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# The impact of early- and late-onset preeclampsia on umbilical cord blood cell populations

Emilie M. Herzog[a], Alex J. Eggink[a], Marten van der Zee[a], Jacqueline Lagendijk[a], Sten P. Willemsen[a,b], Robert de Jonge[c], Eric A.P. Steegers[a], Regine P.M. Steegers-Theunissen[a,d,*]

[a] Department of Obstetrics and Gynaecology, Erasmus MC, University Medical Centre Rotterdam, Postbus 2040, 3000 CA Rotterdam, The Netherlands
[b] Department of Biostatistics, Erasmus MC, University Medical Centre Rotterdam, Postbus 2040, 3000 CA Rotterdam, The Netherlands
[c] Department of Clinical Chemistry, Erasmus MC, University Medical Centre Rotterdam, Postbus 2040, 3000 CA Rotterdam, The Netherlands
[d] Department of Paediatrics, division of Neonatology, Erasmus MC, University Medical Centre Rotterdam, Postbus 2040, 3000 CA Rotterdam, The Netherlands

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ABSTRACT

Pregnancies complicated by preeclampsia (PE) are characterised by an enhanced maternal and fetal inflammatory response with increased numbers of leukocytes in maternal peripheral blood. The impact of PE on newborn umbilical cord blood cell (UCBC) populations however, has been scarcely studied. We hypothesise that PE deranges fetal haematopoiesis and subsequently UCBC populations. Therefore, the objective of this study was to investigate newborn umbilical cord blood cell populations in early- (EOPE) and late-onset PE (LOPE).

A secondary cohort analysis in The Rotterdam Periconceptional Cohort was conducted comprising 23 PE cases, including 11 EOPE and 12 LOPE, and 195 controls, including 153 uncomplicated and 23 fetal growth restriction- and 19 preterm birth complicated controls. UCBC counts and differentials were quantified by flow cytometry and analysed as main outcome measures.

Multivariable regression analysis revealed associations of EOPE with decreased leucocyte- (monocytes, neutrophils, eosinophils, immature granulocytes) and thrombocyte counts and increased NRBC counts (all $p < 0.05$). EOPE remained associated with neutrophil- ($\beta$-0.92, 95%CI −1.27,−0.57, $p < 0.001$) and NRBC counts ($\beta$1.11, 95%CI 0.27,1.95, $p = 0.010$) after adjustment for gestational age and birth weight. LOPE did not reveal any significant association.

We conclude that derangements of fetal haematopoiesis, in particular of neutrophil- and NRBC counts, are associated with EOPE only, with a potential impact for future health of the offspring. This heterogeneity in UCBC should be considered as confounder in epigenetic association studies examining EOPE.



## 1. Introduction

Preeclampsia (PE) is a heterogeneous disease with early-onset (EOPE) and late-onset (LOPE) PE as the main phenotypes.

Due to inadequate spiral artery remodelling with suboptimal placental perfusion, excessive amounts of oxidative stress can lead to an enhanced release of syncytiotrophoblast microparticles and cytokines, which particularly contributes to the pathogenesis of the more severe EOPE phenotype (Raymond and Peterson, 2011). In contrast, LOPE shows a relatively normal initial placentation and is associated with conditions that enhance excessive oxidative stress and placental inflammation later in pregnancy, such as obesity and pre-existing hypertension (Burton et al., 2009;

*Abbreviations:* PE, preeclampsia; EOPE, early- onset preeclampsia; LOPE, late-onset preeclampsia; FGR, fetal growth restriction; PTB, preterm birth; BMI, body mass index; UCB, umbilical cord blood; UCBC, umbilical cord blood cell; *NRBC*, nucleated red blood cell; CI, confidence interval.

* Corresponding author at: Department of Obstetrics and Gynaecology, Erasmus MC, University Medical Centre Rotterdam, Postbus 2040, 3000 CA Rotterdam, The Netherlands.

*E-mail addresses:* e.herzog@erasmusmc.nl (E.M. Herzog), a.eggink@erasmusmc.nl (A.J. Eggink), m.vanderzee@erasmusmc.nl (M. van der Zee), j.lagendijk.2@erasmusmc.nl (J. Lagendijk), s.willemsen@erasmusmc.nl (S.P. Willemsen), r.dejonge@erasmusmc.nl (R. de Jonge), e.a.p.steegers@erasmusmc.nl (E.A.P. Steegers), r.steegers@erasmusmc.nl (R.P.M. Steegers-Theunissen).

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Steegers et al., 2010). Circulating syncytiotrophoblast microparticles can induce an increased maternal systemic inflammatory response with increased numbers of neutrophils and total leukocytes in maternal peripheral blood (Canzoneri et al., 2009; Lurie et al., 1998). The impact of PE on newborn umbilical cord blood cell (UCBC) populations, however, has been scarcely studied.

During pregnancy, haematopoiesis takes place in the yolk sac, liver, bone marrow as well as in the placenta, generating all blood cell types from a small population of pluripotent hematopoietic stem cells as pregnancy advances (Davies et al., 1992; Dzierzak and Robin, 2010; Morrison et al., 1995; Proytcheva, 2009). We hypothesise that PE, in particular EOPE, deranges fetal haematopoiesis resulting in heterogeneity of UCBC populations (Sashida and Iwama, 2012), and investigated the associations between UCBC counts and differentials in early- and late-onset PE.

