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Keikkala, Elina

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Serum Inhibin-A and PAPP-A2 in the prediction of pre-eclampsia during the first and second trimesters in high-risk women

Elina Keikkala [a,b,*], Janina Forstén [c], Olli Ritvos [d], Ulf-Håkan Stenman [e], Eero Kajantie [a,b,f,g], Esa Hämäläinen [e], Katri Räikkönen [h], Pia M. Villa [i,j], Hannele Laivuori [k,l,m]

[a] Oulu University Hospital and University of Oulu, Medical Research Center Oulu, PEDEGO Research Unit, Oulu, Finland
[b] Finnish Institute for Health and Welfare, Population Health Unit, Helsinki and Oulu, Finland
[c] University of Helsinki and Helsinki University Hospital, Childreńs Hospital, Child Psychiatry, Helsinki, Finland
[d] Bacteriology and Immunology and Physiology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
[e] Departments of Clinical Chemistry, University of Helsinki, Helsinki and University of Eastern Finland, Kuopio, Finland
[f] Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
[g] Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway
[h] Department of Psychology and Logopedics, University of Helsinki, Helsinki, Finland
[i] Obstetrics and Gynaecology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
[j] Department of Obstetrics and Gynecology, Hyvinkää Hospital, Hyvinkää, Finland
[k] Medical and Clinical Genetics, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
[l] Institute for Molecular Medicine Finland, Helsinki Institute of Life Science, University of Helsinki, Helsinki, Finland
[m] Department of Obstetrics and Gynecology, Tampere University Hospital and Tampere University, Faculty of Medicine and Health Technology, Tampere, Finland

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ABSTRACT

Objectives: Maternal serum inhibin-A, pregnancy associated plasma protein-A (PAPP-A) and PAPP-A2 together with placental growth factor (PlGF), maternal risk factors and uterine artery pulsatility index (UtA PI) were analysed to study their ability to predict pre-eclampsia (PE).

Study design: Serial serum samples for the nested case-control study were collected prospectively at 12–14, 18–20 and 26–28 weeks of gestation from 11 women who later developed early-onset PE (EO PE, diagnosis $< 34 + 0$ weeks of gestation), 34 women who developed late-onset PE (LO PE, diagnosis $\geq 34 + 0$ weeks) and 89 controls.

Main outcome measures: Gestational age -adjusted multiples of the median (MoM) values were calculated for biomarker concentrations. Multivariate regression analyses were performed to combine first trimester biomarkers, previously reported results on PlGF, maternal risk factors and UtA PI. Area under curve (AUC) values and 95% confidence intervals (CIs) for the prediction of PE and its subtypes were calculated.

Results: A high first trimester inhibin-A predicted PE (AUC 0.618, 95%CI, 0.513–0.724), whereas PAPP-A and PlGF predicted only EO PE (0.701, 0.562–0.840 and 0.798, 0.686–0.909, respectively). At 26–28 weeks PAPP-A2 and inhibin-A predicted all PE subtypes. In the multivariate setting inhibin-A combined with maternal pre-pregnancy body mass index, prior PE and mean UtA PI predicted PE (0.811,0.726–0.896) and LO PE (0.824, 0.733–0.914).

Conclusions: At first trimester inhibin-A show potential ability to predict not only EO PE but also LO PE whereas PlGF and PAPP-A predict only EO PE. At late second trimester inhibin-A and PAPP-A2 might be useful for short-term prediction of PE.

Abbreviations: AUC, area under curve; ASA, acetylsalisylic acid; BMI, body mass index; EO PE, early-onset pre-eclampsia (diagnosis < 34 weeks of gestation); IGF, insulin-like growth factor; IGFBP, insulin growth factor binding protein; IUGR, intrauterine growth restriction; LO PE, late-onset pre-eclampsia (diagnosis ≥ 34 weeks of gestation); MoM, multiples of the median; PAPP-A, plasma protein-A; PAPP-A2, plasma protein-A2; PE, pre-eclampsia; PlGF, placental growth factor; UtA PI, uterine artery pulsatility index; ROC, receiver-operating characteristic; SGA, small for gestational age.

* Corresponding author at: Department of Obstetrics and Gynaecology, PO Box 23, 90029 OYS, Oulu, Finland.
E-mail address: elina.keikkala@oulu.fi (E. Keikkala).

