Published in final edited form as:
J Matern Fetal Neonatal Med. 2009 December ; 22(12): 1122–1139. doi:10.3109/14767050902994838.

A SUBSET OF PATIENTS DESTINED TO DEVELOP SPONTANEOUS PRETERM LABOR HAS AN ABNORMAL ANGIOGENIC/ANTI-ANGIOGENIC PROFILE IN MATERNAL PLASMA: EVIDENCE IN SUPPORT OF PATHOPHYSIOLOGIC HETEROGENEITY OF PRETERM LABOR DERIVED FROM A LONGITUDINAL STUDY

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Abstract

OBJECTIVE—An imbalance between angiogenic and anti-angiogenic factors in maternal blood has been observed in several obstetrical syndromes including preeclampsia, pregnancies with fetal growth restriction, and fetal death. Vascular lesions have been identified in a subset of patients with spontaneous preterm labor (PTL). It is possible that PTL may be one of the manifestations of an anti-angiogenic state. The aim of this study was to determine if patients prior to the clinical diagnosis of PTL leading to preterm delivery had plasma concentrations of angiogenic and anti-angiogenic factors different from normal pregnant women.

STUDY DESIGN—This longitudinal nested case-control study included normal pregnant women (n=208) and patients with PTL leading to preterm delivery (n=52). Maternal blood samples were collected at 6 gestational age intervals from 6-36.9 weeks of gestation. The end point (time of diagnosis) of the study, "True PTL", was defined as patients presenting with PTL and delivered within 1 day. Plasma concentrations of sVEGFR-1, sVEGFR-2, sEng and PlGF were determined by ELISA. Analysis was performed with both cross-sectional and longitudinal (mixed effects model) approaches.

RESULTS—1) Plasma sEng concentration in patients destined to develop PTL was higher than that in normal pregnant women from 15-20 weeks of gestation. The difference became statistical significant at 28 weeks of gestation, or approximately 5-10 weeks prior to the diagnosis of "true

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Presented at the 56th Annual Scientific Meeting of the Society for Gynecologic Investigation, Glasgow, Scotland, March 17-21, 2009.

PTL". 2) Backward analysis suggests that plasma concentrations of PlGF and sVEGFR-2 were lower, and those of sVEGFR-1 were higher in patients with PTL than in normal pregnant women less than 5 weeks prior to the diagnosis of "true PTL"; and 3) Plasma concentrations of sEng and sVEGFR-1 were higher and those of PlGF and sVEGFR-2 were lower in patents diagnosed with PTL and delivery within 1 day than in normal pregnant women who delivered at term.

CONCLUSION—The changes in sEng are demonstrable several weeks prior to the onset of preterm parturition. In contrast, the changes in the other angiogenic proteins are present close to the onset of PTL and delivery. This observation supports the view that an imbalance of angiogenic factors participates in the pathophysiology of spontaneous preterm parturition.

Keywords

preterm labor; preterm parturition; endoglin; VEGFR-1; VEGFR-2; PlGF; angiogenesis; longitudinal study; prematurity; placenta

INTRODUCTION

Spontaneous preterm delivery is the leading cause of perinatal mortality and morbidity worldwide [1-3]. Despite considerable effort, the incidence of preterm birth is still rising.[4] We have proposed that spontaneous preterm parturition is a syndrome[1]resulting from multiple pathological processes including intrauterine infection [5-12], uterine overdistension [13-16], allergic-like reaction [17-19], cervical disease [20-22], endocrine disorders [23-26], maternal or fetal stress[27-31]and uterine ischemia. Evidence in support for a role of utero-placental ischemia as a mechanism of disease leading to preterm labor (PTL) includes: 1) an experimental study showed that, after induced uterine ischemia (designed to generate a primate model for preeclampsia), a proportion of animals went into PTL and delivery [32]; 2) patients with PTL and preterm PROM who delivered preterm had a higher percentage of failure of physiologic transformation in myometrial segment of the spiral arteries than women who delivered at term [33,34]; 3) increased impedance to flow in uterine artery in the second trimester increased risk of preterm delivery [35]. Similarly, patients presenting with PTL who had an abnormal uterine artery Doppler velocimetry were more likely to deliver preterm than those with normal Doppler velocimetry [36]; and 4) the presence of vascular lesions in decidual vessels attached to the placenta was more common in patients with PTL and delivery than in women delivered at term gestation [37]. However, the precise mechanisms responsible for the onset of preterm parturition in cases of ischemia have not been determined. Recently, an imbalance between angiogenic and anti-angiogenic factors in maternal blood has been observed in several obstetrical syndromes with perturbation in utero-placental blood supply including preeclampsia [38-56], fetal growth restriction [57-59], placental abruption [60], "mirror syndrome" [61], twin-to-twin transfusion syndrome(TTTS) [62] and unexplained fetal death [63].

Angiogenesis, a process by which new vessels are formed from pre-existing vasculature, is regulated by several growth factors, cytokines and their receptors, including fibroblast growth factors, transforming growth factors, hepatocyte growth factors, angiogenins, angiopoietins, ephrins, and vascular endothelial growth factors (VEGF) [64,65]. VEGF-signaling represents a critical step in both physiologic and pathologic angiogenesis [65,66]. VEGF is an endothelial cell-specific growth factor with potent angiogenic properties. While VEGF receptor-1 (VEGFR-1) is considered a 'decoy' receptor, VEGFR-2 is the major mediator of the mitogenic, angiogenic, permeability enhancing, and endothelial survival effects of VEGF [67]. Placental growth factor (PlGF), another member of the VEGF family, can also bind to VEGFR-1 on endothelial cells or on macrophages, and enhance the angiogenic response of VEGF on endothelial cells or induce migration of macrophages,

especially in pathological conditions such as limb ischemia or wound healing [68-70]. The soluble form of VEGFR-1 (sVEGFR-1) has potent anti-angiogenic activity since it can bind to VEGF or PlGF and inhibits their biological functions. The natural form of soluble VEGFR-2 (sVEGFR-2) has recently been detected in human plasma [71]. Under experimental conditions, this protein can bind to VEGF[71] and its recombinant form has anti-angiogenic activity [72,73]. The role of sVEGFR-2 in human health and diseases is still unclear. Endoglin (Eng) is a co-receptor of transforming growth factor (TGF)-β and its soluble form (sEng) has anti-angiogenic activity by modulating the actions of TGF-β1 and TGF-β3 [74].

A balance between angiogenic and anti-angiogenic factors is essential for feto-placental development [75-78]. However, there is a paucity of information regarding the changes of these factors in patients destined to develop spontaneous PTL. The aim of this study was to determine if patients prior to the clinical diagnosis of PTL leading to preterm delivery had plasma concentrations of PlGF, sVEGFR-1, sEng and sVEGFR-2 which were different from normal pregnant women.

PATIENTS AND METHODS

Study Design

A retrospective nested longitudinal case-control study was conducted by searching our clinical database and bank of biologic samples from 2002-2006. Patients with spontaneous PTL and delivery (n=52) and normal pregnant women (n=208) were included. Exclusion criteria were 1) patients with chronic hypertension, preeclampsia or gestational hypertension; 2) known major fetal or chromosome anomaly; and 3) multiple gestations. All women were enrolled in the prenatal clinic at the Sotero del Rio Hospital, Santiago, Chile and followed until delivery. Prenatal visits were scheduled at 4-week intervals in the first and second trimester, and every two weeks in the third trimester until delivery. Blood sampling was performed at enrollment and every visit with the patient's consent.

For this study, subjects were included only if they had plasma samples available at least once before and after 24 weeks of gestation. All patients had a minimum of three samples during pregnancy (3-6 samples). Plasma samples were selected once from each patient of the following six intervals: 1) 6-14 weeks; 2) 15-19 weeks; 3) 20-24 weeks; 4) 25-27 weeks; 5) 28-31 weeks; and 6) 32-36 weeks of gestation. The earliest sample for each interval was used.

