Pregnancy Hypertension 16 (2019) 42–47

Contents lists available at ScienceDirect

Pregnancy Hypertension

journal homepage: www.elsevier.com/locate/preghy

CD-34$^+$ and VE-cadherin$^+$ endothelial progenitor cells in preeclampsia and normotensive pregnancies

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ARTICLE INFO

Keywords:
Preeclampsia
Normotensive pregnancy
Endothelial progenitor cells
Vascular endothelial cadherin
Cytokines
Body mass index

ABSTRACT

Objective: The objective of our study was to determine levels of endothelial progenitor cells (EPCs) in preeclampsia and normotensive pregnant women.

Study design: Prospective cohort study of women with preeclampsia and normotensive pregnancies. EPCs were estimated by flow cytometry. Multiple linear regression was used to assess the association of EPCs with preeclampsia adjusting for maternal age, body mass index (BMI), gestation and ethnicity.

Main Outcome Measure: Levels of EPCs in preeclampsia and normotensive pregnancies, with CD-34 and vascular endothelial (VE)-cadherin as markers of EPCs. VE-cadherin is an endothelial cell adhesion molecule used to delineate endothelial lineage of EPCs.

Results: There were thirty women in the preeclampsia group and thirty-three in the normotensive group. The two groups were similar except for the BMI and blood pressures, which were higher in preeclampsia. On multiple linear regression, EPCs numbers were significantly higher by 29 (95% confidence interval 11.7–46.6, p = 0.001) in preeclampsia compared to the normotensive group. There was significant positive correlation between EPCs and systolic blood pressure in preeclampsia (Spearman correlation coefficient 0.39, p = 0.03).

Conclusion: Although widely used in cardiovascular disease other than preeclampsia, this is the first study using VE-cadherin as a marker of endothelial lineage to define EPCs in preeclampsia. Our results suggest the higher number of EPCs in preeclampsia may be a response of the bone marrow to endothelial injury.

1. Introduction

Preeclampsia is a pregnancy specific disorder characterized by new onset hypertension and proteinuria after twenty weeks of gestation. The disorder affects 5–8% of all pregnancies and is a major cause of maternal and neonatal morbidity and mortality worldwide [1]. The deleterious effects of preeclampsia are not confined to pregnancy or the newborn period. Women with a history of preeclampsia and their offsprings are at higher risk of cardiovascular and cerebrovascular disease in later life [2]. Worldwide, hypertensive disorders of pregnancy, particularly preeclampsia account for 76,000 maternal deaths and 500,000 fetal/newborn deaths annually [3]. In the United States, the cost of preeclampsia to the health care system is $2.18 billion annually [4].

Although the exact etiology of preeclampsia remains unknown, an overwhelming large body of evidence suggests that preeclampsia is associated with maternal endothelial activation and dysfunction. Currently, preeclampsia is considered a two-stage disease where initially abnormal placentation results in a hypoxic and hypoperfused placenta, which produces factors affecting maternal endothelium resulting in manifestations of the disease. These factors include anti-angiogenic factors, namely soluble vascular endothelial growth factor 1 (sFlt1) and soluble endoglin (sEng), which bind and lower angiogenic factors, vascular endothelial growth factor (VEGF) and placental growth factor (PlGF). Both VEGF and PLGF are important factors for maintenance of endothelial health and function. In addition, markers of endothelial activation such as von Willebrand factor, plasma cellular fibronectin, endothelin-1, vascular cellular adhesion molecule-1, intercellular adhesion molecule-1, tumor necrosis factor-α (TNF-α) interferon (IFN) -γ and interleukins (IL) – 6 and 1β, are elevated in preeclampsia, some before the onset of the disease [5]. Preeclampsia is

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https://doi.org/10.1016/j.preghy.2019.02.007
Received 18 July 2018; Received in revised form 12 February 2019; Accepted 22 February 2019
Available online 23 February 2019

associated with a distinctive renal lesion termed "glomerular capillary endotheliosis", characterized by glomerular endothelial swelling and vacuolization, considered as markers of endothelial injury and cause of proteinuria [6].