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1. Introduction

Early identification of women destined to develop pre-eclampsia (PE) later in pregnancy would enable preventive interventions such as low-dose acetylsalicylic acid (ASA) and more intensive follow-up of these women [1–3]. The most promising predictive model includes a combination of first trimester serum placental growth factor (PlGF) and the maternal clinical risk factors such as pre-pregnancy body mass index (BMI), maternal age and a history of PE. The accuracy could be slightly improved by adding pregnancy-associated plasma protein-A (PAPP-A) and uterine artery pulsatility index (UtA PI) measured by doppler ultrasound [4]. The ability of this model to predict early-onset PE (EO PE) (diagnosis < 34 weeks of gestation) is fairly good. However, its clinical use is limited because its modest value in predicting late-onset PE (LO PE), which is the most common subtype of PE [4].

Inhibin-A is a glycoprotein hormone produced by placental trophoblasts [5,6]. It is used in the second trimester combined screening of chromosomal abnormalities [7]. Several studies have shown that women who developed PE have higher levels of inhibin-A already in the first trimester [6,8,9]. However, its sensitivity has been found to be too low for use as a single marker to predict PE [10].

PAPP-A is mainly produced by placental syncytiotrophoblasts [11,12] and low levels are associated with pregnancy complications such as PE, small-for-gestational age (SGA), preterm delivery and spontaneous abortion [13–15]. Pregnancy-associated plasma protein-A2 (PAPP-A2) is a homologue of PAPP-A sharing 46% amino acid similarity [16]. PAPP-A2 is also expressed by the syncytiotrophoblasts [17–19] and it cleaves specifically insulin-like growth factor-binding protein 5 (IGFBP-5) while PAPP-A cleaves insulin-like IGFBP-4 [16]. In contrast to PAPP-A, the cleavage of IGFBP-5 by PAPP-A2 does not require the presence of IGF [16], which is essential for normal placentation [20]. Maternal serum concentration of PAPP-A2 has been shown to be significantly higher in manifest PE than in uncomplicated pregnancy [19,21,22]. PAPP-A2 plays a role in normal placentation and alterations in its concentration may be implicated in pregnancy complications [23].

We studied whether inhibin-A, PAPP-A, and PAPP-A2 analysed at three timepoints during first and second trimesters serve as potential biomarkers to predict PE and its subtypes. The results were compared with the previously reported PlGF results [24]. All first trimester biomarkers were also analysed together with the maternal clinical risk factors and UtA PI.

2. Methods

2.1. Subjects

Women in the present nested case-control study participated in the PREDO (Prediction and Prevention of Pre-eclampsia and Intrauterine Growth Restriction) project [25]. Women with clinical risk factors for PE were prospectively recruited between September 2005 and December 2009 at ten participating maternity clinics in Finland. The ethics Committee at Helsinki and Uusimaa Hospital District approved the study and written informed consent was obtained from all participants.

A subcohort of 134 women was included in this study: 45 women diagnosed with PE and 89 controls who did not develop PE (49 with and 40 without known clinical risk factors for PE). In the pre-eclampsia group, 11 had early-onset PE (EO PE, diagnosis < 34 + 0 weeks of gestation) and 34 had late-onset PE (LO PE, diagnosis ≥ 34 + 0 weeks). Eight women who developed PE participated in low-dose ASA trial of the PREDO project. They were treated with low-dose ASA 100 mg/day from 12th to 35th week of gestation. Equal numbers of women who received low-dose ASA and did not develop PE were included as controls. Other controls were chosen based on sample availability.

Inclusion criteria for women with clinical risk factors for PE were as follows: PE, intrauterine growth restriction (IUGR), gestational diabetes or foetal demise in a previous pregnancy; pre-pregnancy obesity (BMI ≥ 30 kg/m2); chronic hypertension; type 1 diabetes; maternal age at childbirth < 20 years or > 40 years; systemic lupus erythematosus (SLE) or Sjögren's syndrome [25].

PE was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg occurring after 20th weeks of gestation and proteinuria (≥300 mg per 24 h or the dipstick equivalent in two consecutive measurements [26]. Women with pre-eclampsia superimposed on chronic hypertension were included in the PE group. SGA was defined as birthweight lower than −2 SD adjusted for sex and gestational age according to Finnish birth weight charts [27].

Blood samples were obtained at three timepoints during pregnancy (12–14, 18–20 and 26–28 weeks of gestation). Mean UtA PI was measured by doppler ultrasound in all participants at 12–14 weeks of gestation [2].

