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Mid-pregnancy levels of angiogenic markers as indicators of pathways to preterm delivery

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Abstract

Objective—To determine if midpregnancy levels of angiogenic markers were associated with increased risk of preterm delivery (PTD).

Methods—We studied a subcohort from the Pregnancy Outcomes and Community Health Study for whom midpregnancy angiogenic markers [(soluble fms-like tyrosine kinase-1 (sFlt-1), soluble endoglin (sEng), and placental growth factor (PlGF)], and covariate data were available (N = 1301). Angiogenic marker levels were grouped as High/Not High (sFlt-1 & sEng), Low/Not Low (PlGF) and High/Intermediate/Low (sFlt-1). Associations between levels of angiogenic markers and PTD/PTD subtype were determined for women who were non-smokers during pregnancy (N=933).

Results—Low PlGF and High sEng were associated with medically-indicated PTD and PTD <35 weeks, largely due to preeclampsia. Excluding preeclampsia and small-for-gestational-age infants, Low sFlt-1 was positively associated with medically-indicated PTD.

Conclusions—Among non-smokers midpregnancy levels of angiogenic markers may mark multiple pathways leading to PTD, only one attributable to preeclampsia.

Keywords

endoglin; placental growth factor; preeclampsia; small-for-gestational age; soluble fms-like tyrosine kinase-1

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Declaration of Interest Statement

Dr. Karumanchi would like to disclose the following: Dr. Karumanchi is a co-inventor on multiple patents related to angiogenic markers for use in preeclampsia. Dr. Karumanchi reports serving as a consultant to Beckman Coulter and Roche Diagnostics and having financial interest in Aggamin LLC.
None of the other co-authors have any declarations of interest.

Introduction

Rates of preterm delivery (PTD), delivery at <37 weeks gestational age, have been rising since 1980[1]. Currently, 1 in 8 US births[1] occurs preterm resulting in substantial economic, social and long-term health costs[2]. The heterogeneous etiology of PTD complicates the identification of causal factors and makes prediction difficult[1]. Identifying the biological pathways involved may improve etiologic studies of PTD[3].

Preeclampsia (PE), characterized by *de-novo* onset of hypertension and proteinuria after 20 weeks' gestation, is an important cause of preterm delivery[4]. Altered angiogenic marker expression is associated with hypertension and proteinuria[5–7]. Increased levels of the angiogenic markers soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) and lower placental growth factor (PlGF) levels may permit detection of PE before symptom onset[4,6]. Associations between these markers and preeclampsia development appear to be stronger and more predictive of risk for severe preeclampsia cases that are early-onset or result in delivery of a small-for-gestational age infant[4]. Altered marker levels may accompany poor infant growth in the absence of PE, however, the marker pattern involved is unclear[8,9].

During pregnancy sFlt-1, PlGF and sEng are produced by trophoblasts, although other cell types may contribute to overall levels[10–12]. Angiogenic marker levels in maternal blood are dynamic[8] and changing levels of markers may influence aspects of placental development and angiogenesis[4,10] including spiral artery remodeling and contribute to maternal pregnancy complications[5,6,13]. While altered marker expression is linked to placental problems, the time order of events is unclear[4].

Relations between angiogenic markers and PTD are important to characterize. In the US slightly more than 40% of medically-indicated PTD <37 weeks[14] and >20% occurring <35 weeks[15] are due to PE. PTD in the absence of PE symptoms is linked to poor placental development and/or function[16,17]. Little is known about angiogenic marker patterns in relation to PTD uncomplicated by PE. PE-like angiogenic marker changes have been reported late in pregnancy among spontaneous preterm labor cases, however similar changes occur in term pregnancies before labor onset[17]. Two previous reports suggest low sFlt-1 levels may mark a subset of spontaneous PTD[17,18].

We measured mid-pregnancy levels of sFlt-1, PlGF, and sEng in maternal blood and assessed their associations with PTD in the Pregnancy Outcomes and Community Health (POUCH) Study. Our objectives were to: 1) determine if altered marker expression was associated with increased risk of PTD (primary); and 2) consider the degree to which PE and small-for-gestational age influenced associations (secondary). Based on prior reports, we examined two patterns of marker expression: PE-like (increased sFlt-1 and sEng, decreased PlGF) and Low sFlt-1.

