



## Plasma concentrations of soluble endoglin in the maternal circulation are associated with maternal vascular malperfusion lesions in the placenta of women with preeclampsia



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### ABSTRACT

We evaluated the association between plasma soluble endoglin (sENG) and maternal vascular malperfusion (MVM) lesions of the placenta in women with preeclampsia. We measured sENG (sCD105) by ELISA in  $N = 70$  women diagnosed with preeclampsia (median [IQR] GA at sampling = 36.4 [6.0] weeks) and available placental pathology. Placental pathology reports were reviewed for evidence of MVM based on the presence of  $\geq 1$  of the following: villous infarct, decidual vasculopathy, accelerated villous maturation, intervillous fibrin deposition, and/or low placental weight ( $< 10$ th percentile for GA). We categorized plasma sENG concentrations into tertiles and used a modified Poisson regression approach to estimate the prevalence of MVM associated with sENG. We separately estimated the association between sENG and accelerated villous maturation, villous infarct, and low placental weight, the three most frequent lesions in the sample. We adjusted all models for age, parity, pre-pregnancy obesity, smoking, and infant sex. The prevalence of MVM in our sample of women with preeclampsia was 74%. Women in the highest sENG tertile had a higher prevalence of MVM (aPR [adjusted prevalence ratio] 1.70, 95% CI 1.15–2.52), low placental weight (aPR 3.26, 95% CI 1.25–8.50), and villous infarcts (aPR 2.93, 95% CI 1.27–6.73) compared with women in the lowest sENG tertile, after adjusting for covariates. Medium (aPR 2.57, 95% CI 1.17–5.66) and high (aPR 3.14, 95% CI 1.47–6.70) tertile concentrations of sENG were associated with higher accelerated villous maturation. Our results suggest that sENG may mark a more severe placental phenotype of preeclampsia, although findings should be replicated in larger cohorts.

### 1. Introduction

Preeclampsia is a heterogeneous, hypertensive syndrome of pregnancy that is characterized by placental dysfunction [1–3]. In women who develop preeclampsia, maternal vascular malperfusion (MVM) lesions of the placenta are common histopathologic findings that provide morphological evidence of a dysfunctional placenta [4–6]. Although MVM lesions are thought to develop in response to placental hypoxia during gestation, these lesions cannot be diagnosed until after delivery [6]. This precludes any clinical utility during pregnancy, and thus the identification of other biologically plausible factors that could serve as antenatal biomarkers of placental health are needed.

Although placental dysfunction is a hallmark feature of preeclampsia, it is believed that placental dysfunction may differ in women who develop early-onset ( $< 34$  weeks) preeclampsia compared to women who develop late-onset preeclampsia ( $\geq 34$  weeks) [7]. For cases of early-onset preeclampsia, the sequence of events depicted in Fig. 1 is thought to be initiated in early pregnancy, and stem from inadequate remodeling of the maternal uterine spiral arteries [8–10]. This lack of remodeling not only sets the stage for placental malperfusion, but it also exposes the syncytiotrophoblast layer of the chorionic villi to the damaging effects of high velocity blood flow [11]. For cases of late-onset preeclampsia, it has been more recently theorized that the sequence of events depicted in Fig. 1 may occur near the end of