2.2. Laboratory methods

Serum was separated by centrifugation within 1 h and aliquots were stored at −80 °C until analyses [25]. Serum inhibin-A, PAPP-A and PAPP-A2 concentrations were measured by ELISA Kits according to manufacturer's instructions (Ansh Labs, Webster, USA) from total of 372 serum samples. Inter-assay coefficients of variation (CV) were 10.9% (at control concentration of 110 pg/ml) and 4.9% (at control concentration of 344 pg/ml) for inhibin-A; 2.3% (at control concentration of 310 ng/ml) and 15.8% (at control concentration of 1150 ng/ml) for PAPP-A, and 8.9% (at control concentration of 0.9 ng/ml) and 19.5% (at control concentration of 3 ng/ml) for PAPP-A2. PlGF concentrations were analysed by a Delfia method Kits as described previously (PerkinElmer, Turku, Finland) [24].

2.3. Statistics

Continuous variables between groups were compared using the Mann-Whitney *U* test and categorical variables were compared using the Chi-Square test. Correlations between continuous variables were analysed using Pearson's correlation. Change of biomarker concentration during advancing pregnancy between two sampling points were analysed using Wilcoxon signed rank test for paired samples. The results were expressed as mean ± SDs, median and 95% confidence intervals (CIs) or interquartile range (IQR) when appropriate. Concentrations were log10 transformed and normality was tested according to the Kolmogorov-Smirnov's test. To adjust the concentrations for gestational age, multiples of the median (MoM) values were calculated for each biomarker: the linear regression equation calculated at three different time points from the samples collected from 40 controls without risk factors from log10 transformed values. For the prediction of PE, clinical background factors (maternal age, pre-pregnancy BMI, prior PE, prior SGA, prior foetus mortus, type 1 diabetes, primiparity, chronic hypertension), biomarkers at 12–14 weeks of gestation and UtA PI were analysed by logistic regression using forward stepwise method where variables with $p < 0.10$ were included in model. The diagnostic accuracy for separate biomarkers and for the combinations of clinical risk factors and biomarkers were analysed using receiver-operating characteristic (ROC) curve analysis and expressed as the area under the curve (AUC). Analyses were performed using SPSS Statistics version 25 (IBM). Two-sided P values < 0.05 were considered statistically significant.

The power of the study was calculated using previous results on PlGF for prediction of EO PE [24]. With these mean MoM values and SDs the power was 98.6% to detect differences between the groups (9 women with EO PE and 67 controls) with two-sided p-value < 0.05.

3. Results

3.1. Clinical characteristics

Clinical characteristics of the study groups are presented in Table 1.

Table 1
Clinical characteristics.

Characteristics	Controls N = 89 Mean / N	SD / %	PE All N = 45 Mean / N	SD / %	EO PE N = 11 Mean / N	SD / %	LO PE N = 34 Mean / N	SD / %
Maternal age at entry, years	31.8	5.1	31.3	5.2	31.8	4.6	31.2	5.4
Pre-pregnancy BMI, kg/m2	25.4	6.4	29.6	7.0[a]	29.4	7.3	29.7	7.0 [a]
Primipara, N	32	36.0	13	28.9	5	45.5	8	23.5
Blood pressure systolic, mmHg	133	19	170	18 [a]	180	17[b]	167	17[b]
Blood pressure diastolic. mmHg	86	12	106	10[b]	110	10[b]	105	11[b]
Proteinuria max, g / 24 h			2.8	3.4	5.3	4.3	1.8	2.4
Gestational age at delivery, weeks	39.4	1.5	37.0	3.4[b]	32.2	3.3[b]	38.5	1.4 [a]
Birth weight, g	3482	450	2941	989 [a]	1744	1052[b]	3328	581
Birth weight, SD	−0.17	0.90	−0.73	1.39 [a]	−1.85	1.55[b]	−0.36	1.12
Placenta, g	594	140	540	155	364	82[b]	594	130
EO PE (<34 wk), N	0	0.0	11	24.4	11	100.0	0	0.0
Preterm PE (<37 wk), N	0	0.0	13	28.9	11	100.0	0	0.0
Chronic hypertension, N	12	13.5	13	28.9 [a]	4	36.4	9	26.5
Gestational hypertension, N	7	7.9	0	0.0	0	0.0	0	0.0
Gestational diabetes, diet, N	14	15.7	11	24.4	2	18.2	9	26.5
Gestational diabetes, insulin, N	4	4.5	1	2.2	0	0.0	1	2.9
HELLP syndrome, N	0	0.0	3	6.7 [a]	2	18.2	1	2.9
SGA, N	1	1.1	8	17.8 [a]	6	54.5[b]	2	5.9

P value [a] < 0.05 or [b] < 0.001 compared to controls based on Mann-Whitney *U* test for continuous variables or Chi-Square test for categorical variables. PE = pre-eclampsia, EO PE = early-onset pre-eclampsia (diagnosis < 34 weeks of gestation), LO PE = late-onset pre-eclampsia (diagnosis ≥ 34 weeks of gestation), BMI = body mass index, HELLP = haemolysis, elevated liver enzymes, low platelets, SGA = small-for-gestational age.