Methods

Cohort

Between September 1998 and June 2004, the POUCH Study recruited pregnant women from 52 prenatal clinics across 5 Michigan communities at 15–27 weeks' gestation[3]. Sampling into the cohort has been described previously[19]. Briefly, participants had maternal serum α-fetoprotein (MSAFP) screening at 15–22 weeks' gestation, were ≥15 years old, spoke English, had singleton pregnancies without known anomalies and did not have pre-pregnancy diabetes. At enrollment, self-completed questionnaires and in-person interviews were administered and maternal biologic samples collected. Of 3038 women recruited, 3019 were followed through delivery and constitute the cohort. Study protocols were approved by institutional review boards at Michigan State University, Michigan Department of Community Health and delivery hospitals.

Study Sample

To study PTD and at-risk groups of women in greater detail, we established a subcohort in which we collected additional information (N=1371). The subcohort included all women delivering preterm or with elevated MSAFP at screening, and a random sample of term births with normal MSAFP oversampled for African-Americans. The Angiogenic Sample included 1301 women who belonged to the subcohort, had angiogenic markers measured and complete information on important covariates (N=1301, Figure 1). The analyses focus on 933 non-smokers within the Angiogenic Sample. To account for oversampling of PTD and at-risk groups into the subcohort, weights based on the probabilities of selection into the cohort and subcohort were applied in all analyses.

Covariates

Race, smoking status and prepregnancy weight were self-reported. Women were grouped as African-American or non-African-American and as smokers or non-smokers based on smoking status at the start of pregnancy. Maternal weight was measured at enrollment. Gestational age (GA) at study enrollment was based on last menstrual period if the estimate was within 2 weeks of estimates based on <25 week ultrasound. Otherwise, ultrasound dates were utilized.

Angiogenic Markers

Maternal serum samples were collected at enrollment and stored at −80°C. Batched samples were analyzed in duplicate using commercially available ELISA kits as described previously[19].

Outcomes

PTD was any delivery occurring <37 weeks GA. Additionally, PTD was grouped into subtypes based on: 1) circumstance initiating delivery: premature rupture of membranes (PPROM), spontaneous preterm labor (sPTL), or medical indications (MI-PTD) or 2) timing of delivery: term, 35–36, 32–34 and <32 weeks. Based on chart review, PE was defined by diagnosis of gestational hypertension (diastolic blood pressure ≥90 or systolic blood

pressure ≥140 on 2 different days) beginning after 20 weeks gestation plus one of the following as evidence of proteinuria in the absence of genitourinary infection: 1) 2+ protein on urine dipstick once after 20 weeks gestation, 2) 1+ protein on two occasions after 20 weeks or 3) if proteinuria was present before 20 weeks, increased levels after 20 weeks[20,21]. Women could also have PE superimposed on chronic hypertension beginning before 20 weeks. SGA was defined as ≤10^{th} centile of weight for gestational age and sex of infant[22]. Analytic Approach: We began by confirming that the well-established PE-like pattern of marker expression (increased sFlt-1 and sEng, decreased PlGF) reviewed in [4] was evident among PE cases in our sample. Since it is unclear whether SGA cases exhibit the entire PE-like pattern or only particular elements[8,9], we compared adjusted-mean biomarker levels among all pregnancies complicated by PE (including PE+SGA cases), the remaining SGA cases and a referent group (no PE/SGA cases). Covariates associated with angiogenic marker levels in the POUCH study were utilized for adjustment: GA at sample collection, maternal age, race, smoking status, parity, and maternal weight at blood sampling, which was highly correlated with self-reported pre-pregnancy weight ($r^2 = 0.92$) [19].

Early in our analysis we found evidence for different relationships among angiogenic markers and pregnancy outcome in smokers vs. nonsmokers. Associations between smoking and angiogenic marker levels[13,23] have been reported but are challenging to interpret in the context of smoking's inverse relation to PE and positive association with SGA[24]. Given these complexities, we removed smokers from all analyses and studied women who were nonsmokers throughout pregnancy (N=933).

The primary analytical objective was to determine if the odds of PTD among non-smokers were increased when either pattern of marker expression was observed at midpregnancy: 1) the PE-like pattern associated with PE in this study and others[4,6] or 2) Low sFlt-1 previously associated with increased odds of spontaneous PTD[17,18].

To create groups representing the PE-like pattern, markers were dichotomized using distributions in term pregnancies among nonsmoking mothers with normal MSAFP: High/Not-High based on the highest quartile (sFlt-1 & sEng) and Low/Not-Low based on the lowest quartile (PlGF). To examine low sFlt-1, a 3-level variable was created: High (highest quartile), Intermediate (middle quartiles) and Low (lowest quartile). Since marker levels change through pregnancy[8] and sampling occurred between 15–27 weeks' GA, we established separate cut-points across three GA windows: 15–<19, 19–<23, and 23–27 weeks.

