# First Trimester CD93 as a Novel Marker of Preeclampsia and Its Complications: A Pilot Study

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## Abstract

**Introduction** CD93 plays a crucial role in endothelial homeostasis and angiogenesis. Recently its role in hypertension has been investigated, holding promise for novel targeted diagnostic and therapeutic strategies.

**Aim** We assessed for the first time differences in first trimester serum CD93 levels in women who lately developed preeclampsia (PE) vs. normotensive pregnancy (NP).

**Methods** First trimester serum CD93 concentrations were assessed in a multicenter cohort of 83 women (34 PE and 49 NP) by ELISA Immunoassay.

**Results** Serum CD93 was lower in women who developed PE vs. NP (111.8 ± 24.4 vs. 137.5 ± 22.3 ng/ml; $p < 0.001$). Serum CD93 was associated with a decreased risk of developing PE (OR 0.950, 95% CI 0.922-0.978) and composite neonatal outcome (OR 0.952, CI 0.923-0.982), after adjustment for confounders.

**Conclusions** PE is accompanied by decreased serum CD93 levels. CD93 might play a role during placentation leading to defective angiogenesis, vascular dysfunction, and PE development.

**Keywords** CD93 · C1qR · Angiogenic marker · Preeclampsia · Pregnancy · Hypertension

## 1 Introduction

The incidence of hypertensive disorders of pregnancy (HDP) is constantly increasing from the 90's, representing a leading cause of maternal morbidity and mortality [1]. Preeclampsia (PE), the most severe form of HDP, presents with new-onset hypertension and a sign of organ damage, including uteroplacental insufficiency, after 20 weeks of pregnancy [2]. To date, no therapies are available with delivery remaining the

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only cure [3, 4]. Therefore, the search for novel biomarkers allowing for early risk assessment and possible therapeutic targeting is one of the pillars of current research on PE. While the etiology of PE is still incompletely understood, growing evidence supports the hypothesis that underlying pathophysiologic mechanisms may involve maternal endothelial dysfunction and angiogenic imbalance [5]. Maternal endothelial and vascular dysfunction not only characterizes PE during pregnancy, but also PE-associated increased cardiovascular risk observed in the entire lifespan of women who suffered from this syndrome [6].

CD93 (also known as complement protein 1 q subcomponent receptor C1qR1 or C1qRp) is a transmembrane glycoprotein predominantly expressed in endothelial cells with a soluble form exerting proangiogenic effects. CD93 promotes endothelial cells migration, and their subsequent tube-like arrangement driving blood vessels remodeling [7]. Recent findings converge on the promising diagnostic and therapeutic role of CD93 for a broad spectrum of cardiovascular diseases, including hypertension [8]. Based on these findings, we hypothesized that CD93 may play a crucial role in maternal endothelial dysfunction and angiogenic imbalance that occur during PE early development and progression.

## 2 Methods

To test the hypothesis that CD93 may play a role in the early phases of PE development, we measured circulating CD93 levels in first trimester serum samples of 34 women who developed PE with severe features and 49 women with normotensive pregnancy (NP). Women were enrolled in three Italian University Hospitals of different geographical areas. Inclusion criteria were: (1) age 18–40 years; (2) singleton pregnancy; (3) absence of fetal genetic abnormalities or major malformations. Exclusion criteria were the presence of chronic hypertension, neoplastic or autoimmune disease or any important infection during pregnancy. Clinical data on baseline characteristics at conception and pregnancy outcomes were retrospectively retrieved from hospital's computerized maternal and neonatal medical records. PE was defined according to the International Society for the Study of Hypertension in Pregnancy criteria. Serum CD93 was measured by Quantikine Human C1q R1 Immunoassay ELISA kit #DCD930 (R&D Systems Inc., MN, USA). Measurements were conducted in duplicates, according to the manufacturer's recommended protocol. Average minimum detectable dose (MDD) of human CD93 is 0.006 ng/mL. Intra-assay coefficient of variation (CV) and inter-assay CV 2.8% and 5.7%, respectively.

All patients provided informed consent, and the study was approved by relevant ethics committees. Comparisons between women with PE and women with normotensive pregnancies were performed using T-student and Chi-square tests for continuous and categorical variables, respectively. The correlation of CD93 concentrations with PE development and neonatal composite outcome (small for gestational age (SGA), low weight at birth, 1-min Apgar score <5) was calculated by logistic regression-derived odds ratios (OR) and 95% confidence intervals (CI). IBM SPSS Statistics for Macintosh (Version 28.0, Armonk, NY) was used for statistical analysis.

## 3 Results

As for baseline characteristics, mean age was 32 ± 4 years in the NP group vs. 33 ± 4 years in the PE group ($p = 0.066$), BMI before conception 21.6 ± 2.4 vs. 24.1 ± 4.8 kg/m$^2$ ($p = 0.002$), the proportion of primiparous individuals was 77.5% vs. 55.9% ($p = 0.054$). In the NP group, 95.9% of the women were White and 4.1% Latino; in the PE group 79.4% were White, 8.8% Latino, 5.9% Black, and 5.9% Asian ($p = 0.075$). Only 3 women used to smoke before conception and 6 were obese (BMI>30 kg/m$^2$), all of these belonging to the PE group (8.8% and 17.6%, respectively).

