Published in final edited form as:

Ultrasound Obstet Gynecol. 2010 February ; 35(2): 155–162. doi:10.1002/uog.7491.

PLASMA SOLUBLE ENDOGLIN CONCENTRATION IN PREECLAMPSIA IS ASSOCIATED WITH AN INCREASED IMPEDANCE TO FLOW IN THE MATERNAL AND FETAL CIRCULATIONS

Tinnakorn Chaiworapongsa, MD^{1,2}, Roberto Romero, MD^{1,2,3}, Juan Pedro Kusanovic, MD^{1,2}, Pooja Mittal, MD^{1,2}, Sun Kwon Kim, MD¹, Francesca Gotsch, MD¹, Nandor Gabor Than¹, Shali Mazaki-Tovi, MD^{1,2}, Edi Vaisbuch, MD^{1,2}, Offer Erez, MD^{1,2}, Lami Yeo^{1,2}, Sonia S Hassan, MD^{1,2}, and Yoram Sorokin²

¹Perinatology Research Branch, NICHD, NIH, DHHS, Detroit, MI, United States

²Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI

³Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI

Abstract

OBJECTIVE—To examine the relationship between abnormalities in uterine (UT) and/or umbilical artery (UA) Doppler velocimetry and maternal plasma concentrations of soluble endoglin (sEng), in patients with preeclampsia (PE).

METHODS—A cross-sectional study was conducted in normal pregnant women (n=135) and patients with PE (n=69). Patients with PE were sub-classified into four groups: 1) those who had Doppler abnormalities in both the UT and the UA; 2) patients who had Doppler abnormalities in the UT alone; 3) those who had Doppler abnormalities in the UA alone; and 4) patients without Doppler abnormalities in either vessel. Plasma concentrations of sEng were determined by ELISA.

RESULTS—Among patients with PE, those with an abnormal UT and UA Doppler velocimetry had the highest median plasma concentration of sEng compared to any other groups (Kruskal Wallis $p<0.001$). Women with PE with normal Doppler velocimetry in both vessels had the lowest median plasma concentration of sEng. There was a significant relationship between plasma concentrations of sEng and mean UT resistance index (Spearman $Rho = 0.5$; $p<0.001$) as well as UA pulsatility index (Spearman $Rho = 0.4$; $p=0.002$). Multiple regression analysis suggested that Doppler abnormalities in the UT/UA as well as gestational age at blood sampling contributed to plasma sEng concentrations ($p<0.001$).

CONCLUSIONS—Abnormalities of impedance to blood flow in the uterine and umbilical arteries are associated with an excess of sEng in the circulation of mothers with PE. These findings suggest that the “anti-angiogenic state” in PE is partially reflected in abnormalities of Doppler velocimetry.

Keywords

Angiogenesis; Preeclampsia; Soluble endoglin; Uterine artery Doppler velocimetry; Umbilical artery Doppler velocimetry

Address correspondence to: Roberto Romero, MD, Perinatology Research Branch, NICHD, NIH, DHHS, Wayne State University/Hutzel Women's Hospital, 3990 John R, Box 4, Detroit, MI 48201, USA, Telephone (313) 993-2700, Fax: (313) 993-2694, prbchiefstaff@med.wayne.edu.

Presented at the 18th World Congress on Ultrasound in Obstetrics and Gynecology. August 24-28, 2008, Chicago, USA.

INTRODUCTION

Preeclampsia, a pregnancy-specific disorder, is clinically characterized by the new-onset hypertension and proteinuria in the second half of pregnancy. Despite considerable research efforts, the causes of this syndrome remain unknown.¹⁻⁴ A central feature in the pathophysiology of preeclampsia is failure of physiologic transformation of the spiral arteries⁵ which is thought to be responsible for increased impedance to blood flow in the uterine arteries.^{6,7} Although the primary etiology for these abnormalities remains elusive,² it has been postulated that the resultant poor placentation and reduced placental perfusion (or ischemic-reperfusion injury to the placenta) in early pregnancy leads to the release of factors into the maternal circulation, which cause systemic endothelial cell dysfunction, intravascular inflammation⁸ and multiple organ damage. Candidates for these unknown factors¹ are cytokines,⁹ syncytiotrophoblast microparticles,¹⁰ apoptotic products,¹¹ reactive-oxygen species,¹² activated leukocytes,¹³ angiotensin II type 1 receptor antibody,¹⁴ soluble vascular endothelial growth factor receptor (sVEGFR)-1¹⁵⁻¹⁹ and soluble endoglin (sEng).¹⁹⁻²¹