Using logistic regression, associations between categories of angiogenic marker expression and odds of PTD were examined. Polytomous regressions were utilized for PTD outcome categorized according to both clinical circumstance and delivery timing. Models were adjusted for covariates used to calculate adjusted means. Since associations between angiogenic markers and PPROM or sPTL did not differ, results are presented for the composite grouping spontaneous PTD (sPTD).

The secondary analytical objective was to determine if PE and SGA, previously associated with altered mid-pregnancy levels of angiogenic markers,[6,8,13] fully account for relations between angiogenic markers and PTD. After removing PE and SGA cases, all analyses were repeated.

Analyses were performed in SAS 9.2 (SAS Institute, Cary, NC) using survey procedures, which incorporate sampling weights to account for study design.

Results

Sample

Weighted percentages describing characteristics of the Angiogenic Sample were not different from those of the entire POUCH subcohort (Table I). Approximately ¼ of were African-American, the average age was 26.6 years, 58.9% were multiparous, and 10% experienced a hypertensive disorder in pregnancy. Preterm deliveries comprised 10.5% of all deliveries. Women who were nonsmokers during pregnancy were like the entire Angiogenic Sample, except for being older and less likely to deliver SGA infants.

Angiogenic Marker Levels Among PE and SGA

We first confirmed the well-established pattern of marker expression among all PE cases[4] (increased sFlt-1 and sEng, decreased PlGF) in POUCH study data. Among nonsmokers, unadjusted and adjusted levels of sFlt-1 and sEng were higher and PlGF concentrations were lower among PE cases (Table II). Comparing women who did not have PE but delivered SGA infants and our referent group, PlGF levels were significantly lower, sEng levels were slightly higher, but not as high as among PE cases, and sFlt-1 levels were not different (Table II).

Since prior reports have suggested that angiogenic marker levels differ by severity of PE, we subdivided PE cases by an indicator of severity (preterm (N=23, weighted frequency 41%) and term (N=12; weighted frequency 59%). We found no statistically significant differences in sFlt-1 levels between groups, but did observe PlGF levels tended to be lower in preterm cases versus term (182.7 vs 259.8 pg/mL, p =0.10) and found higher sEng levels among preterm as compared to term deliveries (2.11 vs 1.85 ng/mL, p = 0.04).

PE-like Patterns and PTD

Tables III and IV describe associations among the PE-like pattern and PTD grouped by clinical circumstance and delivery timing. Low PlGF and High sEng were weakly associated with PTD overall (Table III). Classified by clinical circumstance, Low PlGF (OR = 2.70; 95% CI 1.52–4.80) and High sEng (OR = 2.66; 95% CI 1.59–4.44) were significantly associated with MI-PTD only. A comparison of means confirmed findings; only MI-PTD showed significant alterations in PlGF (265.3 pg/mL vs. 385.1 pg/mL; p <0.0001) and sEng (6.73 vs. 5.47 ng/mL; p <0.0001) vs. term deliveries. Grouped by delivery timing, Low PlGF was associated with PTD <32 weeks (OR = 4.67; 95% CI 2.12–10.27, Table IV). High sEng was associated with a two-fold increased odds of PTD <35 weeks.

Removing PE and SGA cases from the analysis attenuated associations between Low PlGF and High sEng among MI-PTD (Table III). This was mostly due to excluding PE cases. Analyzed by delivery timing, sEng-PTD associations lost significance, most of the effect attributable to PE. Despite removing PE and SGA cases, the association between low PlGF and PTD <32 weeks persisted, OR = 3.35 (95% CI 1.40–8.03, Table IV). Findings were unchanged if pregnancies complicated by other hypertensive disorders (chronic and gestational hypertension) were excluded.

Low sFlt-1 and PTD

Table 5 presents associations between Low sFlt-1 and PTD. Initially, neither Low nor High sFlt-1 was significantly associated with PTD. After removing PE and SGA cases from the analysis, Low sFlt-1 was only associated with increased odds of MI-PTD (OR = 1.88; 95% CI 0.89–3.96; Table V). The OR was similar and strengthened if only PE cases (OR = 2.19; 95% CI 1.08–4.45), all cases of hypertensive disorders (OR = 2.51 (95% CI 1.16–5.46) or if PE/SGA and LGA cases were excluded. After the exclusion of PE and SGA cases, adjusted mean sFlt-1 levels among MI-PTD tended to be lower than term pregnancies (1509 vs. 1819 pg/mL; $p = 0.07$), whereas for sPTD levels were non-significantly 45 pg/mL lower.

