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Impact of early- and late-onset preeclampsia on features of placental and newborn vascular health

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ABSTRACT

Introduction: Offspring exposed to preeclampsia (PE) show an increased risk of cardiovascular disease in adulthood. We hypothesize that this is mediated by a disturbed vascular development of the placenta, umbilical cord and fetus. Therefore, we investigated associations between early-onset PE (EOPE), late-onset PE (LOPE) and features of placental and newborn vascular health.
Methods: We performed a nested case-control study in The Rotterdam Periconceptional Cohort, including 30 PE pregnancies (15 EOPE, 15 LOPE) and 218 control pregnancies (164 uncomplicated controls, 54 complicated controls including 28 fetal growth restriction, 26 preterm birth) and assessed macroscopic and histomorphometric outcomes of the placenta and umbilical cord.
Results: A significant association was observed between PE and a smaller umbilical vein area and wall thickness, independent of gestational age and birth weight. In EOPE we observed significant associations with a lower weight, length and width of the placenta, length of the umbilical cord, and thickness and wall area of the umbilical vein and artery. These associations attenuated after gestational age and birth weight adjustment. In LOPE a significant association with a larger placental width and smaller umbilical vein wall thickness was shown, independent of gestational age and birth weight.
Discussion: Our study suggests that PE is associated with a smaller umbilical cord vein area and wall thickness, independent of gestational age and birth weight, which may serve as a proxy of disturbed cardiovascular development in the newborn. Follow-up studies are needed to ultimately predict and lower the risk of cardiovascular disease in offspring exposed to PE.

1. Introduction

In line with the developmental origins of health and disease paradigm, epidemiological studies substantiated by animal studies strongly suggest that adverse prenatal exposures increase the risk of cardiovascular diseases in child- and adulthood [1,2]. Preeclampsia (PE) occurs in approximately 2–8% of all pregnancies and accounts for one of the major placental-related pregnancy complications. PE is a complex disease characterised by increased

List of abbreviations: ANOVA, Analysis of variance; PE, Preeclampsia; EOPE, Early-onset preeclampsia; LOPE, Late-onset preeclampsia; FGR, Fetal growth restriction; PTB, Preterm birth; BMI, Body mass index; CI, Confidence interval; IEL, Internal elastic lamina; HE, Haematoxylin/eosin staining; EVG, Elastica Von Gieson staining.

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maternal blood pressure and proteinuria during pregnancy and increased risks of fetal growth restriction (FGR) in 12% and preterm birth (PTB) in 20% [3]. Evidence is accumulating that offspring exposed to PE have enhanced risks of increased blood pressure and body mass index in childhood and nearly a double risk of stroke in adulthood [4–7]. Early-onset PE (EOPE) is often more severe than late-onset PE (LOPE) and largely originates from poor first trimester placentation. LOPE seems to be exaggerated by predisposing cardiovascular and metabolic risks for maternal endothelial dysfunction in the second half of pregnancy [4]. It was recently suggested that LOPE may be associated with trophoblast dysfunction due to villous overcrowding in term placentas, leading to diminished intervillous perfusion and increased hypoxia [8]. Both PE phenotypes show enhanced systemic inflammatory responses resulting in exposure of vessels and tissues to excessive oxidative stress [9].

Impaired placentation is a result of inadequate invasion of the maternal spiral arteries by the trophoblast, and has been suggested to affect placental and fetal growth. A lower placental weight at birth has been shown to predict the risk of hypertension in later life, suggesting an association between placental morphological features and offspring vascular health [10]. The umbilical-cord and fetal vasculature share the same embryonic origin and are derived from intra- and extra-embryonic mesodermal layers [11,12]. Therefore umbilical cord vessels are often used as a model for the investigation of non-accessible fetal vessels to reflect newborn vascular health [13–17]. These vessels are essential for prenatal transport of oxygen, nutrient-rich and deoxygenated blood and metabolic waste [11,12]. The development of the umbilical cord is highly influenced by systemic and local haemodynamic conditions of pregnancy, such as blood flow, oxygen tension and oxidative stress [18–20].

Here we hypothesize that a poor placental development particularly in severe EOPE induces excessive inflammatory responses and changes in the intrauterine haemodynamics, contributing to the remodelling of the umbilical cord and fetal vasculature and resulting in a vascular phenotype of the newborn at risk for cardiovascular disease in later life. Therefore, the *objective* of our study was to investigate associations between PE and the phenotypes EOPE and LOPE, and placental and umbilical cord vessel morphology as features of placental and newborn vascular health, in which gestational age and birth weight are taken into account.