Endoglin (Eng), a cell-surface co-receptor of transforming growth factor (TGF)- β 1 and TGF- β 3, is highly expressed on endothelial cells, syncytiotrophoblasts, endometrial stromal cells, monocytes and hematopoietic stem cells.²² This protein modulates the action of TGF- β 1 as well as TGF- β 3, and is essential for vascular homeostasis.^{20,22} The soluble form of this protein, sEng, has potent anti-angiogenic activity.²⁰ The administration of adenovirus encoded for sEng and sVEGFR-1 to pregnant rats induced hypertension, proteinuria, glomeruloendotheliosis, biochemical evidence of HELLP (hemolysis, elevated liver enzyme, low platelets) syndrome, and fetal growth restriction, features similar to those of patients with preeclampsia.²⁰ Moreover, the mean plasma/serum concentration of sEng in preeclampsia both prior to^{19,21,23-25} and at the time of clinical diagnosis^{20,21,26} is higher than that in normal pregnancy and correlates with the disease severity.

If the increased plasma sEng concentrations observed in patients with preeclampsia originates from factors released from a poorly perfused placenta, the plasma concentrations of sEng may be a biomarker for changes in the impedance to flow in the utero-placental circulation at the fetomaternal interface. The objective of this study was to examine the relationship between abnormalities in uterine and/or umbilical artery Doppler velocimetry and maternal plasma concentrations of sEng at the time of clinical diagnosis in patients with preeclampsia.

METHODS

Study Design

This retrospective cross-sectional study was conducted by searching the clinical database and bank of biologic samples of the Perinatology Research Branch. All patients were enrolled at Hutzel Women's Hospital in Detroit, Michigan from September 1999 to June 2002. The following groups were included: 1) women with preeclampsia; and 2) normal pregnant women. The inclusion criteria for women with preeclampsia are 1) singleton gestations; 2) women who had Doppler velocimetry of the uterine and umbilical artery performed within 48 hours of blood sampling; 3) absence of major fetal structural or chromosome abnormality; and 4) absence of chronic hypertension. Patients with preeclampsia had a blood draw upon diagnosis and enrollment. Normal pregnant women were enrolled from either the labor-delivery unit (in cases of scheduled cesarean section) or from the antenatal clinic, had a blood draw, and were followed until delivery. A patient was considered to have a normal pregnancy if she met the following criteria: 1) singleton gestation; 2) no medical, obstetrical or surgical complications; 3) absence of labor at the time of venipuncture; 4) delivery of a normal term (≥ 37 weeks) infant whose birthweight was between the 10th and 90th percentile for gestational age.²⁷

Clinical definition

Preeclampsia was defined as hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg on at least two occasions, 4 hours to 1 week apart) with proteinuria (≥ 300 milligrams in a 24-hour urine collection or one urine dipstick measurement $\geq 2+$).²⁸ Severe preeclampsia was diagnosed as previously described.²⁸ Early-onset and late-onset preeclampsia were defined as those who were diagnosed before and after 34 weeks of gestation respectively.²⁹ All women provided informed consent prior to the collection of plasma samples. The collection of samples and their utilization for research purposes was approved by the IRBs of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development and Wayne State University. Many of these samples have been previously used in other studies.

Doppler velocimetry

Pulsed-wave and color Doppler ultrasound examination of the uterine and umbilical arteries was performed in patients with preeclampsia (Acuson, Sequoia, Mountain View, CA) using a 3.5 or a 5 MHz curvilinear probe. Transducers were directed toward the iliac fossa, the external iliac artery was imaged in a longitudinal section, and the uterine artery was mapped with color Doppler as it crossed the external iliac artery. Pulsed-wave Doppler was performed of both uterine arteries and when three similar consecutive waveforms were obtained, the resistance index (RI) of the right and left uterine arteries was measured and the mean RI of the two vessels was calculated. Uterine artery Doppler velocimetry³⁰ was defined as abnormal if either the mean RI was above the 95th percentile for gestational age³¹ or in the presence of a bilateral early diastolic notch.³² The Doppler signal of the umbilical artery was obtained from a free floating loop of the umbilical cord during the absence of fetal breathing and body movement. When three similar consecutive waveforms were obtained, the pulsatility index (PI) was measured. Umbilical artery Doppler velocimetry was defined as abnormal if either the PI was above the 95th percentile for gestational age using the reference range proposed by Arduini and Rizzo³³ or in the presence of abnormal waveforms (absent or reversed end diastolic velocities) as described by Trudinger et al.³⁴ The patients were classified into the following 4 groups: 1) Normal Doppler velocimetry in the uterine and the umbilical arteries; 2) Doppler abnormalities in the uterine artery alone; 3) Doppler abnormalities in the umbilical artery alone; and 4) Doppler abnormalities in both vessels.