Discussion

In the POUCH Study, both PE-like and Low sFlt-1 patterns of angiogenic markers were associated with elevated odds of MI-PTD among women who did not smoke during pregnancy. First, we confirmed associations between PE and High s-Flt1, Low PlGF, and High sEng. Then we observed that Low PlGF and High sEng were associated with PTD. Most of the effect was attributable to PE, except low PlGF among PTD <32 weeks. Second, Low sFlt-1 levels were associated with MI-PTD unexplained by PE or SGA. These patterns may represent two different pathways, identifiable at midpregnancy, leading to PTD.

The PE-like (Low PlGF and High sEng) associations are largely due to preterm PE cases, consistent with reports of more prominent angiogenic marker changes reported among preterm PE vs. term or less severe PE[8,13]. When mean levels were compared in our study (at an average of 22 gestational weeks'), sEng levels were clearly different between preterm and term PE cases and PlGF levels were suggestive of differences, but not statistically-significant ones. Studies point to a different disease course for preterm and term PE cases, one that we were just beginning to pick up with our midpregnancy measures. In some reports, differences in angiogenic marker levels between groups emerge around 17–23 weeks gestation, but that levels of PlGF do not discriminate between PE subtypes if measured early in gestation [8,13], Differences in disease course reflected in the pattern of angiogenic markers between preterm or term cases could influence clinical decision-making. Because the POUCH Study participants received care from many clinicians within multiple clinics/hospitals across 5 communities, it was not possible to define a set of clinical criteria that lead to medically-indicated delivery among PE pregnancies. In our sample, 12 PE cases were classified as SGA. Comparisons between frequency of SGA among preterm and term PE cases as an indicator of severity are complicated by different meanings of SGA at preterm and term gestations.[25] Additional information that would further help define

severity of PE symptoms was unavailable, but 21 of the 23 PE and MI-PTD cases the main indication for delivery was hypertension, perhaps suggestive of severe disease.

Consistent with the moderate strength of midpregnancy angiogenic marker-PE associations reported in other studies [6,13] and PE-PTD cases driving most of the association between the PE-like pattern of angiogenic markers and PTD, we observed odds ratios ~2–5. The limited contribution of SGA may imply heterogeneity among cases, consistent with varied reports of marker levels[8,9], or reflect fewer SGA cases compared with PE among MI-PTD. The lack of a sFlt-1/PTD association is unsurprising given 1) inconsistent relations between sFlt-1 levels measured before 20 weeks GA and PE[8,13,18] and 2) that sFlt-1 levels change later in pregnancy[8] relative to PlGF and sEng.

Reasons for the persistent association between Low PlGF and PTD <32 weeks are unclear. There were too few observations to determine if factors like placental abruption or previous PTD explain relations. The association may reflect a normal physiological shift as other work suggests a PE-like biomarker pattern occurs in all pregnancies shortly before delivery [8,17].

High levels of sFlt-1 and sEng are causally implicated in the development of hypertension and proteinuria, characteristic signs and symptoms of PE[5,7]. Women with these complications comprise a subset of MI-PTD that exhibits a PE-like marker pattern. Many conditions associated with PE (e.g. hypoxia, immune imbalance, renin-angiotensin system alterations) regulate angiogenic marker expression[4]. Alterations may be adaptive responses to underlying pathology, but it is unclear if they directly contribute. Since some women who develop PE do not exhibit High sFlt-1 and sEng[26,27], future work should explore differences among preeclamptics who show changes in angiogenic markers and those who do not to more fully describe the role of varying levels of angiogenic markers in PE and PTD.

The relation between Low sFlt-1 and MI-PTD became apparent after our analysis was made comparable to previous studies by removing PE/SGA or hypertensive cases. The exclusions are reasonable since the PE-like pattern could mask subtle changes and it does not overlap with the Low sFlt-1 pattern. While previous studies reported associations with sPTD or sPTL[17,18], Low sFlt-1 was associated with a subset of MI-PTD in POUCH, most strongly with cases uncomplicated by hypertension. Several factors could explain varied results across studies. First, the populations studied differ (Chilean[17], Scottish[18], and US-Michigan (POUCH)) and the spectrum of PTD cases may vary accordingly. Second, each study defined sPTD or sPTL differently. Third, clinical practices vary across countries. MI-PTD comprises a smaller proportion of PTD in Scotland and Latin America than in POUCH[28,29]. If US clinical practice results in medical intervention for cases with Low sFlt-1 levels, an association between Low sFlt-1 and MI-PTD would be observed. Perhaps cases marked by Low sFlt-1 end in sPTD if no intervention occurs. Thus, variation in clinical decision making or patient access/adherence to scheduled prenatal care visits might explain inconsistent results across studies.