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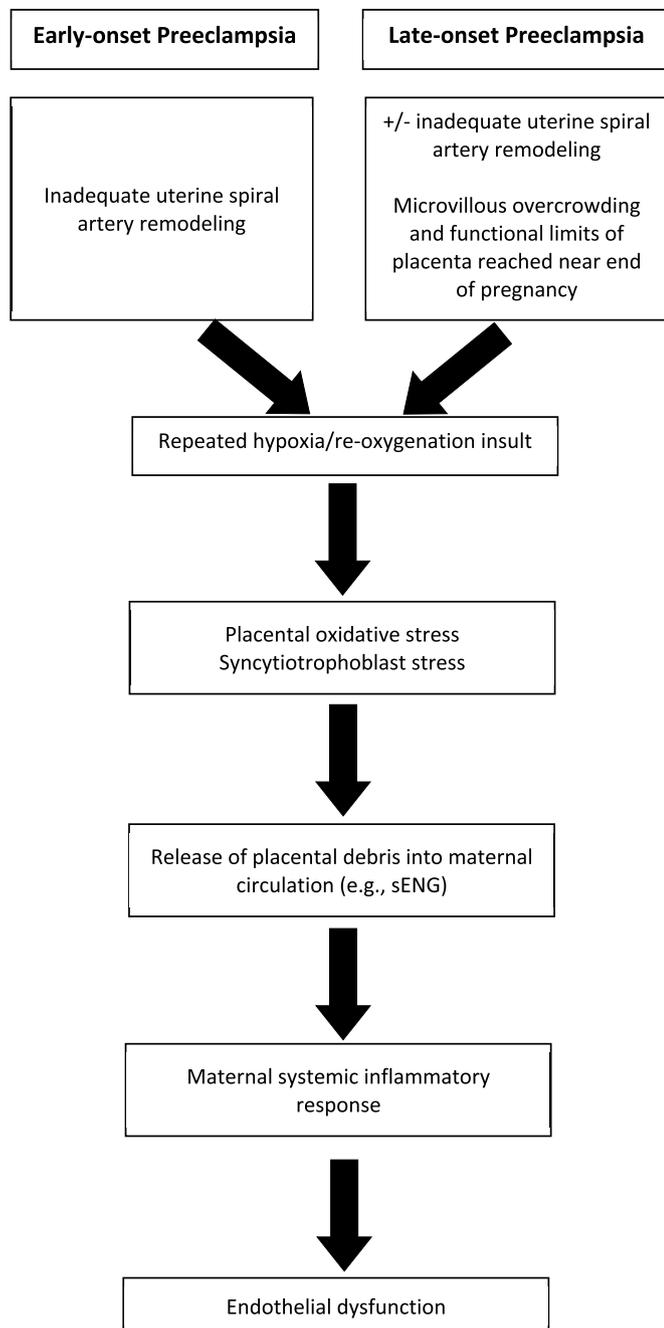


Fig. 1. Origins of placental dysfunction in preeclampsia [7–14].

pregnancy, and more likely stem from microvillous overcrowding and placental senescence as opposed to inadequate spiral artery remodeling [7]. Redman, Sargent, and Staff (2014) posit that microvillous crowding occurs when the expansion of the terminal villi exceeds the increase in the intervillous space in term placenta [7]. However, based on placental bed biopsies, inadequate spiral artery remodeling can also be present in late-onset preeclampsia, but less frequently than in early-onset preeclampsia [12]. Data collectively suggest that if inadequate spiral artery remodeling is present in late-onset preeclampsia, its effects on the placenta are likely less severe [13], and it may take the development of microvillous overcrowding near the end of pregnancy for preeclampsia to develop.

Whether placental dysfunction arises from inadequate spiral remodeling during early gestation or microvillous overcrowding near the end of gestation, both result in an intermittently hypoxic placental

environment. This malperfusion/hypoxic milieu is hypothesized to set the stage for placental damage (e.g., tissue destruction) and the subsequent release of placental debris and other factors into the maternal circulation. Once in the maternal circulation, these placental materials are proposed to activate a maternal response characterized by systemic endothelial dysfunction [3,7–10].

Soluble endoglin (sENG) is a placentally-derived antiangiogenic factor released into the maternal circulation. Once in the maternal circulation, it causes endothelial dysfunction (e.g., stimulation of vascular permeability and hypertension) via interference with the TGF $\beta$  pathway signaling [14,15]. In preeclampsia, plasma concentrations of sENG become significantly elevated in the maternal circulation two-three months prior to the onset of clinical manifestations, and correlate with preeclampsia severity [14,16]. As such, it is biologically plausible that sENG is a blood-based biomarker of placental dysfunction in the antenatal period. Given that MVM lesions are indicative of placental dysfunction, our first step into this line of inquiry was to evaluate the association between maternal plasma concentrations of sENG and prevalence of MVM lesions in the placenta of women diagnosed with preeclampsia.