Clinical definition

Spontaneous preterm labor and delivery was defined by the presence of regular uterine contractions and cervical changes that led to delivery before 37 completed weeks of gestation. Gestational age was determined by the last menstrual period or by ultrasound in case the ultrasonographic determination of gestational age was not consistent with the menstrual dating by >2 weeks. This study included only patients who underwent ultrasound examination for dating before 24 weeks of gestation. An amniocentesis was performed in some patients to assess the microbiologic state of the amniotic cavity at the discretion of the responsible physicians. Some patients may have multiple episodes of preterm contractions or preterm labor without leading to delivery. The end point (time of diagnosis) of the study was "true preterm labor and delivery" defined as preterm labor that leads to preterm delivery within one day. Samples that were taken during episodes of "false preterm labor or preterm contraction" within one day were excluded. Pregnant women were considered normal if they had no medical, obstetrical or surgical complications, and delivered a normal term (> 37 weeks) infant whose birthweight was appropriate for gestational age (10^{th}-90^{th} percentile)

[79]. Acute histologic chorioamnionitis and acute funisitis were diagnosed using previously described criteria [80]. Pathologic findings consistent with maternal hypoperfusion and inflammation were defined according to Redline [81].

The collection and utilization of the samples was approved by both the Human Investigation Committee of the Sotero del Rio Hospital, Santiago, Chile (a major affiliate of the Catholic University of Santiago) and the IRB of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD/NIH/DHHS). Many of these samples have been used in previous studies.

Sample collection and angiogenic factors immunoassays—Venipunctures were performed and the blood was collected into tubes containing EDTA. Samples were centrifuged and stored at −70°C. Maternal plasma concentrations of PlGF, sVEGFR-2, sEng and sVEGFR-1 were determined by sensitive and specific immunoassays obtained from R&D Systems (Minneapolis, MN). All four immunoassays utilized the quantitative sandwich enzyme immunoassay technique and their concentrations in maternal plasma were determined by interpolation from the standard curves. The inter- and intra-assay coefficients of variation (CV) obtained were: PlGF: 6.02% and 4.8%, respectively, sVEGFR-2: 2% and 4%, respectively; sEng: 2.3% and 4.6% respectively; and sVEGFR-1: 1.4% and 3.9%, respectively. The sensitivity of the assays were: PlGF: 9.52 pg/ml; sVEGFR-2: 19.01 pg/ml; sEng: 0.08 ng/ml and sVEGFR-1: 16.97 pg/ml.

Statistical analysis

Cross-sectional analysis—Shapiro-Wilk and Kolmogorov-Smirnov tests were used to test for normal distribution of the data. Kruskal-Wallis and post-hoc Mann-Whitney U tests were utilized to determine the differences of the median among and between groups. Chi-square and Fischer's Exact tests were employed for comparisons of proportions. Multivariate logistic regression was applied to examine the association between high plasma concentrations of sEng or sVEGFR-1 (defined as plasma sEng or sVEGFR-1 concentrations above the third quartile of normal pregnancy), low plasma concentrations of PlGF or sVEGFR-2 (defined as plasma PlGF or sVEGFR-2 concentrations below the first quartile of normal pregnancy) and the development of "true spontaneous preterm parturition" in samples obtained prior to the clinical diagnosis of the disease in various gestational age intervals after adjusting for potential confounders. Cox-regression analysis was applied to determine the association of high or low plasma angiogenic factor concentrations and duration to delivery while adjusting for potential confounders. The statistics package used was SPSS V.15 (SPSS Inc., Chicago, IL). A p value of <0.05 was considered significant.

Longitudinal analysis—Changes in the plasma concentrations of the four angiogenic factors over time and between groups were tested using a linear mixed effects model (fixed effects + random effects). The fixed effects were the diagnosis (a factor with two levels: normal pregnancy and PTL), the linear and quadratic effects of gestational age, the interaction term between the diagnosis and gestational age, and other covariates including: maternal age, body mass index (BMI), smoking, nulliparity, previous preeclampsia, and sample storage time. The random effects were the patient identification numbers, therefore allowing examination of the deviation of each individual from the average profile of each diagnostic group and accounting for the unknown variability among patients. The model was fitted to the transformed plasma concentration [log10 concentration+1)] of the analytes. This logarithmic transformation was employed to achieve normality of the data and stabilize variance across the entire range of gestational age. Statistical significance of fixed effects was assessed using t-scores, and a p value < 0.05 was considered significant. A false discovery rate algorithm was applied to adjust for multiple analytes and multiple covariates.

The analysis was performed using the *nlme* (Nonlinear Mixed Effect Model) package of the R statistical environment (www.r-project.org).

To identify the gestational age at which the difference in the median concentration between groups became significant, a moving window approach was used. Unlike in the cross-sectional study, where the limits of the intervals were predetermined in the moving window approach, the number of data points was fixed to 250 for each window. All observations were sorted as a function of the gestational age in ascending order. A set of 250 data points was chosen starting with the smallest gestation age and moving up the list. A Wilcoxon test was used to determine if there was significant difference between the 2 groups in each window of gestational age. The procedure was repeated until the point when the difference between groups remained significant ($p<0.05$) in the current window and all consecutive ones. The median gestational age in the current window recorded.

RESULTS

Clinical characteristics of the study population are displayed in Table I. As expected, patients with spontaneous PTL and delivery had a history of preterm delivery more frequently than those of normal pregnant women who delivered at term. There were 7 (13.5%) and 13 (25%) patients who delivered before 32 and 34 weeks of gestation, respectively. Eleven patients underwent amniocentesis and 2 (18%) had a positive amniotic fluid culture result for *Candida albican*s.

The gestational age at which patients presented with "true spontaneous preterm labor and delivery" varied. One patient was diagnosed with PTL and delivered at 26 weeks of gestation, five at 28-31 weeks, and forty-six at 32-36 weeks. There was no significant difference in the median gestational age at which venipuncture was performed by the interval window between the group of patients who delivered preterm and the control group (all $p>0.05$, Table II).

Plasma sEng concentrations are elevated prior to the clinical manifestation of "true spontaneous PTL and delivery": forward cross-sectional approach

The median plasma sEng concentrations in women who subsequently experienced PTL and delivery were higher than in normal pregnant women from 15-19 weeks of gestation until delivery ($p<0.05$, except at 32-37 weeks $p=0.06$; Table II). In contrast, no significant difference in the median plasma concentration of sVEGFR-1, PlGF and sVEGFR-2 between patients with PTL before the clinical diagnosis and normal pregnant women was observed in any of the six gestational age intervals, except one. The median plasma concentration of PlGF in patients who subsequently delivered preterm was significantly lower than that of those with a normal pregnancy at 28-31 weeks of gestation ($p=0.03$; Table II).

To examine the association between high plasma concentrations of sEng (above the 3rd quartile of normal pregnancy) and the development of spontaneous PTL and delivery, multivariate logistic regression was applied to adjust for potential confounders. For this analysis, samples obtained at the time of diagnosis were excluded. The dependent variable in the logistic model was the presence of PTL. Different cut-offs for high plasma sEng concentration in various gestational age intervals as well as unadjusted and adjusted odds ratios are displayed in Table III. High plasma sEng concentrations at 28-31 and 32-36 weeks of gestation conferred the risk of spontaneous PTL and delivery with an odds ratio of 3.1 (95% CI 1.3-7.2) and 6.6 (95% CI 1.5-28.6), respectively, after adjusting for maternal age, BMI, smoking, nulliparity, previous preterm labor, gestational age at blood sampling, sample storage time. There was no significant association between the development of spontaneous PTL and high plasma sEng concentrations at any other gestational age intervals

(Table III) after adjusting for potential confounders. Similar analysis was applied to sVEGFR-1, PlGF and sVEGFR-2, all of these high (above the 3rd quartile) or low (below the 1st quartile) plasma angiogenic factor concentrations showed no significant association with the development of spontaneous PTL (data not shown).

Cox-regression analysis was applied to determine the association of high or low plasma angiogenic factor concentrations and the duration from blood sampling to delivery interval while adjusting for gestational age at blood sampling, maternal age, BMI, smoking, nulliparity, previous preterm delivery, sample storage time. Among patients with PTL prior to the diagnosis and normal pregnant women at 32-37 weeks, high plasma sEng concentrations, high plasma sVEGFR-1 concentrations and low plasma PlGF concentrations were associated with shorter duration to delivery [hazard ratio 1.5 (95% CI 1.02-2.2), 1.7 (95% CI 1.1-2.5), and 2.4 (95% CI 1.7-3.6) respectively. In contrast, when the analysis was restricted to normal pregnant women who delivered at term, only plasma sVEGFR-1 and PlGF were associated with shorter duration of the venipuncture to delivery interval with similar hazard ratios. High plasma sEng concentrations and low plasma sVEGFR-2 concentrations were not associated with a shorter duration to delivery interval [hazard ratios of 0.8 (95% CI 0.5-1.1) and 0.7 (95% CI 0.5-1.1), respectively].