2. Methods

2.1. Study design

Between June 2011 and June 2013 mother-child pairs were recruited before delivery and included in a nested case-control study embedded in the Rotterdam Periconceptional Cohort (Predict Study), an ongoing prospective hospital-based cohort conducted at the Erasmus MC, University Medical Centre Rotterdam, The Netherlands [21]. We selected EOPE and LOPE as cases and uncomplicated pregnancies as controls. In order to reduce confounding by FGR and iatrogenic PTB, we also included FGR and PTB as complicated control groups.

2.2. Maternal characteristics

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 [22]. EOPE and LOPE were defined as being diagnosed before and after 34 weeks of gestation, respectively [23]. Uncomplicated control pregnancies were defined as pregnancies without PE, gestational hypertension, FGR or PTB. FGR was defined as an estimated fetal weight below the 10th percentile for gestational age based on ultrasound measurements performed between 20 and 38 weeks gestational age [24]. Birth weight percentiles were calculated using the reference curves of the Dutch Perinatal Registry to validate birth weight <10th percentile [25]. PTB was defined as a spontaneous delivery between 22 and 37 weeks of gestation [26]. Maternal comorbidity was defined by any concurrent presence of cardiovascular-, endocrine-, metabolic-, auto-immune- and/or renal disease. Women with HIV infection, aged <18 years, not able to read and understand the Dutch language, multiple birth pregnancies or women with pregnancies complicated by fetal congenital malformations were excluded.

Maternal and neonatal characteristics were obtained from hospital medical records. All women gave written informed consent before participation and parental informed consent was obtained for the child. The research has been carried out in accordance with the Declaration of Helsinki (2013) of the World Medical Association.

2.3. Data collection

Macroscopic morphological outcomes including weight, length and width of the placenta and length, diameter, number of vessels and number of coils of the umbilical cord, were performed immediately after delivery. The umbilical coiling index was calculated as the total number of coils divided by the total length of the cord in centimetres. After clamping of the umbilical cord, samples of two centimetres for microscopic morphological examination were obtained next to the clamping site within one hour after delivery and immediately fixed in a 4% formaldehyde solution for paraffin sections. All samples and measurements were obtained by trained researchers according to protocol. Between 00:00 h and 07.00 h and during weekends, placental measurements were not performed due to logistic constraints.

2.4. Umbilical cord sample processing

Formalin-fixed umbilical cord samples were cut in transversal slices of four millimetres, perpendicular to the umbilical cord vessels. The slices were dehydrated in graded ethanol series, cleaned in xylene and embedded in paraffin. Paraffin samples were sectioned at 4 micrometre. Sections were deparaffinised and hydrated, before Haematoxylin/eosin (HE) staining and Elastica Von Gieson (EVG) staining was performed. Sections were digitally scanned using a digital slide scanner (NanoZoomer 2.0-HT, C9600-13, Hamamatsu Photonics, Japan) and analysed using the software NDP.view2, U12388-01 (Hamamatsu Photonics, Japan), IMAGE J version 2.0 (National Institutes of Health, US) and KS400 version 3.0 (Carl Zeiss Vision GmbH, Aalen, Germany).

The following parameters of the umbilical cord were measured in digitised HE stained sections; umbilical cord area, Wharton jelly area, vessel area, vessel lumen area and vessel wall thickness. Every individual area was measured manually with Image J [27]. Vessel wall thickness was measured manually in NDP viewer, as the mean of the smallest and largest wall diameter. Artery wall measurements were performed for both the internal and external muscular layer. EVG stained sections were used for the measurement of the percentage of elastin content in the wall of the umbilical artery and umbilical vein and internal elastic lamina (IEL) of the umbilical vein with KS400. The area of elastic fibres in the vessels was digitally marked and divided by the total vessel area to calculate the percentage of elastin content in the vein and in one artery separately.

All measurements were performed blinded by one researcher. Only sections without artefacts were used for analysis. Accuracy of

the digital slices and measurements were checked by an experienced pathologist at the Erasmus MC, University Medical Centre in Rotterdam.

2.5. Statistical analysis

For normal distributed maternal and neonatal characteristics and outcomes of the umbilical cord and placenta Analysis of Variance (ANOVA) was applied to assess overall differences between the groups, followed by the post hoc Dunnett *t*-test for pairwise comparisons between EOPE, LOPE and uncomplicated and complicated control groups. Kruskall-Wallis was applied to skewed variables, followed by pairwise Mann-Whitney tests for posthoc comparisons.

In the comparisons between PE and the total control group, multivariable linear regression analysis with adjustment for gestational age and birth weight was applied. We additionally applied the multivariable linear regression model to compare EOPE and LOPE with the total control group.

Differences were considered statistically significant at $p < 0.05$. All analyses were performed with Statistical Package for Social Sciences (SPSS, version 21.0, SPSS Inc, Chicago, IL, USA).