Endothelial progenitor cells (EPCs) are bone marrow derived cells critical for maintenance of endothelial health and function [7]. They have the potential to differentiate into mature endothelial cells, form new capillaries and produce paracrine factors helpful for vessel wall repair [7]. They are mobilized from the bone marrow into the circulation in response to a number of chemotactic factors and cytokines released in response to endothelial injury and ischemia. Such conditions include but are not limited to trauma, atherosclerosis, coronary artery disease, stroke, retinal disease, arthritis, cancer growth and metastasis, psoriasis and hemangiomas [8]. Currently, circulating EPCs levels can be assessed by flow cytometry or *in vitro* culture methods. Using flow cytometry EPCs are quantified as percentage of mononuclear cells expressing at least one marker of prematurity and at least one marker of endothelial lineage [9]. Although the combination of markers that constitute an EPC is controversial, the most common markers to delineate progenitor status are CD-34 and CD-133. More recently, the presence of CD-34 without CD-133 has been recommended as marker for progenitor status. VEGFR-2 is the most commonly used marker for endothelial cell lineage [9–11]. Almost all studies in preeclampsia have used CD-34 and CD-133, either in combination or separately, along with VEGFR-2 to delineate EPCs [11]. The results are, however, variable with higher, lower and similar numbers of EPCs being reported in preeclampsia compared to normotensive pregnant women [11]. Another marker for endothelial lineage is vascular endothelial (VE) – cadherin, an adhesion molecule specific to endothelial cells. In recent studies on cardiovascular disease, VE-cadherin is reported to be a better marker than VEGFR-2 to delineate endothelial lineage with better reproducibility [12,13]. Although widely used in cardiovascular disease to delineate EPCs, VE-Cadherin, to our knowledge, has not been used in reports of EPCs in preeclampsia. The objective of our study was to investigate levels of EPCs in preeclampsia compared to normotensive pregnant women using flow cytometry with CD-34 and VE-cadherin as cell surface markers to delineate EPCs. We also investigated levels of cytokines and angiogenic factors that are associated with endothelial damage and preeclampsia and involved in mobilization of EPCs from the bone marrow [5,14,15].

2. Methods

This was a prospective cohort study, approved by the Institutional Review Board of the University of Calgary and conducted at the Foothills Medical Center, a tertiary care center in Calgary. Women with preeclampsia and singleton pregnancy were recruited from the Obstetrics floor of the hospital between January 2014 and March 2015. Preeclampsia was defined according to the Society of Obstetrics and Gynecology Canada definition [16]. Hypertension was systolic blood pressure ≥than 140 mm Hg or a diastolic level of (Korotkoff 5) ≥ 90 mm Hg on two or more occasions at least 4–6 h (but not more than 7 days) apart, ≥ 20 weeks gestation in a woman with previously normal blood pressure. Blood pressure should have been taken in the hospital or office setting with the women sitting and the arm at the level of the heart. Proteinuria was defined as ≥0.3 g protein in a 24 h urine sample. When a 24 h urinary sample was not feasible, ≥0.3 g/l protein or ≥1 + on a dipstick test-strip on two random urine samples taken at least 4–6 h apart were considered proteinuria. Healthy normotensive pregnant women with singleton pregnancy were recruited from the obstetrics triage area of the Hospital. Exclusion criteria include multiple pregnancy, chronic hypertension, smoking, substance use, malignancies, fever, diabetes mellitus, maternal renal, cardiovascular, hematological or autoimmune disease, premature rupture of membranes, preterm premature rupture of membranes and any congenital malformations. All participants gave written informed consent.

Five ml of venous blood from the ant-cubital vein was collected in serum separator tubes followed by collection of five ml of blood in sodium heparin tubes. Blood from the first tube was centrifuged and serum stored at −80 °C for analysis of cytokines and angiogenic factors. Blood from the second tube was used for flow cytometric analysis of EPCs. We chose the second tube for flow cytometry to avoid contamination of the sample with endothelial cells that may have sloughed off following venipuncture.