Plasma sEng concentrations are elevated prior to the clinical manifestation of PTL: longitudinal approach

Patients who subsequently delivered preterm had a significantly different profile (plasma concentration over time) of plasma sEng concentration from patients with normal pregnancies after adjusting for gestational age at blood sampling, maternal age, BMI, nulliparity, history of preterm delivery, smoking and duration of sample storage ($p=0.04$; Table IV). Plasma sEng concentrations were higher in women destined to develop PTL than in normal pregnant women from approximately 15 weeks of gestation. The difference became statistically significant ($p<0.05$) at 27.7 weeks of gestation (see Statistics section), and was more pronounced as delivery approached (Figure 1).

Patients who subsequently delivered preterm also had a significantly different profile of plasma sVEGFR-1 concentration from that of normal pregnant women ($p=0.003$; Table IV). Plasma sVEGFR-1 concentrations in patients with PTL were lower than those in normal pregnant women from 10-28 weeks of gestation. After that gestational age, plasma concentrations of sVEGFR-1 became higher until the time of delivery (Figure 2). In contrast, there was no significant difference in the profiles of plasma concentrations of PlGF and sVEGFR-2 between patients with PTL and normal pregnant women ($p>0.05$; Table V). However, upon observations of the profiles of sVEGFR-1, PlGF and sVEGFR-2, there was an increase in plasma sVEGFR-1 concentrations, but a decrease in plasma PlGF and sVEGFR-2 concentrations just prior to the time of diagnosis of “true PTL” (Figure 2, 3 and 4 respectively). Individual changes in maternal plasma concentration of sEng, sVEGFR-2, sVEGFR-1 and PlGF in normal pregnant women and patients destined to develop preterm labor and delivery in relation to gestational age were displayed in Figure 5 and 6.

Plasma concentrations of sEng are elevated prior to the clinical manifestation of PTL: (backward analysis)

To examine the relationship between plasma concentrations of angiogenic / antiangiogenic factors and the interval-to-clinical diagnosis of PTL leading to preterm delivery, plasma samples of PTL patients at different gestational ages were stratified according to the interval from blood sampling to clinical diagnosis into seven groups: 1) at clinical diagnosis; 2) 2 to 14 days before diagnosis; 3) 15 to 35 days before diagnosis; 4) 36 to 70 days before diagnosis; 5) 71 to 105 days before diagnosis; 6) 106-140 days before diagnosis; and 7)

more than 140 days before diagnosis. Plasma samples from normal pregnant women were matched for gestational age with the plasma samples of PTL patients at different gestational ages according to the intervals pre-specified above (e.g. 2 to 14 days before diagnosis, etc.).

The median plasma sEng concentration was significantly higher in patients who developed PTL than in normal pregnant women at clinical diagnosis, at 2 to 14 days, 15 to 35 days, 36 to 70 days, and at 106 to 140 days before the clinical diagnosis (all $p<0.05$; Table VI). No significant differences in the median plasma sEng concentration were observed between patients who developed PTL and the normal control group, both at 71 to 105 days before the diagnosis and more than 140 days before clinical diagnosis of PTL (both $p>0.05$, Table VI).

In contrast, the median plasma concentration of sVEGFR-1 was higher and that of sVEGFR-2 was lower in patients with PTL than that of normal pregnant women at clinical diagnosis and at 15 to 35 days (median 27 days) before the clinical diagnosis (all $p<0.05$ Table VI). Women with PTL had a median plasma concentration of PlGF lower than that of normal pregnant women at clinical diagnosis ($p<0.001$) and at 2-14 days (median days) before the diagnosis. However, the difference did not reach statistical significance at interval 2-14 days ($p= 0.054$; Table VI).

Women with high plasma sEng concentration (above the 3rd quartile) had a high rate of placental pathologic findings consistent with maternal underperfusion

Among patients with PTL, placental pathology results were available in 30 cases (57%). Four (13%) cases of pathologic findings in the placenta were consistent with lesions indicative of maternal hypoperfusion, and 5 (17%) cases were consistent with histologic chorioamnionitis and/or funisitis. Pathologic findings consistent with maternal hypoperfusion, but not inflammation, were associated with high plasma concentrations of sEng at gestational age intervals of 15-19 and 28-31 weeks ($p<0.05$; Table VII). Either high plasma concentrations of sVEGFR-1 or low plasma concentrations of PlGF as well as sVEGFR-2 were not associated with pathologic findings consistent with maternal hypoperfusion (all $p>0.05$; data not shown).

DISCUSSION

Principal findings

1) A subset of mothers destined to develop spontaneous preterm labor and delivery had higher plasma sEng concentrations than those who had a normal pregnancy. This difference was detectable several weeks prior to the clinical diagnosis of preterm labor; 2) the median plasma sEng concentration in patients destined to develop preterm labor/delivery was elevated from 15-20 weeks of gestation onwards, and became significantly different from normal pregnant women at 28 weeks of gestation or approximately 5-10 weeks prior to the diagnosis of preterm labor, and the difference was more pronounced as preterm labor and delivery approached; 3) among normal pregnant women and patients destined to develop preterm labor and delivery, high plasma sEng, high plasma sVEGFR-1, and low plasma PlGF concentrations at 32-37 weeks were associated with a shorter duration to delivery interval after adjusting for potential confounders including gestational age at blood sampling; 4) backward analysis suggests that plasma concentrations of PlGF and sVEGFR-2 are lower and those of sVEGFR-1 are higher than those of normal pregnant women less than 5 weeks prior to the diagnosis of preterm labor and delivery; and 5) plasma concentrations of sEng and sVEGFR-1 were higher and those of PlGF and sVEGFR-2 were lower in patents diagnosed with preterm labor and delivery within 1 day than in normal pregnant women who delivered at term.

Plasma concentrations of pro-angiogenic and anti-angiogenic factors change prior to the diagnosis of spontaneous preterm labor and delivery

The findings that women destined to develop preterm labor and delivery had an increase in plasma sEng and sVEGFR-1 concentrations, a decrease in plasma PlGF and sVEGFR-2 concentrations several weeks prior to the diagnosis of PTL and delivery are consistent with a study of Tsai et al. (published in an abstract form) who reported that plasma concentrations of sEng and sVEGFR-1 concentrations were elevated and those of PlGF were decreased before the diagnosis of spontaneous PTL and delivery [82]. These observations suggest that an imbalance of angiogenesis is involved in the pathophysiology of a subset of patients with spontaneous preterm parturition. Moreover, these findings support the view that the differential expression of angiogenesis–related genes or proteins in gestational tissues observed in studies comparing patients with and without labor is not a consequence of wound healing or tissue remodeling associated with the physical stress of labor, but is more likely to be casual in the preparation for labor [83].

Plasma sEng concentrations are elevated 5-10 weeks prior to the diagnosis of spontaneous preterm labor and delivery

Among the studied angiogenic factors, plasma sEng concentration is the earliest one to increase in patients destined to deliver preterm. Although forward cross-sectional analysis suggested that this change began at 15-20 weeks of gestation, it became statistically different from that of normal pregnant women at 28 weeks of gestation after adjusting for potential confounders. Backward analysis indicated that the pattern of elevation of plasma sEng concentration was bimodal. The first elevation was at 15-20 weeks prior to preterm labor and a second elevation was observed at 5-10 weeks prior to preterm delivery. It is likely that the elevation in plasma sEng concentrations in patients with preterm labor is not a signal to initiate labor, but rather reflects perturbation in blood supply to the placenta.

Eng is expressed on vascular endothelial cells, vascular smooth muscle cells, cytotrophoblasts, syncytiotrophoblasts, uterine stromal cells, monocytes and hematopoietic stem cells [74,84]. Elevated plasma sEng concentrations have been reported in obstetrical conditions with perturbation of blood supply to the uterus such as preeclampsia [38-45,48,49,51,52,56], and pregnancies with fetal growth restriction [57-59]. Evidence suggests that over-expression of Eng on trophoblasts could inhibit trophoblast differentiation and invasion during placentation [85,86]. Systemic elevation of plasma sEng concentrations in patients with PTL might reflect the changes of this protein locally in the feto-maternal interface and prevent the remodeling of spiral arteries resulting in reduced blood supply to the placenta. The remodeling process of the spiral arteries, although begins in early pregnancy, continues until the third trimester as late normalization of abnormal uterine artery Doppler velocimetry has been documented [87]. Consistent with this hypothesis, in the current study we observed an association between high plasma sEng concentrations and the presence of pathological lesions in the placenta which were consistent with poor maternal perfusion. The finding of an elevation of plasma sEng concentrations in patients destined to develop preterm labor observed herein was of a lesser magnitude than that observed in patients destined to develop preeclampsia and that of pregnancies destined to be diagnosed to have an SGA neonate. Thus, it seems that all these conditions have in common a degree of utero-placental ischemia. This interpretation is consistent with the findings in the placental bed of patients with preterm labor [33].