After removing PE and SGA, 15 women in our sample had Low sFlt-1 and MI-PTD. Study clinicians recorded indication for MI-PTD by reviewing hospital charts. Indications for the above 15 women were: 4 maternal hypertension, 2 intrauterine growth restriction (IUGR), 1 placental abruption, 1 oligohydramnios, 3 complications in previous pregnancy, 2 placenta previa and 2 non-reassuring fetal signs. The latter group had the lowest average sFlt-1 levels, but their removal only moderately attenuated the elevated risk of MI-PTD among the Low sFlt-1 group. No single clinical circumstance linked low sFlt-1 and MI-PTD. One underlying explanation might involve changes in placental oxygen levels, which partially regulate sFlt-1 expression. Hypoxia increases expression[4], but placental hyperoxia, such as that associated with fetal hypoxia in IUGR[30] may decrease sFlt-1 expression.

This study had several strengths. First, the use of a multiethnic, multi-community sample demonstrated the strength of angiogenic marker-PTD associations in a diverse population of nonsmokers with singleton pregnancies. Second, by not excluding women who had PE or SGA at the design stage, we were able to selectively remove these women from the analysis and determine their influence on findings in this population-based sample. Third, our definition of PE was broader than some and may hint at the strength of angiogenic marker associations within the spectrum of PE[4,6]. Finally, we were able to examine relations among PTD subdivided by clinical circumstances and timing. Had MI-PTD been excluded at the design stage, we would not have detected the Low sFlt-1/MI-PTD association or been able to posit why our findings differ from others.

A limitation is the lack of repeated angiogenic marker measurements during pregnancy. Recent work has demonstrated the utility of longitudinal measurements[8,17], showing stronger associations between changes in biomarker concentrations and PE risk[31,32] and SGA[33]. We did not adjust for multiple comparisons given our *a-priori*-stated hypothesis that the PE-like pattern would be associated with PE and PTD. For comparisons with less established angiogenic biomarker patterns, findings are noted as potentially indicative of a relation and meriting further study.

This report confirms, extends and unifies previous work describing associations between angiogenic markers and PTD, showing that angiogenic marker levels may reveal two pathways leading to PTD. Additionally, the findings underscore the importance of considering the overlap and interrelatedness of pregnancy conditions when studying angiogenic markers. Finally, our results raise the possibility that variation in obstetric practice might influence studies examining the utility of biomarkers for predicting and understanding PTD.

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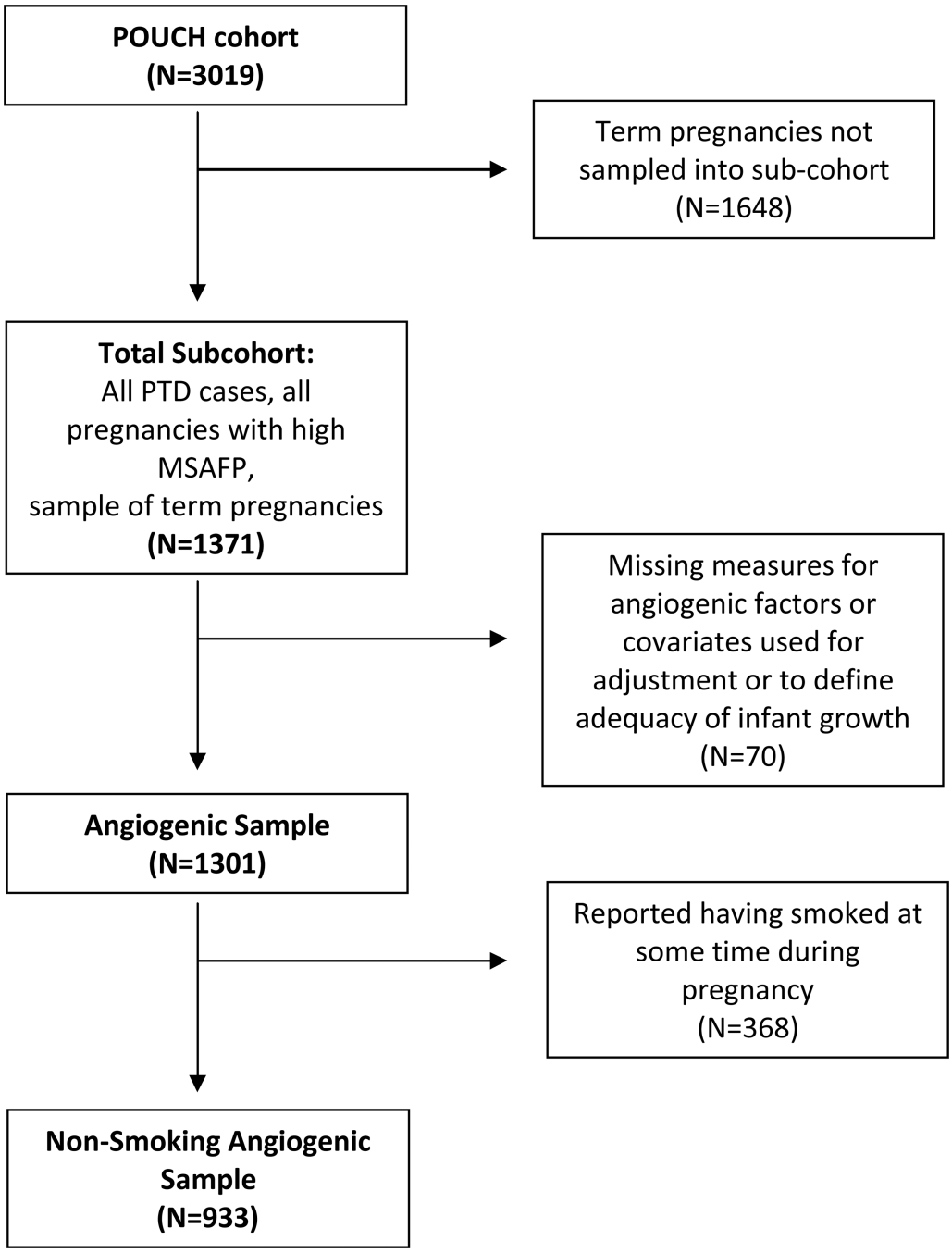