There were no significant differences in gestational age at sampling between the study groups (data not shown). Eight women who developed both subsequent PE and SGA were analysed as a separate subgroup (data not shown).

3.2. MoMs for biomarker concentrations

Inhibin-A concentration decreased from 12 to 14 to 18–20 weeks of gestation and increased after that ($p < 0.001$ for controls and $p = 0.001$ for PE) in all groups ($p < 0.001$ for both) (Supplementary Fig. 1A). Inhibin-A concentration was higher than in controls at 18–20 and 26–28 weeks (Table 2A) and the MoM values were higher than in controls at all studied gestational weeks in women with subsequent PE (Table 2B). Inhibin-A MoM values were higher at 18–20 and 26–28 weeks in women with subsequent EO PE and at 26–28 weeks in women with LO PE compared to controls (Table 2B).

PAPP-A concentrations increased during advancing pregnancy in all groups ($p < 0.001$ for all) (Supplementary Fig. 1B). Women developing EO PE had lower PAPP-A concentrations and MoM values than controls at 12–14 weeks of gestation (Table 2A and 2B),

PAPP-A2 concentrations increased during advancing pregnancy in all groups ($p < 0.001$ for all) (Supplementary Fig. 1C). PAPP-A2 concentrations and MoM values were higher in women with subsequent PE as compared to controls at 26–28 weeks. Higher PAPP-A2 MoM values were observed already at 18–20 weeks in women with subsequent EO PE (Table 2A and 2B).

Inhibin-A concentrations and MoM values were higher in women with subsequent PE with SGA already at 12–14 weeks of gestation. Higher PAPP-A2 MoM values were observed already at 18–20 weeks in women with subsequent PE with SGA ($p < 0.001$) in addition to 26–28 weeks of gestation (data not shown).

Table 2A
Concentrations of serum Inhibin-A, pregnancy associated plasma protein-A (PAPP-A) and pregnancy associated plasma protein-A2 (PAPP-A2).

			Controls	PE			EO PE			LO PE		
	Weeks	N	Median IQR	N	Median IQR	P	N	Median IQR	P	N	Median IQR	P
Inhibin-A (pg/mL)	12–14	88	277 200–410	41	367 251–499	0.058	10	378 274–378	0.127	31	341 248–496	0.138
	18–20	89	195 139–253	43	227 186–353	0.008	11	340 216–340	0.001	32	213 178–283	0.148
	26–28	88	346 269–466	40	507 355–839	<0.001	8	1250 554–1250	<0.001	32	430 344–695	0.006
PAPP-A2 (ng/mL)	12–14	88	21.3 16.3–27.4	41	22.4 13.6–34	0.925	10	22.1 12.9–22.1	0.814	31	22.4 13.6–34.1	0.993
	18–20	89	22.6 16.7–31.1	43	25.8 16.3–40.8	0.213	11	57.6 19.2–57.6	0.007	32	24.4 15.3–30.1	0.937
	26–28	88	36.1 25.2–51.6	41	46.6 32–132.7	0.002	9	236 133–236	<0.001	32	41 30.4–71	0.058
PAPP-A (ng/mL)	12–14	88	129 72.9–185	41	99.9 67.3–163	0.275	10	71.7 62.6–71.7	0.038	31	121 74–182	0.811
	18–20	89	621 464–938	43	653 387–934	0.582	11	569 355–569	0.796	32	661 391–895	0.597
	26–28	88	1392 1006–2271	41	1236 767–2052	0.251	9	1170 537–1170	0.494	32	1276 790–2005	0.307

P value based on Mann-Whitney *U* test as compared to controls. PE = pre-eclampsia, EO PE = early-onset pre-eclampsia (diagnosis < 34 weeks of gestation), LO PE = late-onset pre-eclampsia (diagnosis ≥ 34 weeks of gestation), IQR = interquartile range.

Table 2B
Multiples of the median (MoM) values of serum Inhibin-A, pregnancy associated plasma protein-A (PAPP-A) and PAPP-A2.