Sample collection and human sEng immunoassay

Maternal blood was collected in tubes containing EDTA. Samples were centrifuged and stored at -70° C. Maternal plasma concentrations of sEng were measured using an enzyme-linked immunoassay (ELISA; R&D Systems, Minneapolis, MN) employing a quantitative sandwich immunoassay technique as previously described.¹⁹ The concentrations of sEng in maternal plasma samples were determined by interpolation from individual standard curves composed of purified human sEng. The inter- and intra-assay coefficients of variation for sEng immunoassays were 3.3% and 2.7% respectively. The sensitivity was 0.08 ng/mL.

Statistical analysis

Shapiro-Wilk and Kolmogorov-Smirnov tests were used to test for normal distribution of the data. Analysis of Variance (ANOVA) with post-hoc (Bonferroni or Dunnett's T3) corrections for multiple comparisons or Kruskal Wallis with post-hoc Mann-Whitney U tests were utilized to determine the differences of the mean or the median among groups according to the data distribution. Contingency tables and Chi-square tests were employed for comparisons of proportions. Spearman correlation was used to assess the relationship between continuous variables. Multivariate linear regression analysis was applied to examine the contribution of Doppler abnormalities on the plasma concentration of log (sEng+1), while adjusting for

potential confounders. Analysis was conducted with SPSS V.12 (SPSS Inc., Chicago, IL). A p value of <0.05 was considered significant.

RESULTS

Demographic and obstetric characteristics are displayed in Table I. Among patients with preeclampsia, 57 (82%) were diagnosed with severe preeclampsia, and 34 (49%) had an early-onset disease (Table II).

Among patients with preeclampsia, the mean $\pm$ SD uterine artery RI was 0.69 ± 0.16 and the mean $\pm$ SD umbilical artery PI was 1.3 ± 0.9 . Eleven (15.9 %) patients had Doppler abnormalities in both uterine and umbilical circulations, while 44 (63.7%) patients had abnormal uterine artery Doppler velocimetry alone. Abnormal umbilical artery Doppler velocimetry alone was observed in only 3 (4.3%) patients and all neonates delivered from these patients did not have any signs and symptoms of congenital heart disease or chromosomal abnormalities. There was no significant difference in the rate of abnormal umbilical artery Doppler velocimetry between those with normal [21.4% (3/14)] and those with abnormal uterine artery Doppler velocimetry [20% (11/55); $p=0.9$].

When patients with preeclampsia and normal pregnant women were stratified according to gestational age at which blood sampling was performed, patients with early and late-onset preeclampsia had median plasma sEng concentrations higher than normal pregnant women (both $p<0.001$; Figure 1). The median plasma sEng concentration in the early-onset group was higher than that of the late-onset group ($p<0.001$; Figure 1). While all patients in the early-onset group had abnormal Doppler velocimetry in either the uterine and/or umbilical arteries, only three (8.5%) patients in the late-onset group had Doppler abnormalities in both circulations, and 21 (60%) had abnormal uterine artery Doppler velocimetry alone.

Among patients with preeclampsia, there was a relationship between the plasma concentrations of sEng and the mean uterine artery RI (spearman $Rho = 0.5$; $p<0.001$; Figure 2.). A similar relationship was observed between plasma sEng concentrations and the umbilical artery PI (spearman $Rho = 0.4$; $p=0.002$; Figure 3). Plasma sEng concentration in normal pregnant women increased as a function of gestational age (spearman $Rho = 0.4$; $p<0.001$; Figure 4). In contrast, among patients with preeclampsia, the earlier the diagnosis of preeclampsia, the higher the concentration of plasma sEng (spearman $Rho = -0.5$; $p<0.001$; Figure 4). There was an inverse relationship between plasma sEng concentrations and gestational age at delivery, unadjusted and adjusted (multiples of median) neonatal birthweight for gestational age (spearman's $\rho = -0.6$, -0.6 and -0.5 respectively; all $p < 0.001$).

