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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- (β-0.92, 95%CI −1.27,−0.57, $p < 0.001$) and NRBC counts (β1.11, 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

Table 1
Maternal and newborn characteristics.

Maternal characteristics				Uncomplicated	Complicated controls		
		EOPE (n = 11)	LOPE (n = 12)	controls (n = 153)	FGR (n = 23)	PTB (n = 19)	Overall p-value
Age (years)		27.1 (5.2)	34.1* (3.8)	32.2* (5.1)	29.3,** (5.8)	30.6 (5.2)	0.002
Nulliparous, n (%)		8 (72.7)	7 (58.3)	66 (43.1)	11 (47.8)	6 (31.3)	0.204
Ethnicity, n (%)	Western	9 (81.8)	7 (58.3)	122 (80.3)	14 (60.9)	15 (83.3)	0.152
	Non-Western	1 (18.2)	5 (41.7)	30 (19.7)	9 (39.1)	3 (16.7)	
Preconception BMI[a] (kg/m^2)		26.8 (9.3)	26.4 (2.8)	24.1 (7.1)	23.6 (5.6)	24.1 (7.1)	0.785
Smoking during pregnancy (yes), n (%)		1 (12.5)	0 (0.0)	8 (5.4)	2 (9.1)	1 (6.3)	0.813
Co-morbidity (yes), n (%)		1 (9.1)	3 (25.0)	61 (39.9)	4 (17.4)	3 (15.8)	0.022
Newborn characteristics							
Male gender, n (%)		9 (81.8)	7 (58.3)	77 (50.3)	14 (60.9)	9 (47.4)	0.210
Gestational age at birth[a] (weeks)		31.0 (3.7)	37.4* (1.9)	39.6*,** (1.7)	39.0*,** (2.6)	35.9*,** (4.9)	<0.001
Birth weight[a] (grams)		1155 (353)	3238* (1689)	3515* (563)	2625*,** (600)	2650*,** (1455)	<0.001
Birth weight <10th percentile, n (%)		2 (18.2)	2 (16.7)	0*,** (0.0)	23*,** (100.0)	0 (0.0)	<0.001

Data are presented as mean (standard deviation) with corresponding ANOVA testing to examine overall differences between the groups, followed by the post hoc Dunnett *t*-test for pairwise comparisons of EOPE and LOPE with (un)complicated controls.
Data are presented as number (%) with corresponding Chi^2/Fischer's exact testing.
ANOVA analysis of variance; *BMI* body mass index; *EOPE* early onset preeclampsia; *LOPE* late onset preeclampsia; *FGR* fetal growth restriction; *PTB* preterm birth.
[a] Non-parametric data are presented as median (interquartile range) with corresponding Kruskall-Wallis testing and posthoc Mann-Whitney testing.
* $p < 0.05$ versus EOPE pregnancies.
** $p < 0.05$ versus LOPE pregnancies.

shown (27.1 year, versus 34.1 and 32.2 year respectively, overall p = 0.002). EOPE pregnancies ended more often in a caesarean section compared to LOPE and (un)complicated controls (90%, versus 25% and up to 35% respectively, overall p = 0.003). Comorbidity was significantly different between the groups and highest in uncomplicated controls, but no significant differences were observed in the posthoc analysis. Neonatal temperature at birth was similar for each group.

By ANOVA testing, we observed significantly lower cell counts for all UCBC populations and a significantly higher NRBC count in EOPE versus (un)complicated controls. In LOPE only significantly higher neutrophil- and erythrocyte counts and lower reticulocyte counts compared to PTB complicated controls were observed (Supplemental Table S2).

In Table 2 we show the results of the linear regression analyses of the UCBC counts and differentials of both EOPE and LOPE versus the pooled (un)complicated controls. The crude estimates revealed that EOPE was associated with the decreased counts of total leucocytes (β −7.6, 95% CI −10.53, −4.76, p < 0.001); monocytes (β −0.88, 95% CI −1.26, −0.51, p < 0.001); neutrophils (β −1.92, 95% CI −2.25, −1.60, p < 0.001); eosinophils (β −0.67, 95% CI −1.16, −0.18, p = 0.007); immature granulocytes (β −1.82, 95% CI −2.60, −1.03, p < 0.001) and thrombocytes (β −80.19, 95% CI −126.7, −33.7, p = 0.001). EOPE was associated with increased NRBC count (β 1.20, 95% CI 0.57, 1.84, p < 0.001). After adjustment for gestational age and birth weight, EOPE remained associated with decreased neutrophil count (β −0.92, 95% CI −1.27, −0.57, p < 0.001) and increased NRBC count (β 1.11, 95% CI 0.27, 1.95, p = 0.010). The linear regression analyses did not reveal any significant association with LOPE versus the (un)complicated control group.