			Controls	PE			EO PE			LO PE		
		N	Median	N	Median	P	N	Median	P	N	Median	P
	Weeks		IQR		IQR			IQR			IQR	
Inhibin-A	12–14	87	1.01	41	1.16	0.031	10	1.26	0.169	31	1.15	0.062
MoM			0.68–1.37		0.91–1.8			0.84–1.59			0.96–1.83	
	18–20	88	0.96	43	1.12	0.008	11	1.77	0.001	32	1.06	0.144
			0.69–1.28		0.94–1.77			1.08–2.44			0.9–1.39	
	26–28	87	0.9	40	1.3	<0.001	8	3.31	<0.001	32	1.13	0.004
			0.7–1.2		0.93–2.08			1.43–9.56			0.9–1.8	
PAPP-A2	12–14	87	1.03	41	1.02	0.826	10	1.11	0.713	31	1.02	0.651
MoM			0.76–1.33		0.63–1.44			0.58–1.71			0.63–1.37	
	18–20	88	1.06	43	1.17	0.237	11	2.59	0.008	32	1.11	0.995
			0.77–1.43		0.73–1.84			0.88–4.57			0.68–1.36	
	26–28	87	1.08	41	1.67	<0.001	9	6.63	<0.001	32	1.39	0.015
			0.68–1.53		1.02–3.77			4.15–13.46			1–2.03	
PAPP-A	12–14	87	0.82	41	0.65	0.072	10	0.57	0.038	31	0.73	0.278
MoM			0.53–1.12		0.45–0.98			0.42–0.63			0.46–1.06	
	18–20	88	0.83	43	0.88	0.447	11	0.73	0.73	32	0.89	0.462
			0.63–1.26		0.54–1.26			0.49–1.35			0.54–1.09	
	26–28	87	0.85	41	0.78	0.299	9	0.77	0.517	32	0.8	0.362
			0.63–1.36		0.49–1.15			0.32–2.11			0.52–1.09	
PlGF	12–14	67	0.98	39	0.89	0.25	9	0.73	0.004	30	0.93	0.975
MoM			0.76–1.22		0.71–1.15			0.55–0.84			0.81–1.22	
	18–20	70	0.89	38	0.77	0.011	10	0.36	<0.001	28	0.84	0.436
			0.74–1.2		0.44–1.05			0.19–0.59			0.7–1.21	
	26–28	68	0.93	34	0.55	<0.001	8	0.14	<0.001	26	0.73	0.009
			0.65–1.32		0.23–0.98			0.07–0.23			0.41–1.01	

P values are based on Mann-Whitney *U* test as compared to controls. PE = pre-eclampsia, EO PE = early-onset pre-eclampsia (diagnosis < 34 weeks of gestation), LO PE = late-onset pre-eclampsia (diagnosis ≥ 34 weeks of gestation), IQR = interquartile range. MoM values are based on concentrations of markers analysed from women without risk factors of PE and who did not develop PE (n = 40) by linear regression analysis using log10 transformed values.

3.3. Prediction of PE, EO PE and LO PE

The AUC values for all studied biomarkers including previous data on PlGF [24] are shown in Table 3. The AUC value for the prediction of all subtypes of PE at 12–14 weeks of gestation was 0.618 for inhibin-A and it improved during advancing pregnancy (0.643 at 18–20 weeks and 0.717 at 26–28 weeks of gestation). PAPP-A2 predicted PE at 26–28 weeks (AUC 0.700) and PlGF at 18–20 and 26–28 weeks (AUC 0.648 and 0.733, respectively) (Table 3).

Only PAPP-A and PlGF showed utility to predict EO PE at 12–14 weeks of gestation (AUC 0.701 and 0.798, respectively). Inhibin-A, PAPP-A2 and PlGF showed ability to predict EO PE at 18–20 weeks and 28–26 weeks of gestation. Inhibin-A, PAPP-A2 and PlGF predicted LO PE at 26–28 weeks of gestation (Table 3).

3.4. Multivariate analyses at 12–14 weeks of gestation

The combinations of the studied biomarkers, previous data on PlGF, maternal clinical risk factors and UtA PI were studied using stepwise forward logistic regression analysis (Table 4). The MoM for Inhibin-A and PAPP-A showed capacity to predict PE with an AUC value for the combination of 0.694. AUC value for BMI, prior PE and UtA PI was 0.796. Combination of Inhibin-A MoM with pre-pregnancy BMI, prior PE, and UtA PI gave an AUC value of 0.811 (Table 4, Fig. 1).

Of the studied biomarkers, PAPP-A MoM and PlGF MoM predicted EO PE (AUC 0.701 and 0.798, respectively). AUC value for BMI, prior SGA and UtA PI was 0.825.Combining PlGF MoM with information of prior SGA, pre-pregnancy BMI, and UtA PI gave an AUC value 0.906 for prediction of EO PE. PlGF MoM and clinical risk factors were superior to PAPP-A MoM to predict EO PE in the multivariate setting (Table 4, Supplementary Fig. 2).