Table III displays the clinical characteristics of patients with preeclampsia sub-classified according to the results of uterine and umbilical artery Doppler velocimetry. Although patients with abnormal Doppler velocimetry in both the uterine and the umbilical artery had the lowest gestational age at blood sampling (Table III), they had the highest median plasma sEng concentration (Kruskal Wallis $p<0.001$; Figure 5) among all groups. Patients with abnormal uterine, but normal umbilical artery Doppler velocimetry, had a median plasma sEng concentration higher than those with normal Doppler velocimetry in both vessels ($p=0.001$). It is noteworthy that patients with preeclampsia and normal Doppler velocimetry in both the uterine and umbilical arteries still had a median plasma sEng concentration two-fold higher than normal pregnant women ($p=0.002$; Figure 5).

Multiple regression analysis was applied to examine the contribution of Doppler abnormalities to the plasma concentration of sEng in patients with preeclampsia, while adjusting for gestational age at blood sampling, nulliparity, smoking, and duration of sample storage. Factors entered into the regression model are displayed in Table IV. The final regression model

suggested that Doppler abnormality in the uterine artery, Doppler abnormality in the umbilical artery, and gestational age at blood sampling were associated with an increased plasma sEng concentration ($p < 0.001$).

DISCUSSION

The principal findings of this study are: 1) Patients with preeclampsia with Doppler velocimetry abnormalities in the uterine and umbilical arteries had the highest median plasma sEng concentration of all the groups; 2) Among patients with preeclampsia, there was a relationship between the plasma sEng concentrations and the mean uterine artery RI as well as the umbilical artery PI; and 3) Gestational age at diagnosis, Doppler abnormalities in the uterine artery and in the umbilical artery contributed to the increased plasma sEng concentrations in preeclampsia.

The finding that there was a relationship between the mean uterine artery RI as well as umbilical artery PI and plasma concentrations of sEng is consistent with previous observations that there was a relationship between Doppler abnormalities in both circulations and plasma sVEGF-R1 concentrations.³⁵ Moreover, in a study of patients with preeclampsia and isolated fetal growth restriction, there was an inverse relationship between both the mean uterine artery PI, and umbilical artery PI and serum concentrations of PlGF.³⁶ These findings suggest that Doppler abnormalities in the uterine and the umbilical artery are associated with the postulated “anti-angiogenic state”, defined as an increase in sEng and sVEGFR-1 concentrations, with a decrease in PlGF concentrations in the maternal circulation of patients with preeclampsia.^{15, 20}

In the current study, the median plasma sEng concentration was increased when abnormalities in Doppler velocimetry involving both uterine and umbilical circulations were documented. These findings can be interpreted as suggesting that pathologic conditions, affecting both the fetoplacental and maternal circulations, represent a greater stimulus for the release of sEng into the maternal circulation than when only one vascular territory is affected.

Patients with preeclampsia who had normal Doppler velocimetry in both the uterine and umbilical arteries still had a median plasma sEng concentration two-fold higher than normal pregnant women, reflecting a perturbation in angiogenic state, but of a lower magnitude than that of those who had Doppler abnormalities. These results indicate that factors, other than the increased impedance to blood flow in the uterine/umbilical artery, are responsible for these findings. Alternatively, Doppler abnormalities detected by pulsed-wave Doppler ultrasound are not as sensitive as the changes in anti-angiogenic factor concentrations in maternal circulation when there is a reduction of flow in uteroplacental circulation.

Uterine artery Doppler velocimetry at 20-24 weeks has been proposed to identify a subset of patients at risk to develop preeclampsia. However, the results have been disappointing.³⁷ The sensitivity of uterine artery Doppler velocimetry or plasma angiogenic factor concentrations, individually, for the prediction of early-onset preeclampsia has been 80-90%, while the detection rate for preeclampsia at any gestational age has been only 41-45% for false positive rates between 5% and 7%.³⁷ The combination of uterine artery Doppler with plasma angiogenic factor concentrations in the second trimester could improve the diagnostic performance for early-onset preeclampsia,^{23,38} but not for late-onset disease.^{26,39} Several studies have demonstrated that early and late-onset preeclampsia have different pathophysiology and clinical features.^{29,40-42} The high rate of patients who had abnormal Doppler velocimetry in the early-onset preeclampsia group could explain why the diagnostic performance of uterine artery Doppler velocimetry as well as plasma concentration of anti-angiogenic factors in the second trimester was better for the early-onset than that for the late-onset disease.