3. Results

298 women met the inclusion criteria and after exclusion of 50 pregnancies because of missing umbilical cord samples or macroscopic outcomes, 248 pregnancies remained for further analysis (Fig. 1). PE cases comprised 15 EOPE and 15 LOPE, and controls included 164 uncomplicated and 54 complicated pregnancies (28 FGR, 26 PTB). Microscopic outcomes were assessed in all EOPE, 14 LOPE, a random selection of 24 uncomplicated- and in 52 complicated control pregnancies.

Maternal and neonatal characteristics are depicted in Table 1. Except for the case specific parameters, such as blood pressure, proteinuria, gestational age and birth weight, we observed a higher frequency of nulliparous women in EOPE (80.0%) and LOPE (86.7%) versus uncomplicated controls (40.9%) and PTB (46.2%, overall $p < 0.001$). The frequency of caesarean section was higher in EOPE (80%) compared to LOPE and (un)complicated controls (23.1–37.5%, overall $p = 0.004$). In EOPE, one pregnancy was complicated by FGR (6.7%) versus two in LOPE (13.3%, $p = 1.000$) and all pregnancies were complicated by PTB (100%) versus two in LOPE (13.3%, $p < 0.001$).

Table 2 depicts the results of the multivariable linear regression analyses with gestational age and birth weight adjustments for the associations between the macroscopic morphological placental and umbilical cord outcomes and total PE, EOPE and LOPE versus the total control group. A negative association with placental weight, length and width and umbilical cord length was observed in total PE and EOPE, which attenuated after adjustment for birth weight and gestational age. LOPE was only positively associated with placental width, which remained statistically significant after adjustment for birth weight and gestational age ($p = 0.009$).

Macroscopic morphological outcomes of the placenta and umbilical cord are also depicted in Supplemental table 1. In EOPE we observed negative associations with placental weight, length and width and umbilical cord length versus (un)complicated controls (overall $p<0.001$). In LOPE Dunnett t-testing revealed a significantly higher placental weight compared to FGR, and a higher placental width compared to FGR and PTB complicated controls.

The multivariable linear regression analyses for the microscopic umbilical cord outcomes are depicted in Table 3. Since there were no significant differences between the two individual artery measurements, the mean of both measurements was used for further analysis. In PE we revealed a negative association with umbilical cord length, total vessel area, vein area and wall thickness, total artery area, outer artery wall area and artery wall thickness (all $p < 0.05$). After adjustment for gestational age and birth weight, PE remained inversely associated with the vein area (β −1.05, 95% CI -2.05, 0.04, $p = 0.041$) and vein wall thickness (β −0.15, 95% CI -0.26, −0.04, $p = 0.006$). EOPE was negatively associated with total umbilical cord vessel area, vein area, wall thickness and artery areas and wall thickness, which attenuated after adjustment for gestational age and birth weight. A negative association was observed between LOPE and vein wall thickness, only after adjustment for

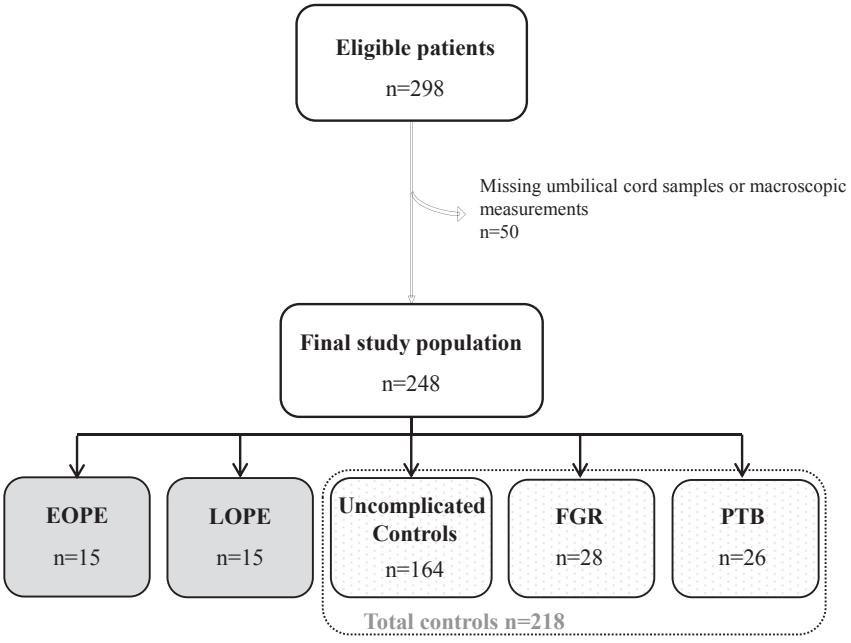

Fig. 1. Flowchart of the study groups.

Table 1
Maternal and neonatal characteristics.