None of the studied biomarkers alone predicted LO PE. Combining

Table 3
The area under the curve (AUC) values from receiver operating characteristic (ROC) curve and 95% confidence intervals (CIs) of different biomarkers in the prediction of pre-eclampsia and its subgroups.

		PE				EO PE				LO PE			
	Weeks	AUC	95% CI		P	AUC	95% CI		P	AUC	95% CI		P
Inhibin-A	**12–14**	0.618	0.513	0.724	0.031	0.633	0.456	0.810	0.169	0.613	0.495	0.732	0.062
MoM	**18–20**	0.643	0.542	0.745	0.008	0.807	0.668	0.946	0.001	0.587	0.472	0.703	0.144
	26–28	0.717	0.621	0.813	<0.001	0.889	0.764	1.000	<0.001	0.674	0.567	0.782	0.004
PAPP-A2	**12–14**	0.488	0.374	0.602	0.826	0.536	0.310	0.762	0.713	0.473	0.347	0.598	0.651
MoM	**18–20**	0.564	0.452	0.675	0.237	0.748	0.529	0.967	0.008	0.500	0.382	0.619	0.995
	26–28	0.700	0.596	0.804	<0.001	0.891	0.698	1.000	<0.001	0.646	0.532	0.761	0.015
PAPP-A	**12–14**	0.599	0.496	0.701	0.072	0.701	0.562	0.840	0.038	0.566	0.451	0.680	0.278
MoM	**18–20**	0.541	0.431	0.651	0.447	0.532	0.320	0.744	0.730	0.544	0.423	0.665	0.462
	26–28	0.557	0.447	0.667	0.299	0.566	0.320	0.812	0.517	0.555	0.437	0.672	0.362
PlGF	**12–14**	0.567	0.452	0.682	0.250	0.798	0.686	0.909	0.004	0.498	0.371	0.625	0.975
MoM	**18–20**	0.648	0.535	0.761	0.011	0.921	0.846	0.997	<0.001	0.551	0.421	0.680	0.436
	26–28	0.733	0.628	0.837	<0.001	0.925	0.792	1.000	<0.001	0.674	0.555	0.793	0.009

The AUC and P values are based on receiver operating characteristics (ROC) curve compared to controls (n = 89). PE = pre-eclampsia, EO PE = early-onset pre-eclampsia (diagnosis < 34 weeks of gestation), LO PE = late-onset pre-eclampsia (diagnosis ≥ 34 weeks of gestation), MoM = multiples of median, PAPP-A = pregnancy associated plasma protein-A, PlGF = placental growth factor.

Table 4
The area under the curve (AUC) values from receiver operating characteristic (ROC) curve for combinations of maternal clinical risk factors, studied biomarkers and uterine artery pulsatility index (UtA PI) at 12–14 weeks of pregnancy in predicting of pre-eclampsia (PE), early-onset pre-eclampsia (EO PE) and late-onset pre-eclampsia (LO PE).

	Model	Variables	N	AUC	95%CI	P value
PE	Clinical risk factors[a]	BMI + prior PE	44	0.769	0.684–0.853	<0.001
		BMI	44	0.684	0.587–0.782	0.001
		Prior PE	45	0.666	0.563–0.769	0.002
	Doppler US	UtA PI	39	0.629	0.520–0.739	0.023
	Combination	BMI + prior PE + UtA PI	38	0.796	0.715–0.878	<0.001
	Biomarkers[b]	Inhibin-A + PAPP-A	41	0.694	0.597–0.792	<0.001
	All[c]	BMI + prior PE + Inhibin-A + UtA PI	31	0.811	0.726–0.896	<0.001
EO PE	Clinical risk factors[a]	Prior SGA + BMI	11	0.734	0.599–0.869	0.012
		BMI	11	0.687	0.530–0.844	0.044
		Prior SGA	11	0.591	0.397–0.786	0.324
	Doppler US	UtA PI	8	0.780	0.670–0.890	0.009
	Combination	Prior SGA + BMI + UtA PI	8	0.825	0.724–0.926	0.003
	Biomarkers[b]	PlGF	9	0.798	0.686–0.909	0.004
	All[c]	Prior SGA + BMI + PlGF + UtA PI	6	0.906	0.824–0.988	0.001
LO PE	Clinical risk factors[a]	BMI + prior PE	33	0.777	0.685–0.869	<0.001
		BMI	33	0.684	0.573–0.794	0.002
		Prior PE	34	0.683	0.569–0.796	0.002
	Doppler US	UtA PI	31	0.590	0.467–0.714	0.142
	Combination	BMI + prior PE + UtA PI	30	0.807	0.723–0.892	<0.001
	Biomarkers[b]	Inhibin-A	31	0.613	0.495–0.732	0.062
	All[c]	BMI + prior PE + Inhibin-A + UtA PI	27	0.824	0.733–0.914	<0.001
	All (excluding UtA PI)[c]	BMI + prior PE + Inhibin-A	30	0.815	0.730–0.900	<0.001