Alternatively, an elevation of plasma sEng concentrations, particularly for the second elevation, in patients with preterm labor could indicate the changes in gestational tissues in preparation for labor. TGF-β plays a pivotal role in cyclic growth and remodeling in uterine epithelial [88], stromal [89], endothelial [90] and myometrial cells [91,92]. Since Eng is a

co-receptor of TGF-β, it can modulate the function of TGF-β1 as well as TGF-β3. TGF-β1 has been proposed to participate in human parturition by up-regulating the ryanodine-sensitive intra-cellular Ca2+ release channel, a contraction-associated protein [93,94]. By triggering the synthesis of this protein, the smooth muscle cells of the uterus are transformed from quiescent (phase 0) into activated state (phase I) and primed to response to endogenous uterotonin (eg: oxytocin, prostaglandins) [95]. Moreover, the protein expression of TGF-β1 in myometrium is elevated during pregnancy and further increased after labor, while TGF-β receptor type I and II protein expression in myometrium increased before labor and down-regulated after term labor [94]. The temporal change of TGF-β and its receptor protein expression in myometrium suggests that the TGF-β system plays a role in preparation of myometrium at term [91,94]. Moreover, TGF-β1 has been demonstrated to inhibit the production of prostaglandin E2 by human decidual [96] and amnion cells [97]. Although Eng is highly expressed at the surface of mouse uterine stromal cells and has been shown to modulate the TGF-β-induced cell proliferation [89], it remains to be determined if Eng is expressed in human myometrium, uterine cervix, choriodecidua and amnion cells, components of the common pathways of parturition [1].

Plasma concentration of PlGF, sVEGFR-2 is decreased and that of sVEGFR-1 is increased less than 5 weeks prior to the diagnosis of spontaneous preterm labor and delivery

Although longitudinal analysis suggested that the profile of plasma sVEGFR-1 concentration in patients destined to deliver preterm was different from that of normal pregnant women, we did not observe a statistically significant difference between the two groups at any gestational age intervals using a forward cross-sectional analysis. Moreover, longitudinal and forward cross-sectional analysis also suggests that there was no difference in the profiles of plasma PlGF and sVEGFR-2 between the two groups. In contrast, backward analysis demonstrated that plasma concentrations of PlGF and sVEGFR-2 are lower and those of sVEGFR-1 are higher than normal pregnant women less than 5 weeks prior to the diagnosis of PTL and delivery. We suspect that the reason for this apparent discrepancy is related to the study design. In this study, maternal blood was sampled every 4 weeks, and this interval may have been too long to detect any rapid changes in plasma concentrations of these angiogenic/anti-angiogenic factors which start a short period of time prior to delivery. By the time “true preterm labor” was diagnosed, the plasma concentrations of sVEGFR-1 were already higher and those of PlGF and sVEGFR-2 were already lower in patents with preterm labor and delivery within 1 day than in normal pregnant women who delivered at term. Interestingly, among normal pregnant women at 32-36 weeks of gestation, high plasma sVEGFR-1 and low plasma PlGF concentrations, but not high plasma sEng and low plasma sVEGFR-2 concentrations, were associated with shorter duration to delivery after adjusting for gestational age at blood sampling. These observations indicate that the changes in plasma sVEGFR-1 and PlGF, but not those of sEng and sVEGFR-2, observed in patients destined to deliver preterm in the last 4-5 weeks might follow the same pattern as those in normal pregnancy. Future studies should be designed to allow blood sampling once a week in the last 4-6 weeks before term (longitudinal study) to confirm this hypothesis. We have previously reported that there was no significant difference in the median delta plasma sVEGFR-1 concentration between patients presenting with preterm labor and intact membranes and normal pregnancy as well as among preterm labor subgroups including patients with preterm labor who delivered at term gestation, those who delivered preterm without intra-amniotic infection (IAI) and those who delivered preterm with IAI [63]. The difference in the study design (cross-sectional vs. longitudinal) and the endpoint of both studies (preterm delivery at any gestational age after blood sampling vs. preterm delivery within 1 day of diagnosis) could explain the apparent discrepancy in the results of our studies.

Clinical implication

The observations in this study could explain, at least in part, the false positive results of angiogenic/anti-angiogenic markers in the prediction of preeclampsia, especially when using plasma sEng concentration in the second trimester. It is of interest that unlike the angiogenic/anti-angiogenic profile observed in preeclampsia, there was no significant change in the maternal plasma concentration of other angiogenic (PlGF) or anti-angiogenic factors (sVEGFR-1 or sVEGFR-2) in the second trimester in patients who subsequently had spontaneous preterm delivery. Therefore, we propose that the changes in maternal plasma angiogenic and anti-angiogenic factors are stereotypic for each of the "Great Obstetrical Syndromes"[98], and that the profile and magnitude of different angiogenic and anti-angiogenic factor concentrations at a specific time in gestation is associated with the development of different complications of pregnancy, such as: 1) fetal death [99]; 2) early onset preeclampsia [44,46,49,51]; 3) pregnancies with small for gestational age fetuses (SGA) [49,51] ; 4) term preeclampsia [44,46,49,51], and 5) preterm labor.

Preeclampsia, preterm labor with intact membranes, preterm premature rupture of membranes (PROM), fetal death and pregnancies with SGA fetuses are all considered the "Great Obstetrical Syndromes" resulting from multiple etiologies [98]. Moreover, it is now apparent that the same mechanisms of disease (e.g. intravascular inflammation, an anti-angiogenic state, etc.) may be shared by several of the "Great Obstetrical Syndromes" at the time of the onset of the disease or even before. Intravascular inflammation (previously implicated in the pathophysiology of preeclampsia [99-104]) has subsequently been reported in SGA [105], preterm labor with intact membranes [106] and preterm PROM [107]. Moreover, unexplained fetal death was associated with changes in the adaptive limb of the maternal immune response consistent with prior antigenic exposure [108]. Thus, there is similarity between the observations made with flow cytometry in these "Great Obstetrical Syndromes", and those that we report by examining the concentrations of angiogenic and anti-angiogenic factors (e.g. preeclampsia [38-45,47-56], pregnancies with SGA fetuses and abnormal Doppler velocimetry [57-59], "mirror syndrome"[61], TTTS [62] and unexplained fetal death [63].

Strengths and limitations

Several studies have documented the differentially regulated gene or protein expression in myometrium [83,109-112], cervix [113-115], and chorioamniotic membranes[116-119] or terminal villi in the placenta[120] after term[111,112,121] and preterm parturition [110,114,122]. Other than genes encoding proteins involved in prostaglandin synthesis and in the control of the inflammatory response (chemokines, cytokines, etc.), angiogenesis-related genes have been identified as differentially regulated in labor (in the common pathway of parturition) supporting a role of angiogenesis in parturition [83,113]. Although the changes of angiogenic factors in maternal plasma might not reflect protein expression in gestational tissue, the advantage of studying maternal plasma is that we can examine the temporal changes in angiogenic factors systemically prior to spontaneous parturition. Longitudinal sampling of tissue could not be performed in human gestational tissue in an on-going pregnancy due to an obvious reason.

The limitation of this study is that patients with PTL were carefully selected and might not represent PTL patients in general population. We applied strict criteria for the diagnosis of "true PTL" as an endpoint and excluded blood samples that were obtained within one day of an episode of false labor. This was done to avoid the possibility of changes in the plasma angiogenic factor determination related to an episode of false preterm labor or preterm contractions, since either one of these two may change the profile of these angiogenic factors. Further studies are required to test whether this is the case or not. We also excluded

patients who delivered by induction of labor and included only cases with at least three serial samples of plasma. The majority (87%) of patients with PTL in this study delivered after 32 weeks, which represents the majority of preterm birth in general population [4]. However, it can be argued that an abnormality of blood vessel development may be more relevant as a mechanism of disease in late preterm birth than in early preterm birth. Previous studies by our group [123-126], as well as those reported by others [3, 127-130], indicate that the lower the gestational age at delivery, the higher the rate of infection/inflammation.