The median plasma sEng concentration of patients with late-onset preeclampsia was also higher than that of normal pregnant women, although, as in the case for sVEGFR-1, at a lower magnitude than that of early-onset disease. This observation suggests that a subset of patients with late-onset disease also have an “anti-angiogenic state”. It is possible that the diagnostic performance of uterine artery Doppler velocimetry combined with plasma anti-angiogenic factor concentrations in the identification of patients with late-onset preeclampsia could be improved if these tests were performed closer to the time of clinical manifestations. However, these strategies may be too late to implement therapy, if one existed.⁴³ In any case, it is likely that incorporation of other diagnostic markers that are not involved in similar pathological process (such as anti-angiogenic factors combined with uterine artery Doppler, both of which reflect poor placental perfusion) would be needed.

A reduction in utero-placental blood flow has been implicated in the pathogenesis of preeclampsia. The findings that there was a significant relationship between Doppler abnormalities in the uterine/umbilical circulations and plasma sEng concentrations add further evidence to support this view. Consistent with our findings, reduced uterine perfusion pressure by clamping the aorta above the iliac bifurcation and branches of ovarian arteries in pregnant rats led to increased plasma concentrations and placental expression of sEng as well as hypoxic inducible factor (HIF)-1 α .⁴⁴ Moreover, an increase in plasma concentrations of anti-angiogenic factors has been demonstrated in several obstetrical syndromes that have evidence of perturbation in the blood supply to the placenta including pregnancies with small for gestational age neonates,^{19,35,36,45} fetal death,⁴⁶ “mirror syndrome”,⁴⁷ and twin-to-twin transfusion syndrome.⁴⁸

Experimental studies examining the effect of hypoxia on Eng expression by trophoblast, however, yielded conflicting results. Some have reported an increase⁴⁹ and others, no change⁵⁰ noted. Redman and Sargent emphasized that although hypoxia is one trigger for the release of anti-angiogenic factors, inflammatory mechanisms may contribute or even predominate since HIF-1 α can also be stimulated by inflammatory triggers (eg: lipopolysaccharide, thrombin, growth factors and cytokines) under normoxic conditions.^{1,51}

Strengths and limitations

This study is the first that has examined the relationship between plasma sEng concentrations and uterine/umbilical artery Doppler velocimetry simultaneously at the time of diagnosis of preeclampsia. The findings from this study will improve the understanding of the behaviors of anti-angiogenic factors and their participation in the pathophysiology of preeclampsia. However, since this was a cross-sectional study, the temporal relationship between the increased impedance to blood flow in uterine/umbilical arteries and the plasma sEng concentrations in preeclampsia could not be determined.

In conclusion, this study provides evidence that an increased plasma sEng concentration in preeclampsia is associated with abnormalities in uterine and/or umbilical artery Doppler velocimetry. These findings suggest that an “anti-angiogenic state” (defined by maternal concentrations of sEng) in preeclampsia is associated with poor perfusion in the feto-maternal circulations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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.