## 2. Materials and methods

### 2.1. Participants

For this secondary data analysis, we obtained data from a subset of women who participated in the Prenatal Exposures and Preeclampsia Prevention (PEPP) cohort study (1997–2007; Magee-Womens Hospital; Pittsburgh, PA) and its associated nested, case-control genetic association study [17]. Both studies received ethics committee approval from the University of Pittsburgh Institutional Review Board, and participants provided written informed consent for participation in the parent and follow-up studies. The PEPP cohort study utilized two recruitment/enrollment approaches to identify factors that predispose women to the development of preeclampsia. Pregnant women were either enrolled during early pregnancy and followed through delivery or they were enrolled when they presented to the labor and delivery unit for suspected preeclampsia. Women with a history of chronic hypertension, chronic renal disease, and pre-gestational diabetes were excluded from participation in the PEPP cohort study. A detailed description of the source cohort, along with the nested, case-control study of Caucasian and African American women is provided by Bell et al. [17].

Inclusion in this secondary analysis required that participants met the following four criteria: (1) Caucasian (self-report), (2) diagnosis of preeclampsia, (3) available sENG measurement from a third trimester plasma sample, and (3) available placenta pathology report. Due to the small sample of African American women diagnosed with preeclampsia ( $n = 30$ ), and in an attempt to minimize variation by race/ethnicity, we did not measure sENG concentrations in this subgroup. As such, we restricted our analyses to Caucasian women with a diagnosis of preeclampsia ( $n = 178$ ). Because women with uncomplicated pregnancies do not routinely have their placentas evaluated, it was not feasible to include this group. Moreover, participants missing smoking status ( $n = 6$ ) were also excluded. After the above exclusions, the study population included 70 women with placental data (Fig. 2). Of these,  $n = 50$  (71%) were enrolled in the PEPP cohort when they presented to the labor and delivery unit for suspected preeclampsia. As such, plasma samples collected around the time of delivery were used for the measurement of sENG in this study.

#### 2.1.1. Preeclampsia phenotyping criteria

A panel of clinicians and researchers with expertise in preeclampsia systematically reviewed clinical data for each participant to determine pregnancy outcome diagnosis. The diagnosis of preeclampsia was determined based on blood pressure, proteinuria, and uric acid criteria. The presence of new-onset hypertension after 20 weeks' gestation was

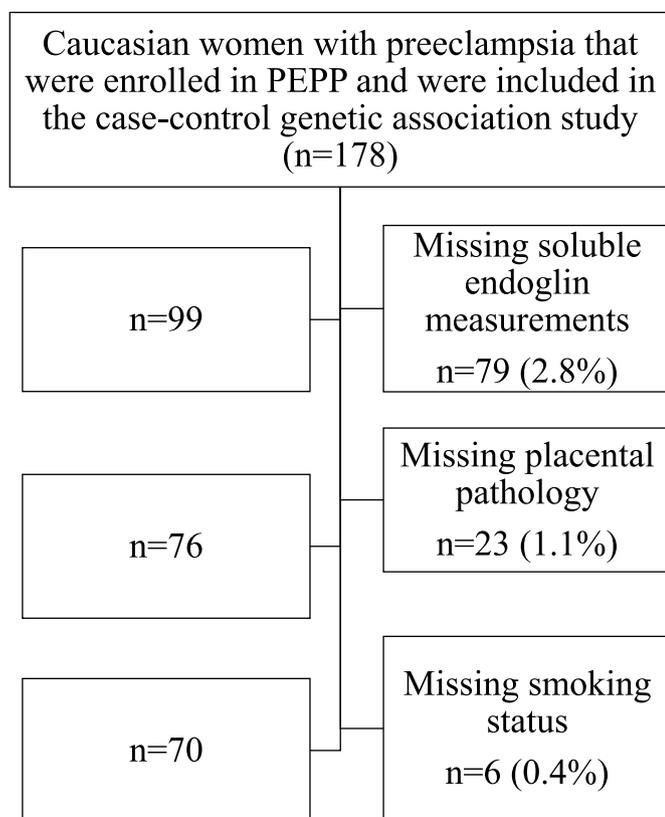


Fig. 2. Flowchart of exclusions for analysis of soluble endoglin and maternal vascular malperfusion lesions.