Maternal characteristics	Cases		Uncomplicated	Complicated controls		Overall
	EOPE	LOPE	Controls	Normotensive FGR	Normotensive PTB	
	($n = 15$)	($n = 15$)	($n = 164$)	($n = 28$)	($n = 26$)	p-value
Age (years)	30.4 (5.2)	33.3 (4.7)	32.3 (4.9)	29.7 (5.9)	30.9 (5.1)	0.051
Nulliparous, n (%)	12 (80.0)	13 (86.7)	67*,† (40.9)	17 (60.7)	12*,† (46.2)	<0.001
Geographical origin, n (%)						0.172
Western	13 (86.7)	8 (53.3)	130 (79.8)	18 (64.3)	22 (84.6)	
Non-Western	2 (13.3)	7 (46.7)	33 (20.2)	10 (35.7)	4 (15.4)	
Preconception BMI‡ (kg/m^2)	24.1 (9.1)	24.2 (4.3)	24.1 (7.1)	22.5 (6.2)	23.9 (6.4)	0.620
Smoking in pregnancy (yes), n (%)	2 (15.4)	0 (0.0)	9 (5.7)	2 (7.7)	2 (8.7)	0.549
Co-morbidity (yes), n (%)	1 (6.7)	7 (46.7)	49 (30.1)	7 (25.0)	5 (19.2)	0.115
Neonatal characteristics						
Gender (male), n (%)	5 (33.3)	6 (40.0)	85 (51.8)	15 (53.6)	13 (50.0)	0.622
Gestational age at birth‡ (weeks)	30.7 (3.4)	37.4* (1.9)	39.6*,† (1.7)	38.9* (2.6)	35.1*,† (6.6)	<0.001
Birth weight‡ (grams)	1185 (435)	3200* (1250)	3560*,† (565)	2628*,† (593)	2568*,† (1674)	<0.001
Birth weight <10th percentile, n (%)	1 (6.7)	2 (13.3)	0† (0.0)	28*,† (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 between EOPE, LOPE and the uncomplicated and complicated control groups.
Data are presented as number (%) with corresponding Chi2/Fischer's exact testing.
‡Skewed 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. ANOVA analysis of variance; BP blood pressure; BMI body mass index; EOPE early onset preeclampsia; LOPE late onset preeclampsia; FGR fetal growth restriction; PTB preterm birth.

Table 2
Multivariable linear regression analysis of the macroscopic morphology of the placenta and umbilical cord in EOPE and LOPE versus the total control group.

	PE ($n = 30$)		EOPE ($n = 15$)		LOPE ($n = 15$)	
	Crude β	Adjusted β (GA + BW)	Crude β	Adjusted β (GA + BW)	Crude β	Adjusted β (GA + BW)
Macroscopy of the placenta						
Weight (gr)	−148.0 (−236, −60)*	34.0 (−31, 98)	−300.1 (−408.7,−191.5)*	6.7 (−86.6, 100.1)	41.9 (−81.7, 165.5)	55.5 (−28.8, 139.8)
Length (cm)	−2.38 (−3.84, −0.92)*	−0.52 (−1.99, 0.95)	−3.9 (−5.8, −2.0)*	−0.2 (−2.4, 2.1)	−0.7 (−2.7, 1.3)	−0.8 (−2.6, 1.0)
Width (cm)	−1.51 (−2.85, −0.18)*	0.99 (−0.22, 2.21)	−4.8 (−6.4, −3.2)*	−0.8 (−2.6, 1.0)	2.1 (0.3, 3.8)*	2.0 (0.5, 3.6)*
Macroscopy of the umbilical cord						
Length (cm)	−6.92 (−11.75, −2.08)*	−0.33 (−5.50, 4.84)	−15.6 (−22.1, −9.2)*	−5.2 (−13.4, 3.0)	1.8 (−4.7, 8.4)	−2.4 (−4.0, 8.7)
Diameter (mm)	0.69 (−0.71, 2.09)	1.02 (−0.54, 2.58)	0.2 (−1.8, 2.1)	1.1 (−1.4, 3.6)	1.2 (−0.7, 3.1)	1.1 (−0.7, 3.0)
Coiling index (coils/cm)	−0.01 (−0.04, 0.03)	−0.02 (−0.06, 0.02)	0.0 (−0.0, 0.1)	0.0 (−0.0, 0.1)	−0.0 (−0.1, 0.0)	−0.0 (−0.1, 0.0)

Data are presented as β (95% Confidence Interval) with corresponding multivariate linear regression analysis of both EOPE (on the left) and LOPE (on the right) versus the total control group ($n = 218$), crude and with adjustment for gestational age and birth weight. The regression coefficient (β) indicates the increase or decrease (−) change per unit.
*$p < 0.05$. *GA* gestational age; *BW* birth weight; *PE* preeclampsia; *EOPE* early-onset preeclampsia; *LOPE* late-onset preeclampsia. Missings are depicted in Supplemental table 1.

