Published in final edited form as:

Am J Obstet Gynecol. 2009 June ; 200(6): 630.e1–630.e8. doi:10.1016/j.ajog.2009.01.033.

MATERNAL PLASMA CONCENTRATIONS OF THE SOLUBLE TUMOR NECROSIS FACTOR RECEPTOR 2 ARE INCREASED PRIOR TO THE DEVELOPMENT OF PREECLAMPSIA

Baha Sibai, MD^{1,2}, Roberto Romero, MD^{3,4}, Mark A. Klebanoff, MD⁴, Madeline Murguia Rice, PhD⁵, Steve Caritis, MD⁶, John Hauth, MD⁷, Marshall D. Lindheimer, MD⁸, Peter Van Dorsten, MD⁹, Mark Landon, MD¹⁰, Menachem Miodovnik, MD², Richard Paul, MD¹¹, Mitchell Dombrowski, MD³, Paul Meis, MD¹², Gary Thurnau, MD¹³, and for the National Institute of Child Health Human Development Maternal-Fetal Medicine Units Network

¹ Departments of Obstetrics and Gynecology at the University of Tennessee, Memphis, TN

² University of Cincinnati, Cincinnati, OH

³ Wayne State University, Detroit MI

⁴ Perinatology Research Branch, Division of Intramural Research, National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services, Bethesda, MD

⁵ The George Washington University Biostatistics Center, Washington, DC

⁶ University of Pittsburgh, Pittsburgh, PA

⁷ University of Alabama at Birmingham, Birmingham, AL

⁸ University of Chicago, Chicago, IL

⁹ Medical University of South Carolina, Charleston, SC

¹⁰ Ohio State University, Columbus, OH

¹¹ University of Southern California, Los Angeles, CA

¹² Wake Forest University, Winston-Salem, NC

¹³ University of Oklahoma; Oklahoma City, OK

Abstract

OBJECTIVE—Circulating tumor necrosis factor-alpha (TNF- α), a potent pro-inflammatory cytokine, capable of activating endothelial cells, as well as its soluble receptors (sTNF-R1 and sTNF-R2), is increased during overt preeclampsia, consistent with hypotheses that enhanced systemic inflammatory response and endothelial cell dysfunction are important in the pathophysiology of the preeclamptic syndrome. If so, such increases in levels should precede the onset of the disease. This study was designed to examine whether plasma concentrations of sTNF-R1 and sTNF-R2 are elevated prior to the onset of preeclampsia.

METHODS—This was a retrospective biomarker study of stored maternal plasma from an NICHD preeclampsia prevention trial conducted in patients with risk factors for developing preeclampsia to test the effectiveness of low dose aspirin compared with placebo. The first sample was collected at

13–26 weeks' gestation and the second at 24–28 weeks' gestation. Serial sTNF-R1 and sTNF-R2 concentrations were assessed using sensitive and specific immunoassays in 1,004 patients in whom both samples were collected.

RESULTS—The incidence of preeclampsia was 21.3% (214/1004). Median plasma levels of the sTNF-R2, but not sTNF-R1, were significantly higher at 13–26 weeks (sample 1) and at 24–28 weeks (sample 2) in patients who developed preeclampsia than in those who did not (sample 1: sTNF-R2: median 2,678 pg/ml, range 934–7,835 vs. median 2,535 pg/ml, range 1,022–13,000, $p=0.02$; sTNF-R1: median 936 pg/ml, range 449–3,239 vs. median 913 pg/ml, range 359–5,060, $p=0.19$). There was a significantly increased odds of preeclampsia for an increase in sTNF-R2 from sample 1 to sample 2 (OR=1.23 per 1,000 unit increase). Women in the fourth quartile of sTNF-R2 at 24–28 weeks, had a significantly increased adjusted odds of preeclampsia (OR=1.55, 95% CI=1.02–2.35, $p=0.04$), compared with women in the first quartile. This association, however, varied by treatment group (aspirin or placebo). No association was observed for sTNF-R1. The sensitivity and positive predictive values were low for sTNF-R2, as well as sTNF-R1.

CONCLUSION—An increase in maternal plasma sTNF-R2 concentration precedes clinical manifestation of preeclampsia. These observations demonstrate that levels of proinflammatory cytokines rise well before development of overt disease and could be operative in the pathogenic mechanisms responsible for preeclampsia.