In the PE group, 13 women were prescribed with acetylsalicylic acid during pregnancy (38.2%) and 11 with any antihypertensive medication (32.3%); 15 women had SGA babies (44.1%), and 8 had early-onset PE (25%). Neonates were all born alive, with 48.9% males in the NP group and 61.7% in the PE group ($p = 0.272$). In the PE group, 47% of neonates were premature (born <37 weeks gestation), 58.8% had low weight at birth (< 2500 g), and 8.8% had 1-min Apgar score <5. As for delivery, mean neonatal birth weight was 3429g in the NP group vs. 2397g in the PE group ($p < 0.001$). Gestational week at delivery was 39.7 weeks vs. 35.8 weeks ($p < 0.001$); urgent C-section only occurred in PE group (35.3%), while vaginal delivery occurred in 79.6% of women in the NP group vs. 38.2% in the PE group ($p < 0.001$).

We found that CD93 was lower in the first trimester sera of women who developed PE compared to women with NP (111.8 ± 24.4 vs. 137.5 ± 22.3 ng/ml; $p < 0.001$). The adjusted and unadjusted logistic regression models of CD93 for both PE development and composite neonatal outcome are reported in Table 1.

## 4 Discussion

Endothelial dysfunction is a key pathogenic feature of PE, being characterized by altered endothelial cells proliferation, migration, invasion, and angiogenesis [9].

CD93 plays a crucial role in all these processes in addition to efferocytosis and immune regulation [7]. At present,

**Table 1** Logistic regression models of serum CD93 for maternal and neonatal outcomes

| | O.R. | S.E. | Exp(B) with 95% CI | p value |
|---|---|---|---|---|
| **Development of PE (unadjusted)** | | | | |
| sCD93 (ng/ml) | 0.951 | 0.013 | 0.927–0.975 | **<0.001*****|
| **Development of PE (adjusted)** | | | | |
| sCD93 (ng/ml) | 0.950 | 0.015 | 0.922–0.978 | **<0.001*****|
| Age (years) | 1.156 | 0.081 | 0.987–1.354 | 0.073 |
| BMI (kg/m$^2$) | 1.244 | 0.084 | 1.055–1.466 | **0.009*** |
| Neonate female sex (yes/no) | 1.659 | 0.618 | 0.494–5.576 | 0.413 |
| Primiparous (yes/no) | 0.445 | 0.622 | 0.132–1.504 | 0.192 |
| **Composite neonatal outcome (unadjusted)** | | | | |
| sCD93 (ng/ml) | 0.955 | 0.014 | 0.929–0.980 | **<0.001*****|
| **Composite neonatal outcome (adjusted)** | | | | |
| sCD93 (ng/ml) | 0.952 | 0.016 | 0.923–0.982 | **0.002**** |
| Age (years) | 1.455 | 0.134 | 1.118–1.893 | **0.005*** |
| BMI (kg/m$^2$) | 1.217 | 0.093 | 1.015–1.459 | **0.034*** |
| Neonate female sex (yes/no) | 2.096 | 0.664 | 0.571–7.694 | 0.265 |
| Primiparous (yes/no) | 5.217 | 0.824 | 1.038–26.218 | **0.045*** |

* $p < 0.05$; ** $p < 0.005$; *** $p < 0.001$

the only study that investigated CD93 in pregnancy has been performed by part of our group. We examined CD93 expression in human placenta and cell lines, hypothesizing that CD93 can guide extravillous cytotrophoblast migration through β1-integrin in uterine spiral arteries during placentation in the first trimester of pregnancy, and that decreased CD93 expression in third trimester and PE placentas could be linked to poor extravillous cytotrophoblast cells migration [10].

In the present study we assessed for the first time serum CD93 concentrations during the first trimester of pregnancy in women who developed PE with severe features as well as those with NP. Women with PE were found to have lower CD93 at first trimester of pregnancy, with an increased risk of developing PE and adverse neonatal outcomes.

To our best knowledge there are no data on literature on the differences between serum concentrations of CD93 in pregnant women vs. non-pregnant women or non-pregnant women vs. men. However, in a large study involving more than 2000 controls and 901 individuals with type 2 diabetes (IMPROVE cohort) average serum CD93 in controls was 164 ± 45 ng/ml and 157 ± 40 ng/ml in diabetic subjects. CD93 levels did not correlate with sex in both healthy controls and individuals with diabetes [11]. It must be noted that mean age of both study groups was 64 years, thus most women were in menopause. The manufacturer of the ELISA kit declared a normal range for soluble CD93 in apparently healthy volunteers of approximately 90–223 ng/ml (average 146 ± 33 ng/ml).

Other angiogenic factors such as placental growth factor (PlGF), soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin, have been previously studied for their predictive value for PE and related adverse outcomes [12]. During first trimester of pregnancy only PlGF, together with clinical risk factors and ultrasound markers, has shown to hold predictive capacity for PE. Angiogenesis and endothelial homeostasis are complex processes regulated by various pro- and anti-angiogenic molecules. In the future, a combination of different angiogenic markers, including CD93, may help reaching a good predictive capacity for PE from the very beginning of pregnancy. Furthermore, unveiling the complex pathophysiology of PE-associate impaired angiogenesis and endothelial dysfunction may facilitate the development of multiple-target therapy, increasing therapeutic efficacy and avoiding possible drug evasion by alternate pathways of anti-angiogenesis. Unfortunately, in the present study we do not have data on other angiogenic markers.

In conclusion, the results of the present study support the hypothesis that low levels of the proangiogenic glycoprotein CD93 may play a role in PE, holding promise for future personalized diagnostic and therapeutic strategies. Indeed, a novel target drug derepressing anti-miR-92a and consequently increasing CD93 levels was able to induce substantial cardiovascular benefits in several studies [13–15]. Future studies are mandatory to unveil the clinical implications of this novel biomarker and the potential benefits of its targeting in both prevention and treatment of PE.

## Declarations

**Conflict of Interest** The authors declare no competing interests regarding this work.

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