Table 3
Multivariable linear regression analysis of the microscopic morphology of the umbilical cord in EOPE and LOPE versus the total control group.

	PE ($n = 29$)		EOPE ($n = 15$)		LOPE ($n = 14$)	
	Crude β	Adjusted β (GA + BW)	Crude β	Adjusted β (GA + BW)	Crude β	Adjusted β (GA + BW)
Total cord area (mm^2)	−8.92 (−21.39, 3.55)	−5.17 (−17.77, 7.43)	−12.1 (−28.7, 4.5)	6.9 (−12.5, 26.3)	−5.8 (−22.8, 11.3)	−11.1 (−27.5, 5.3)
Wharton jelly area (mm^2)	−2.53 (−16.00, 10.93)	−1.41 (−15.09, 12.27)	−3.7 (−22.1, 14.7)	12.4 (−9.4, 34.2)	−1.5 (−19.2, 16.1)	−7.3 (−24.1, 9.5)
Total vessel area (mm^2)	−2.01 (−3.49, −0.52)*	−1.25 (−2.64, 0.14)	−3.3 (−5.3, −1.2)*	−0.3 (−2.7, 2.0)	−0.9 (−2.8, 1.1)	−1.6 (−3.4, 0.2)
Total lumen area (mm^2)	−0.10 (−0.50, 0.31)	−0.32 (−0.46, 0.39)	−0.4 (−1.0, 0.1)	−0.3 (−1.0, 0.4)	0.2 (−0.3, 0.7)	0.1 (−0.4, 0.7)
Umbilical vein						
Total area (mm^2)	−1.67 (−2.71, −0.63)*	−1.05 (−2.05, 00.04)*	−2.6 (−4.0, −1.2)*	−0.8 (−2.4, 0.9)	−0.7 (−2.1, 0.7)	−1.1 (−2.5, 0.2)
Wall thickness (mm)	−0.19 (−0.29. −0.09)*	−0.15 (−0.26, −0.04)*	−0.3 (−0.4, −0.1)*	−0.1 (−0.3, 0.0)	−0.1 (−0.3, 0.0)	−0.1 (−0.3, −0.0)*
Lumen area (mm^2)	−0.11 (−0.44, 0.23)	0.04 (−0.30, 0.37)	−0.4 (−0.8, 0.0)	−0.1 (−0.6, 0.5)	0.2 (−0.3, 0.7)	0.1 (−0.3, 0.6)
Elastin content %	−0.73 (−1.75, 0.29)	−0.26 (−1.31, 0.78)	−1.2 (−2.6, 0.1)	0.4 (−1.2, 2.0)	−0.2 (−1.6, 1.2)	−0.5 (−1.9, 0.9)
Umbilical artery						
Total area (mm^2)	−0.46 (−0.88, −0.05)*	−0.23 (−0.64, 0.18)	−0.7 (−1.3, −0.2)*	−0.0 (−0.7, 0.6)	−0.2 (−0.8, 0.4)	−0.4 (−0.9, 0.2)
External wall layer area (mm^2)	−0.41 (−0.71, −0.11)*	−0.24 (−0.54, 0.06)	−0.6 (−1.0, −0.2)*	−0.2 (−0.6, 0.3)	−0.2 (−0.6, 0.2)	−0.3 (−0.7, 0.1)
Internal wall layer area (mm^2)	−0.14 (−0.29, 0.02)	−0.05 (−0.20, 0.10)	−0.2 (−0.4, −0.0)*	0.1 (−0.2 0.3)	−0.1 (−0.3, 0.2)	−0.1 (−0.3, 0.10)
Wall thickness (mm)	−0.07 (−0.13, −0.00)*	−0.03 (−0.09, 0.03)	−0.1 (−0.2, −0.0)*	0.0 (−0.1, 0.1)	−0.0 (−0.1, 0.0)	−0.1 (−0.1, 0.0)
Lumen area (mm^2)	0.05 (−0.03, 0.13)	0.03 (−0.57, 0.12)	0.1 (−0.0, 0.2)	0.1 (−0.1, 0.2)	−0.0 (−0.1, 0.1)	0.0 (−0.1, 0.1)
Elastin content %	−0.06 (−0.90, 0.77)	0.13 (−0.74, 1.00)	−0.1 (−1.3, 1.0)	0.4 (−1.0, 1.8)	0.0 (−1.0, 1.1)	−0.0 (−1.1, 1.1)

Data are presented as β (95% Confidence Interval) with corresponding multivariate linear regression analysis of both EOPE (on the left) and LOPE (on the right) versus the total control group ($n = 76$), crude and with adjustment for gestational age and birth weight. The regression coefficient (β) indicates the increase or decrease (−) change per unit.
*$p < 0.05$. *GA* gestational age; *BW* birth weight; *PE* preeclampsia; *EOPE* early-onset preeclampsia; *LOPE* late-onset preeclampsia. Missings are depicted in Supplemental table 2.