In this study, we examined the association between the maternal plasma concentration of angiogenic/anti-angiogenic factors and covariates [eg: pregnancy outcome (normal pregnancy or PTL), gestational age, previous preterm delivery, etc.] using longitudinal data. The analysis cannot be performed via classical generalized linear models or repeated measure analysis of variance. Using a simple linear model on these longitudinal data would over-state the significance of the covariates, while the repeated measure analysis could not be used due to missing samples in some patients. In contrast, the linear mixed-effects model analysis, used herein, is among the statistical tools able to handle the repeated, yet correlated data, with occasional missing observations from the same individuals by allowing each individual to have its own “random effect” on the baseline analyte concentrations. The ability of these models to fit the observed data is improved over classical linear models (F test p-value <0.0001 for all 4 analytes). Both the group effect magnitude and significance presented in Tables (IV and V) were extracted from the linear mixed-effects model analysis.

We have incorporated all data points included in the linear mixed-effects model analysis in the figures to enable the reader to visualize the main trends in the raw data of each study group on a logarithmic scale. The curves in the figures (one for each clinical group) represent a quadratic fit of the analyte concentrations based on the gestational age alone. The purpose of these over all curves is to depict each group’s average concentration at a given gestational age.

Finally, we have presented the changes of plasma concentrations of angiogenic/anti-angiogenic factors in PTL across all gestational age ranges. However, this was based on the assumption that the early (eg: < 32 weeks of gestation) and the late PTL groups had similar profiles of angiogenic/anti-angiogenic factor concentrations. Of note, 87% of patients with PTL in this study delivered after 32 weeks of gestation. A larger study that is specifically designed to address the question of whether early PTL and delivery has a different profile of angiogenic/anti-angiogenic factor concentrations compared to late preterm delivery is warranted.

In conclusion, our study demonstrates that plasma sEng concentration in women destined to develop preterm labor and delivery was elevated from 15-20 weeks of gestation and became significantly higher compared to normal pregnant women approximately 5-10 weeks prior to the clinical manifestation of the disease. In contrast, the changes in plasma concentrations of sVEGFR-1, PlGF and sVEGFR-2 were detectable within 5 weeks prior to spontaneous preterm labor and delivery. These changes were more pronounced as the patient approached the time of preterm labor and delivery. These observations support the view that an imbalance of angiogenic/anti-angiogenic factors participates in the pathophysiology of preterm parturition in a subset of patients.

Acknowledgments

This research was supported (in part) by the Perinatology Research Branch, Division of Intramural Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, DHHS.

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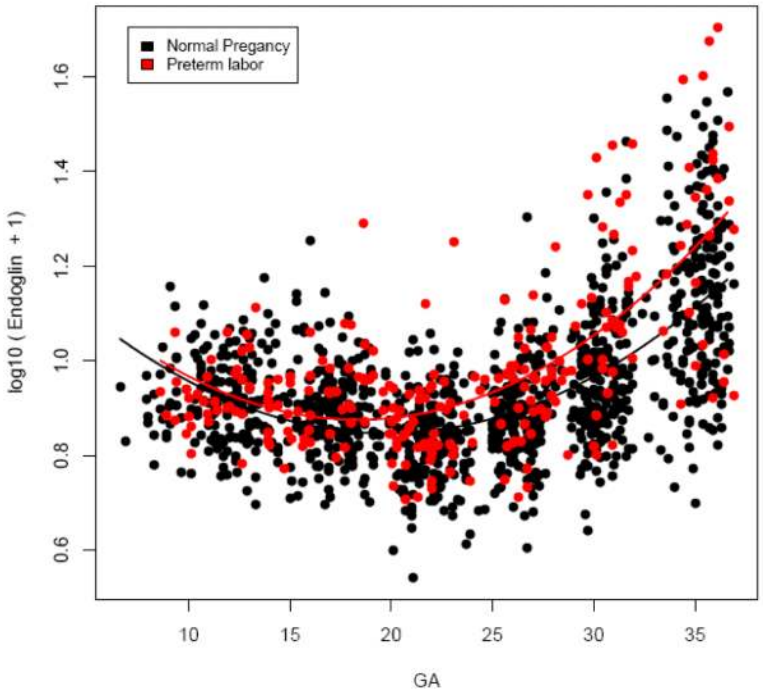

Figure 1.

Profile of plasma soluble endoglin (sEng) concentrations (ng/ml) in relation to gestational age in normal pregnant women and patients with spontaneous preterm labor and delivery (PTL). Patients destined to developed PTL had a significantly different profile (plasma concentration over time) of plasma sEng concentration from patients with normal pregnancies after adjusting for gestational age at blood sampling, maternal age, body mass index, nulliparity, a history of preterm delivery, smoking and duration of sample storage (p=0.04). Plasma sEng concentration was higher in patients destined to develop PTL than in normal pregnant women from 15-20 weeks of gestation. The difference became statistical significance at 27.7 weeks and was more pronounce as delivery approached.

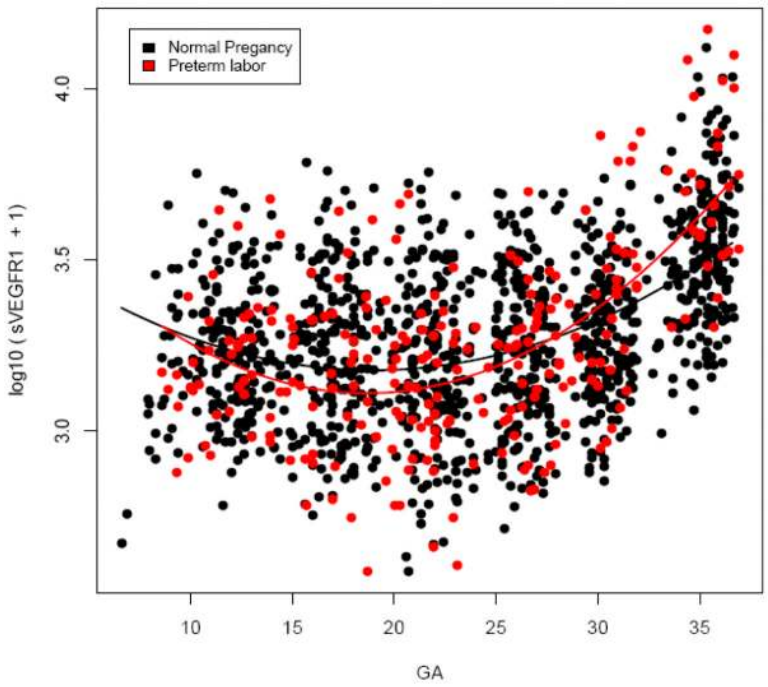

Figure 2.

Profile of plasma sVEGFR-1 concentrations (pg/ml) in relation to gestational age in normal pregnant women and patients with spontaneous preterm labor (PTL) and delivery. Patients destined to developed PTL had a significantly different profile (plasma concentration over time) of plasma sVEGFR-1 concentration from patients with normal pregnancies after adjusting for gestational age at blood sampling, maternal age, body mass index, nulliparity, a history of preterm delivery, smoking and duration of sample storage (p=0.003). Plasma sVEGFR-1 concentrations in patients with PTL were slightly lower than those in normal pregnant women from 10-28 weeks of gestation. After this gestational age, plasma sVEGFR-1 concentrations became higher until delivery.

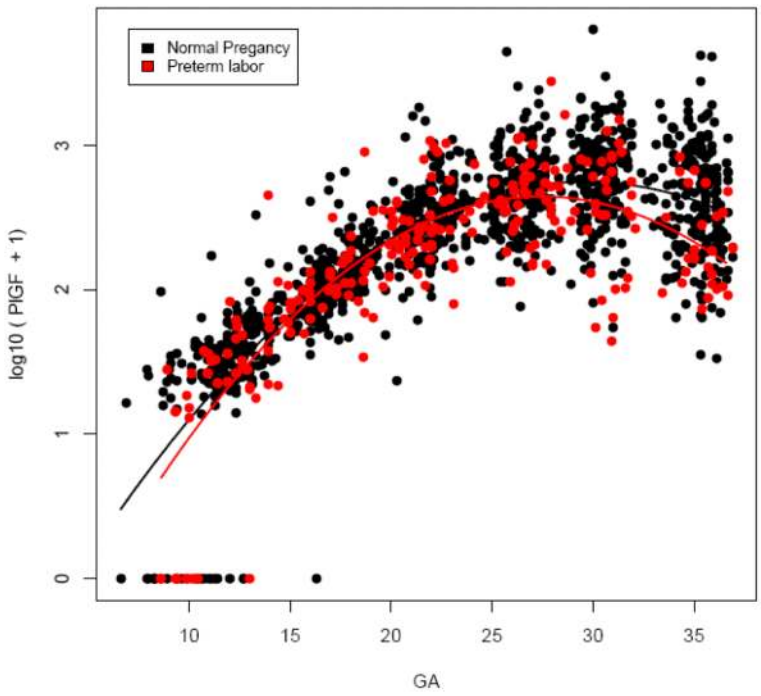

Figure 3.