2.1. Flow cytometry

Following collection, blood was stored at 4° C. Mononuclear cells (MNCs) were separated from blood using standard Ficoll gradient separation method within 2 h of sample collection. 2 million mononuclear cells were stained for 30 min at 4 °C with the panel of fluorochrome-labeled mouse/rabbit monoclonal antibodies against human cell surface markers that included CD34 conjugated with Phycoerythrin (CD34-PE, BD Biosciences, San Jose, CA, USA), and VE-Cadherin conjugated with fluorescein isothiocyanate (VE-Cadherin-FITC, Abcam Inc, Cambridge, MA, USA). Fluorescent isotype-matched antibodies (Beckman Coulter, Mississauga, Ontario, Canada) were used as controls. CD3 conjugated with cyanine-5 (CD3-PC5) Beckman Coulter, Mississauga, Ontario, Canada) was used as a dump channel surface marker. After washing stained cells were resuspended in PBS with 1% bovine serum albumin and 0.1% sodium azide. Immediately before flow cytometry, a known number of fluorospheres (50,000) (Flow Count, Beckman Coulter) were added to each sample. The cells were analyzed by two colors -flow cytometry (FACS Canto II, BD Biosciences, San Jose, CA, USA).

EPCs were identified as $CD34^+$ and VE-$cadherin^+$ cells when gated from lymphocyte gate in the forward vs size scattered 2D plot. The number of EPCs acquired was set to at least 2,000,000 nucleated cells in the lymphocyte gate. The absolute count of EPCs were expressed as cells per 10^6 MNCs analyzed where number of MNCs analyzed were enumerated using fluorosphere counts and initial quantity of MNCs.

2.2. Cytokines and angiogenic factors

PlGF and VEGFR-1 were analyzed by means of enzyme-linked immunoassays (ELISA, R & D systems, Minneapolis, USA). All samples were assayed in duplicates. IL-6, 8 and 10 and granulocyte colony stimulations factor (G-CSF) were assayed by Multiplex Laser Bead Technology. All samples were assayed in duplicates. Persons performing flow cytometry, ELISAs or Multiplex assays were blinded to the group of the patient.

2.3. Statistics

Continuous variable are reported as mean and SD or median and interquartile range depending on the distribution. Mann-Whitney or *t* tests or χ^2 were performed for bivariate associations as appropriate. Spearman rank correlation coefficient were calculated to assess the association between EPCs and cytokines and angiogenic factors. After assumptions for linear assumptions were met, linear regression was performed to adjust for *a priori* factors including gestation, BMI, maternal age and ethnicity to investigate the association between EPCs levels and the two groups. A two-tailed *p* value below 0.05 was considered as significant. All statistical tests were performed using SPSS version 24 for Windows (IBM Inc, NY, USA).

3. Results

Of the 35 women with preeclampsia approached, 33 consented to the study. Three were later found to have hypertension before pregnancy and were excluded from the study. Of the 36 normotensive women approached, 33 consented to the study. Table 1 shows the

Table 1
Demographic variables of the cohort.

	Preeclampsia $n = 30$	Normotensive $n = 33$	P-value
Age (yrs)	31 ± 6	32 ± 5	0.60
Primigravida n (%)	21 (70)	19 (57.6)	0.30
BMI	27 ± 5	23 ± 4	< 0.01
Gestation (wks)	31 ± 3	30 ± 3	0.09
Systolic Blood Pressure (mmHg)	149 ± 14	113 ± 8	< 0.01
Diastolic Blood Pressure (mmHg)	97 ± 11	71 ± 21	< 0.01
Caucasian n (%)	20 (66.7)	26 (78.8)	0.425

Table 2
Levels of cytokines and angiogenic factors in preeclampsia and normotensive women.

	Preeclampsia $n = 30$	Normotensive $n = 33$	P-value
G-CSF (pg/ml)	88 ((70–121)	87 (63–105)	0.478
IL-10 (pg/ml)	2.4 (1.2–3.3)	1.9 (1.2–3.2)	0.635
IL-6 (pg/ml)	5.9 (2.8–8.2)	1.4 (0.6–2.4)	< 0.001
TNF-α (pg/ml)	16.7 (11–20.4)	11 (7.4–13.0)	< 0.001
PlGF (pg/ml)	32.5 (2.7–47.4)	515 (288–702)	< 0.01
IL-8 (pg/ml)	13.5 (7.1–22)	5.9 (2.4–8.8)	0.02
VEGFR1 (pg/ml)	7349 (6341–7935)	1799 (1518–2225)	< 0.01

Data as median and interquartile range.

demographic variables of the two groups. BMI, systolic and diastolic blood pressures were significantly higher in the preeclampsia group. There were more primigravidas and less Caucasians in the preeclampsia group but the difference was statistically non-significant. There was no difference in gestation and maternal age between the two groups. Table 2 shows levels of cytokines and angiogenic factors in the two groups. IL-6, 8, TNF-α, and VEGF-R1 were significantly higher in the preeclampsia group while PlGF was significantly lower. There was no difference in levels of G-CSF and IL − 10 between the two groups.

