal and fetal/neonatal complications) and the novel biomarkers and traditional predictors. Traditional predictors concerned gestational age (GA) at biomarker measurement, parity, proteinuria (urinary protein-to-creatinine ratio), and DBP. Because of a high correlation between systolic blood pressure and DBP, systolic blood pressure was not included in the model. PAPP-A2 was divided by PlGF to generate the PAPP-A2 to PlGF ratio. The markers sFlt-1, PlGF, sFlt-1/PlGF ratio, inhibin A, PAPP-A2, and PAPP-A2/PlGF ratio were assessed either alone or added to the traditional predictors, and were assessed in all women or women with a GA <37 weeks.

To test the added value of new markers on top of the sFlt-1/PlGF ratio (or PlGF) we fitted a logistic

regression model containing sFlt-1/PlGF ratio (or PlGF) and a logistic regression model containing both sFlt-1/PlGF ratio (or PlGF) and either PAPP-A2 or inhibin A. PAPP-A2 or inhibin A were considered to have additional value if the likelihood ratio test comparing both models was statistically significant.

To assess the discriminative ability of the prediction models we used the C-index, which is equivalent to the area under the ROC curve for dichotomous outcomes. SPSS Statistics 21 (IBM Corporations) and R Software were used for the statistical analysis.

RESULTS

Patient Demographics According to Clinical Diagnosis

In this secondary analysis, 524 women with a median age of 31 (27–35) years were included (Table 1). Of these women, 249 (48%) had suspected preeclampsia, 105 (20%) had gestational hypertension, and 170 (32%) met the clinical criteria for preeclampsia and/or HELLP syndrome. Median GA at inclusion was 35 weeks of which 205 (39%) women were <34 weeks. Women with preeclampsia/HELLP syndrome displayed higher systolic blood pressure and DBP, more proteinuria and higher levels of sFlt-1/PlGF ratio in comparison with women only suspected of preeclampsia. As expected, women with preeclampsia/HELLP syndrome delivered earlier, while their newborns were more premature (GA <34 weeks) and had lower birth weight percentiles. In total, 68 maternal complications (in some cases; ≥1 complication) developed in 64 (13%) women after inclusion, while 206 (39%) of all pregnancies had ≥1 fetal/ neonatal complications (Table S1).

Inhibin A and PAPP-A2 Levels According to Clinical Diagnosis

Women with preeclampsia/HELLP syndrome displayed higher levels of inhibin A (2248 [1374–4071] versus 1165 [595–1965] pg/mL) and PAPP-A2 (380 [171–555] versus 151 [75–300] ng/mL) when compared with women only suspected of preeclampsia (Table 1 and Figure 1).

Inhibin A and PAPP-A2 levels in Placental Perfusate

Eleven healthy and four women with preeclampsia were included in the placental studies. Their clinical characteristics are depicted in Table 2. All healthy women underwent elective caesarean section because of previous caesarean section. All patients with preeclampsia underwent a caesarean section because of maternal illness and fetal distress. As expected, the placentas from preeclamptic pregnancies were born at an earlier GA (<34 weeks) and were associated with higher maternal DBP, lower birth weight, and lower placental weight. In total, 12 healthy cotyledons (1 placenta yielded 2 cotyledons) were used and perfused with either endothelin receptor antagonists (n=6), sildenafil (n=3), or no drug (n=3). The data on the maternal-to-fetal transfer of these drugs have been reported before.[16,17] Since no difference in biomarker levels was observed between the different drugs in the healthy placentas, all results were combined. Two of the four experiments with preeclamptic placentas were stopped after 90 minutes of perfusion because of fetal-to-maternal leakage. As shown in Figure 2, the concentrations of inhibin A, PAPP-A2, sFlt-1, and PlGF gradually increased with time in the maternal perfusate. The biomarkers were not detectable in the fetal perfusate (data not shown). The biomarkers PAPP-A2, inhibin A, and sFlt-1 showed increased levels in all 4 preeclamptic placental perfusates when compared with