Reference List

1. Redman CW, Sargent IL. Placental Stress and Pre-eclampsia: A Revised View. *Placenta*. 2009
2. Roberts JM, Hubel CA. The Two Stage Model of Preeclampsia: Variations on the Theme. *Placenta*. 2008
3. Sibai BM. Hypertensive disorders of pregnancy: the United States perspective. *Curr Opin Obstet Gynecol* 2008;20:102–106. [PubMed: 18388806]
4. Romero R, Lockwood C, Oyarzun E, Hobbins JC. Toxemia: new concepts in an old disease. *Semin Perinatol* 1988;12:302–323. [PubMed: 3065943]
5. Pijnenborg R, Anthony J, Davey DA, Rees A, Tiltman A, Vercruysse L, van Assche A. Placental bed spiral arteries in the hypertensive disorders of pregnancy. *Br J Obstet Gynaecol* 1991;98:648–655. [PubMed: 1883787]
6. Voigt HJ, Becker V. Doppler flow measurements and histomorphology of the placental bed in uteroplacental insufficiency. *J Perinat Med* 1992;20:139–147. [PubMed: 1501057]
7. Olofsson P, Laurini RN, Marsal K. A high uterine artery pulsatility index reflects a defective development of placental bed spiral arteries in pregnancies complicated by hypertension and fetal growth retardation. *Eur J Obstet Gynecol Reprod Biol* 1993;49:161–168. [PubMed: 8405630]
8. Sacks GP, Studena K, Sargent K, Redman CW. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. *Am J Obstet Gynecol* 1998;179:80–86. [PubMed: 9704769]
9. Conrad KP, Benyo DF. Placental cytokines and the pathogenesis of preeclampsia. *Am J Reprod Immunol* 1997;37:240–249. [PubMed: 9127646]
10. Goswami D, Tannetta DS, Magee LA, Fuchisawa A, Redman CW, Sargent IL, von DP. Excess syncytiotrophoblast microparticle shedding is a feature of early-onset pre-eclampsia, but not normotensive intrauterine growth restriction. *Placenta* 2006;27:56–61. [PubMed: 16310038]
11. Huppertz B, Frank HG, Reister F, Kingdom J, Korh H, Kaufmann P. Apoptosis cascade progresses during turnover of human trophoblast: analysis of villous cytotrophoblast and syncytial fragments in vitro. *Lab Invest* 1999;79:1687–1702. [PubMed: 10616217]
12. Sedeek M, Gilbert JS, LaMarca BB, Sholook M, Chandler DL, Wang Y, Granger JP. Role of reactive oxygen species in hypertension produced by reduced uterine perfusion in pregnant rats. *Am J Hypertens* 2008;21:1152–1156. [PubMed: 18670418]
13. Mellembakken JR, Aukrust P, Olafsen MK, Ueland T, Hestdal K, Videm V. Activation of leukocytes during the uteroplacental passage in preeclampsia. *Hypertension* 2002;39:155–160. [PubMed: 11799095]
14. Walther T, Wallukat G, Jank A, Bartel S, Schultheiss HP, Faber R, Stepan H. Angiotensin II type 1 receptor agonistic antibodies reflect fundamental alterations in the uteroplacental vasculature. *Hypertension* 2005;46:1275–1279. [PubMed: 16260641]
15. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 2003;111:649–658. [PubMed: 12618519]
16. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* 2004;350:672–683. [PubMed: 14764923]
17. Chaiworapongsa T, Romero R, Espinoza J, Bujold E, Mee KY, Goncalves LF, Gomez R, Edwin S. Evidence supporting a role for blockade of the vascular endothelial growth factor system in the pathophysiology of preeclampsia. Young Investigator Award. *Am J Obstet Gynecol* 2004;190:1541–1547. [PubMed: 15284729]