The area under the curve (AUC) and P values are based on receiver operating characteristics (ROC) curve compared to controls (n = 89). Combinations are analysed using logistic regression analysis stepwise forward method where variables with p < 0.10 are included to model. 95% CI = confidence interval, PE = pre-eclampsia, US = ultrasound, EO PE = early-onset pre-eclampsia (diagnosis < 34 weeks of gestation), LO PE = late-onset pre-eclampsia (diagnosis ≥ 34 weeks of gestation).

[a] Clinical background factors include: maternal age, pre-pregnancy body-mass-index (BMI), prior pre-eclampsia, prior small-for-gestational age, prior foetus mortus, type 1 diabetes, primiparity, chronic hypertension.

[b] Inhibin-A, pregnancy associated plasma protein-A2 (PAPP-A2), pregnancy associated plasma protein-A (PAPP-A) and placental growth factor (PlGF)

[c] Clinical risk factors, Inhibin-A, PAPP-A2, PAPP-A and PlGF and uterine artery pulsatility index (UtA PI).

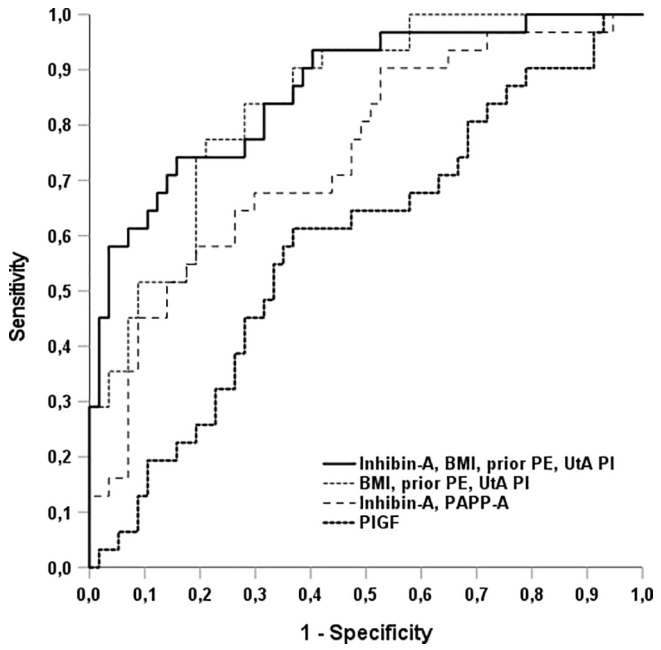

Fig. 1. Receiver operating characteristics (ROC) curve for the prediction of pre-eclampsia (PE) using biomarkers alone or combined with clinical risk factors and uterine artery pulsatility index (UtA PI) at 12–14 weeks of gestation. BMI = body-mass-index, PE = pre-eclampsia, UtA PI = uterine artery pulsatility index, PAPP-A = pregnancy associated plasma protein-A, PlGF = placental growth factor.

inhibin-A MoM with pre-pregnancy BMI, information of prior PE, and UtA PI the AUC value to predict LO PE was 0.824. Combination of clinical risk factors and UtA PI without inhibin-A gave an AUC value of 0.807 and when UtA PI was removed the AUC value for the combination of inhibin-A and clinical risk factors was 0.815 (Table 4, Supplementary Fig. 3).

In women who developed PE with SGA Inhibin-A MoM combined with information about chronic hypertension and UtA PI gave an AUC value of 0.857 (95% CI 0.724–0.990; p = 0.001). In a multivariate setting with clinical risk factors Inhibin-A performed better than PlGF in predicting PE with SGA (data not shown).