Figure 1.
Flow diagram showing sample derivation, Pregnancy Outcomes and Community Health Study

Table I

Weighted frequencies of maternal and pregnancy characteristics in the POUCH Samples

					Angiogenic Sample, Non-Smokers by Pregnancy Outcome					
	Angiogenic Sample (N=1301)		Angiogenic Sample, Non-Smokers (N=933)		Spontaneous PTD (N=151)		Medically Indicated PTD (N=71)		Term (N=711)	
Characteristics	**N**	**Weighted %**	**N**	**Weighted %**	**N**	**Weighted %**	**N**	**Weighted %**	**N**	**Weighted %**
Race										
Non-African American	765	76.1	552	76.9	98	65.2	49	68.3	405	78.1
African American	536	23.9	381	23.17	53	34.8	22	31.7	306	21.9
Maternal Age										
<20 years	227	14.5	155	12.8	27	18.9	11	15.2	177	12.2
20–29 years	733	56.8	497	54.4	73	48.1	38	52.7	386	54.9
≥30years	341	28.7	281	32.9	51	33.0	22	32.1	208	32.9
Parity										
Primiparous	543	41.0	407	42.5	73	49.5	29	40.5	305	42.1
Multiparous	758	59.0	526	57.5	78	50.5	42	59.5	406	57.9
Mean Enrollment Weight (lb)	1301	174.9	933	173.4	151	170.5	71	180.8	711	173.4
Mean GA at enrollment	1301	22.4	933	22.4	151	21.9	71	22.6	711	22.4
Hypertension in Pregnancy										
Yes	145	10.0	97	8.8	8	5.3	39	46.2	57	7.7
No	1156	90.0	836	91.2	143	94.7	32	53.8	654	92.3
Smoking during Pregnancy										
Yes	368	27.5	-	-	-	-	-	-	-	-
No	933	72.5	-	-	-	-	-	-	-	-
Gestational Age at Delivery										
<32 weeks	38	1.3	29	1.3	22	14.5	7	9.7	-	-
32–34 weeks	69	2.3	50	2.3	28	18.3	22	29.8	-	-
35–36 weeks	207	7.0	143	6.7	101	67.2	42	60.6	-	-
Term (≥37 weeks)	987	89.5	711	89.7	-	-	-	-	711	100