Fig. 1 shows levels of EPCs between the two groups [preeclampsia 22, interquartile range (IQR) 6–71, normotensive 2.5 IQR 1–6, $p < 0.01$].

There was a significant positive correlation between the systolic blood pressure and EPC number in the preeclampsia group (Spearman correlation coefficient 0.35, $p = 0.04$). Fig. 2 shows the scatter plot of the EPC number and systolic blood pressure in the preeclampsia group. There was no correlation between the systolic blood pressure and EPC

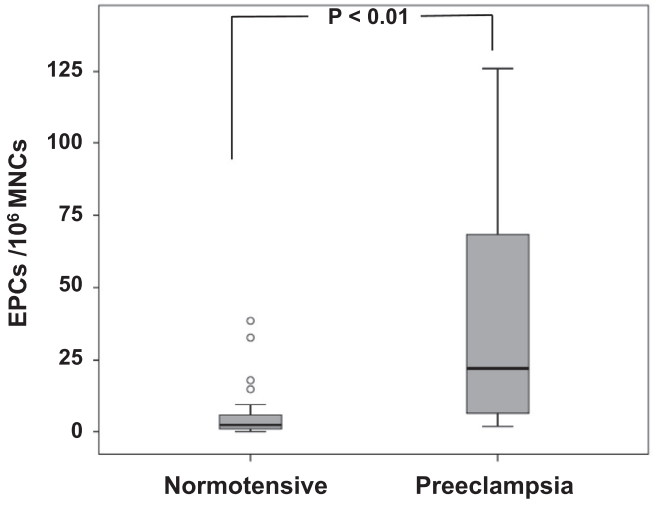

Fig. 1. Endothelial progenitor cells in preeclampsia and normotensive pregnancies.

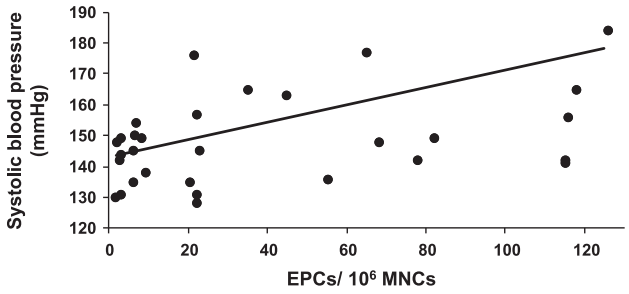

Fig. 2. Correlation between endothelial progenitor cells and systolic blood pressure in preeclampsia.

number in the normotensive group. The diastolic blood pressure did not correlate with the EPC number in either of the groups.

BMI is reported to be a major confounding factor for EPC number during pregnancy, with higher BMI associated with higher EPC number [17,18]. To address if the higher BMI in the preeclampsia group was a confounding factor for the difference in EPCs, we assessed the relationship between BMI and EPCs in the two groups by categorizing the study participants into normal weight and overweight using BMI < 25 kg/m^2 and ≥ 25 kg/m^2 respectively. Normotensive women, but not women with preeclampsia, had significantly higher EPCs if the BMI was ≥25 (Table 3a). Compared to normotensive women, women with preeclampsia had higher number of EPCs irrespective of a BMI < 25 or ≥ 25 (Table 3b).

There was no correlation, either positive or negative, between the EPC number and cytokines or angiogenic factors tested (data not shown).

Using multiple linear regression and after controlling for *a priori* factors (maternal age, gestation, BMI, ethnicity), EPCs numbers were significantly higher by 29 (95% confidence interval 11.7–46.6, $p = 0.001$) in preeclampsia compared to the normotensive group (Table 4).

Table 5 is a summary of studies reporting EPC numbers in preeclampsia and normotensive pregnancies.

4. Discussion

Using CD-34$^+$ and VE-cadherin$^+$ cells on flow cytometry to delineate EPCs, our study demonstrates that preeclampsia is associated with higher number of EPCs compared to normotensive women. This

Table 3
Levels of EPCs in preeclampsia and normotensive women with BMI < 25 and ≥ 25.