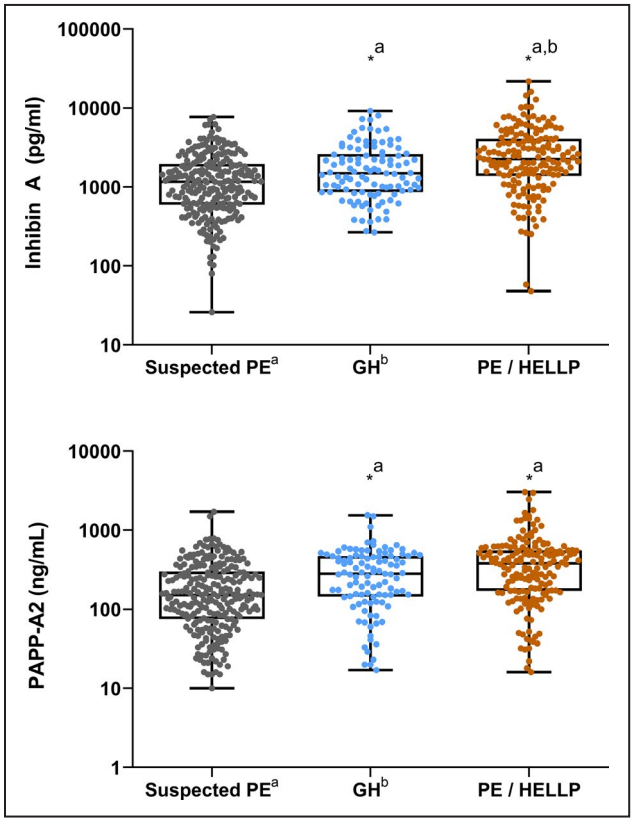

Figure 1. Inhibin A and PAPP-A2 (pregnancy-associated plasma protein-A2) levels in 524 women according to clinical diagnosis.

GH indicates gestational hypertension; HELLP; hemolysis, elevated liver enzymes and low platelets *indicates $P<0.05$ versus the other groups (a, versus suspected preeclampsia; b, versus GH); *a*indicates significance at $P<0.05$ level when compared with suspected preeclampsia; *b* indicates significance at $P<0.05$ level when compared with GH.

Table 2. Characteristics of Healthy and Preeclamptic Placentas Used for Determination of Biomarkers in Placental Perfusion Studies

	Healthy	Preeclampsia
No.	11	4
Maternal age, y	34 (28–36)	31 (29–33)
Gestational age, wks, d	39.0 (38.5–39.1)	31.4 (31.2–31.6)
Nulliparity, n	0	3
Current smoker, n	1	0
White, n	6	3
Highest DBP (mm Hg)	80 (73–80)	110 (107–113)
Fetal sex (Male/Female)	5/6	3/1
Birth weight (g)	3580 (3338–3815)	1138 (1119–1228)
Birth weight (centile)	61 (53–85)	3 (0–6)
Placental weight (g)	659 (622–753)	333 (318–341)

Values are median (interquartile range) or number. DBP indicates diastolic blood pressure.

the healthy placentas. With regard to PlGF, biomarker levels were within the low range of levels in healthy placentas. Results remained unchanged after adjustment for cotyledon weight (data not shown).

Correlations Between Inhibin A and PAPP-A2 with the Angiogenic Markers

Inhibin A and PAPP-A2 were positively correlated with sFlt-1 (r=0.51 and r=0.59, respectively) and showed a negative correlation with PlGF (r=−0.43 and r=−0.51, respectively) (Table S2).

Prediction of Maternal Complications

In univariable analysis, PAPP-A2 showed the highest ability to discriminate between women with and without maternal complications when compared with inhibin A (C-index=0.71 versus 0.69), but was inferior to PlGF and sFlt-1/PlGF ratio (Table 3). A combination of PAPP-A2 divided by PlGF (PAPP-A2/PlGF ratio) showed the highest value in univariable analysis to predict maternal complications, even when compared with the sFlt-1/PlGF ratio. When inhibin A, PAPP-A2 and PAPP-A2/PlGF ratio were added to a model with traditional predictors (traditional model), the C-index improved from 0.60 for a model without biomarkers to 0.73, 0.75, and 0.76, respectively, in comparison with 0.72, 0.73, and 0.77 for sFlt-1, PlGF, and sFlt-1/