defined by the presence of absolute blood pressure (SBP  $\geq$  140 mmHg and/or DBP  $\geq$  90 mmHg). Blood pressure determinations were made based on the average of the last four blood pressures measured prior to therapeutic intervention. The presence of proteinuria was defined as either (1) 24-h urinary protein  $\geq$  300 mg, (2) protein: creatinine ratio  $\geq$  0.3, (3) random urine dipstick  $\geq$  2+, or (4) catheterized urine specimen  $\geq$  1+. A serum uric acid concentration of  $>$  1 standard deviation from normal for gestational age defined hyperuricemia [18]. The PEPP cohort principal investigators included hyperuricemia in their preeclampsia phenotyping criteria as a way to identify a group of women with a more severe form of preeclampsia [19,20].

## 2.2. Plasma soluble endoglin (sENG) measurement

We measured plasma sENG concentrations with the Human Endoglin/CD105 Quantikine<sup>®</sup> ELISA kit from R&D Systems (R&D Systems Inc., Minneapolis, MN). A plasma sample was obtained from each participant during clinically evident preeclampsia (median gestational age at sample collection = 36.4 weeks [minimum; maximum: 27.9; 41.4 weeks]), which was around the time of delivery. Inter-assay and intra-assay coefficients of variation were 4.0% and 3.9%, respectively.

## 2.3. Placental pathology data abstraction protocol

We used a University of Pittsburgh Medical Center (UPMC) honest broker to retrieve and de-identify clinical placental pathology reports associated with the index pregnancy. All placental pathology reports were generated from the Pathology Department at Magee-Womens Hospital. The morphological diagnostic criteria used at the time the clinical reports were generated (1997–2007) were based on the 1997 guidelines of the Placental Pathology Practice Guideline Development Task Force of the College of American Pathologists [21], and

supplemented with MVM-specific guidelines published by the Society for Pediatric Pathology, Perinatal Section, Maternal Vascular Perfusion Nosology Committee in 2004 [22]. All diagnoses predate the Amsterdam criteria [23], but all have been converted to Amsterdam terminology for the study. For the current study, de-identified placental pathology reports were manually abstracted by two independent reviewers (MS and JC). Discrepancies identified between the two reviewers were adjudicated by a third reviewer (WTP). Each placental pathology report was reviewed for the presence of keywords associated with evidence of maternal vascular malperfusion (MVM: yes/no). MVM was a combined outcome consisting of one or more of the following lesions: villous infarct, decidual vasculopathy, accelerated villous maturation, intervillous fibrin deposition, and/or low placental weight (placental weight  $<$  10th percentile for gestational age). The following keywords were also grouped under accelerated villous maturation: advanced maturation, increased syncytial knots, Tenney Parker change, and focal villous crowding.

As previously reported, we conducted a small validation study to assess the reliability/reproducibility of MVM lesion diagnoses between clinical pathology reports and blinded research review of histopathology slides. Individual MVM lesions were assessed for concordance between the clinical pathology report and blinded review of histopathology slides (decidual vasculopathy, infarct, accelerated villous maturation, perivillous fibrin deposition, and intervillous fibrin deposition.) The clinical pathology report and blinded review were deemed concordant when each recorded the same type and number of lesions. Any single discordant finding rendered the entire case discordant. For the MVM lesions, we found 62% agreement between the clinical pathology report diagnoses and findings from a blinded review (excluding gestational age) of the associated histology slides by a single pathologist (WTP). Despite the moderate level of agreement [24], we found that the clinical reports tended to underreport cases of MVM [25]. As such, the results generated from our current study may more likely underestimate the magnitude of the relationship between sENG and MVM.

## 2.4. Statistical analysis

The normality of sENG was tested by visual inspection of histograms and the Shapiro-Wilk W-test. sENG was not normally distributed and was categorized into tertiles (low, medium, and high). Maternal characteristics across sENG concentrations were compared using Fisher's exact tests, one-way ANOVA, or Kruskal-Wallis tests, as appropriate. Covariates were determined *a priori* based on existing literature [5,26] and included: maternal age at delivery ( $<$  35 or  $\geq$  35), parity (nulliparous or multiparous), pre-pregnancy obesity (not obese,  $<$  30 kg/m<sup>2</sup> or obese  $\geq$  30 kg/m<sup>2</sup>), smoking status during pregnancy (yes or no), infant sex (male or female), and gestational age at sample collection. Given that women with early-onset preeclampsia, or preeclampsia with severe features, are more likely to deliver preterm, we considered gestational age at delivery to be on the causal pathway and did not consider this a covariate. Rather we conducted a subgroup analysis of women who delivered preterm ( $<$  37 weeks).