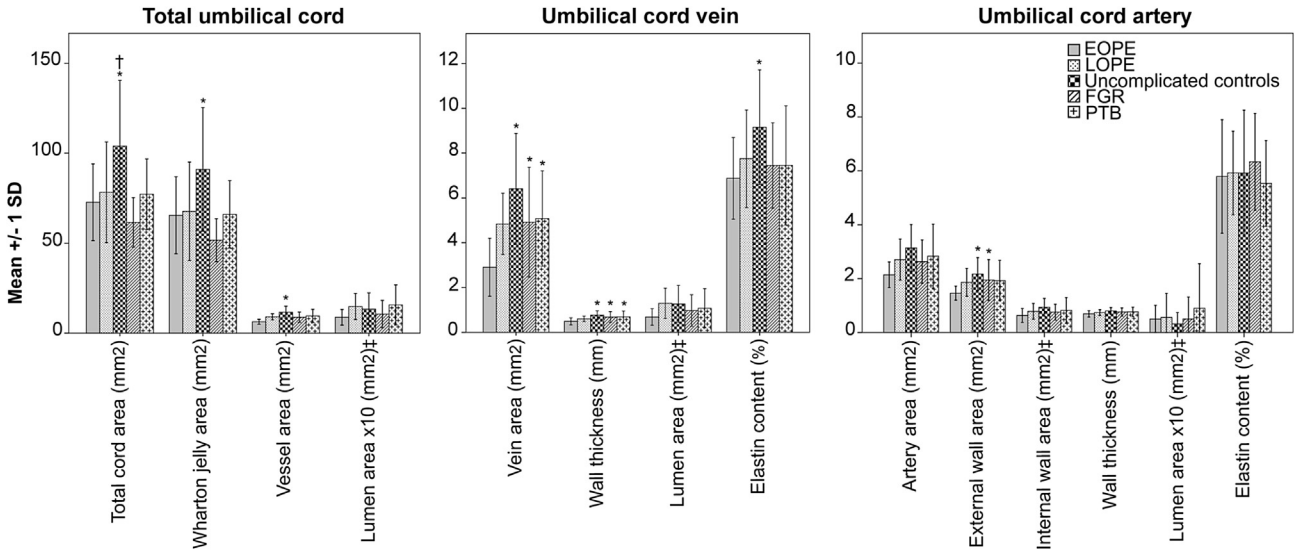

Fig. 2. Microscopic morphological outcomes of the umbilical cord.
Microscopic morphological outcomes of the umbilical cord are presented as mean (+/− standard deviation) with corresponding ANOVA testing to examine overall differences between the groups, followed by the post hoc Dunnett *t*-test for pairwise comparisons of EOPE and LOPE with the (un)complicated control groups. ‡ Skewed data are analysed by Kruskall-Wallis testing.
*p < 0.05 versus EOPE pregnancies. †p < 0.05 versus LOPE pregnancies. *ANOVA* analysis of variance; *EOPE* early onset preeclampsia; *LOPE* late onset preeclampsia; *FGR* fetal growth restriction; *PTB* preterm birth.