INTRODUCTION

Tumor necrosis factor alpha (TNF- α) is a 17kDa polypeptide pro-inflammatory pleiotropic cytokine, produced by many cells in response to a wide range of stimuli such as microbial products (i.e., bacterial endotoxin), viruses, and immune complexes.¹ This cytokine can induce endothelial cell activation,^{2–4} production of tissue factor,^{5,6} and hypertriglyceridemia,⁷ as well as induce/potentiate oxidative stress.^{8,9} TNF- α exerts its effects by interacting with two receptors which have distinct biological effects: the 55kDa TNF-R1 that induces apoptosis, and the 75-kDa TNF-R2 that induces proliferation through the activation of the transcription factor, nuclear factor κ B (NF κ B).¹⁰ Shedding of the soluble receptors of TNF- α from the cell membranes plays a role in the regulation of TNF- α 's biological functions by decreasing its availability as a ligand.^{11,12} Moreover, plasma or serum concentrations of sTNF-R are considered markers for excessive TNF biological activity, as these receptors have longer half lives than the ligand.

Most, but not all studies,^{13–24} report circulating maternal TNF- α , TNF-R1 and TNF-R2 levels as elevated during overt preeclampsia. We could locate only two studies reporting elevations in TNF-R1 levels, and none evaluating sTNF-R2, prior to clinical manifestations of the disease. Our study was designed to test whether elevations in circulating sTNF-R1 and sTNF-R2 do, in fact, occur prior to the clinical manifestations of preeclampsia, for if so it would implicate these cytokines, further, in plausible explanations of the cause of this disease or its phenotypes.

MATERIAL AND METHODS

Subjects

The NICHD Maternal Fetal Medicine Network conducted a placebo-controlled randomized trial in 2539 pregnant women at risk for the development of preeclampsia to test the effectiveness of low dose aspirin (60mg/day) in preventing this serious hypertensive disorder of pregnancy.²⁵ Randomized women had conditions that placed them into one of the following risk groups: pregestational insulin-treated diabetes mellitus, chronic hypertension, multifetal gestation, and preeclampsia during a previous pregnancy. The criteria for subject selection, enrollment, study protocol, and results have been reported elsewhere.²⁵ Of the 2539 randomized women, 1004 had serial samples available for this biomarker study. Collection of

serial samples from only 40% of the trial population reflected the fact that collection of maternal blood to establish a repository for future secondary studies was initiated after the trial was well underway.

Blood samples

During the NICHD trial, blood samples were obtained at enrollment (13–26 weeks; Sample 1), 24–28 weeks (Sample 2), and 34–37 weeks (Sample 3)²⁵. Due to the small number of samples obtained at 34–37 weeks, this analysis focuses only on women with Samples 1 and 2. The blood was collected into EDTA-containing tubes, centrifuged, and the plasma fraction aliquotted and stored at -70°C for future studies.

TNF soluble receptor assays

TNF-R1 and TNF-R2 concentrations were determined using commercially available enzyme linked immunoassay (R&D Systems, MN). The sensitivity of the assays for TNF-R1 and TNF-R2 were 5.2 pg/ml and 4.5 pg/ml, respectively (intra-assay coefficients of variation: 3.1% and 2.0%, respectively; inter-assay coefficients of variation: 2.8% and 2.7%, respectively).

Outcome variables

The primary outcome variable was preeclampsia, defined in women with neither hypertension nor proteinuria at baseline as the development of hypertension plus one of the following: proteinuria, thrombocytopenia, or pulmonary edema. Hypertension was defined as a systolic and/or diastolic blood pressure ≥ 140 mm Hg and/or ≥ 90 mm Hg, respectively, on two occasions at least four hours apart. Abnormal protein excretion was defined as $\geq 300\text{mg/day}$, or two dipstick determination of $\geq 2+$ (≥ 100 mg/dL), recorded at least 4 hours apart, with no evidence of urinary tract infection. Thrombocytopenia was defined as a platelet count of less than $100,000/\text{ml}^3$.