Since MVM was a common outcome (prevalence  $>$  10%), the odds ratio from a binary logistic regression will not be a direct estimate of the true risk ratio [27]. Therefore, we used a modified Poisson regression approach (e.g., Poisson regression with robust error variance) [27,28] to directly estimate the prevalence of MVM lesions associated with concentrations of sENG in maternal circulation, after adjusting for age, parity, pre-pregnancy obesity, smoking status, infant sex, and gestational age at sample collection. We then separately estimated the prevalence of low placental weight, accelerated villous maturation, and villous infarcts, the three most predominant lesions in our sample. Due to insufficient sample size, we were unable to separately estimate the prevalence ratios for decidual vasculopathy (n = 7) or intervillous fibrinoid deposition (n = 4). Women with the lowest tertile

**Table 1**  
Frequency of placental lesions among women with preeclampsia (n = 70).

Lesion type	n (%)
Maternal Vascular Malperfusion	52 (74.3)
Low placental weight <sup>a</sup>	26 (37.1)
Decidual vasculopathy	7 (10.0)
Accelerated villous maturation	36 (51.4)
Villous infarctions (Y/N)	29 (41.4)
1	12 (41.4)
≥1	17 (58.6)
Intervillous fibrinoid deposition	4 (5.7)
Number of lesions	
0	20 (28.6)
1	28 (40.0)
≥2	22 (31.4)

<sup>a</sup> Placental weight < 10th percentile for gestational age.

concentrations of sENG were the referent for all analyses. A *P* value < 0.05 was considered to be statistically significant in all analyses. We did not make adjustment for multiple comparisons, given the modest sample size. From each model, we report the unadjusted and adjusted prevalence ratios and 95% confidence intervals. We used SAS version 9.4 (©SAS Institute, Inc. Cary, NC) for all analyses.

### 3. Results

The overall prevalence of MVM in women with preeclampsia was 74.3%, with accelerated villous maturation being the most common MVM lesion affecting 36/70 (51.4%) women (Table 1). Obese women and women with preterm deliveries were more likely to have their placenta submitted for a pathological examination (Supplemental Table 1). When limited to the group with available placental pathology reports, there were no significant differences in the distributions of age, parity, pre-pregnancy obesity, smoking status, or infant sex among women with low, medium, or high tertile concentrations of sENG (Table 2). As expected, women with medium or high tertile concentrations of sENG were more likely to deliver at an earlier median gestational age as compared with women who had the lowest tertile concentrations of sENG. There were also differences in median gestational age at sample collection, placental weight, and birthweight percentile across sENG concentrations, with medium and high tertiles having earlier gestational ages, smaller placentas, and lower birthweight centiles than those in the lowest tertile (Table 2).

The prevalence of MVM in women with preeclampsia was greatest in those in the highest sENG tertile (91.3%) as compared with women in medium (75.0%) or the lowest (56.5%) tertile (*p* = 0.026). This pattern also persisted when we examined the prevalence by type of individual MVM lesion (Fig. 3). For instance, there were significant differences in the proportions of low placental weight (*p* = 0.023), villous infarcts (*p* = 0.045), and accelerated villous maturation (*p* = 0.009) across the sENG tertiles. However, there were no significant differences in the proportion of decidual vasculopathy (*p* = 0.319) or intervillous fibrinoid deposition (*p* = 0.754) across sENG concentrations.