Profile of plasma placenta growth factor (PlGF) concentrations (pg/ml) in relation to gestational age in normal pregnant women and patients with spontaneous preterm labor (PTL) and delivery. There was no significant difference in the profile (plasma concentration over time) of plasma PlGF concentration between patients with PTL and normal pregnant women after adjusting for gestational age at blood sampling, maternal age, body mass index, nulliparity, a history of preterm delivery, smoking and duration of sample storage (p=0.3).

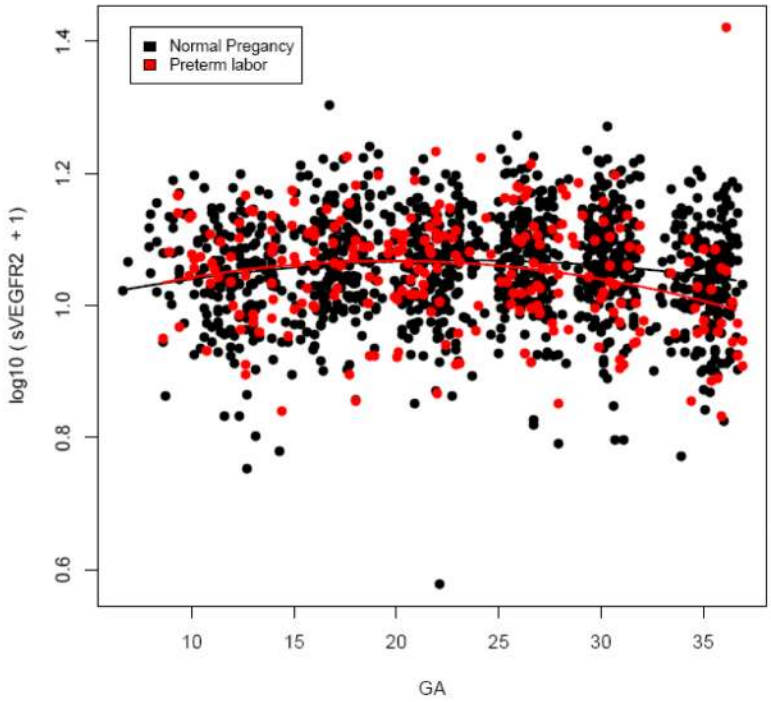

Figure 4.

Profile of plasma sVEGFR-2 concentrations (ng/ml) in relation to gestational age in normal pregnant women and patients with spontaneous preterm labor (PTL) and delivery. There was no significant difference in the profile (plasma concentration over time) of plasma sVEGFR-2 concentration between patients with PTL and normal pregnant women after adjusting for gestational age at blood sampling, maternal age, body mass index, nulliparity, a history of preterm delivery, smoking and duration of sample storage (p=0.1).

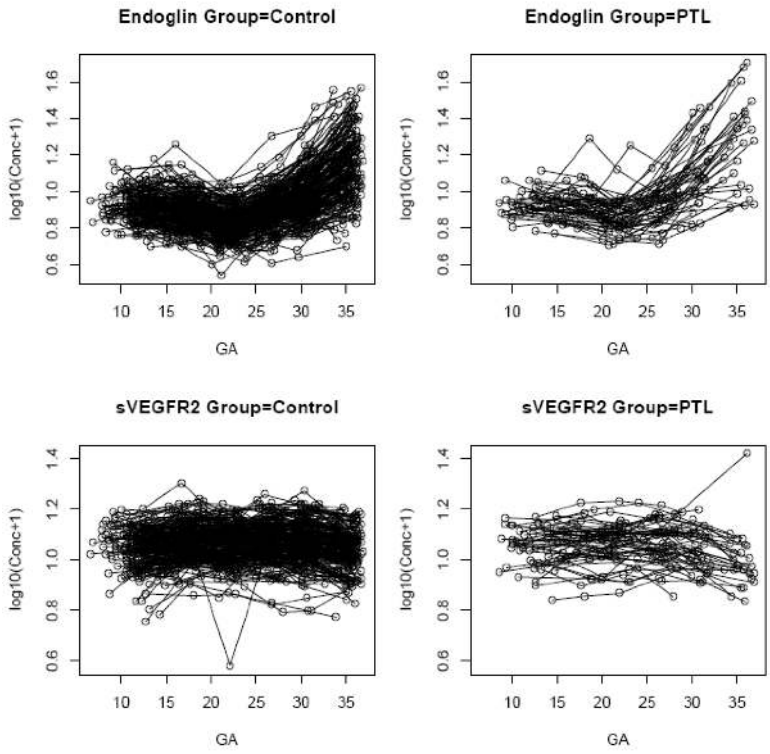

Figure 5.
Individual changes in maternal plasma concentration (Log10 (conc+1)) of soluble endoglin (sEng) and soluble vascular endothelial growth factor receptor-2 (sVEGFR-2) in normal pregnant women (control; n= 208) and patients destined to develop preterm labor and delivery (PTL; n=52) in relation to gestational age (GA).

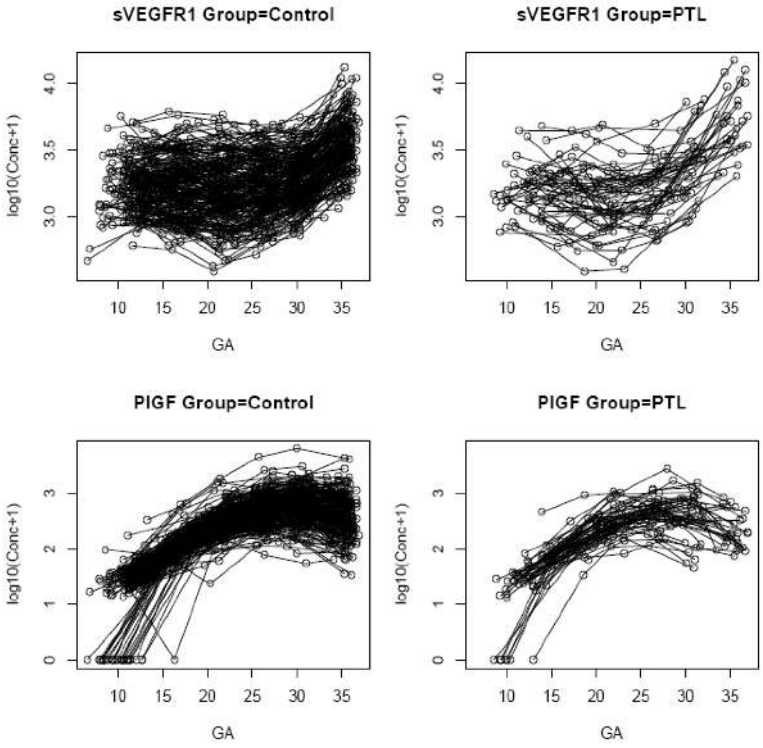

Figure 6.

Individual changes in maternal plasma concentration (Log10 (conc+1)) of soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) and placental growth factor (PlGF) in normal pregnant women (control; n= 208) and patients destined to develop preterm labor and delivery (PTL; n=52) in relation to gestational age (GA).