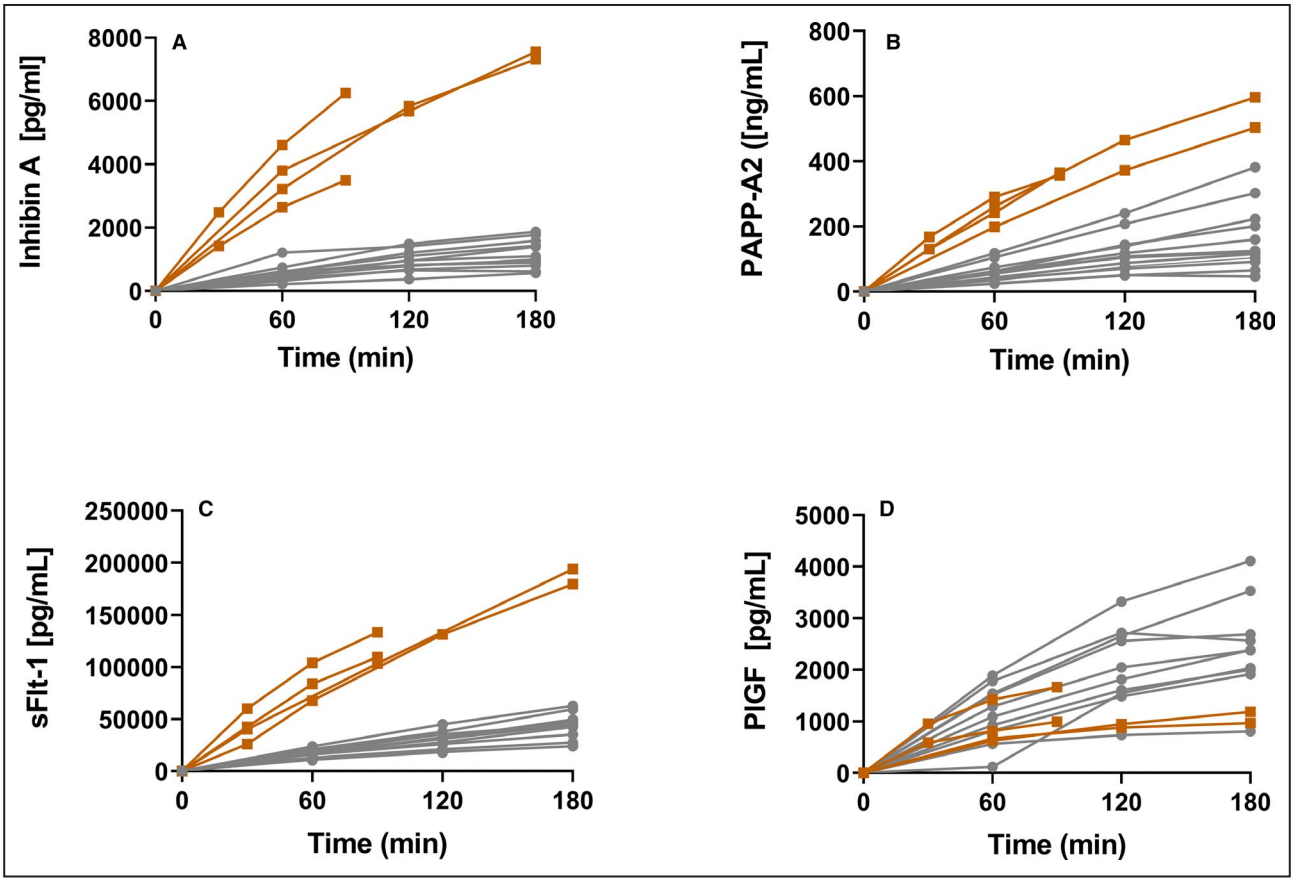

Figure 2. Placental perfusate levels of inhibin A (A), PAPP-A2 (pregnancy-associated plasma protein-A2) (B), sFlt-1 (C), and placental growth factor (D) on the maternal side in healthy placentas (grey circle) and preeclamptic placentas (orange squares). PAPP-A2 indicates pregnancy-associated plasma protein-A2; PlGF, placental growth factor; and sFlt-1, soluble Fms-like tyrosine kinase-1.