4. Discussion

In this study we observed that pregnancies complicated by EOPE are associated with decreased leucocyte- (monocytes, neutrophils, eosinophils, immature granulocytes) and thrombocyte counts and with increased NRBC counts in umbilical cord blood. After adjustment for gestational age and birth weight, EOPE remained associated with decreased neutrophil and increased NRBC counts.

Our findings demonstrate that the associations of most UCBC counts (total leucocytes, monocytes, eosinophils, immature granulocytes and thrombocytes) with EOPE are confounded by gestational age and birth weight, which is in agreement with previous studies (Davies et al., 1992; Proytcheva, 2009). It revealed that LOPE compared to EOPE has a marginally impact on UCBC populations, which may be explained by its milder phenotype as well as the absence of FGR and PTB in this group. The 4–7-fold decrease of neutrophil count and 5-fold increase of NRBC count in association with EOPE however, were independent of gestational age and birth weight. Because the innate immune-system matures during pregnancy, this system of the newborn is prepared to be fully functional at birth by a sudden neutrophil rise during the late third trimester (Davies et al., 1992). The excessive oxidative stress from early pregnancy onwards might have affected UCB neutrophil counts in EOPE by generating enhanced inflammation in the fetal circulation, as demonstrated earlier by higher activated neutrophils and monocytes as well as increased CRP, α-1-antitrypsin and plasma chemokine levels (Catarino et al., 2012; Mellembakken et al., 2001). As a consequence, fetal endothelial cell dysfunction might occur, by which the maturation and development of fetal haematopoiesis can be affected. Fetal haematopoiesis originates from endothelial cells in the ventral aorta of the developing embryo and is thus extremely sensitive to endothelial damage (Adamo and Garcia-Cardena, 2012). It has been suggested that the maternal endothelial cell damage is of more importance in EOPE than LOPE and that the excessive oxidative stress develops only towards the end of gestation in LOPE (Raymond and Peterson, 2011). This is in line with the observed difference in leucocyte counts between EOPE and LOPE. The association between PE and decreased UCB leucocyte count has been described before and is in agreement with our findings (Aali et al., 2007; Davies et al., 1992). Low neutrophil counts might result in a temporarily reduced immune capacity of the newborn, especially if the child is also born preterm. This might increase the vulnerability for infections.

The observed increase of UCB NRBC in EOPE pregnancies is in line with earlier studies (Aali et al., 2007; Akercan et al., 2005; Catarino et al., 2009; Hebbar et al., 2014; Bayram et al., 2010). However, Akercan- and Catarino et al. did not observe this increase independent of gestational age, which can be explained by the lack of separate analysis for EOPE and LOPE. High numbers of circulating NRBC can reflect an activation of erythropoiesis as a response to the placental ischemia-reperfusion phenomenon resulting from diminished and intermittent perfusion of the intervillous space or a compensation of the erythrocyte-damage, both more profoundly

Table 2
Linear regression analysis of UCBC count and differentials in EOPE and LOPE versus the (un)complicated control group (n = 195).

	EOPE (n = 11)		LOPE (n = 12)	
	Crude β	Adjusted β (GA + BW)	Crude β	Adjusted β (GA + BW)
Haemoglobin (mmol/L)	−0.51 (−1.22, 0.21)	0.37 (−0.54, 1.29)	0.36 (−0.28, 1.01)	0.48 (−0.15, 1.12)
Haematocrit (L/L)	0.00 (−0.03, 0.04)	0.05* (0.00, 0.09)	0.02 (−0.01, 0.05)	0.03 (−0.01, 0.06)
Leucocytes				
Total leucocytes (x10^9/L)	−7.6* (−10.53, −4.76)	0.32 (−3.14, 3.78)	−0.75 (−3.66, 2.16)	−0.07 (−2.68, 2.55)
Lymphocytes (10^9/L)	−0.62 (−1.68, 0.44)	0.98 (−0.32, 2.28)	−0.40 (−1.29, 0.50)	−0.19 (−1.05, 0.66)
Monocytes[a] (10^9/L)	−0.88* (−1.26, −0.51)	−0.05 (−0.49, 0.39)	−0.10 (−0.41, 0.22)	−0.05 (−0.33, 0.23)
Granulocytes				
Neutrophils[a] (10^9/L)	−1.92* (−2.25, −1.60)	−0.92* (−1.27, −0.57)	0.05 (−0.22, 0.32)	0.14 (−0.08, 0.36)
Eosinophils[a] (10^9/L)	−0.67* (−1.16, −0.18)	−0.37 (−1.01, 0.26)	−0.08 (−0.49, 0.34)	−0.10 (−0.51, 0.31)
Basophils[a] (10^9/L)	−0.51 (−1.12, 0.09)	0.59 (−0.15, 1.32)	−0.05 (−0.56, 0.45)	0.04 (−0.44, 0.52)
Immature gran[a] (10^9/L)	−1.82* (−2.60, −1.03)	−0.15 (−0.78, 1.07)	−0.26 (−0.90, 0.37)	−0.16 (−0.73, 0.41)
Erythroid cells				
NRBC[a] (10^9/L)	1.20* (0.57, 1.84)	1.11* (0.27, 1.95)	0.07 (−0.54, 0.68)	0.08 (−0.53, 0.70)
Reticulocytes (x10^9/L)	18.93 (−9.72, 47.58)	−24.25 (−60.40, 11.91)	1.12 (−25.36, 27.60)	−5.34 (−30.48, 19.80)
Erythrocytes (x10^{12}/L)	−0.63* (−0.96, −0.30)	0.04 (−0.37, 0.44)	0.12 (−0.17, 0.42)	0.21 (−0.07, 0.49)
Thrombocytes				
Thrombocytes (x10^9/L)	−80.19* (−126.7, −33.7)	−40.4 (−11.6, 19.8)	−32.05 (−72.25, 8.16)	−28.93 (−69.12, 11.27)