Table I

Clinical characteristics of the study population

	Normal pregnancy n = 208	Preterm labor n = 52	p
Age (y)	24 (16-47)	25.5 (16-45)	0.4
Body mass index (Kg/m^2)	23.9$^+$ (16-39)	23.7^{++} (17-32)	0.6
Nulliparity	93 (44.7%)	21 (40.4%)	0.6
Previous preterm delivery	1 (0.5%)	10 (19.2%)	<0.001 *
Smoking	27 (13.0%)	7 (13.5%)	0.9
GA at first ultrasound	12.3 (6-20)	12.5 (7-23.6)	0.2
GA at delivery (weeks)	39.9 (37-41.9)	35.8 (26.1-36.9)	<0.001 *
Birthweight (grams)	3,475 (2,540-4,150)	2,665 (966-3,420)	<0.001 *
Delivery ≤32 weeks	0	7 (13.5%)	<0.001 *
Delivery ≤34 weeks	0	13 (25%)	<0.001 *
Amniotic fluid culture positive	0	2/11 (18.2%)	<0.001 *

Value expressed as median (range) or number (percent) ; GA: gestational age; +: n=197; ++: n=51

* p<0.05

Table II

Plasma concentrations of angiogenic factors in normal pregnancy and preterm labor who delivered preterm

	Normal pregnancy	p	Pre-clinical samples Preterm labor	p	Clinical samples Preterm labor	p[β]
1st blood sampling (6-14.9 weeks)						
sVEGFR-1 (pg/ml)	1,636 (467-5653)	0.2	1,428 (758-4755)			
PlGF (pg/ml)	33 (0-329)	0.2	27 (0-451)			
sEng (ng/ml)	7.1 (3.9-13.9)	0.5	7.2 (4.9-12.0)			
sVEGFR-2 (ng/ml)	10.2 (4.7-14.8)	0.5	10.5 (5.9-13.9)			
Gestational age (weeks)	12 (6.6-14.9)	0.8	12.3 (8.6-14.9)			
	n=208		n=48			
2nd blood sampling (15-19.9 weeks)						
sVEGFR-1 (pg/ml)	1,684 (564-6112)	0.2	1,551 (388-4374)			
PlGF (pg/ml)	114 (0-651)	0.4	102 (32-905)			
sEng (ng/ml)	6.8 (3.9-16.9)	0.002 *	7.6 (5.3-18.6)			
sVEGFR-2 (ng/ml)	10.8 (6.2-19.2)	0.7	10.9 (6.2-19.2)			
Gestational age (weeks)	17.3 (15-19.9)	0.8	17.4 (15-19.9)			
	n=208		n=42			
3rd blood sampling (20-24.6 weeks)						
sVEGFR-1 (pg/ml)	1,470 (388-5723)	0.07	1,297 (403-4920)			
PlGF (pg/ml)	316 (22-1833)	0.3	274 (78-1087)			
sEng (ng/ml)	5.7 (2.5-10.4)	0.03 *	6.0 (4.1-16.8)			
sVEGFR-2 (ng/ml)	10.8 (2.8-15.7)	0.9	10.9 (6.3-16.1)			
Gestational age (weeks)	22.0 (20-24.6)	0.3	21.9 (20-24.6)			
	n=207		n=52			
4th blood sampling (25-27.9 weeks)						
sVEGFR-1 (pg/ml)	1,659 (518-4962)	0.5	1,753 (668-5015)	0.9	1,788	0.8
PlGF (pg/ml)	460 (75-4437)	0.4	465 (113-2783)	0.3	255	0.3
sEng (ng/ml)	6.6 (3.0-19.2)	0.01 *	7.6 (4.1-12.7)	0.8	8.2	0.3
sVEGFR-2 (ng/ml)	10.9 (5.2-17.1)	0.3	10.3 (6.1-15.4)	0.2	8.8	0.2
Gestational age (weeks)	26.3 (25-27.9)	0.07	26.6 (25-27.9)	0.4	26.0	0.7

	Normal pregnancy	p	Pre-clinical samples Preterm labor	p	Clinical samples Preterm labor	pβ
	n=208		n=38		n= 1	
5th blood sampling (28-31.9 weeks)						
sVEGFR-1 (pg/ml)	1,862 (715-5481)	0.2	2,236 (893-7282)	0.9	1,885 (1563-3401)	0.6
PlGF (pg/ml)	604 (53-6343)	0.03 *	493 (53-1647)	0.06	297 (43-298)	0.02 *
sEng (ng/ml)	7.9 (3.4-28.1)	<0.001 *	10.8 (5.3-27.8)	0.8	8.5 (7.2-27.6)	0.4
sVEGFR-2 (ng/ml)	10.8 (5.3-17.7)	0.2	10.1 (7.1-14.7)	0.6	12.3 (7.0-14.3)	0.7
Gestational age (weeks)	30.3 (28-31.9)	0.3	30.6 (28.1-31.9)	0.2	28.8 (28.1-30.9)	0.1
	n=208		n=36		n=3	
6th blood sampling (32-36.9 weeks)						
sVEGFR-1 (pg/ml)	3,254 (979-13163)	0.3	3,806 (2012-10516)	0.2	5,614 (1979-14878)	0.001 *
PlGF (pg/ml)	388 (32-4225)	0.3	299 (87-835)	0.2	165 (72-546)	<0.001 *
sEng (ng/ml)	11.7 (3.9-36.1)	0.06	17.0 (7.1-49.6)	0.2	17.9 (7.2-46.4)	0.06
sVEGFR-2 (ng/ml)	9.9 (4.9-15.0)	0.9	9.9 (6.7-25.4)	0.2	8.2 (5.8-11.2)	<0.001 *
Gestational age (weeks)	35.3 (32.4-36.9)	0.8	35.0 (34.3-36.1)	0.2	35.7 (32.1-36.9)	0.3
	n=208		n=10		n=19	

pβ :compared between samples at clinical manifestation of preterm labor and normal pregnancy

* p<0.05; value expressed as median (range).

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Table III

Unadjusted and adjusted odds ratio for the identification of spontaneous preterm labor by plasma sEng concentrations above the 3st quartile for various gestational age intervals in pre-clinical samples

	Normal pregnancy	Pre-clinical preterm labor	unadjusted Odds ratio (95% CI)	Adjusted Odds ratio (95% CI)
1st blood sampling (6-14.9 weeks)				
sEng ≥8.3 ng/ml (n=256)	52/208 (25.0%)	10/48 (20.8%)	0.8 (0.4-1.7)	0.6 (0.2-1.4)
2nd blood sampling (15-19.9 weeks)				
sEng ≥7.7 n/ml (n=250)	53/208 (25.5%)	19/42 (45.2%)	2.4 (1.2-4.8)	1.9 (0.9-4.4)
3rd blood sampling (20-24.9 weeks)				
sEng ≥6.6 ng/ml (n=259)	51/207 (24.6%)	20/52 (38.5%)	1.9 (1.01-3.6)	1.5 (0.7-3.2)
4th blood sampling (25-27.9 weeks)				
sEng ≥7.7 ng/ml (n=246)	53/208 (25.5%)	19/38 (50.0%)	2.9 (1.5-5.9)	1.8 (0.8 -2.4)
5th blood sampling (28-31.9 weeks)				
sEng ≥9.7 ng/ml (n=244)	52/208 (25.0%)	21/36 (58.3%)	4.2 (2.0-8.6)	3.1* (1.3-7.2)
6th blood sampling (32-36.9weeks)				
sEng ≥16.4 ng/ml (n=218)	52/208 (25.0%)	6/10 (60.0%)	4.5 (1.3-15.4)	6.6* (1.5-28.6)

Adjusted for previous preterm delivery (yes/no), gestational age at sampling (weeks), duration of sample storage (days), maternal age (years), BMI (Kg/m^2), smoking (yes/no), nulliparity (yes/no).

CI: confidence interval;

* $p<0.05$

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Table IV

Mixed-effect model comparing the profiles of plasma sEng and sVEGFR-1 concentrations in relation to gestational age between patients with PTL and normal pregnant women adjusting for confounders (see text).

	sEndoglin			sVEGFR-1		
	Coefficient	**p**	**P+**	**Coefficient**	**p**	**P+**
(Gestational age)2	0.0012	<0.0001 *	<0.0001 *	0.0013	<0.0001 *	<0.0001 *
Preterm labor and delivery (yes/no)	−0.0579	0.0169 *	0.0381 *	−0.1359	0.0012 *	0.0028 *
Gestational age (weeks)	−0.0479	<0.0001 *	<0.0001 *	−0.0521	<0.0001 *	<0.0001 *
Age (years)	0.0027	0.0233 *	0.0442 *	−0.0002	0.9180	0.9542
Body mass index (Kg/m^2)	−0.0053	0.0003 *	0.0009 *	−0.0064	0.0181 *	0.0384 *
Nulliparity (yes/no)	0.0339	0.0212 *	0.0423 *	0.1026	0.0002 *	0.0006 *
Previous preterm delivery (yes/no)	0.0279	0.3643	0.4684	−0.0295	0.6035	0.6789
Smoking (yes/no)	0.0045	0.7956	0.8679	−0.0017	0.9568	0.9568
Group * Gestational age (interaction)	0.0045	<0.0001 *	<0.0001 *	0.0058	<0.0001 *	<0.0001 *

P+: Adjusted for false discovery rate;

* p<0.05

Table V

Mixed-effect model comparing the profiles of plasma PlGF and sVEGFR-2 concentrations in relation to gestational age between patients with PTL and normal pregnant women adjusting for confounders (see text).