Table 3. Associations Between Maternal Complications (n=64) and Biomarkers in Women With Suspected or Confirmed Preeclampsia Without Hemolysis, Elevated Liver Enzymes, Low Platelet ount Syndrome at Time of Inclusion (n=487) and Restricted to GA < 37 weeks (n=309)

	Univariable		Multivariable	
Model/biomarker	Odds Ratio	C-Index	Odds Ratio	C-Index
Traditional model				0.60
sFlt-1	1.9 (1.4–2.5)	0.69	1.9 (1.5–2.5)	0.72
PlGF	0.2 (0.1–0.4)	0.73	0.2 (0.1–0.5)	0.73
sFlt-1/PlGF ratio	5.3 (2.9–9.9)	0.75	5.9 (3.1–11)	0.77
Inhibin A	3.9 (2.0–7.8)	0.69	6.3 (2.9–13)	0.73
PAPP-A2	4.5 (2.3–9.1)	0.71	7.8 (3.6–17)	0.75
PAPP-A2/PlGF ratio	5.9 (2.9–12)	0.76	6.4 (3.1–13)	0.76

GA at inclusion < 37 wks (n=309)	Odds Ratio	C-index
sFlt-1	1.9 (1.4–2.5)	0.70
PlGF	0.1 (0.0–0.4)	0.80
sFlt-1/PlGF ratio	5.6 (2.9–11)	0.80
Inhibin A	5.4 (2.2–13)	0.74
PAPP-A2	10 (3.5–25)	0.79
PAPP-A2/PlGF ratio	18 (7.0–48)	0.85

Traditional model consists of gestational age at time of biomarker measurement, parity, diastolic blood pressure and proteinuria at inclusion. Multivariable includes traditional model with one of the biomarkers. Interquartile odds ratio and associated 95% CI was calculated to aid interpretation of continuous predictors. It is defined as comparing the risk of maternal complications at the 75th percentile of the marker value versus the 25th percentile. GA indicates gestational age; PlGF, placental growth factor; PAPP-A2, pregnancy-associated plasma protein-A2; and sFlt-1, soluble Fms-like tyrosine kinase-1.

PlGF ratio. When restricting the univariable analysis to women with a GA <37 weeks, the PAPP-A2/PlGF ratio showed the highest ability to predict maternal complications, followed by PlGF, sFlt-1/PlGF ratio, and PAPP-A2 (Table 3). Multivariable analysis in this group was not performed, because of the limited number of maternal complications. When PAPP-A2 was added on top of the sFlt-1/PlGF ratio alone, the C-index significantly improved (Table S3).

Prediction of Fetal/Neonatal Complications

For the prediction of fetal/neonatal complications, inhibin A and PAPP-A2 showed poor discriminative ability with C-indices of 0.63 and 0.64, but the PAPP-A2/PlGF ratio yielded a C-index of 0.74. When added on top of traditional predictors, the C-index increased from 0.79 for the traditional model only, to 0.80, 0.82, and 0.83 for inhibin A, PAPP-A2, and PAPP-A2/PlGF ratio, respectively. This implies that PAPP-A2 and PAPP-A2/PlGF ratio reached a value almost similar to that of PlGF and sFlt-1/PlGF ratio (Table 4). Restricting the calculations to women with GA <37 weeks improved the predictive value of all biomarkers in multivariable analysis, with PAPP-A2 and PAPP-A2/PlGF ratio reaching the highest value when compared with inhibin A (C-index = 0.85 and 0.85 versus 0.81), but not when compared with sFlt-1/PlGF ratio (C-index=0.86) or PlGF alone (C-index=0.87) (Table 4). Inhibin A had added value beyond PlGF in predicting fetal/neonatal complications, however, the increase in discriminative ability was negligible (Table S4).

DISCUSSION

In this secondary analysis, we evaluated inhibin A and PAPP-A2 as novel biomarkers to predict adverse pregnancy outcome in women with suspected or confirmed preeclampsia, and compared their predictive value to that of the established angiogenic markers sFlt-1, PlGF, and sFlt-1/PlGF ratio. We found that PAPP-A2 showed the highest value of the 2 biomarkers to predict maternal and fetal/neonatal complications, particularly when added to a model with traditional clinical predictors (GA at biomarker measurement, DBP, protein-to-creatinine ratio, and parity). Conversely, inhibin A was a relatively weak predictor in univariable analysis but showed additive value when added on top of traditional variables for the prediction of maternal complications. When compared with the angiogenic markers, PAPP-A2 performed nearly as well as the sFlt-1/PlGF ratio in multivariable analysis to predict maternal complications, while in the case of fetal/neonatal complications the 2 biomarkers showed a predictive value marginally inferior to that of sFlt-1/PlGF ratio or PlGF. Strikingly, when we incorporated the ratio of PAPP-A2/PlGF ratio in our prediction model, this model performed even

