

# First Trimester Markers for Pre-Eclampsia: Placental vs. Non-Placental Protein Serum Levels

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## Key Words

Pre-eclampsia · First trimester · Biochemistry · Inhibin A · Ultrasound

## Abstract

**Background/Aim:** Parallel investigation, in a matched case-control study, of the association of different first-trimester markers with the risk of subsequent pre-eclampsia (PE). **Method:** The levels of different first trimester serum markers and fetal nuchal translucency thickness were compared between 52 cases of PE and 104 control women by non-parametric two-group comparisons and by calculating matched odds ratios. **Results:** In univariable analysis increased concentrations of inhibin A and activin A were associated with subsequent PE ( $p < 0.02$ ). Multivariable conditional logistic regression models revealed an association between increased risk of PE and increased inhibin A and translucency thickness and respectively reduced pregnancy-associated plasma protein A (PAPP-A) and placental lactogen. However, these associations varied with the gestational age at sample collection. For blood samples taken in pregnancy weeks 12 and 13 only, increased levels of activin A, inhibin A and nuchal translucency thickness, and lower levels of placenta growth factor and PAPP-A were associated with an increased

risk of PE. **Conclusions:** Members of the inhibin family and to some extent PAPP-A and placental growth factor are superior to other serum markers, and the predictive value of these depends on the gestational age at blood sampling. The availability of a single, early pregnancy ‘miracle’ serum marker for PE risk assessment seems unlikely in the near future.

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## Introduction

Pre-eclampsia (PE) is a major pregnancy disorder and, in spite of its incidence of around 5%, its pathogenesis is still poorly understood. Though the disease can only be treated by the delivery of the placenta, the abnormal development originates in early pregnancy by restricted trophoblast invasion and insufficient adaptation of the spiral arteries leading to placental hypoxia. The maternal response is characterised by increased blood pressure, oedema, proteinuria and abnormal clotting, liver and renal function; these symptoms, however, are not seen until later in pregnancy and are associated with endothelial dysfunction in an overreacting inflammatory response [1]. After the onset of the maternal symptoms, the serum

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concentrations of soluble endothelial markers were reported to be elevated [2], together with the placentally derived inhibin A, activin A [3] and pregnancy-associated plasma protein A (PAPP-A) [4]. We were recently able to confirm this latter finding and to demonstrate a positive correlation between PAPP-A and the soluble endothelial (sE-) selectin [5].

Therefore, it became interesting to identify circulating markers whose serum concentrations in the first or second trimester of pregnancy would differ between women with normally proceeding pregnancies and women who subsequently develop PE. Placental factors play an early role, and a logical approach consisted in searching for serum markers from this source for distinguishing between high- and low-risk pregnancies regarding PE. Since the availability of dimer specific assays, inhibin A and activin A have been the most intensely investigated factors, and raised inhibin A levels have been reported for the late first [6] or the early second [7] trimester in women who later developed severe PE. One publication, however, did not find such an elevation [8], but a different antibody and method was used in that study. Early pregnancy PAPP-A was only recently investigated in the context of PE and, unexpectedly, reduced serum levels were found to increase the risk [9]. We were able to confirm this observation in a cross-sectional study at 17 pregnancy weeks [10], while the longitudinal investigation revealed that this reduction was disappearing thereafter, thus leading into the previously shown increased levels later in gestation [5]. Concentrations of pregnancy-specific  $\beta$ 1-glycoprotein (SP1) [11] and placental lactogen (hPL) [12] were not found to be related to the risk of PE, but information on these markers is scarce. On the other hand, placenta growth factor (PLGF) is the only documented marker found to be reduced in the serum of pre-eclamptic patients [10, 13], and this already in the second [14] but apparently not in the first trimester [15].