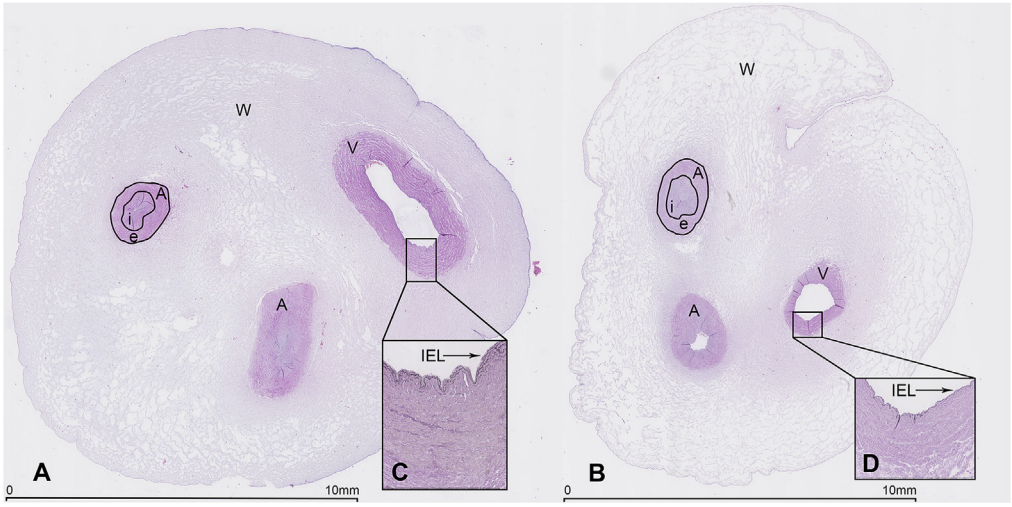

Fig. 3. Microscopic transversal sections of the umbilical cord in an uncomplicated control.(A) and EOPE (B).
Legend: Umbilical cord paraffin section with Haematoxylin Eosin staining. Veins consist of a single layer circular smooth muscle cells and an internal elastic lamina (IEL). The umbilical arteries have a double-layered muscular wall divided in an internal layer of longitudinal smooth muscle cells and an external layer of crossing spiralled smooth muscle cells. Umbilical arteries do not contain an IEL.
A and **B**. Umbilical cord transversal section of an uncomplicated control (A) and EOPE (B) with a smaller vein area versus (un)complicated pregnancies.
C and **D**. Umbilical cord transversal paraffin sections with Elastic Van Gieson staining in an uncomplicated control (C) and EOPE (D) with a lower total vein elastin content versus uncomplicated pregnancies (x5). *W* Wharton jelly; *V* vein; *A* artery; *i* internal muscular layer; *e* external muscular layer; *IEL* internal elastic lamina.

gestational age and birth weight.

In Figs. 2 and 3 and Supplemental table 2 the microscopic morphological outcomes of the umbilical cord are depicted per group. In EOPE we observed smaller total umbilical cord areas, vein area, wall thickness, external artery wall area and vein elastin content compared to uncomplicated controls. In EOPE vein total area and vein wall thickness were also significantly smaller compared to FGR and PTB (overall $p = 0.001$ and $p = 0.004$ respectively). In LOPE we only observed a smaller Wharton jelly area versus uncomplicated controls ($p = 0.030$).

4. Discussion

4.1. Main findings

This study shows a negative association between the umbilical vein area and vein wall thickness in the total group of PE pregnancies, independent of gestational age and birth weight. In EOPE, the negative associations with placental weight, length and width and umbilical cord length, vein and artery wall area and wall thickness are largely explained by a shorter gestational age and lower birth weight. This is in contrast to LOPE showing a positive

association with placental width and a negative association with umbilical vein wall thickness independent of gestational age and birth weight.

4.2. Strengths and limitations

Strengths of our study are that we established different associations between EOPE and LOPE and microscopic morphological outcomes in umbilical cord vessels and independent of FGR and PTB. The digital qualitative elastin measurement-technique is novel and accurate and seems feasible for future examinations.

4.3. Interpretation

The different associations observed in EOPE and LOPE with placental and umbilical cord morphology can partly be explained by a shorter gestational age and lower birth weight in the more severe EOPE phenotype. In addition, the positive association between LOPE and placental width is even opposite to the association with EOPE, as observed by others [28]. Kajantie et al. described an association between a smaller placental width and the risk of PE severity, which is in line with our observation in EOPE suggesting that placental width is a marker of placental development [10]. The discrepancy of placental measurements in EOPE and LOPE may be explained by the concept of initial poor and restricted placental development in EOPE versus microvillous overcrowding in term placentas without prior pathology in LOPE [8].

The association of an approximately 2-fold smaller vein area and approximately 1.5-fold smaller vein wall thickness in EOPE compared to the total control group was much stronger than in LOPE, but appeared only independent of gestational age and birth weight in LOPE. This may be due to the exposure to maternal cardiovascular and metabolic risk factors associated with LOPE (47% in LOPE versus 7% in EOPE pregnancies). However, in the total PE group we also demonstrated that the EOPE and LOPE subgroups may in fact be underpowered, by revealing a strong significant association with a smaller vein area and wall thickness in PE, independent of gestational age and birth weight. Moreover, the low rate of FGR cases within the EOPE pregnancies indicates that a relatively small number of pregnancies complicated with severe placental dysfunction leading to FGR were included, which cannot exclude that the observed association is underestimated.

We do believe that the significantly smaller vein wall thickness is related to the adverse influences originating from PE itself and possibly the additional complications of PTB and FGR. Both gestational age and birthweight are highly related to the severity and moment of onset of PE, suggesting that the most evident findings are appearing in the most severe EOPE cases, who suffered from excessive oxidative stress from the beginning of pregnancy. PTB and FGR have indeed been associated with alterations of cardiovascular risk factors in offspring [29,30]. However, as suggested by our data and that of others, the intrauterine PE environment seems to be unique and exacerbates or acts in synergy with any risks inferred by PTB or FGR [31]. During normal fetal development, the morphology of the vessel walls changes due to a thickening of the elastic lamellae of the media [32]. PE however deranges the haemodynamic characteristics of the materno-fetal circulation with fluctuations in shear stress, which affects the fetal vascular development [33,34]. It has been shown that umbilical perfusion and elastin content of the umbilical veins are decreased in neonates born after PE [13,16]. Additionally, episodes of placental hypoxia or reperfusion result in excessive oxidative stress and the production of inflammatory cytokines in both maternal and fetal circulation [35]. A disbalance between angiogenic factors and pro-inflammatory chemokines is associated with maternal endothelial cell dysfunction as pregnancy advances with possible consequences for the structure and content of the vessel walls [4]. In PE pregnancies a reduced prostacyclin production in endothelial cells of the umbilical cord vessels resulting in decreased placental perfusion and umbilical cord blood flow has been observed [36]. This substantiates our finding of the smaller umbilical cord vein wall thickness and tendency of decreased elastin content being related to the haemodynamic fluctuations and excessive oxidative stress.