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					Angiogenic Sample, Non-Smokers by Pregnancy Outcome					
	Angiogenic Sample (N=1301)		Angiogenic Sample, Non-Smokers (N=933)		Spontaneous PTD (N=151)		Medically Indicated PTD (N=71)		Term (N=711)	
Characteristics	**N**	**Weighted %**	**N**	**Weighted %**	**N**	**Weighted %**	**N**	**Weighted %**	**N**	**Weighted %**
Delivery Type										
Spontaneous PTL	217	7.3	151	6.9	151	100	-	-	-	-
Medically Indicated	97	3.2	71	3.3	-	-	71	100	-	-
Term (≥37 weeks)	987	89.5	711	89.7	-	-	-	-	711	100
Preeclampsia/SGA[a]										
PE + PTD	27	1.0	23	1.1	-	-	23	33.6	-	-
PE + Term	17	1.7	12	1.6	-	-	-	-	12	1.8
SGA + PTD	27	0.8	22	1.0	11	7.2	11	14.3	-	-
SGA + Term	125	8.6	75	6.4	-	-	-	-	75	7.1
No PE + non-SGA PTD	260	8.7	177	8.2	140	92.8	37	52.1	-	-
No PE + non-SGA Term	845	79.2	624	81.7	-	-	-	-	624	91.0
Missing	0		0							

GA = Gestational Age; PE = Preeclampsia; PTD = Preterm Delivery; PTL = Preterm Labor; SGA = Small-for-Gestational Age [ref 22]

[a]Preeclampsia cases include any case that meets the definition for preeclampsia, including cases in which the infant is SGA. SGA cases were normotensive.

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Table II

Angiogenic marker concentrations among non-smokers in POUCH (N=933, by pregnancy complication)

	Other Pregnancies			SGA Only[c]			All Preeclampsia Cases[d]		
Angiogenic Factor[a]	**N**	**LS-mean Concentration[b] (95% CI)**	**p-value**	**N**	**LS-mean Concentration[b] (95% CI)**	**p-value**	**N**	**LS-mean Concentration[b] (95% CI)**	**p-value i**
sFlt-1	801	1808 (1731–1887)	Ref	97	1889 (1689–2112)	0.48	35	**30332(2393–3843)**	*<0.0001*
PlGF	801	397.3 (381.2–414.1)	Ref	97	**293.4 (256.8–335.2)**	*<0.0001*	35	**224.4 (183.0–275.1)**	*<0.0001*
sEng	801	5.41 (5.29–5.53)	Ref	97	**6.08 (5.48–6.74)**	0.04	35	**7.06 (6.21–8.04)**	*<0.0001*

[a] Adjusted odds ratios (aOR) for each angiogenic factor (sFlt-1: soluble FMS-like tyrosine kinase-1; PlGF: placental growth factor; sEng: soluble endoglin) are adjusted for the following covariates: race, parity, weight at enrollment, gestational age at enrollment, maternal age at enrollment

[b] The adjusted least-squares mean (LS-mean) log angiogenic marker value has been back-transformed to normal scale and concentrations are in pg/mL for sFlt-1 and PlGF, ng/mL for endoglin

[c] SGA=Small-for-Gestational Age [ref 22]

[d] All Preeclampsia Cases=Any case that meets the definition for preeclampsia, including cases in which the infant is SGA.

Table III

Associations between angiogenic markers and PTD among non-smokers in POUCH (N=933, by clinical circumstance)

		Angiogenic sample, non-smokers (N=933)			Angiogenic sample, non-smokers (N=933)					Angiogenic sample, non-smokers excluding PE and SGA[d] (N=801)				
		Term	All PTD		Term	sPTD[d]		MI-PTD[d]		Term	sPTD		MI-PTD	
Angiogenic Factor[a]	Level[b]	N[c]	N	aOR (95% CI)	N	N	aOR (95% CI)	N	aOR (95% CI)	N	N	aOR (95% CI)	N	aOR (95% CI)
sFlt-1	Not High	507	163		507	111		52		450	102		31	
	High	204	59	0.91 (0.63–1.32)	204	40	0.97 (0.68–1.39)	19	0.99 (0.59–1.67)	174	38	0.89 (0.56–1.41)	6	0.63 (0.25–1.57)
PlGF	Not Low	565	156		565	116		40		506	110		26	
	Low	146	66	**1.48 (1.01–2.16)**	146	35	1.06 (0.67–1.68)	31	**2.70 (1.52–4.80)**	118	30	1.12 (0.69–1.83)	11	1.32 (0.56–3.09)
sEng	Not High	499	140		499	103		37		453	99		27	
	High	212	82	**1.60 (1.14–2.24)**	212	48	1.22 (0.81–1.82)	34	**2.66 (1.59–4.44)**	171	41	1.23 (0.80–1.89)	10	1.28 (0.60–2.72)
Total		711	222		711	151		71		624	140		37	

[a] Adjusted odds ratios (aOR) for each angiogenic factor (sFlt-1: soluble FMS-like tyrosine kinase-1; PlGF: placental growth factor; sEng: soluble endoglin) are adjusted for the following covariates: race, parity, weight at enrollment, gestational age at enrollment, maternal age at enrollment

[b] High: concentrations > 75th percentile of the distribution among term pregnancies having normal MSAFP. Low: PlGF concentration < 25th percentile of the distribution among term pregnancies having normal MSAFP.