	BMI < 25	BMI ≥ 25	P-value
a			
Normotensive	$n = 25$	$n = 8$	0.007
EPCs	1.5 (1–3.5)	6 (3–17)	
Preeclampsia	$n = 12$	$n = 18$	0.42
EPCs	9 (4–60)	22.5 (7–90)	
	Preeclampsia	**Normotensive**	**P-value**
b			
BMI < 25	$n = 12$	$n = 25$	< 0.01
EPCs	21 (3.5–60)	1.5 (1–3.5)	
BMI ≥ 25	$n = 18$	$n = 8$	0.03
EPCs	22.5 (6–90)	6 (3–17)	

Data presents as median and interquartile range.
BMI = Body Mass Index.
EPC = Endothelial progenitor cells (per 10 ^{6} mononuclear cells).

Table 4
Multiple regression analysis with preeclampsia predicting EPC numbers adjusted for maternal age, gestation, BMI and ethnicity.

	B[b]	Standard error of *B*	ß[c]	t	p	95% CI for *B*	
EPCs[a]							
Intercept	31.185	54.415		0.573	0.569	−77.778	140.149
Preeclampsia	29.203	8.734	0.425	3.343	0.001	11.713	46.693
Age	−0.819	0.671	−0.140	−1.221	0.227	−2.163	0.525
Gestation	−0.916	1.227	−0.088	0.746	0.459	−3.374	1.542
BMI	0.890	0.890	0.137	1.110	0.272	−0.715	2.495
Caucasian	7.389	8.937	0.096	0.827	0.412	−10.508	25.285

EPCs = Endothelial progenitor cells, BMI = Body mass index.
[a] Dependent variable.
[b] Non-standard Co-efficient.
[c] Standardized Co-efficient.

association persisted using linear regression and after controlling for *apriori* factors. Although widely used in cardiovascular disease, to our knowledge, this is the first study to use VE-cadherin positive cells to define EPCs in preeclampsia [12,19].

We used VE-Cadherin to define EPCs for several reasons. Described first in 1991, VE-Cadherin is a vascular endothelial cell specific adhesion molecule vital for endothelial cell-to-cell communication in both developing and mature blood vessels [12,20–22]. In contrast, VEGFR-2 is expressed in cells other than endothelial cells including early non-committed stem cells [7,9,23,24]. On *in vitro* culture, VE-Cadherin is preferentially expressed in outgrowth endothelial cells (OECs) and endothelial colony forming cells (ECFCs), two subsets of EPCs that have greater potential for proliferation and blood vessel formation [10,12,25–27]. Importantly, results using VE-Cadherin positive cells to define EPCs on flow cytometry are much more reproducible [13].

Using VEGFR-2 in combination with CD-34 and CD-133, either separately or together, other investigators have also reported higher number of EPCs in preeclampsia compared to normotensive pregnant women [28–31]. In eight patients with preeclampsia, Parsanezhad reported higher number of EPCs compared to normotensive women but with less potential to form colonies on culture [28]. Similarly, Murphy et al reported higher EPCs in preeclampsia two months postpartum but with reduced capacity to form colonies [30]. Buemi reported higher number of EPCs in gestational hypertension but lower in mothers with diabetes [29]. Using flow cytometry, Al-Sweedan reported higher number of CD-34 positive cells in umbilical cord blood in preeclampsia [32]. In one of the earliest studies on EPCs in preeclampsia, Matsubara et al reported no difference in EPC numbers on flow cytometry but increased proliferative capacity on culture in preeclampsia compared to normotensive women [33]. The increased EPCs in preeclampsia are considered a response by the bone marrow to endothelial injury [28,29]. However, as reported by Parasenzhad and Murphy, these cells may not be functionally competent, failing to participate in endothelial repair and further stressing the bone marrow [11,28,30]. Other conditions associated with endothelial injury such as myocardial infarction and coronary artery disease are also reported to be associated with

Table 5
Studies of EPC numbers in preeclampsia and normotensive pregnancies using flow cytometry.