An overexpression of type III and down regulation of type I collagen has been described in umbilical cord veins, arteries and Wharton jelly of neonates exposed to PE, which decreases solubility (water-binding capacity) and may explain the smaller vessel wall thickness and also the decreased elastin content of the umbilical vein walls [15,16].

Our findings are consistent with Inan et al., who reported a significant reduction in the umbilical vein and artery wall areas of 70 neonates exposed to PE [14]. In contrast, two smaller studies reported an increased umbilical artery wall thickness, which may be due to differences in the definition of PE and umbilical cord sampling methods (at the placental side instead of next to the umbilical cord clamping site) and sample sizes [13,17].

5. Conclusion

Our study suggests that PE is associated with a smaller umbilical cord vein area and wall thickness, independent of gestational age and birth weight, which may be considered as a proxy for early features of disturbed cardiovascular development in the newborn.

Periconceptional follow-up studies are needed to ultimately predict the risk of cardiovascular disease in offspring exposed to PE which may create opportunities for early prediction, prevention and treatment in the future.

6. Contribution to authorship

EH contributed to the study design, data collection, laboratory work, statistical analysis and writing of the first draft and all revisions of the manuscript. AE contributed to the study design and writing of the manuscript. AR and MK were involved in the data collection and laboratory work. RK and AJMR contributed to the study design and supervised the laboratory work. IR contributed to the study design and writing of the manuscript. AN was involved in the laboratory data analysis. PE supervised the statistical analysis. ES was responsible for the clinical data collection. RS initiated the study and supervised all aspects of the study and contributed to all versions of the manuscript. All authors contributed to the writing and revision of the manuscript and approved the final version.

Details of ethics approval

Ethical approval for the study was given by the Erasmus MC, University Medical Centre Research Ethics Board (MEC-2004-227).

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Authors' contributions

Emilie M. Herzog. I declare that I participated in the study by taking part in the study design, data collection, laboratory work, statistical analysis and writing of all versions of the manuscript. I

have seen and approve the final version of the manuscript. I have no conflicts of interest.

Alex. J. Eggink. I declare that I participated in the study by taking part in the study design and writing of the manuscript. I have seen and approve the final version of the manuscript. I have no conflicts of interest.

Anniek Reijnierse. I declare that I participated in the study by taking part in the data collection, laboratory work and contributing to the final version of the manuscript. I have seen and approve the final version of the manuscript. I have no conflicts of interest.

Martina A.M. Kerkhof. I declare that I participated in the study by taking part in the data collection and contributing to the final version of the manuscript. I have seen and approve the final version of the manuscript. I have no conflicts of interest.

Ronald R. de Krijger. I declare that I participated in the study by taking part in the study design, supervising the laboratory analysis and contributing to the final version of the manuscript. I have seen and approve the final version of the manuscript. I have no conflicts of interest.

Anton J.M. Roks. I declare that I participated in the study by taking part in the study design, supervising the laboratory analysis and contributing to the final version of the manuscript. I have seen and approve the final version of the manuscript. I have no conflicts of interest.

Irwin K.M. Reiss. I declare that I participated in the study by taking part in the study design and contributing to the final version of the manuscript. I have seen and approve the final version of the manuscript. I have no conflicts of interest.

Alex L. Nigg. I declare that I participated in the study by taking part in the laboratory data analysis and contributing to the final version of the manuscript. I have seen and approve the final version of the manuscript. I have no conflicts of interest.

Paul H.C. Eilers. I declare that I participated in the study by supervising the statistical analysis and contributing to the final version of the manuscript. I have seen and approve the final version of the manuscript. I have no conflicts of interest.

Eric A.P. Steegers. I declare that I participated in the study by supervising the clinical data collection and contributing to the final version of the manuscript. I have seen and approve the final version of the manuscript. I have no conflicts of interest.

Régine P.M. Steegers-Theunissen. I declare that I participated in the study by supervising all aspects of the study and contributing to all versions of the manuscript. I have seen and approve the final version of the manuscript. I have no conflicts of interest.

Disclosure of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.placenta.2016.11.014.

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