[c] N represents number of observations for that category which had high sFlt-1, low PlGF, or high sEng levels

[d] sPTD=spontaneous preterm delivery, MI-PTD=medically-indicated PTD, SGA=Small-for-Gestational Age [Ref 22]

Table IV

Associations between angiogenic markers and PTD among non-smokers in POUCH (N=933, by delivery timing)

		Angiogenic sample, non-smokers (N=933)						Angiogenic sample, non-smokers, excluding PE and SGA[d] (N=801)					
		Term	35–36 weeks		32–34 weeks		<32 weeks	Term	35–36 weeks		32–34 weeks		<32 weeks
Angiogenic Factor[a]	**Level[b]**	**N[c]**	**N**	**aOR (95% CI)**	**N**	**aOR (95% CI)**	**N**	**aOR (95% CI)**	**N**	**N**	**aOR (95% CI)**	**N**	**aOR (95% CI)**	**N**	**aOR (95% CI)**
sFlt-1	**Not High**	507	109		37		17		450	92		26		15	
	High	204	34	0.80 (0.51–1.26)	13	0.94 (0.47–1.88)	12	1.52 (0.64–3.61)	174	27	0.78 (0.47–1.29)	9	0.86 (0.36–2.03)	8	1.14 (0.42–3.14)
PlGF	**Not Low**	565	102		40		14		506	90		32		14	
	Low	146	41	1.36 (0.85–2.15)	10	0.88 (0.41–1.90)	15	**4.67 (2.12–0.27)**	118	29	1.17 (0.70–1.96)	3	0.33 (0.09–1.20)	9	**3.35 (1.40–8.03)**
Endoglin	**Not High**	499	98		26		16		453	88		22		16	
	High	212	45	1.28 (0.85–1.93)	24	**2.70 (1.49–4.89)**	13	**1.84 (0.82–4.12)**	171	31	1.14 (0.71–1.82)	13	1.90 (0.92–3.90)	7	1.01 (0.40–2.55)
Total		711	143		50		29		624		119		35		23

[a] Adjusted odds ratios (aOR) for each angiogenic factor (sFlt-1: soluble FMS-like tyrosine kinase-1; PlGF: placental growth factor; sEng: soluble endoglin) are adjusted for the following covariates: race, parity, weight at enrollment, gestational age at enrollment, maternal age at enrollment

[b] High: concentrations > 75th percentile of the distribution among term pregnancies having normal MSAFP. Low: PlGF concentration < 25th percentile of the distribution among term pregnancies having normal MSAFP.

[c] N represents number of observations for that category which had high sFlt-1, low PlGF, or high sEng levels

[d] SGA=Small-for-Gestational Age [Ref 22]

Table V

Associations between High and Low sFlt-1 and PTD among non-smokers in POUCH (N=933)

		Angiogenic sample, non-smokers (N=933)					Angiogenic sample, non-smokers, excluding PE and SGA[d] (N=801)				
		Term	sPTD		MI PTD		Term	sPTD		MI PTD	
Angiogenic Factor[a]	Level[b]	N[c]	N	aOR (95% CI)	N	aOR (95% CI)	N	N	aOR (95% CI)	N	aOR (95% CI)
sFlt-1	**High**	204	40	0.81 (0.52–1.27)	19	1.14 (0.62–2.10)	174	38	0.83 (0.52–1.34)	6	0.80 (0.38–2.13)
	Intermediate	352	83		33		303	76		16	
	Low	155	28	0.83 (0.50–1.37)	19	1.22 (0.67–2.29)	147	26	0.77 (0.45–1.29)	15	**1.88 (0.89–3.96)**
Total		711	151		71		624	130		37	

[a] Adjusted odds ratios (aOR) for each angiogenic factor (sFlt-1: soluble FMS-like tyrosine kinase-1) are adjusted for the following covariates: race, parity, weight at enrollment, gestational age at enrollment, maternal age at enrollment

[b] High: concentrations > 75th percentile of the distribution among term pregnancies having normal MSAFP. Low: concentration < 25th percentile of the distribution among term pregnancies having normal MSAFP.

[c] N represents number of observations for that category which had high sFlt-1, low PlGF, or high sEng levels

[d] sPTD=spontaneous preterm delivery, MI-PTD=medically-indicated PTD, SGA=Small-for-Gestational Age [Ref 22]