Pro-inflammatory cytokines have also been investigated. Serum interleukin-6 (IL-6) levels were found not to differ between normotensive women and PE of varying degree of severity [16] while, in an older report on severe cases, increased concentrations were observed [17, 18]. Information on cytokines in early pregnancy is scarce, which is also due to the very low serum levels and an insufficient sensitivity of some assay methods. As IL-8 is acting further down the pro-inflammatory cascade (benefit of an amplification effect), we decided to look at this cytokine instead of IL-6 or TNF- $\alpha$ . More recently, C-reactive protein (CRP) has been intensively studied in the context of inflammation-related events including PE; in

a third-trimester study increased serum CRP levels were found but with a loss of statistical significance after correcting for the body mass index [19].

Leptin is the product of the *ob* gene [20] and normally produced by peripheral adipocytes; its serum levels correlate with the body mass index. In pregnancy, leptin is also produced to some extent by the placenta [21], resulting in increased serum concentrations as a function of advancing gestation until the mid-third trimester [22]. In PE serum, leptin was found to be increased after the onset of maternal symptoms [23]. More recent studies, however, did not find elevated serum leptin levels in the first [24] or second [25] trimester of subsequently pre-eclamptic pregnancies.

The aim of this project was to investigate, in a carefully matched case-control study, the association of the most promising serum markers, measured in the first trimester of pregnancy, and the sonographically determined nuchal translucency thickness (NT) with the risk of subsequent PE. This ultrasound parameter is now routinely determined in the first trimester in the context of fetal trisomy screening together with PAPP-A. NT in the first trimester was found to be positively correlated to the maternal diastolic pressure at the end of pregnancy, and significantly increased in subsequently pre-eclamptic pregnancies when compared to controls [26].

## Patients, Materials and Methods

### Subjects

The study was conducted retrospectively with women who had a first trimester serum sample for routine Down syndrome screening between December, 1999 and June, 2002 and for whom complete information on pregnancy outcome (gestational age at delivery, pregnancy complications, etc.) was available. For this project, our University facility had a close collaboration with an external laboratory (MCL, Medizinische Laboratorien, Düringen, Switzerland). All sera referred for screening were stored by this certified laboratory at  $-30^{\circ}\text{C}$  for follow-up and additional testing. Amongst these pregnancy sera, there was a total of 52 women fulfilling the criteria of a condition with PE. This was defined as a diastolic blood pressure  $>90$  mm Hg or a systolic pressure  $>140$  mm Hg together with proteinuria of at least 300 mg in a 24-hour period [27]. For women meeting these criteria, the referring obstetricians were reporting back to the MCL laboratory and providing the gestational age at birth and the birthweight of the baby (table 1). Each of these cases was matched with sera from two pregnant women who did not develop PE. The matching included not only the gestational age by the day, the maternal age and the duration of storage, but also the maternal weight since not only leptin, but also CRP levels in the serum are positively correlated to the body mass [28]. The clinical variables for the two groups cases and controls are given in table 1.

**Table 1.** Clinical parameters of the study populations

	Pre-eclampsia	Controls
Number of women	52	104
Maternal age, years	30.7 ± 5.0	31.1 ± 4.7
Maternal weight, kg	72.7 ± 16.1	72.3 ± 14.5
Gestational age at sampling, days	86.4 ± 5.1	86.6 ± 4.9
Nuchal translucency thickness, mm	1.47 ± 0.51	1.35 ± 0.52
Calculated DS risk, 1/ratio	3,521 ± 3,064	3,498 ± 2,811
Gestational age at birth, weeks (median, range)	38.6 (28.0–41.8)	N/A <sup>a</sup>
Birthweight, g (median, range)	3,108 (720–4,870)	N/A <sup>a</sup>

Values are mean and standard deviations unless stated otherwise.

<sup>a</sup> N/A = Not available (see section Patients, Materials and Methods).

#### *Handling of Blood Samples and Determination of Serum Analyte Levels*

All blood samples were taken between 11 + 2 and 13 + 6 weeks of gestation (late first trimester, gestational days 79–97). Blood samples were centrifuged in the laboratory at room temperature, PAPP-A and free  $\beta$ -hCG were then determined immediately by means of an automated method (Kryptor<sup>®</sup>, CIS Bio, France) as part of the Down syndrome screening protocol. The sera were then stored at  $-30^{\circ}\text{C}$  until their retrieval for this study.