Reference	Number of Preeclamptic patients	Gestation (weeks)	Blood Source	Markers	Cell Count
Studies with EPC numbers higher in preeclampsia					
Buemi et al, ref# 29	7	36	Maternal	CD34+,VEGFR-2+	Percent of MNCs
Murphy et al, ref# 30	7	35.9 ± 3.6 (at delivery)	Maternal (2 months post-partum)	CD34+,VEGFR-2+ CD133+,VEGFR-2+	Percent of MNCs
Parsanezhad et al, ref# 28	8	32.29 ± 3	Maternal	CD133+,VEGFR-2+	Cells/10^6 MNCs
Monga et al, ref# 31	14	23–42	Umbilical cord	CD45dim CD34-ive, CD133+, VEGFR-2	Percent of MNCs
Studies with EPC numbers lower in preeclampsia					
Luppi et al, ref# 35	14	34.5 ± 2.9	Maternal	CD34+,VEGFR2+ CD133+,VEGFR2+	Percent of MNCs
Lagana et al, ref# 36	13	10.3 ± 0.31	Maternal	CD34+,CD133+, VEGFR-2+	Cells/μL
Xia et al, ref# 37	14	36 ± 2	Umbilical Cord	CD133+, VEGFR2+	Cells/mL blood
Gumina et al, ref# 38	13	33.5 ± 3.2	Umbilical cord	CD45dim,CD34+, CD31+,CD133+	Percent of MNCs
Szpera-Gozdziewicz et al, ref# 39	21	33.2 ± 2.6	Maternal	CD133+,CD 34 + CD146+	Cells/10^6 MNCs
Monga R et al, ref# 31	14	23–42	Umbilical cord	CD45dim,CD34+, CD133+, VEGFR-2+	Percent of MNCs
Wang Y et al, ref# 40	48	20	Maternal	CD45-ive, CD34+ VEGFR2+	Cells/mL blood
Studies with no difference in EPC numbers between preeclampsia and normotensive pregnancies					
Parsanezhad et al, ref# 28	8	32.29 ± 3	Maternal	CD34+,CD33+, VEGFR-2+, CD45+	Cells/10^6 MNCs&cells/mL blood
Parsanezhad et al, ref# 28	8	32.29 ± 3	Maternal	CD34+,CD133-ive, VEGFR-2+,CD45-ive	Cells/10^6 MNCs& cells/mL blood
Matsubara et al, ref# 33	10	34 ± 4	Maternal	CD34+, CD133+ VEGFR-2+	Cells/mL blood

MNCs = Mononuclear cells.

higher EPC numbers [34]. However, there are as many studies, if not more, which have reported decreased number of EPCs in preeclampsia [31,35–40]. Whether the low number of EPCs is a consequence of preeclampsia or women with low EPCs are at a higher risk of preeclampsia is not established. Studies have also reported no difference in EPC numbers between preeclamptic and normotensive pregnancies [28,33]. There are many reasons for the disparate results of EPC numbers in preeclampsia. Foremost among these is the lack of consensus on the cell surface markers to define EPCs with investigators using a variety of markers [41]. Data are reported in two different ways, either as cell numbers in a volume of blood or cells in a defined number of mononuclear cells, making comparisons difficult [28]. Similarly, no comparison can be made between studies using flow cytometry and *in vitro* culture and studies using umbilical cord blood versus maternal blood [10]. In addition, studies have not taken into account clinical factors such as BMI, gestation, smoking and medications which may affect EPC numbers [11]. Importantly, these studies have small numbers, especially in the preeclampsia group. Our number of thirty in the preeclampsia group is one of the largest in studies on EPCs in preeclampsia.

The EPC numbers in our study correlated positively with maternal systolic blood pressure in the preeclampsia group but not in the normotensive group. One other study has reported a similar correlation. Szpera-Gozdziewicz reported a positive correlation between a ratio of EPCs, defined as cells expressing CD-34$^+$, CD-146$^+$ and CD-133$^+$, and circulating endothelial cells, defined as cells expressing CD-34$^+$, CD146$^+$ and CD-133$^-$ and systolic blood pressure in preeclampsia [39]. The higher EPC number with increased systolic blood pressure may be a reflection of more severe endothelial injury as higher systolic blood pressure is associated with more severe preeclampsia [42]. However, further studies are needed before EPC numbers can be designated as markers of severity of preeclampsia.

BMI was higher in the preeclampsia group. In both the preeclampsia and normotensive group, EPCs numbers were higher in women with BMI ≥ 25 compared to women with BMI < 25 but were statistically significant only in the normotensive group. Similar results are reported in normotensive pregnant women and in non-pregnant individuals [18,43]. EPCs were higher in the preeclampsia group compared to the normotensive group whether the maternal BMI was < 25 or ≥ 25, suggesting that the increase in EPCs in the preeclampsia group was independent of BMI. Importantly, on linear regression and after adjusting for BMI and other factors, EPCs remained significantly higher in the preeclampsia group. Taken together our results suggest that BMI is a confounding factor for EPC numbers and the increase in EPC that we demonstrate in preeclampsia in our study is independent of BMI.