Table 4. Associations Between Fetal/Neonatal Complications and Biomarkers in All Women (n=524) and Restricted for GA <37 Weeks (n=343)

Model/biomarker	Univariable		Multivariable	
	Odds Ratio	C-Index	Odds Ratio	C-Index
Traditional model				0.79
sFlt-1	1.9 (1.5–2.4)	0.65	1.9 (1.5–2.6)	0.81
PlGF	0.1 (0.0–0.2)	0.77	0.2 (0.0–0.3)	0.83
sFlt-1/PlGF ratio	2.2 (1.8–2.8)	0.74	2.2 (1.6–2.9)	0.83
Inhibin-A	1.6 (1.3–1.9)	0.63	1.6 (1.2–2.0)	0.80
PAPP-A2	2.2 (1.5–3.2)	0.64	4.9 (2.9–8.3)	0.82
PAPP-A2/PlGF ratio	2.8 (2.1–3.5)	0.74	2.3 (1.8–3.1)	0.83
GA at inclusion < 37 wks (n=343)				
Traditional model				0.78
sFlt-1	2.5 (1.8–3.5)	0.70	2.7 (1.8–4.2)	0.83
PlGF	0.0 (0.0–0.1)	0.83	0.1 (0.0–0.1)	0.87
sFlt-1/PlGF ratio	3.9 (2.5–6.4)	0.80	4.3 (2.4–7.6)	0.86
Inhibin A	2.1 (1.5–2.8)	0.68	2.1 (1.5–3.1)	0.81
PAPP-A2	4.2 (2.6–6.8)	0.70	10 (5–21)	0.85
PAPP-A2/PlGF ratio	5.6 (3.5–9.1)	0.80	4.6 (2.7–7.9)	0.85

Traditional model consists of gestational age at time of biomarker measurement, parity, diastolic blood pressure and proteinuria at inclusion. Multivariable includes traditional model with one of the biomarkers. Interquartile odds ratio and associated 95% CI was calculated to aid interpretation of continuous predictors. It is defined as comparing the risk of fetal/neonatal complications at the 75th percentile of the marker value vs the 25th percentile. GA indicates gestational age; PAPP-A2, pregnancy-associated plasma protein-A2; PlGF, placental growth factor; and sFlt-1, soluble Fms-like tyrosine kinase-1.

better (univariable analysis), if not similar (multivariable analysis) to the sFlt-1/PlGF ratio to predict maternal complications, whereas for predicting fetal/neonatal complications, PAPP-A2/PlGF ratio showed similar value to that of PlGF or sFlt-1/PlGF ratio in multivariable analysis.

Few studies have been performed to investigate inhibin A and PAPP-A2 as biomarkers, while most of them focused on preeclampsia diagnosis rather than adverse pregnancy outcome. For inhibin A, its predictive value has mostly been studied in early pregnancy, around 11 to 18 weeks of gestation. Whereas some studies reported a weak value of inhibin A as a biomarker for preeclampsia diagnosis,[21–23] others have shown area under the curve values ranging between 0.71 and 0.79, suggesting it was a relatively good predictor, either alone or in the presence of other maternal biomarkers.[10,24] Yet, inhibin A remained inferior to sFlt-1 and PlGF, in agreement with our observations.[10,21,24] PAPP-A2 has been studied less extensively than inhibin A, although it has gained increasing interest over the past years. Early studies reported elevated PAPP-A2 levels both before and during the clinical onset of preeclampsia,[8,25] which we were able to confirm in a larger cohort of women. Consequently, previous studies hypothesized that PAPP-A2 could be a potential biomarker in women with preeclampsia to predict adverse outcomes, and to our knowledge we are the first to investigate this concept.

When we limited our analysis to women with GA <37 weeks, the predictive value of the novel biomarkers substantially increased, with PAPP-A2 and PAPP-A2/PlGF ratio showing the highest increase in C-index, suggesting that PAPP-A2 might be a better predictive biomarker when measured earlier in pregnancy. In a study by Kramer et al.,[13] placental expression of PAPP-A2 was downregulated in the second trimester of healthy pregnancy, which might explain the higher predictive value when PAPP-A2 is elevated. Likewise, circulating inhibin A levels have shown to remain relatively low in the second trimester of a healthy pregnancy.[26] Because of the limited number of maternal complications, we were unable to investigate whether this was also true for women with GA <4 weeks.