#### *Placental Proteins*

SP1 and hPL were determined using polyclonal double-antibody ELISA protocols developed in our laboratory and published previously [29, 30]. SP1 was calibrated against the international reference preparation 78-610 (WHO, Geneva), and for hPL ready-to-use diluted standards were obtained from DPC, Los Angeles, USA, supplied by Buhmann Laboratories, Switzerland. Dimeric inhibin A (ultra-sensitive) and activin A were quantified using amplified alkaline phosphatase based kits from Serotec, Oxford, England after a denaturation step.

#### *Leptin, IL-8, CRP*

These markers were similarly quantified with ELISA methods developed in our laboratory using 96-well microplate ELISA technology. For the determination of leptin and of IL-8 specially designated, 'matched-pair' (capture and detection) antibodies, obtained from R&D Systems, Abingdon, England, were used; the protocols have been described in detail elsewhere [31, 32]. For IL-8, the standard curve ran between 200 and 3.125 pg/ml in serial 1:2 dilutions, and the sensitivity of the assay for IL-8 was 2 pg/ml. CRP was similarly determined with a high sensitivity method published recently [33]. The initial sample dilution was 1:250, but it had to be readjusted for some samples to a higher or to a lower setting. The sensitivity of the CRP assay was 0.5 ng/ml.