Women in the highest sENG tertile had a higher prevalence of MVM (aPR 1.70, 95% CI 1.15–2.52) compared with women in the lowest tertile after adjusting for maternal age, pre-pregnancy obesity, parity, smoking status, and infant sex (Table 3). Increased prevalence ratios for the association between sENG and low placental weight (aPR 3.26, 95% CI 1.25–8.50) and villous infarcts (aPR 2.93, 95% CI 1.27–6.73) were

observed in women in the highest compared with the lowest tertile. We also observed a higher prevalence of accelerated villous maturation in women in the medium (aPR 2.57, 95% CI 1.17–5.66) or highest (aPR 3.14, 95% CI 1.47–6.70) sENG tertiles (Table 3). Restriction of our analyses to preterm birth resulted in effect estimates that were similar in direction as the main analyses, but estimates were imprecise as evidenced by the width of the confidence intervals. Additionally, the prevalence ratios for women in the highest sENG tertile were no longer statistically significant in this preterm group after adjusting for covariates (Supplemental Table 2).

### 4. Discussion

We conducted a cross-sectional, secondary analysis of data collected from women with preeclampsia to evaluate the association between circulating plasma concentrations of sENG and MVM lesions in the placenta in women with preeclampsia. Overall, we found that higher plasma concentrations of sENG were significantly associated with an increased prevalence of MVM. Separate assessment of individual lesions further demonstrated that the prevalence of low placental weight, villous infarcts, and accelerated villous maturation increased as concentrations of plasma sENG increased. Moreover, higher concentrations of sENG were significantly associated with other indicators of placental dysfunction, including small placentas and lower birthweight centiles. Taken together, our findings suggest that sENG may mark a more severe placental phenotype of preeclampsia.

In the current study of women with preeclampsia, the overall prevalence of MVM was 74% (early-onset: 95% [20/21]; late-onset: 65% [32/49]), and the two most common MVM lesions were accelerated villous maturation (51%) and villous infarcts (41%; both central and peripheral infarcts are included in this percentage). In a previous study by Soto et al., which was restricted to late-onset preeclampsia (≥34 weeks), the prevalence of MVM was 47% [5], which was slightly lower than the prevalence of MVM detected in the current subset of women who delivered at ≥34 weeks' gestation. In another study, Moldenhauer et al. [4] reported on the prevalence of individual vascular malperfusion lesions across pregnancy. The prevalence of accelerated villous maturation (referred to as “hypermaturation of villi”) was 33% and the prevalence of villous infarcts, which was limited to central infarctions, was 37% in their sample of women with early and late-onset preeclampsia [4]. Furthermore, our subgroup analysis demonstrated that women with late-onset preeclampsia and evidence of MVM may have higher concentrations of sENG compared to women with late-onset preeclampsia and no evidence of MVM (late-onset preeclampsia with MVM: *n* = 32, 32.4 [24.7] ng/mL vs. late-onset without MVM: *n* = 17, 21.2 [21.3] ng/mL, *p* = 0.1). Although this finding was similar to what was previously reported by Soto et al. [5], neither study was able to demonstrate statistical significance.

The higher prevalence of overall MVM, and accelerated villous maturation, in our study compared to previous reports may be related to the more severe preeclampsia phenotype studied. Our study's classification of preeclampsia was based on blood pressure, urinary protein, and serum uric acid criteria, while neither the studies by Soto et al. [5] or Moldenhauer et al. [4] included hyperuricemia as a diagnostic criterion for preeclampsia.

Inter-observer variability amongst pathologists may have also contributed to differences in the prevalence of MVM lesions reported across studies. In our study, placental pathology reports were generated by several pathologists as part of clinical care. As such, pathologists were not blinded to clinical outcomes. Because knowledge of clinical outcomes may introduce surveillance bias, this represents a limitation of studies that utilize clinically-generated pathology reports for research purposes. Lastly, the use of different classification systems, as well as the implementation of new classification systems may impact the ability to truly compare findings across studies. For example, the morphological diagnostic criteria used in our study predated the