	PlGF			sVEGFR-2		
	Coefficient	**p**	**P+**	**Coefficient**	**p**	**P+**
(Gestational age)2	−0.0045	<0.0001*	<0.0001*	−0.0002	<0.0001*	<0.0001*
Preterm labor and delivery (yes/no)	0.0889	0.1911	0.2752	0.0265	0.0838	0.1312
Gestational age (weeks)	0.2648	<0.0001*	<0.0001*	0.0096	<0.0001*	<0.0001*
Age (years)	−0.0039	0.2122	0.2938	−0.0016	0.0826	0.1312
Body mass index (Kg/m^2)	−0.0035	0.3626	0.4684	0.0010	0.3815	0.4736
Nulliparity (yes/no)	−0.0214	0.5825	0.6764	−0.0206	0.0661	0.1133
Previous preterm delivery (yes/no)	0.0074	0.9277	0.9542	−0.0161	0.4898	0.5878
Smoking (yes/no)	0.0879	0.0562	0.1012	0.0198	0.1327	0.1991
Group * Gestational age (interaction)	−0.0082	0.0005*	0.0015*	−0.0014	0.0009*	0.0022*

P+: Adjusted for false discovery rate;

* p<0.05

Table VI

Plasma concentrations of angiogenic factors in normal pregnancy and preterm labor who delivered preterm

	Normal pregnancy	Pre-clinical samples Preterm labor	p
Interval to delivery 0-1 day			
sVEGFR-1 (pg/ml)	2,684 (787-8685)	4,568 (1563-14878)	0.001 *
PlGF (pg/ml)	459 (68-2432)	184 (43-546)	<0.001 *
sEng (ng/ml)	9.9 (3.9-32.2)	14.3 (7.2-46.4)	0.03 *
sVEGFR-2 (ng/ml)	9.7 (4.9-15.6)	8.4 (5.8-14.3)	0.003 *
Gestational age (weeks)	34.7 (25.1-36.9)	35.4 (26.0-36.9)	0.1
	n=92	n=23	
Interval to delivery 2-14 days			
sVEGFR-1 (pg/ml)	3,238 (1392-10845)	3,777 (2012-10,516)	0.3
PlGF(pg/ml)	367 (34-4225)	189 (87-668)	0.054
sEng (ng/ml)	11.4 (3.9-20.4)	17.4 (7.1-49.6)	0.016 *
sVEGFR-2 (ng/ml)	9.8 (6.9-13.1)	9.7 (6.7-25.4)	0.8
Gestational age (weeks)	35.2 (29.7-36.6)	35.0 (31.7-36.1)	0.8
Interval to delivery (days)		7 (2-13)	
	n=36	n=9	
Interval to delivery 15 -35 days			
sVEGFR-1 (pg/ml)	1,750 (654-4363)	2,772 (797-7282)	0.01 *
PlGF (pg/ml)	571 (137-6343)	518 (53-900)	0.08
sEng (ng/ml)	7.5 (3.9-20.3)	11.1(5.6-27.8)	0.002 *
sVEGFR-2 (ng/ml)	10.6 (5.3-15.6)	9.5 (7.3-15.4)	0.02 *
Gestational age (weeks)	30.1 (22.3-36.0)	30.9 (24.0-34.7)	0.1
Interval to delivery (days)		27 (15-35)	
	n=84	n=21	
Interval to delivery 36-70 days			
sVEGFR-1 (pg/ml)	1,764 (518-5481)	1,791 (605-5015)	0.4
PlGF (pg/ml)	451 (53-4437)	414 (83-2783)	0.3
sEng (ng/ml)	7.0 (3.4-28.1)	8.3 (4.6-21.5)	0.01 *
sVEGFR-2 (ng/ml)	10.8 (2.8-17.7)	10.2 (6.1-14.7)	0.2
Gestational age (weeks)	28.0 (17.6-31.9)	27.9 (18.6-31.3)	0.9
Interval to delivery (days)		48 (37-66)	
	n=164	n=41	
Interval to delivery 71-105 days			
sVEGFR-1 (pg/ml)	1,380 (388-5723)	1,204 (403-4920)	0.052
PlGF (pg/ml)	332 (60-1935)	298 (78-1253)	0.8
sEng (ng/ml)	5.9 (2.5-12.6)	6.0 (4.1-16.8)	0.2
sVEGFR-2 (ng/ml)	10.8 (6.2-19.2)	11.1 (6.3-15.8)	0.4
Gestational age (weeks)	22.1 (15.6-27.9)	22.3 (15.6-26.4)	0.9

	Normal pregnancy	Pre-clinical samples Preterm labor	p
Interval to delivery (days)		90.5 (71-105)	
	n=168	n=42	
Interval to delivery 106-140 days			
sVEGFR-1 (pg/ml)	1,563 (465-6112)	1,487 (388-4622)	0.2
PlGF (pg/ml)	135 (0-1596)	121 (0-905)	0.6
sEng (ng/ml)	6.3 (2.9-12.9)	7.5 (4.4-18.6)	0.001 *
sVEGFR-2 (ng/ml)	10.8 (6.4-15.8)	10.9 (6.2-14.8)	0.8
Gestational age (weeks)	17.9 (8.0-24.0)	18.0 (8.6-21.4)	0.6
Interval to delivery (days)		122 (106-137)	
	n=171	n=43	
Interval to delivery >140 days			
sVEGFR-1 (pg/ml)	1,676 (467-5653)	1,670 (758-4755)	0.4
PlGF(pg/ml)	38.6 (0-396)	34.9 (0-451)	0.8
sEng (ng/ml)	7.1 (3.9-13.9)	7.1 (4.9-12.0)	0.5
sVEGFR-2 (ng/ml)	10.4 (5.3-15.2)	10.6 (6.9-13.9)	0.8
Gestational age (weeks)	12.4 (6.6-19.4)	12.6 (9.3-16.0)	0.8
Interval to delivery (days)		161 (141-183)	
	n=171	n=43	

p^{β} :compared between samples at clinical manifestation of preterm labor and normal pregnancy;

* $p<0.05$; value expressed as median (range).

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Table VII

Proportions of PTL patients who had plasma sEng concentrations above the 3rd quartile stratified by placental pathology

	Pathology consistent with maternal under-perfusion			Pathology consistent with inflammation		
	No (n=26)	**Yes (n=4)**	**p**	**No (n=25)**	**Yes (n=5)**	**p**
1st blood sampling (6-14.9 weeks)						
sEng ≥8.3 ng/ml	2/25 (8.0%)	0/4 (0%)	1.0	2/24 (8.3%)	0/5 (0%)	1.0
2nd blood sampling (15-19.9 weeks)						
sEng ≥7.7 n/ml	5/23 (21.7%)	3/3 (100%)	0.02*	2/22 (22.7%)	3/4 (75%)	0.07
3rd blood sampling (20-24.9 weeks)						
sEng ≥6.6 ng/ml	7/26 (26.9%)	3/4 (75.0%)	0.09	9/25 (36%)	1/5 (20%)	0.6
4th blood sampling (25-27.9 weeks)						
sEng ≥7.7 ng/ml	7/21 (33.3%)	3/4 (75.0%)	0.3	8/20 (40%)	2/5 (40%)	1.0
5th blood sampling (28-31.9 weeks)						
sEng ≥9.7 ng/ml	9/23 (69.2%)	4/4 (100%)	0.04*	10/23 (43.5%)	3/4 (75%)	0.3
6th blood sampling (32-36.9weeks)						
sEng ≥16.4 ng/ml	8/17 (47.1%)	2/2 (100%)	0.5	7/15 (46.7%)	3/4 (75%)	0.6

Fisher's Exact test.;

* p<0.05