18. Chaiworapongsa T, Romero R, Kim YM, Kim GJ, Kim MR, Espinoza J, Bujold E, Goncalves L, Gomez R, Edwin S, Mazor M. Plasma soluble vascular endothelial growth factor receptor-1 concentration is elevated prior to the clinical diagnosis of pre-eclampsia. *J Matern Fetal Neonatal Med* 2005;17:3–18. [PubMed: 15804781]
19. Romero R, Nien JK, Espinoza J, Todem D, Fu W, Chung H, Kusanovic JP, Gotsch F, Erez O, Mazaki-Tovi S, Gomez R, Edwin S, Chaiworapongsa T, Levine RJ, Karumanchi SA. A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. *J Matern Fetal Neonatal Med* 2008;21:9–23. [PubMed: 18175241]
20. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, Bdolah Y, Lim KH, Yuan HT, Libermann TA, Stillman IE, Roberts D, D'Amore PA, Epstein FH, Sellke FW, Romero R, Sukhatme VP, Letarte M, Karumanchi SA. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med* 2006;12:642–649. [PubMed: 16751767]
21. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, Sibai BM, Epstein FH, Romero R, Thadhani R, Karumanchi SA. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med* 2006;355:992–1005. [PubMed: 16957146]
22. ten Dijke P, Goumans MJ, Pardali E. Endoglin in angiogenesis and vascular diseases. *Angiogenesis* 2008;11:79–89. [PubMed: 18283546]
23. Stepan H, Geipel A, Schwarz F, Kramer T, Wessel N, Faber R. Circulatory soluble endoglin and its predictive value for preeclampsia in second-trimester pregnancies with abnormal uterine perfusion. *Am J Obstet Gynecol* 2008;198:175–176. [PubMed: 18226617]
24. Savvidou MD, Noori M, Anderson JM, Hingorani AD, Nicolaides KH. Maternal endothelial function and serum concentrations of placental growth factor and soluble endoglin in women with abnormal placentation. *Ultrasound Obstet Gynecol* 2008;32:871–876. [PubMed: 18814185]
25. Baumann MU, Bersinger NA, Mohaupt MG, Raio L, Gerber S, Surbek DV. First-trimester serum levels of soluble endoglin and soluble fms-like tyrosine kinase-1 as first-trimester markers for late-onset preeclampsia. *Am J Obstet Gynecol* 2008;199:266. [PubMed: 18771978]
26. Hirashima C, Ohkuchi A, Matsubara S, Suzuki H, Takahashi K, Usui R, Suzuki M. Alteration of serum soluble endoglin levels after the onset of preeclampsia is more pronounced in women with early-onset. *Hypertens Res* 2008;31:1541–1548. [PubMed: 18971528]
27. Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. *Obstet Gynecol* 1996;87:163–168. [PubMed: 8559516]
28. Sibai BM, Ewell M, Levine RJ, Klebanoff MA, Esterlitz J, Catalano PM, Goldenberg RL, Joffe G. Risk factors associated with preeclampsia in healthy nulliparous women. The Calcium for Preeclampsia Prevention (CPEP) Study Group. *Am J Obstet Gynecol* 1997;177:1003–1010. [PubMed: 9396883]
29. von Dadelszen P, Magee LA, Roberts JM. Subclassification of preeclampsia. *Hypertens Pregnancy* 2003;22:143–148. [PubMed: 12908998]
30. Harrington KF, Campbell S, Bewley S, Bower S. Doppler velocimetry studies of the uterine artery in the early prediction of pre-eclampsia and intra-uterine growth retardation. *Eur J Obstet Gynecol Reprod Biol* 1991;42(Suppl):S14–S20. [PubMed: 1809604]
31. Kurmanavicius J, Florio I, Visser J, Hebisch G, Zimmermann R, Muller R, Huch R, Huch A. Reference resistance indices of the umbilical, fetal middle cerebral and uterine arteries at 24–42 weeks of gestation. *Ultrasound Obstet Gynecol* 1997;10:112–120. [PubMed: 9286020]
32. Bower S, Schuchter K, Campbell S. Doppler ultrasound screening as part of routine antenatal scanning: prediction of pre-eclampsia and intrauterine growth retardation. *Br J Obstet Gynaecol* 1993;100:989–994. [PubMed: 8251470]
33. Arduini D, Rizzo G. Normal values of Pulsatility Index from fetal vessels: a cross-sectional study on 1556 healthy fetuses. *J Perinat Med* 1990;18:165–172. [PubMed: 2200862]
34. Trudinger BJ, Cook CM, Giles WB, Ng S, Fong E, Connelly A, Wilcox W. Fetal umbilical artery velocity waveforms and subsequent neonatal outcome. *Br J Obstet Gynaecol* 1991;98:378–384. [PubMed: 2031896]