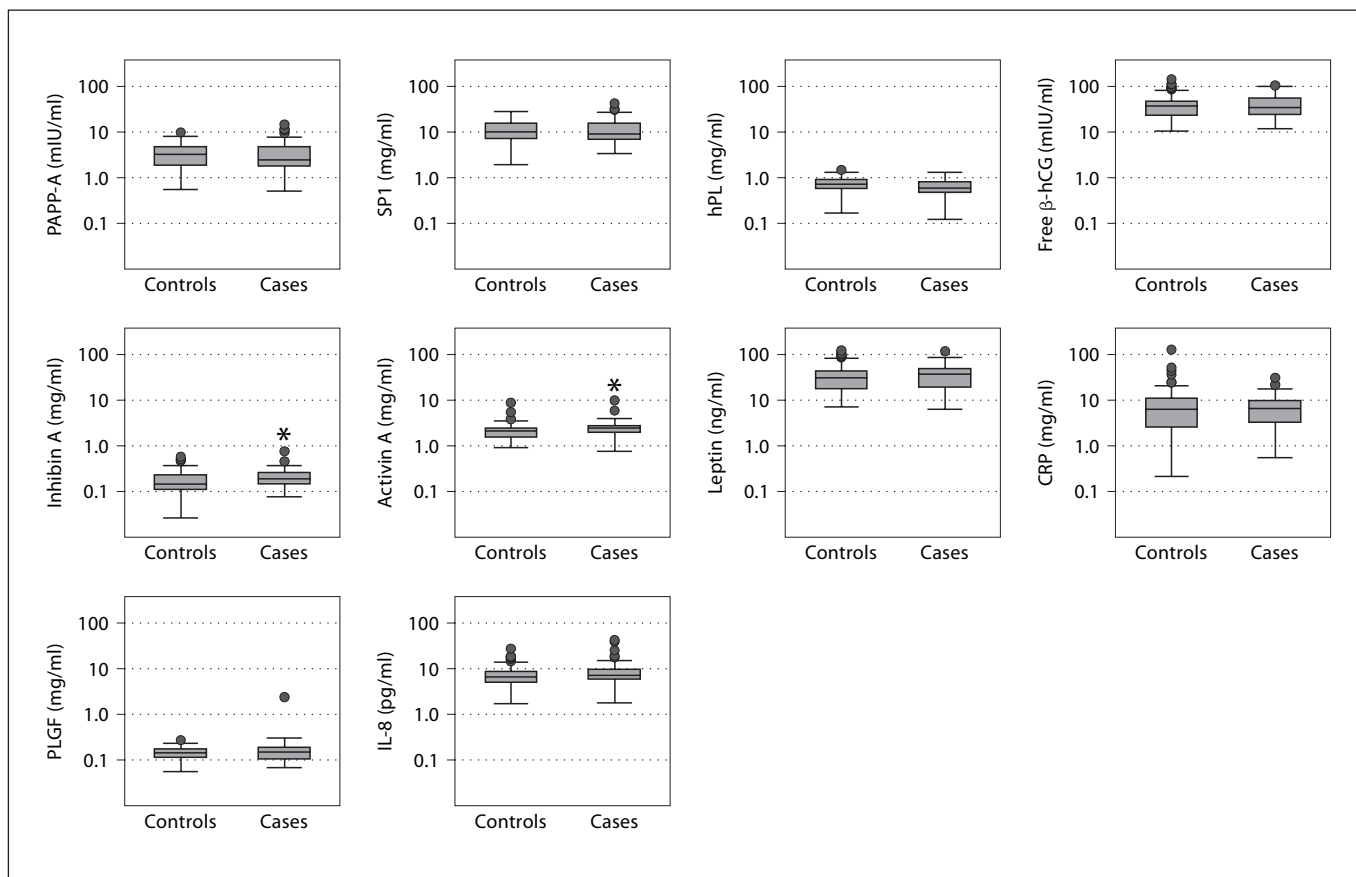
#### *Nuchal Translucency Thickness*

Ultrasound data were obtained from the referring obstetricians at the time of blood sampling. Training for the measurement of NT was ascertained by a quality control programme under the auspices of the Fetal Medicine Foundation, London, England.

#### *Data Analysis*

*Descriptive Analysis.* Measured serum levels of placental and non-placental markers were descriptively summarised for cases and controls by calculating means, standard deviation, medians and range. Simple two-sample comparisons were performed by calculating the Wilcoxon rank-sum test statistics and *p* values derived from these.

*Conditional Logistic Regression Models.* As the study design was that of a matched case-control study, we used, in a second step, conditional logistic regression models to analyse the association between having developed PE and the measured serum levels. In order to avoid strong assumptions about the functional relationship between the measured serum markers and the risk of developing PE, the measured levels were dichotomised using the median value of the control sample and indicator variables were constructed accordingly and entered in the conditional logistic regression models. Firstly, each measurement was entered separately, providing estimates of the crude odds ratio and 95 % confidence intervals. Secondly, multivariable conditional logistic regression models were explored and constructed. To obtain a reduced set of predictors stepwise procedures to include (forward selection) or remove (backward selection) exposure variables were used with a decision rule set at *p* values smaller than 0.2. Backward and forward selection resulted in the same model. In a further step, effect modification with gestational week was explored (week 11 vs. week 12 or 13). Even though the case-control sets were closely matched for gestational age, effect modification by gestational age can still be explored, either by performing stratified analyses or by incorporating appropriate interaction terms in the conditional logistic regression models. Indeed, effect modification was found when interaction terms were included. Therefore, a stratified analysis was performed and the results were summarised for the subset of case-control triples with gestational week 12 or 13 (combined) only. On this subset, again the measured serum levels were dichotomised using the median values observed in the control set (of only week 12 or 13) and odds ratios were estimated by conditional logistic regression. All analyses were performed using STATA version 8.2 (StataCorp, College Station, Tex., www.stata.com).



**Fig. 1.** Box-plots of various marker protein concentrations (on a logarithmic scale) in the first trimester serum of pregnant women who did not (controls) or who did (cases) develop PE later in gestation. The boxes represent the median (solid horizontal line within the box) and the 25/75th centiles (begin and end of box), and data points outside the lower and upper adjacent values. \* Indicates a p value lower than 0.02 when testing with the Wilcoxon rank-sum test for no difference in mean between cases and controls.