The diagnosis of preeclampsia in women proteinuric but normotensive at enrollment required the development of thrombocytopenia, a serum aspartate aminotransferase concentration of ≥ 70 U/L, or hypertension accompanied by severe headaches, epigastric pain, or a sudden increase in proteinuria (to ≥ 5 -fold increase over baseline or, if baseline values exceeded 5g/day, to ≥ 2 -fold increase). In nonproteinuric hypertensive women, the diagnosis of preeclampsia required the development of proteinuria or thrombocytopenia. In the women both hypertensive and proteinuric at baseline, at least one of the following criteria were required to diagnose preeclampsia: thrombocytopenia; an elevated serum concentration of aspartate aminotransferase (≥ 70 U per liter); or acceleration of the hypertension (defined by two diastolic readings ≥ 110 mm Hg taken 4 hours apart during the week preceding delivery) combined with either exacerbation of proteinuria (see above), severe headaches, or epigastric pain.²⁵

Other criteria for a diagnosis of preeclampsia were an eclamptic convulsion and manifestation of the HELLP syndrome, the latter defined as hemolysis (serum total bilirubin concentration, ≥ 1.2 mg/dL [$20 \mu\text{mol/L}$], elevated serum aspartate aminotransferase levels (≥ 70 U/L), lactate dehydrogenase concentration of ≥ 600 U/L, or evidence of microangiopathic hemolytic anemia (presence of schistocytes in the peripheral smear), and thrombocytopenia.

Consistency of diagnosis was further controlled by a committee comprised of three physicians unaware of the treatment group assignments who reviewed the records of women with new or accelerating hypertension, new onset proteinuria, or a baseline dipstick 1+ protein recording. Unanimous concurrence on the diagnosis was achieved. Forty-eight percent (478) of the charts required review.

Statistical analysis

Statistical analysis was conducted using SAS software, version 8 (SAS Institute). Continuous variables were compared using the Wilcoxon rank sum test. Categorical variables were analyzed using Chi-square or Fisher's exact test, where appropriate. Spearman correlation coefficients were estimated to summarize the association between the continuous biomarker variables. Logistic regression models included sTNF-R1 and sTNF-R2 from Samples 1 and 2, categorized into quartiles. Logistic regression models also examined change per 1,000-unit increase in sTNF-R1 and sTNF-R2 from Sample 1 to Sample 2. Multivariable logistic regression was used to adjust for potential confounding maternal clinical characteristics when evaluating morbidities and outcomes. Stratified analyses and models with interaction terms examined whether associations varied by baseline risk group, singleton or twin gestation, smoking status or treatment group (aspirin or placebo). Analyses were also conducted to determine the sensitivity, specificity, and positive and negative predictive values for the 75th percentile of sTNF-R1 and sTNF-R2. A nominal 2-tailed p-value of < 0.05 was considered significant. No adjustments were made for multiple comparisons.

RESULTS

Plasma sTNF levels were measured in 1,004 of the 2639 women (40%) enrolled in the randomized clinical trial. Table I describes the distribution of patients by baseline preeclampsia risk group and by those who developed preeclampsia. The samples analyzed here (n=1004) represent all women with Samples 1 and 2 available for analysis. The baseline characteristics of the women included in this analysis were similar to those who did not participate in this biomarker study (data not shown).

The incidence of preeclampsia in this study was 21.3% (214/1,004), which was similar to that of the trial as a whole (19.4%). Demographic and clinical characteristics are described in Table II.

A significant correlation was found between plasma concentrations of sTNF-R1 and sTNF-R2 (sample 1: $r=0.76$; $p<0.0001$ and sample 2: $r=0.77$; $p<0.0001$). The median plasma concentrations of sTNF-R2, but not sTNF-R1, in the first and second samples were significantly higher in patients who subsequently developed preeclampsia than in those who did not (Table III). The relationship between plasma concentration of sTNF-R2, and the subsequent development of preeclampsia remained significant after adjusting for variables known to be associated with preeclampsia in this population: body mass index, black race and chronic hypertension at baseline. Table IV depicts these associations by quartiles of sTNF-R2. Women in the fourth quartile of sTNF-R2 at 24–28 weeks, had a significantly increased adjusted odds of preeclampsia (OR=1.55, CI=1.02–2.35, $p=0.04$), compared with women in the first quartile, with a similar, but non-significant trend at an earlier measured sTNF-R2 at 13–26 weeks (OR=1.50, 95% CI=0.97–2.31, $p=0.07$). The associations observed were consistent by baseline risk group, singleton or twin gestation, and smoking status. An interaction was observed between sTNF-R2 and treatment group (aspirin or placebo), which showed a stronger association between sTNF-R2 and outcome among the patients assigned to placebo (Table V).