We measured a number of cytokines, angiogenic and anti-angiogenic factors in both preeclampsia and normotensive pregnancies and confirmed results reported previously. These include higher levels of VEGFR-1 and lower levels of PlGF in preeclampsia.[42] Preeclampsia is an inflammatory state and inflammatory cytokines IL-6, 8 and TNF-α were elevated in preeclampsia, results which have been reported by other investigators [44]. We did not find any difference in levels of G-CSF and IL-10 between the two groups. These factors also have role in EPC mobilization from the bone marrow [14,29]. However, there was no correlation between any of the factors that we investigated and EPC numbers in preeclampsia. This could be due to two reasons. One, we did not estimate levels of other known factors that are involved in mobilization of EPCs from the bone marrow. These include but are not limited to stromal derived factor, estrogens, erythropoietin, angiopoetin-1, granulocyte macrophage colony stimulating factor, IFN-γ and fibroblast growth factor basic [14,29,45,46]. It is currently unknown which factor is the most potent in mobilizing EPCs [46]. Second, although our number of preeclampsia patients is larger than almost all studies on EPCs in preeclampsia, it may not have been large enough to detect statistically significant correlations. Other investigators have also reported on a lack of correlation between EPCs and angiogenic factors [35,47].

The surface markers CD-133 and CD-45^{-ive}, which we did not measure, have also been recommended for definition of EPCs. Both are, however, controversial [10,48]. CD 133$^+$CD34$^+$VEGFR-2$^+$ do not form endothelial colonies on *in vitro* culture and maintain their hematopoietic status [7,10,49]. In addition, the presence of CD-133, along with the other two markers, makes the population of cells extremely scarce, increasing variability and decreasing reproducibility of results [9]. It has also been suggested that EPCs reside in the CD-45$^-$ fraction of cells.[50] However, hematopoietic stem cells can be CD-45$^-$ as well [48,51]. Investigators have used CD-45$^-$, CD-45^{dim}, or CD-45^{med} gating strategies to define EPCs making any meaningful comparisons difficult [49,52,53]. Like CD-133, CD-45$^-$ cells also from an extremely small population of cells [9].

There are several strengths to our study. Our sample of thirty women in the preeclampsia group is larger than almost all studies on the subject. Preeclampsia was defined according to the most recent guidelines. In addition, we had detailed demographic information on women in both groups, key information that is missing from a number of previous studies. This allowed us to control for factors such as BMI and gestation that may affect EPC numbers. We also, for the first time, use VE-cadherin as a marker to define EPCs in preeclampsia. VE-cadherin is reported to be a better marker of EPCs delineation than VEGFR-2 in a number of recent studies. Importantly, results are reported to be much more reproducible which can be a concern with flow cytometry. There are also limitations to our study. We did not perform any functional assays on the EPCs. However, there is controversy on what test best describes the functionality of EPCs. Investigators have used the formation of cobblestones and tube like structure on *in vitro* cultures, ability to form angiogenic factors and also alleviation of tissue ischemia after treatment by the cells [7]. The culture protocols used to isolate EPCs are even more complex, intricate and variable as compared to flow cytometry protocols [9]. In addition, much larger volumes of blood are needed for these protocols, something that may be difficult to consent during pregnancy. Results may also not be available for several weeks. The other limitation of our study is our population comprised mostly Caucasian women from a single hospital, making our results less generalizable.

In summary, using CD-34$^+$ and VE-cadherin$^+$ to delineate EPCs in a well-defined cohort of women, we report higher number of EPCs in preeclampsia as compared to normotensive women.

Although the higher number of EPCs correlated with higher systolic blood pressure, a marker of severity of preeclampsia, further studies are needed before EPCs can be used to define severity of preeclampsia. Given the confusion and lack of consensus in the field, there is an urgent need for characterization of EPCs on flow cytometry with better cell surface markers, which are reproducible and correlate with functional assays.

Declaration of interest

None

Funding

We are grateful to the Alberta Children's Hospital Research Institute, Calgary, Canada for providing the funding for this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2019.02.007.

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