To further establish the etiology of these biomarkers, we measured their levels along with those of sFlt-1 and PlGF in maternal and fetal placental perfusate. While none of the biomarkers were detectable in fetal perfusate, the biomarkers sFlt-1, inhibin A, and PAPP-A2 were all elevated in the maternal perfusate of preeclamptic placentas in comparison with healthy placentas. This demonstrates that these biomarkers originate maternally, and that their synthesis is upregulated in preeclampsia. Median serum levels of these biomarkers during preeclampsia amount up to ≈5641 (range, 696–83 967) pg/mL, 2248 (range, 48–21 696) pg/mL, and 380 (range, 16–3024) ng/mL for sFlt-1, inhibin A, and PAPP-A2, respectively

(Table 1). When comparing our cotyledon effluent levels with these levels (Figure 2), it is likely that placental release of these 3 biomarkers has contributed to the increased biomarker levels in the maternal circulation during preeclampsia. The >99% drop of sFlt-1 after birth confirms this view.[27]

As expected, maternal perfusate levels of PlGF were not elevated nor markedly reduced in preeclamptic placentas when compared with healthy placentas, indicating its downregulation in preeclampsia is predominantly caused by additional factors such as the binding of excess circulating sFlt-1. Importantly, caution is granted when interpreting these findings, since the number of preeclamptic placentas remains relatively small. Moreover, it was not possible to match these placentas for gestational age, which might affect the effluent levels observed. However, this reflects the reality that successfully perfusing preterm placentas, particularly from preeclamptic patients, remains extremely difficult.

The fact that both inhibin A and PAPP-A2 were also positively correlated with sFlt-1 in the maternal circulation, raises the question whether similar mechanisms account for their upregulation during preeclampsia. Indeed, in a study by Macintire et al.,[28] PAPP-A2 expression in placental explants was significantly upregulated during hypoxia, a well-known trigger for sFlt-1 synthesis and secretion,[3] while another study has shown that inhibin A expression in differentiated cytotrophoblasts is upregulated by hypoxia-inducible factor.[29] Nevertheless, PAPP-A2 did display added value on top of the sFlt-1/PlGF ratio to predict maternal complications, suggesting that there may still be additional regulatory mechanisms. Our observation that the PAPP-A2/PlGF ratio showed even better value than the sFlt-1/PlGF ratio alone to predict maternal complications, also agrees with this concept. Hence, these novel biomarkers, particularly PAPP-A2, might improve risk prediction models in which the sFlt-1/PlGF ratio is already included. Comparing the predictive value of PAPP-A2/PlGF ratio versus the sFlt-1/PlGF ratio is an interesting area for future research.

The present study has limitations. Since this is a secondary analysis, future prospective trials are necessary to validate our findings. Here, determining specific thresholds and estimating the sensitivity, specificity, negative and positive predictive values of these novel biomarkers might be of use to further estimate their predictive performance. Also, we were not able to assess the predictive value of these biomarkers in early pregnancy, since most women were already diagnosed with preeclampsia and in ≈60% of women blood was taken at ≥34 weeks GA. Lastly, we did not perform repeated measurements of the biomarkers during pregnancy, which could have given more insight into their predictive value.

PERSPECTIVES

Our data illustrate that inhibin A and PAPP-A2 are not only good alternate biomarkers for the prediction of adverse pregnancy outcome but could have additional value on top of the well-known angiogenic factors (sFlt-1, PlGF, and their ratio). Moreover, combining PAPP-A2 with PlGF (by calculating the PAPP-A2/PlGF ratio) might further improve prediction beyond the sFlt-1/PlGF ratio. These findings emphasize the need to investigate their value prospectively alone and combined with the established angiogenic markers.

ARTICLE INFORMATION

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Supplementary Material

Table S1–S4

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Supplemental Material

Table S1. Occurrence of adverse maternal outcomes in women without (partial) HELLP at time of inclusion (n = 487) and fetal / neonatal outcome in all pregnancies (n = 524).