When using the 75th percentile of plasma concentrations of sTNF-R1 and sTNF-R2 as a cut-point, the sensitivity and positive predictive values for elevated plasma levels were low (sTNF-R1 Sample 1: sensitivity 29.0%, specificity 74.8%, positive predictive value 23.8%, negative predictive value 79.5%; sTNF-R1 Sample 2: sensitivity 33.6%, specificity 74.9%, positive predictive value 26.7%, negative predictive value 80.7%; sTNF-R2 Sample 1: sensitivity 31.3%, specificity 74.9%, positive predictive value 25.3%, negative predictive value 80.11%; sTNF-R2 Sample 2: sensitivity 35.5%, specificity 81.1%, positive predictive value 27.7%,

negative predictive value 81.1%). The diagnostic indices were higher among the women assigned to placebo, although they remained relatively low (data not shown).

An increase in the plasma concentration of sTNF-R2, but not sTNF-R1, between the first and second sample was associated with an increased odds for preeclampsia (for sTNF-R2, odds ratio for 1,000 unit increase: 1.23, CI=1.03–1.48, $p=0.02$).

There was a modest but significant correlation between the concentrations of sTNF-R1 and sTNF-R2 and duration of sample storage (sTNF-R1: $r=-0.05$, $p=0.01$; sTNF-R2: $r=0.05$, $p=0.02$). This did not affect the association between the plasma sTNF-R2 levels and the development of preeclampsia, which was still significant when adjusted for length of storage.

COMMENT

This study demonstrated the following: 1) The median plasma concentration of sTNF-R2, but not sTNF-R1, was significantly higher at 13–26 weeks of gestation in women who subsequently developed preeclampsia than in women who did not; 2) The median plasma concentration of TNF-R2 was also elevated at 24–28 weeks' gestation in patients subsequently manifesting the disorder; 3) Women in the fourth quartile of sTNF-R2 at 24–28 weeks, had a significantly increased adjusted odds of preeclampsia (OR=1.5), compared with women in the first quartile; this association was null among the women assigned to aspirin, and stronger in the women assigned to placebo (OR=2.54); 4) There was a significantly increased odds of preeclampsia when sTNF-R2 increased from Sample 1 to Sample 2 (OR=1.2 per 1,000 unit increase). However, diagnostic indices and predictive values derived from these observations do not support use of sTNF-R2 receptor's circulating levels alone as a test to predict preeclampsia. Finally our observations suggest the presence early in pregnancy of an increased systemic inflammatory response, reflected by increased sTNF-R2 levels, in patients destined to develop preeclampsia.

Increments in the systemic inflammatory response, a feature of healthy pregnancy, are exaggerated in preeclampsia.^{24–30} Based upon studies conducted with flow cytometry in which the phenotypic and metabolic changes of neutrophils and monocytes have been examined as well, as observational studies of the concentrations of maternal proinflammatory cytokines in peripheral blood,^{16–24} this exaggeration has been offered as a plausible hypothesis for the pathogenesis of preeclampsia and/or its phenotypes.^{28,29}

If, as some suggest, the cause of the preeclampsia syndrome is multifactorial it would be naïve to ascribe the disease in all patients to a single mechanism. Also multiple causes could result in biochemical markers varying among subjects with different risk factors.³⁰ The patients enrolled in our study included a heterogeneous group of women, though all were at increased risk to develop preeclampsia. Although the associations observed in this study were consistent across baseline risk groups, further research may be required to determine whether inflammation is operative in the genesis of preeclampsia in concert with other risk factors.

Recently, Schipper and Associates²⁴ studied sTNF-R1 levels throughout pregnancy in 68 women with a history of severe preeclampsia, fetal growth retardation or chronic hypertension. The authors found that serum levels of sTNF-R1 were elevated in the second trimester only in those who subsequently developed preeclampsia complicated by fetal growth restriction. The authors suggested that the “increased TNF- λ production in women with preeclampsia is related to impaired placentation rather than to the maternal syndrome.”²⁴

What is the clinical significance of elevated sTNF-R2 concentrations at 13–26 weeks of pregnancy in women who ultimately developed preeclampsia? Using a cut-off value of the 75th percentile, we found that elevated levels of this soluble receptor had poor sensitivity

(31.3%) and a limited positive predictive value (25.3%) for subsequent development of preeclampsia. Therefore our findings indicate that measurement of sTNF-R2 early in pregnancy alone has limitations.