35. Chaiworapongsa T, Espinoza J, Gotsch F, Kim YM, Kim GJ, Goncalves LF, Edwin S, Kusanovic JP, Erez O, Than NG, Hassan SS, Romero R. The maternal plasma soluble vascular endothelial growth factor receptor-1 concentration is elevated in SGA and the magnitude of the increase relates to Doppler abnormalities in the maternal and fetal circulation. *J Matern Fetal Neonatal Med* 2008;21:25–40. [PubMed: 18175242]
36. Schlenbach D, Wallner W, Sengenberger R, Stiegler E, Mortl M, Beckmann MW, Lang U. Angiogenic growth factor levels in maternal and fetal blood: correlation with Doppler ultrasound parameters in pregnancies complicated by pre-eclampsia and intrauterine growth restriction. *Ultrasound Obstet Gynecol* 2007;29:407–413. [PubMed: 17330322]
37. Papageorgiou AT, Leslie K. Uterine artery Doppler in the prediction of adverse pregnancy outcome. *Curr Opin Obstet Gynecol* 2007;19:103–109. [PubMed: 17353676]
38. Espinoza J, Romero R, Nien JK, Gomez R, Kusanovic JP, Goncalves LF, Medina L, Edwin S, Hassan S, Carstens M, Gonzalez R. Identification of patients at risk for early onset and/or severe preeclampsia with the use of uterine artery Doppler velocimetry and placental growth factor. *Am J Obstet Gynecol* 2007;196:326–13. [PubMed: 17403407]
39. Crispi F, Dominguez C, Llurba E, Martin-Gallan P, Cabero L, Gratacos E. Placental angiogenic growth factors and uterine artery Doppler findings for characterization of different subsets in preeclampsia and in isolated intrauterine growth restriction. *Am J Obstet Gynecol* 2006;195:201–207. [PubMed: 16545329]
40. Valensise H, Vasapollo B, Gagliardi G, Novelli GP. Early and late preeclampsia: two different maternal hemodynamic states in the latent phase of the disease. *Hypertension* 2008;52:873–880. [PubMed: 18824660]
41. Egbor M, Ansari T, Morris N, Green CJ, Sibbons PD. Morphometric placental villous and vascular abnormalities in early- and late-onset pre-eclampsia with and without fetal growth restriction. *BJOG* 2006;113:580–589. [PubMed: 16579806]
42. Moldenhauer JS, Stanek J, Warshak C, Khoury J, Sibai B. The frequency and severity of placental findings in women with preeclampsia are gestational age dependent. *Am J Obstet Gynecol* 2003;189:1173–1177. [PubMed: 14586374]
43. Li Z, Zhang Y, Ying MJ, Kapoun AM, Shao Q, Kerr I, Lam A, O'Young G, Sannajust F, Stathis P, Schreiner G, Karumanchi SA, Protter AA, Pollitt NS. Recombinant vascular endothelial growth factor 121 attenuates hypertension and improves kidney damage in a rat model of preeclampsia. *Hypertension* 2007;50:686–692. [PubMed: 17724276]
44. Gilbert JS, Gilbert SA, Arany M, Granger JP. Hypertension produced by placental ischemia in pregnant rats is associated with increased soluble endoglin expression. *Hypertension* 2009;53:399–403. [PubMed: 19075097]
45. Savvidou MD, Yu CK, Harland LC, Hingorani AD, Nicolaides KH. Maternal serum concentration of soluble fms-like tyrosine kinase 1 and vascular endothelial growth factor in women with abnormal uterine artery Doppler and in those with fetal growth restriction. *Am J Obstet Gynecol*. 2006
46. Espinoza J, Chaiworapongsa T, Romero R, Kim YM, Kim GJ, Nien JK, Kusanovic JP, Erez O, Bujold E, Goncalves LF, Gomez R, Edwin S. Unexplained fetal death: another anti-angiogenic state. *J Matern Fetal Neonatal Med* 2007;20:495–507. [PubMed: 17674262]
47. Espinoza J, Romero R, Nien JK, Kusanovic JP, Richani K, Gomez R, Kim CJ, Mittal P, Gotsh F, Erez O, Chaiworapongsa T, Hassan S. A role of the anti-angiogenic factor sVEGFR-1 in the 'mirror syndrome' (Ballantyne's syndrome). *J Matern Fetal Neonatal Med* 2006;19:607–613. [PubMed: 17118734]
48. Kusanovic JP, Romero R, Espinoza J, Nien JK, Kim CJ, Mittal P, Edwin S, Erez O, Gotsch F, Mazaki-Tovi S, Than NG, Soto E, Camacho N, Gomez R, Quintero R, Hassan SS. Twin-to-twin transfusion syndrome: an antiangiogenic state? *Am J Obstet Gynecol* 2008;198:382–388. [PubMed: 18395032]
49. Yinon Y, Nevo O, Xu J, Many A, Rolfo A, Todros T, Post M, Caniggia I. Severe intrauterine growth restriction pregnancies have increased placental endoglin levels: hypoxic regulation via transforming growth factor-beta 3. *Am J Pathol* 2008;172:77–85. [PubMed: 18156205]
50. Munaut C, Lorquet S, Pequeux C, Blacher S, Berndt S, Frankenne F, Foidart JM. Hypoxia is responsible for soluble vascular endothelial growth factor receptor-1 (VEGFR-1) but not for soluble endoglin induction in villous trophoblast. *Hum Reprod* 2008;23:1407–1415. [PubMed: 18413304]

51. Gotsch, F.; Kim, YM.; Kim, CJ.; Edwin, S.; Holtra, J.; Tarca, AL.; Chaiworapongsa, T.; Kusanovic, JP.; Mor, G.; Redman, C.; Romero, R. A Link Between Inflammation/Infection and Anti-Angiogenic State in Preeclampsia: Inflammatory Mediators Mimic Effect of Hypoxia on Trophoblast by Increasing sFlt-1 and Decreasing Placental Growth Factor Production. International Society for the Study of Hypertension in Pregnancy; Proceedings World Congress XVI; Washington D.C.. 2008; 2009. p. p46Abstract 1-7