Data are presented as β (95% Confidence Interval) with corresponding multivariable linear regression analysis of EOPE and LOPE versus the (un)complicated controls, crude and with adjustment for gestational age and birth weight. The regression coefficient (β) indicates the increase or decrease (−) change per unit.
GA gestational age; *BW* birth weight; *NRBC* nucleated red blood cells; *UCBC* umbilical cord blood cell; *EOPE* early- onset preeclampsia; *LOPE* late- onset preeclampsia; Immature gran. Immature granulocytes.
[a] Log-transformed data.
* $p < 0.05$.

present in EOPE than in LOPE (Aali et al., 2007; Catarino et al., 2009; Jauniaux et al., 2006; Raymond and Peterson, 2011). The suboptimal placental perfusion results in a relatively hypoxic placental environment, which is beneficial for early invasion of the cytotrophoblast into the maternal decidua. However a prolonged hypoxic placental state may lead to an over-expression of hypoxia-inducible factor 1α (HIF-1α), regulating several processes such as erythropoiesis (Rath et al., 2016). Placental over-expression of HIF-1α has been described in PE pregnancies, which may explain our finding of enhanced NRBC counts in umbilical cord blood, being a result of HIF-1α-induced erythropoietin-release (Rath et al., 2016; Sezer et al., 2013).

4.1. Strengths and limitations

A strength of the study is that we investigated associations between PE and UCBC counts and differentials in EOPE and LOPE separately, which revealed a much stronger association between UCBC counts and EOPE, and is relevant concerning the different aetiologies of both. Moreover, associations were investigated independent of gestational age and birth weight.

Pregnancies complicated by EOPE in our study population ended more often in a Caesarean section. This unfortunately resulted in more missing blood samples (n = 25, 59.5%) compared to LOPE (n = 7, 26.9%, p = 0.009) due to the emergency of the Caesarean sections. Because of the sample size we were not able to adjust for many confounders and therefore inherent to an observational study residual confounding cannot be excluded. The wide confidence intervals demonstrate that the sample size also resulted in a limited power of the study. This implies that certain UCBC values with seemingly clinical relevant UCBC differences between groups might have failed to achieve statistical significance because of lack of power. Additionally, a selection bias due to the relatively high percentage of EOPE and LOPE pregnancies with missing data might be present, but this is an often occurring problem in high-risk patients where medical care is a priority. Another limitation of our study is the tertiary university hospital-setting, in which uncomplicated pregnancies presented with a relatively high percentage of concurrent comorbidity (40%), for which they were referred. These patients were mostly included in the cohort study in the first trimester of pregnancy. Complicated PE, FGR and PTB pregnancies were more often included as late cohort inclusions after the first trimester. They presented with less additional comorbidity, as visualised in Supplemental Fig. 1. Two neonates only in EOPE and LOPE were complicated by FGR. Therefore, future studies may address differences in UCBC populations in a subgroup of early- and late-onset PE complicated by FGR.

5. Conclusion

Derangements of fetal haematopoiesis, in particular of neutrophil- and NRBC counts, are associated with EOPE only. These findings imply potential impact on the future health of offspring, and that heterogeneity in UCBC should be considered as confounder in epigenetic association studies examining EOPE. Further investigation is needed to establish the potential impact for future health of offspring.

Conflicts of interest

The authors report no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jri.2016.05.002.

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