The strength of this study is that it provides information about sTNF-R₂ plasma concentrations early in pregnancy in a large number of women considered at very high risk for development of preeclampsia. In this respect we could locate no similar data from this large of a population. Limitations include the retrospective nature of the measurement of the biomarkers, failure to obtain samples from all trial participants, as well as the added limitation of restricting the cohort to patients who had at least two blood samples available for analysis. The latter resulted in the evaluation of only 40% of the women enrolled in the original trial. Even among these 1004 women, we only consistently examined two blood samples per woman per pregnancy, precluding repeated measurements in the same patient to search for trends, as might be done in clinical practice.

We did not determine whether there are changes in plasma concentration in sTNF-R1 or sTNF-R2 in women developing gestational hypertension nor in pregnancies complicated by fetal growth restriction with or without preeclampsia. Finally, one must be circumspect in interpreting changes occurring before overt disease in relation to causation. Preeclampsia has a pre-clinical phase, and there is a substantial literature describing small but discernable differences among populations developing the disease, and those remaining normotensive, that are present long before the disorder becomes obvious.

These findings should be verified in a prospectively designed study with serial measurements of sTNF-R1 and sTNF-R2 concentrations. In summary, our results reveal that among women at high-risk for preeclampsia, the plasma concentrations of sTNF-R2 at 13–26 or 24–28 weeks are higher in a subset of women who develop preeclampsia than in women who do not develop preeclampsia. This finding provides support for the hypothesis that intravascular inflammation is a mechanism of disease in a subset of patients.

Acknowledgments

Supported by grants from the National Institute of Child Health and Human Development (HD19897, HD36801, HD21410, HD21414, HD21434, HD27860, HD27861, HD27869, HD27883, HD27889, HD27905, HD27915, and HD27917)

This work was funded, in part, by the Division of Intramural Research of the National Institutes of Health

Reference List

1. Vassalli P. The pathophysiology of tumor necrosis factors. *Annu Rev Immunol* 1992;10:411–52. [PubMed: 1590993]
2. Taylor RN, de Groot CJ, Cho YK, Lim KH. Circulating factors as markers and mediators of endothelial cell dysfunction in preeclampsia. *Semin Reprod Endocrinol* 1998;16:17–31. [PubMed: 9654604]
3. Pober JS. Activation and injury of endothelial cells by cytokines. *Pathol Biol (Paris)* 1998;46:159–63. [PubMed: 9769910]
4. Rahman A, Kefer J, Bando M, Niles WD, Malik AB. E-selectin expression in human endothelial cells by TNF-alpha-induced oxidant generation and NF-kappaB activation. *Am J Physiol* 1998;275:L533–L544. [PubMed: 9728048]
5. Clauss M, Grell M, Fangmann C, Fiers W, Scheurich P, Risau W. Synergistic induction of endothelial tissue factor by tumor necrosis factor and vascular endothelial growth factor: functional analysis of the tumor necrosis factor receptors. *FEBS Lett* 1996;390:334–38. [PubMed: 8706889]
6. Mechtcheriakova D, Schabbauer G, Lucerna M, Clauss M, De Martin R, Binder BR, et al. Specificity, diversity, and convergence in VEGF and TNF-alpha signaling events leading to tissue factor up-regulation via EGR-1 in endothelial cells. *FASEB J* 2001;15:230–42. [PubMed: 11149911]