## 2. Materials and methods

Study design Between June 2011 and June 2013 we included pregnant women in a prospective hospital-based periconceptional birth cohort: The Rotterdam Periconceptional Cohort (Predict Study), at the Erasmus MC, University Medical Centre Rotterdam, The Netherlands (Steegers-Theunissen et al., 2015). For the current secondary cohort analysis, we selected EOPE and LOPE as cases and uncomplicated pregnancies as controls. To adjust for the often accompanied fetal growth restriction (FGR) and iatrogenic preterm birth (PTB) in PE, we oversampled the uncomplicated control group with FGR and PTB as complicated controls. Pregnancies were included in the cohort during the first trimester (early cohort inclusions) or after the first trimester when they were referred to our hospital (late cohort inclusions).

PE was defined according to the International Society for the Study of Hypertension in Pregnancy as gestational hypertension of at least 140/90 mmHg accompanied by an urine protein/creatinine ratio of $\geq$30 mg/mmol, arising *de novo* after the 20th week of gestation (Brown et al., 2001). EOPE was defined when PE was diagnosed before 34 weeks of gestation, LOPE when diagnosed after 34 weeks of gestation (Tranquilli et al., 2013). Uncomplicated control pregnancies were defined as pregnancies without the presence of PE, gestational hypertension, FGR or PTB. FGR inclusion was based on an estimated fetal weight below the 10th percentile for gestational age based on ultrasound measurements performed between 20 and 38 weeks of gestation (Battaglia and Lubchenco, 1967). Birth weight percentiles were calculated using the reference curves of the Dutch Perinatal Registry to validate birth weight $\leq$10th percentile and exclude those newborns with birth weight >10th percentile (Visser et al., 2009). Spontaneous preterm deliveries between 22 and 37 weeks of gestation were defined as PTB (2013). Women with HIV infection, age <18 years and insufficient knowledge of the Dutch language could not participate and pregnancies complicated with a fetal congenital malformation and twins were excluded for the current study. Maternal comorbidity was defined by any concurrent cardiovascular-, heamatologic-, endocrine-, metabolic-, auto-immune- or renal disease. Maternal and fetal characteristics were obtained from hospital medical records. All women gave written informed consent before participation and written parental informed consent was obtained for the child. Ethical approval was given by the Erasmus MC, University Medical Centre Research Ethics Board (MEC-2004-227).

### 2.1. Collection and handling of blood samples

Umbilical cord blood (UCB) samples from the umbilical vein were obtained in vacutainer tubes (Ethylenediaminetetraacetic acid as anticoagulant), immediately after delivery and clamping of the umbilical cord. Samples were transported at room temperature and subjected to flow cytometric analysis within 48 h after delivery (Sysmex XE-5000, Sysmex XN-3000 and XS-800i, Etten-Leur, The Netherlands) to quantify erythrocytes, thrombocytes and leucocyte differentials. Between arrival at the Clinical Chemistry Laboratory and time of analysis, samples were stored at 4–8 °C. Quality of the blood cell counts was guaranteed by a manual check whereby flow cytometric data of suspect plots or reported system errors were excluded for further analysis.

### 2.2. Statistical analysis

We used cell numbers/L for the analysis of leucocyte differentials and nucleated red blood cells (NRBC), which is preferable to the widely used percentages of total leucocyte count, since the largely variable total leucocyte count could result in misleading percentages (Perrone et al., 2005). The normal distributed maternal and newborn characteristics were tested using Analysis of Variance (ANOVA) to detect overall differences between the groups, followed by the posthoc Dunnett *t*-test for pairwise comparisons of EOPE and LOPE with uncomplicated controls and FGR and PTB complicated controls. The Dunnett *t*-test limits the multiple testing problem by comparing each group to one reference group only. The Kruskall-Wallis-test was applied to all non-parametric maternal and newborn characteristics, followed by pairwise Mann-Whitney tests for posthoc comparisons.

Log-transformation was applied to the non-parametric UCBC to achieve normal distributions of neutrophils, monocytes, eosinophils, basophils, NRBC and immature granulocytes. We converted zero values of neutrophils and NRBC into half of the lowest detectable value of the Sysmex haematology system, prior to log-transformation. Linear regression analysis was performed to investigate the association between UCBC counts and differentials and EOPE/LOPE versus the pooled group of (un)complicated controls. In the crude linear regression analyses, UCBC counts were estimated with group (case-control) as the only predictive variable. In the adjusted multivariable analyses, gestational age and birth weight were additionally entered to the model as covariates, in formula: $[\mathrm{UCBC}] = \beta_0 + \beta_1 \mathrm{group} + \beta_2 \mathrm{GA} + \beta_3 \mathrm{BW} + \varepsilon$. Here group is an indicator variable that is 1 for EOPE or LOPE and 0 for the pooled group of (un)complicated controls. [UCBC] represents the concentration of a certain UCBC population. All measurements were performed with IBM SPSS Statistics version 21.0 (SPSS Inc, Chicago, IL, USA).

## 3. Results

From the Predict Study we included all eligible women for this secondary cohort analysis that met the inclusion criteria as described earlier (n = 412). After exclusion of 194 pregnancies due to missing blood samples (n = 117) or poor quality of blood cell counts (n = 77), 218 pregnancies were included for analysis. Patients with missing data were characterised by a shorter gestational age (38.2 versus 39.1 weeks, $p < 0.001$) and lower birth weight (3065 versus 3363 g, $p < 0.001$) as compared to the final study population, and contained twice as much EOPE- (10.3% versus 5.0%) and LOPE pregnancies (9.3% versus 5.5%, $p = 0.076$), as depicted in Supplemental Table 1. The final study population comprised 23 cases of PE including 11 EOPE and 12 LOPE, and 195 controls, including 153 uncomplicated controls and 23 FGR and 19 PTB complicated controls (Supplemental Fig. 1).

Maternal and newborn characteristics are shown in Table 1. In addition to the case specific parameters blood pressure, proteinuria, gestational age and birth weight, a significant lower mean maternal age in EOPE versus LOPE and uncomplicated controls was