**Table 2**  
Maternal characteristics by soluble endoglin tertile (n = 70).

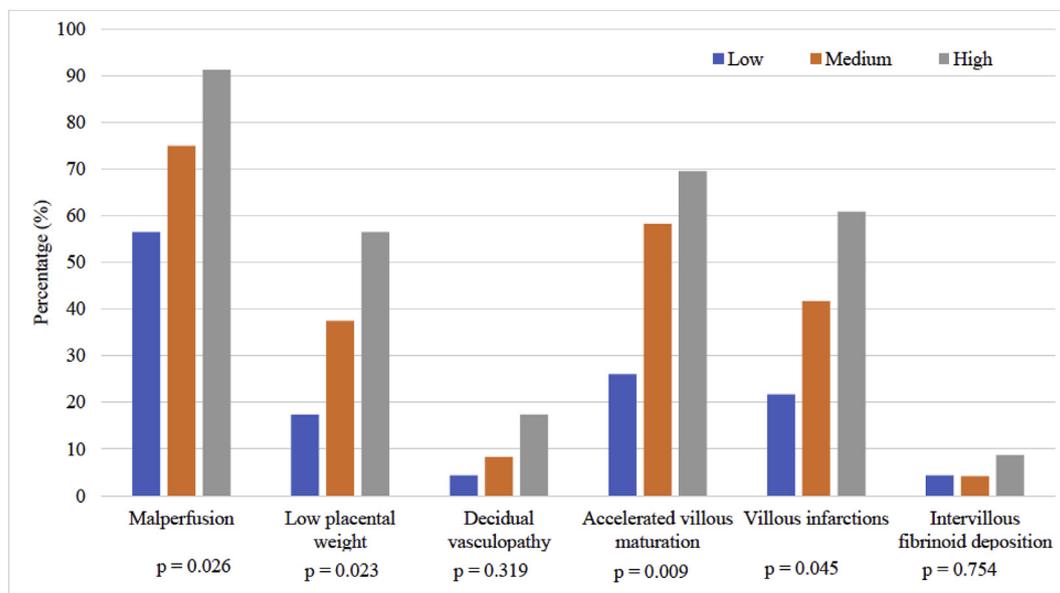
Characteristics	Low < 23.6 ng/mL	Medium 23.6–41.1 ng/mL	High ≥ 41.2 ng/mL	P
	n = 23	n = 24	n = 23	
Age				0.38
< 35 years	20 (87)	17 (71)	17 (74)	
≥ 35 years	3 (13)	7 (29)	6 (26)	
Parity				1.00
Nulliparous	18 (78)	19 (79)	18 (78)	
Multiparous	5 (22)	5 (21)	5 (22)	
Pre-pregnancy obesity				0.71
Not obese (< 30)	16 (70)	14 (58)	15 (65)	
Obese (≥ 30)	7 (30)	10 (42)	8 (35)	
Smoking status				0.94
Non-smoker	19 (83)	19 (79)	19 (83)	
Smoker	4 (17)	5 (21)	4 (17)	
Infant sex				0.49
Male	13 (57)	12 (50)	9 (39)	
Female	10 (43)	12 (50)	14 (61)	
Gestational age at delivery, median (interquartile range)	38.6 (2.7)	36.2 (5.2)	33.6 (4.8)	< 0.0001
Gestational age at sample collection, median (interquartile range)	38.3 (8.8)	35.9 (11)	32.6 (11.1)	< 0.0001
Placental weight, median (interquartile range)	440 (155)	355 (170)	260 (130)	< 0.0001
Birthweight percentile, median (interquartile range)	72.7 (53.5)	14.8 (38.1)	17.3 (26.5)	< 0.0001

Notes: ng/mL (nanograms/milliliter).

current Amsterdam criteria, and were supplemented with MVM-specific guidelines published by the Society for Pediatric Pathology, Perinatal Section, Maternal Vascular Perfusion Nosology Committee in 2004 [22]. Soto et al. [5] followed the same MVM-specific guidelines [22,29], but did not include low placental weight in their MVM construct. Ultimately, inter-observer variability amongst pathologists is a limitation that is present in many research studies, including our own. This limitation underscores the need for prospective research studies that utilize current and standardized diagnostic criteria.

Other limitations of our study include the use of pre-existing data

and samples, the cross-sectional sample collection (can't assess temporality), small sample size, lack of ethnic diversity, lack of personal and family past medical history of hypertension and previous preeclampsia, and lack of a comparison group. Despite these limitations, our study had several strengths, including the use of stringent preeclampsia phenotyping criteria, the use of a systematic placental pathology abstraction protocol, and the use of multivariate modeling to control for potential confounders. Importantly, our results provide support for larger, prospective studies to examine if sENG may be a robust marker of poor placental function in preeclampsia.