7. Lanza-Jacoby S, Phetteplace H, Sedkova N, Knee G. Sequential alterations in tissue lipoprotein lipase, triglyceride secretion rates, and serum tumor necrosis factor alpha during *Escherichia coli* bacteremic sepsis in relation to the development of hypertriglyceridemia. *Shock* 1998;9:46–51. [PubMed: 9466473]
8. Hubel CA. Oxidative stress in the pathogenesis of preeclampsia. *Proc Soc Exp Biol Med* 1999;222:222–35. [PubMed: 10601881]
9. Walsh SW. Maternal-placental interactions of oxidative stress and antioxidants in preeclampsia. *Semin Reprod Endocrinol* 1998;16:93–104. [PubMed: 9654611]
10. Bazzoni F, Beutler B. The tumor necrosis factor ligand and receptor families. *N Engl J Med* 1996;334:1717–25. [PubMed: 8637518]
11. Van Zee KJ, Kohno T, Fischer E, Rock CS, Moldawer LL, Lowry SF. Tumor necrosis factor soluble-receptors circulate during experimental and clinical inflammation and can protect against excessive tumor necrosis factor alpha in vitro and in vivo. *Proc Natl Acad Sci U S A* 1992;89:4845–49. [PubMed: 1317575]
12. Aderka D, Sorkine P, Abu-Abid S, Lev D, Setton A, Cope AP, et al. Shedding kinetics of soluble tumor necrosis factor (TNF) receptors after systemic TNF leaking during isolated limb perfusion. Relevance to the pathophysiology of septic shock. *J Clin Invest* 1998;101:650–59. [PubMed: 9449699]
13. Kupferminc MJ, Peaceman Am, Wigton TR, Rehnberg KA, Socol ML. Tumor necrosis factor-alpha is elevated in plasma and amniotic fluid of patients with severe preeclampsia. *Am J Obstet Gynecol* 1994;170:1752–57. [PubMed: 8203436]
14. Orvieto R, Ben Rafael Z. The role of cytokines in early detection of preeclampsia. *Med Hypotheses* 1994;43:315–18. [PubMed: 7877526]
15. Conrad KP, Benyo DF. Placental cytokines and the pathogenesis of preeclampsia. *Am J Reprod Immunol* 1997;37:240–49. [PubMed: 9127646]
16. Conrad KP, Miles TM, Benyo DF. Circulating levels of immunoreactive cytokines in women with preeclampsia. *Am J Reprod Immunol* 1998;40:102–11. [PubMed: 9764352]
17. Vince GS, Starkey PM, Austgulen R, Kwiatkowski D, Redman CW. Interleukin-6, tumour necrosis factor and soluble tumour necrosis factor receptors in women with preeclampsia. *Br J Obstet Gynaecol* 1995;102:20–25. [PubMed: 7833306]
18. Visser W, Beckmann I, Bremer HA, Lim HL, Wallenburg HC. Bioactive tumour necrosis factor alpha in pre-eclamptic patients with and without the HELLP syndrome. *Br J Obstet Gynaecol* 1994;101:1081–82. [PubMed: 7826964]
19. Williams MA, Farrand A, Mittendorf R, Sorensen TK, Zingheim RW, O'Reilly GC, et al. Maternal second trimester serum tumor necrosis factor-alpha-soluble receptor p55 (sTNFp55) and subsequent risk of preeclampsia. *Am J Epidemiol* 1999;149:323–29. [PubMed: 10025474]
20. Kupferminc MJ, Peaceman AM, Aderka D, Wallach D, Socol ML. Soluble tumor necrosis factor receptors and interleukin-6 levels in patients with severe preeclampsia. *Obstet Gynecol* 1996;88:420–27. [PubMed: 8752252]
21. Meekins JW. Interleukin-6, tumour necrosis factor and soluble tumour necrosis factor receptors in women with preeclampsia. *Br J Obstet Gynaecol* 1995;102:842–43. [PubMed: 7547749]
22. Opsjon, SI; Novick, D.; Wathen, NC.; Cope, AP.; Wallach, D.; Aderka, D. Soluble tumor necrosis factor receptors and soluble interleukin-6 receptor in fetal and maternal sera, coelomic and amniotic fluids in normal and pre-eclamptic pregnancies. *J Reprod Immunol* 1995;29:119–34. [PubMed: 7500319]
23. Williams MA, Mahomed K, Farrand A, Woelk GB, Mudzamiri S, Madzime S, et al. Plasma tumor necrosis factor-alpha soluble receptor p55 (sTNFp55) concentrations in eclamptic, preeclamptic and normotensive pregnant Zimbabwean women. *J Reprod Immunol* 1998;40:159–73. [PubMed: 9881743]
24. Schipper EJI, Bolte AC, Schalkwijk CE, Van Geijn HP, Dekker GA. TNF-receptor levels in preeclampsia – results of a longitudinal study in high-risk women. *Journal of Maternal-Fetal and Neonatal Medicine* 2005;18:283–287. [PubMed: 16390786]