3.5. Correlations

At 12–14 weeks, PAPP-A and PAPP-A2 correlated negatively with maternal pre-pregnancy BMI and PAPP-A2 correlated positively with chronic hypertension status. Otherwise, the markers studied were independent of maternal age, parity, pre-pregnancy BMI and chronic hypertension. When comparing inhibin-A, PAPP-A2 and PAPP-A concentrations between ASA and placebo users in women with subsequent PE, EO PE or LO PE, among women with subsequent PE, inhibin-A at 18–20 weeks of gestation was slightly lower in women having ASA than in women receiving placebo (MoM values: 0.94, 0.76 – 1.26; median, IQR; vs. 1.58, 1.17–2.86; p = 0.006). Other biomarker concentrations did not differ between ASA and placebo users (data not shown).

4. Discussion

We found that inhibin-A together with clinical risk factors and UtA PI performed well in the prediction of PE, especially LO PE was predicted already at 12–14 weeks of gestation. Previously established biomarkers,

PlGF and PAPP-A, showed advantage only for the prediction of EO PE.

In line with earlier studies, inhibin-A concentration decreased after the first trimester and increased after midgestation [5] and was higher in women with subsequent PE compared to controls [6,9,28]. Our study revealed that inhibin-A was better than PlGF and PAPP-A for prediction of LO PE at 12–14 weeks of gestation but it did not predict EO PE. In a previous *meta*-analysis, the sensitivity of inhibin-A to predict EO PE was only 32% (95% CI, 25–39%) with a specificity of 90% [10] and to the best of our knowledge its utility to predict LO PE has not been examined in earlier studies. Inhibin-A is an endocrine regulator of the gonadal and placental function [5,6,29]. In pre-eclamptic pregnancies the production of inhibin-A in placental trophoblasts have been shown to be increased compared to normal placentas with unknown mechanism [30,31].

In the present study concentrations of PAPP-A and PAPP-A2 increased during advancing gestation in women with or without subsequent PE. This is in agreement with the results of earlier studies [32,33]. Our study demonstrates that low levels of PAPP-A at 12–14 weeks of gestation are associated with increased risk of EO PE. This has also been seen in earlier studies [9,14,34,35]. In a multivariate analysis PAPP-A did not improve the prediction of all PE or EO PE when using the combination of maternal serum PlGF or inhibin-A, maternal characteristics, obstetric history and uterine artery PI. This is in line with several earlier studies [4,36,37]. Other studies have shown association of early pregnancy low PAPP-A levels with other placental disease such as SGA and preterm delivery [14,15,34]. Low PAPP-A levels also correlate more strongly to EO PE than LO PE [35]. These studies support the notion of PAPP-A rather being a serum marker for placental disease in general than being specific for PE because most of the women with EO PE have placental dysfunction and SGA foetus. PAPP-A is an IGFBP-4 proteinase and the presence of IGF is obligatory for its proteolytic function [38]. IGFs have an important role in the regulation of trophoblast invasion [34]. Low PAPP-A levels may rather be a cause than a consequence in early placental dysfunction.

In the present study increased concentration of PAPP-A2 was associated with all studied PE subtypes at 18–20 and 26–28 weeks of gestation. Increased concentrations have been shown previously in subsequent [22] and established PE [19,32]. In contrast to PAPP-A, the cleavage of IGFBP-5 by PAPP-A2 does not require the presence of IGF [16]. Hypoxia, which is known to occur in the PE placenta, has been shown to upregulate the expression of PAPP-A2 mRNA in placental explants [21] and placental cell lines (BeWo cells) in cell culture [39]. These findings suggest that increased PAPP-A2 serum levels are more likely to be a consequence of placental pathology than vice versa. Thus, PAPP-A2 may be more sensitive and specific for PE than PAPP-A.

In the prediction of all PE and EO PE, Inhibin-A [40] and PAPP-A2 showed similar ability with PlGF at 26 to 28 weeks of gestation. PlGF can be used in short-term prediction of PE with or without soluble fms-like tyrosine kinase-1 (sFlt-1) [41,42]. Our study suggests that inhibin-A and PAPP-A2 may, as well, have potential for short-term prediction of PE among women having suspected symptoms of PE.

The strength of our study is the prospectively recruited, well-characterised cohort with three consecutive serum samples from the same individuals. Its main weakness is the relatively small sample size and the phenomenon that ASA might have had an effect on biomarker concentrations at 18–20 and 26–28 weeks of gestation and development of PE. However, the difference between ASA and placebo users was observed only in concentrations of inhibin-A at 18–20 weeks in women with subsequent PE with unknown mechanism.

In conclusion, this study showed that inhibin-A with maternal clinical risk factors and UtA PI show potential ability to predict all PE and LO PE at 12–14 weeks of gestation whereas PlGF was superior for predicting EO PE. PAPP-A2 and inhibin-A show potential capacity for short-term prediction of PE at 26–28 weeks of gestation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2021.05.024.

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