## Results

Medians and interquartile ranges of the serum concentrations are given in figure 1. In comparison to the control group, the levels of inhibin A and activin A were found to be significantly increased in the subsequently pre-eclamptic group (Mann-Whitney U test;  $p = 0.017$  and  $0.012$ , respectively) though there was a considerable overlap of the values. None of the other analytes, including PAPP-A and PLGF, was yielding a significant difference in concentration between the two groups. Similarly, the increase in the NT in the cases compared to the controls (1.47 vs. 1.35 mm, table 1) did not quite reach statistical significance ( $p = 0.069$ ).

Univariable analysis with conditional logistic regression models, taking account of the matched case-control

design, suggested that increased values of NT and inhibin A were associated with a 1.95- and 3.5-fold increase of the odds of developing PE. However, multivariable conditional logistic regression models revealed that the associations of developing PE with measured values of NT, PAPP-A, hPL, and inhibin A were confounded, and stronger associations were estimated on the odds ratio scale in multivariable models (table 2). Including interaction terms in the model provided evidence that effect modification was present with regard to the number of gestation weeks at which the blood sample were collected (weeks 12 and 13 vs. week 11,  $p = 0.006$ ). Analysing case-control-sets of women who provided their blood samples in gestation weeks 12 and 13 separately in multivariate models, we found strong associations between increased levels of NT, inhibin A and activin A (odds ratios all 5 or higher

**Table 2.** Association (odds ratio and 95% confidence interval) of serum levels\* of placental and non-placental proteins in the first trimester of pregnancies with or without later occurring pre-eclampsia

Serum measurement	Cut-off value	Crude odds ratio comparing higher vs. lower <sup>a</sup>	Adjusted odds ratio comparing higher vs. lower <sup>b</sup>
Nuchal translucency thickness, mm	1.40	1.95 (0.97–3.92)	2.89 (1.23–6.78)
PAPP-A, mIU/ml	3.11	0.61 (0.28–1.29)	0.48 (0.19–1.25)
Free $\beta$ -hCG, mIU/ml	36.35	0.88 (0.44–1.78)	
SP1, g/ml	9.81	0.86 (0.45–1.65)	
hPL, g/ml	0.70	0.48 (0.21–1.08)	0.50 (0.18–1.39)
Inhibin A, pg/ml	148	3.47 (1.52–7.90)	4.79 (1.82–12.65)
Activin A, pg/ml	2,109	1.71 (0.85–3.45)	
Leptin, ng/ml	30.6	1.88 (0.79–4.50)	
IL-8, pg/ml	6.72	1.48 (0.75–2.92)	
CRP, $\mu$ g/ml	6.32	1.51 (0.72–3.17)	
PLGF, pg/ml	142.9	1.20 (0.58–2.50)	

\* Based on median value that were defined using the distribution observed in the control group.

<sup>a</sup> Derived from conditional logistic regression.

<sup>b</sup> Based on stepwise selection of variables.

**Table 3.** Analysis in subsample of case-control sets with gestational weeks 12 or 13: associations (odds ratio and 95% confidence interval) of serum levels based on median value that were defined using the distribution observed in the control group of placental and non-placental proteins in pregnancies with or without later occurring pre-eclampsia

Serum measurement	Cut-off value	Crude odds ratio comparing higher vs. lower <sup>a</sup>	Adjusted odds ratio comparing higher vs. lower <sup>b</sup>
Nuchal translucency thickness, mm	1.4	1.85 (0.78–4.38)	6.68 (1.53–29.2)
PAPP-A, mIU/ml	3.46	0.55 (0.21–1.40)	0.20 (0.04–1.03)
Free $\beta$ -hCG, mIU/ml	34.3	0.72 (0.30–1.71)	
SP1, g/ml	10.02	0.94 (0.42–2.13)	
hPL, g/ml	0.82	0.75 (0.31–1.86)	
Inhibin A, pg/ml	137.95	4.92 (1.60–15.1)	13.4 (1.97–91)
Activin A, pg/ml	2,003	2.80 (1.13–6.91)	6.14 (1.25–30.1)
Leptin, ng/ml	30.7	2.34 (0.68–8.06)	
IL-8, pg/ml	6.66	1.71 (0.73–4.02)	
CRP, $\mu$ g/ml	6.7	2.19 (0.85–5.63)	
PLGF, pg/ml	158.4	0.80 (0.31–2.05)	0.33 (0.07–1.47)

<sup>a</sup> Derived from conditional logistic regression.