Parameter	n (%)
Maternal Complication	
Eclampsia	0 (0)
(Partial) HELLP syndrome	25 (5)
Placental abruption	2 (0.4)
Pulmonary edema	3 (0.6)
Renal insufficiency	2 (0.4)
Visual disturbances	2 (0.4)
Postpartum hemorrhage	34 (7)
All women with 1 or more complication	64 (13)
Fetal / Neonatal Complication	
Admission to NICU	135 (26)
Endotracheal intubation	38 (7)
Birth weight percentile <10	76 (15)
Intraventricular hemorrhage	5 (1)
Intracranial hemorrhage	1 (0.2)
Other intracerebral abnormalities	8 (2)
Sepsis	38 (7)
Respiratory distress syndrome	64 (12)
Bronchopulmonary dysplasia	11 (2)
Posthemorrhagic ventricular dilatation	1 (0.2)
Periventricular leukomalacia	3 (0.6)
Necrotizing enterocolitis	1 (0.2)
Fetal or neonatal death	20 (4)
All pregnancies with 1 or more complication	206 (39)

Values are number (percentage). Other intracerebral complications include stroke, cysts, developmental anomalies, meningitis and vasculopathy. HELLP syndrome indicates hemolysis, elevated liver enzymes, low platelet count; NICU indicates neonatal intensive care unit.

Table S2. Correlations between inhibin A and PAPP-A2 with the angiogenic markers.

Biomarker	sFlt-1	PlGF	sFlt-1/PlGF ratio
Inhibin A	0.512*	- 0.426*	0.540*
PAPP-A2	0.590*	- 0.510*	0.633*

sFlt-1 indicates soluble Fms-like tyrosine kinase-1; PlGF, placental growth factor; PAPP-A2, pregnancy associated plasma protein-A2. *P<0.01.

Table S3. Value of inhibin A and PAPP-A2 on top of sFlt-1/PlGF ratio to predict maternal complications in all women without HELLP syndrome at time of inclusion (n = 487).

Biomarkers	Univariable Odds Ratio	C-index	Multivariable Odds Ratio	C-index	P-Value
sFlt-1/PlGF ratio	5.3 (2.9 - 9.9)	0.75	3.7 (1.9 - 7.4)		0.0001
Inhibin A	3.9 (2.0 - 7.8)	0.69	2.2 (1.0 - 4.8)	0.76	0.10
sFlt-1/PlGF ratio	5.3 (2.9 - 9.9)	0.75	3.4 (1.6 - 7.2)		0.0001
PAPP-A2	4.5 (2.3 - 9.1)	0.71	2.1 (0.9 - 4.9)	0.77	0.01

Multivariable includes sFlt-1/PlGF ratio with either inhibin A or PAPP-A2. Interquartile odds ratio and associated 95% confidence interval was calculated to aid interpretation of continuous predictors. It is defined as comparing the risk of maternal complications at the 75^{th} percentile of the marker value versus the 25^{th} percentile. sFlt-1 indicates soluble Fms-like tyrosine kinase-1; PlGF, placental growth factor; PAPP-A2, pregnancy associated plasma protein-A2.

Table S4. Value of inhibin A and PAPP-A2 to predict fetal/neonatal complications on top of PlGF in in all women (n = 524).

Biomarkers	Univariable Odds Ratio	C-index	Multivariable Odds Ratio	C-index	P-Value
PlGF	0.1 (0 - 0.2)	0.77	0.1 (0.1 - 0.2)		0.0001
Inhibin A	1.6 (1.3 - 1.9)	0.63	1.2 (1.0 - 1.5)	0.77	0.04
PlGF	0.1 (0 - 0.2)	0.77	0.1 (0.1 - 0.2)		0.0001
PAPP-A2	2.2 (1.5 - 3.2)	0.64	0.9 (0.6 - 1.6)	0.77	0.12

Multivariable includes PlGF with either inhibin A or PAPP-A2. Interquartile odds ratio and associated 95% confidence interval was calculated to aid interpretation of continuous predictors. It is defined as comparing the risk of fetal/neonatal complications at the 75th percentile of the marker value versus the 25th percentile. sFlt-1 indicates soluble Fms-like tyrosine kinase-1; PlGF, placental growth factor; PAPP-A2, pregnancy associated plasma protein-A2.