**Fig. 3.** Prevalence of type of placental lesion by concentration of soluble endoglin.

**Table 3**

Association between concentration of soluble endoglin and maternal vascular malperfusion lesions in women with preeclampsia by tertile (n = 70).

Outcome	n (%)	Unadjusted		Adjusted <sup>a</sup>	
		PR	95% CI	PR	95%CI
<b>Malperfusion</b>					
Low tertile	13/23(56.5)	1.00	–	1.00	–
Medium tertile	18/24 (75.0)	1.33	0.87, 2.03	1.42	0.94, 2.15
High tertile	21/23 (91.3)	1.62	1.10, 2.36	1.70	1.15, 2.52
<b>Low placental weight</b>					
Lowest tertile	4/23 (17.4)	1.00	–	1.00	–
Medium tertile	9/24 (37.5)	2.16	0.77, 6.04	2.23	0.82, 6.14
Highest tertile	13/23 (56.5)	3.25	1.24, 8.49	3.26	1.25, 8.50
<b>Villous infarcts</b>					
Lowest tertile	5/23 (21.7)	1.00	–	1.00	–
Medium tertile	10/24 (41.7)	1.92	0.77, 4.75	2.00	0.82, 4.90
Highest tertile	14/23 (60.9)	2.80	1.21, 6.50	2.93	1.27, 6.73
<b>Accelerated villous maturation</b>					
Lowest tertile	6/23 (26.1)	1.00	–	1.00	–
Medium tertile	14/24 (58.3)	2.24	1.04, 4.81	2.57	1.17, 5.66
Highest tertile	16/23 (69.6)	2.67	1.27, 5.58	3.14	1.47, 6.70

<sup>a</sup> Adjusted for maternal age, pre-pregnancy obesity, parity, maternal smoking status, and infant sex.

#### 4.1. Conclusions and next steps

This secondary data analysis represents the first step in evaluating the association between sENG concentrations and risk of MVM in women with preeclampsia. Overall, we found that elevated plasma concentrations of sENG were significantly associated with MVM lesions of the placenta in women with preeclampsia. In the setting of placental dysfunction, sENG is released from the placenta into the maternal circulation. Because MVM lesions of the placenta cannot be diagnosed until after delivery, sENG may represent a useful antenatal surrogate of MVM and underlying placental dysfunction; however, large longitudinal studies of diverse ethnicities and pregnancy outcomes (e.g., uncomplicated, preterm birth) that measure sENG concentrations at several time points across pregnancy are needed to determine the utility of sENG as a population-based screening tool for MVM.

#### Conflicts of interest

The authors have no conflicts of interest to declare.

#### Author contributions

**Mandy Schmella:** I declare that I participated in the study design, data collection, and interpretation of study results. I also drafted the manuscript and I approved the final version of the manuscript.

**Vanessa Assibey-Mensah:** I declare that I completed the statistical analysis, interpreted study results, and assisted with drafting of the manuscript. I have critically reviewed and approved the final version of the manuscript.

**W. Tony Parks:** I declare that I participated in protocol development, and interpretation of data study results. I have critically reviewed and approved the final version of the manuscript.

**James Roberts:** I declare that I participated in the design and execution of the parent study (PEPP cohort). I assisted in the interpretation of the study results, and I have critically reviewed and approved the final version of the manuscript.

**Arun Jeyabalan:** I declare that I participated in the design and execution of the parent study (PEPP). I assisted in the interpretation of the study results, and I have critically reviewed and approved the final version of the manuscript.

**Carl Hubel:** I declare that I participated in the design and execution of the parent study (PEPP). I also participated in the collection of sENG measurements. I assisted in the interpretation of the study results, and I have critically reviewed and approved the final version of the manuscript.

**Janet Catov:** I declare that I participated in the study design, data collection, and interpretation of study results. I have critically reviewed and approved the final version of the manuscript.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.placenta.2019.02.014>.

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