<sup>b</sup> Based on stepwise selection of variables.

and statistically significant) and subsequent PE, and a reduction of the risk of PE with increased levels of PAPP-A and PLGF (borderline significant odds ratios of 0.2 and 0.33). These estimates translate to a 5- and 3-fold risk increase for low levels of these two markers from blood samples in gestation weeks 12 and 13 (table 3).

## Discussion

In this study, we were able to confirm, using descriptive non-parametric statistical analysis, significantly increased first trimester serum concentrations of inhibin A and activin A in women subsequently developing PE, to-

gether with a trend towards reduced levels for PAPP-A and PLGF for which statistical significance was not reached by this method (Mann-Whitney) while this was found to be the case in the second trimester in other studies [10, 14]. This trend to slightly reduced first-trimester PLGF is in agreement between the more strongly decreased second trimester levels and a study reporting unchanged concentrations in the first trimester [15]. In univariable analysis the other markers investigated in this study were not significantly associated with subsequent PE. However, analysis in multivariable conditional logistic regression models revealed an association between increased inhibin A and translucency thickness and increased PE risk with a suggestion of a reduced risk with increased PAPP-A and hPL. Furthermore, the association of the serum levels with PE risk varied by gestational age at sample collection. Restricting the analysis to blood samples taken in the pregnancy weeks 12 and 13, the multivariable model included activin A and PLGF levels in addition to NT, PAPP-A, and inhibin A.

This matched case-control study measured a wide array of serum markers thought to be associated with the risk of developing PE using state-of-the-art laboratory methods. The serum samples were prospectively collected in the first trimester by the MCL laboratory and, due to the provision of comprehensive marker assessment for a non-selective sample of pregnancies, there are no indications that this study suffered selective ascertainment of PE events. However, due to the limited sample size of this study, the functional relationship of the measured potential risk factors with the developing PE was not estimated. This analysis was designed as a pilot study for finding out whether it would be worth looking further into the question of first trimester PE screening and with which marker. For this reason, the number of studied markers was high but the number of cases small. In a follow-on project, the investigation will be prospective and with a larger number of cases and selected markers only. In addition, the gestational age at the onset of the maternal symptoms will have to be known more precisely in order to distinguish between mild and severe cases. Variations in this parameter are at least in part responsible for the disagreeing reports in the literature. This study contained mostly mild cases (table 1).

In this project, the most potent first trimester serum markers associated with the risk to later develop PE were inhibin A and activin A, followed by PAPP-A and PLGF. It is interesting to note that these are at least partially, but not exclusively produced by the placenta. The observed differences of risk association by gestational age clearly

indicate that these serum markers might not be of easy use to guide decisions regarding PE risk in clinical settings. Leptin, for which we did not observe an association with subsequent PE, would also fall into this partially placental category, but the non-placental (adipose tissue) production is predominant and increasing with body mass. The same is the case for CRP, for which we were recently able to demonstrate a small placental production by real-time PCR [34]. Leptin, CRP and IL-8 are related to inflammatory processes [35] such as PE, but this may play a role only later in gestation. The exclusively placental markers SP1 and free  $\beta$ -hCG are no indicators for an increased risk of PE, thus illustrating that the pathogenetic mechanism of this disease is complex.

## Conclusion

In conclusion, this carefully matched case-control study showed that members of the inhibin family and to some extent PAPP-A and PLGF were superior to other markers to predict PE, but that the prognostic information varied with the gestational age at the time of blood sampling. Therefore, it seems unlikely that a single 'miracle' serum marker in early pregnancy will be available in the near future for reliably predicting which women would subsequently develop PE.

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