25. Caritis S, Sibai B, Hauth J, Lindheimer MD, Klebanoff M, Thom E, et al. Low-dose aspirin to prevent preeclampsia in women at high risk. National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. *N Engl J Med* 1998;338:701–05. [PubMed: 9494145]
26. Sacks GP, Studena K, Sargent K, Redman GW. Normal pregnancy and preeclampsia both produce inflammatory changes in the peripheral blood leukocytes akin to those of sepsis. *Am J Obstet Gynecol* 1998;179:80–86. [PubMed: 9704769]
27. Naccasha N, Gervasi M-T, Chaiworapongsa T, Berman S, Yoon BH, Maymon E, Romero R. Phenotypic and metabolic characteristics of monocytes and granulocytes in normal pregnancy and maternal infection. *Am J Obstet Gynecol* 2001;185:1118–23. [PubMed: 11717644]
28. Sargent IL, Germain SJ, Sacks GP, Kumar S, Redman CW. Trophoblast deportation and the maternal inflammatory response in preeclampsia. *J Reprod Immunol* 2003;59:153–60. [PubMed: 12896819]
29. Sibai BM. Preeclampsia: An inflammatory syndrome? *Am J Obstet Gynecol* 2004;191:1061–26. [PubMed: 15507921]
30. Sibai B, Dekker G, Kupferminc M. Preeclampsia. *Lancet* 2005;365:785–99. [PubMed: 15733721]

Appendix

The following subcommittee members participated in protocol development and coordination between clinical research centers (M. Cotroneo RN and B. Collins PhD), and protocol/data management and statistical analysis (Elizabeth Thom PhD and Cora MacPherson PhD).

In addition to the authors, other members of the National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network are as follows:

University of Pittsburgh — J. Kuller, M. Cotroneo, and T. Kamon

University of Tennessee — B. Mercer and R. Ramsey

University of Southern California — Y. Rabello, D. McCart, and E. Mueller

University of Alabama at Birmingham — R. Goldenberg and R. Copper

Wayne State University — Y. Sorokin, G. Norman, and A. Millinder

Medical College of Virginia — J.T. Christmas, S. McCoy, and S. Elder

University of Cincinnati — N. Elder, B. Carter, and V. Pemberton

University of Oklahoma — A. Meier and V. Minton

Wake Forest University — M. Swain

University of Chicago — A.H. Moawad and P. Jones

Ohio State University — J.D. Iams, S. Meadows, and S. Brenner

Medical University of South Carolina — B. Collins, R.B. Newman, and S.G. Carter

The George Washington University Biostatistics Center — E.A. Thom, M. McNellis, C. MacPherson, D. Johnson, and M.L. Fischer

National Institute of Child Health and Human Development, National Institutes of Health — D. McNellis, C. Spong, C. Catz and S. Yaffe.

TABLE I

Distribution of patients according to baseline risk group.

	Total		Preeclampsia	
	N		N	%
Diabetes	192		46	21.5
Chronic hypertension	310		79	36.9
Multiple gestation	240		39	18.2
Previous history of preeclampsia	262		50	23.4
Total	1004		214	21.3

Demographic and clinical characteristics of the study population by patients who did and did not develop preeclampsia.

TABLE II

Characteristics	No Preeclampsia (n=790)	Preeclampsia (n=214)	P value
Maternal age in years (mean and SD)	26.3 (6.3)	27.4 (6.5)	0.024
Primigravida	146 (18.5%)	56 (26.2%)	0.013
Nullipara	214 (27.1%)	70 (32.7%)	0.11
Body mass index (mean and SD)	28.9 (8.2)	30.6 (9.1)	0.013
Race or ethnic groups			0.38
	White	72 (33.6%)	
	Black	128 (59.8%)	
	Hispanic/Other	14 (6.5%)	
Blood pressure (mmHg) at enrollment			
	Systolic (mean and SD)	115 (14)	122 (15)
	Diastolic (mean and SD)	68 (11)	73 (12)
			<0.0001
			<0.0001
Marital status			
	Single	407 (51.5%)	90 (42.1%)
	Married	297 (37.6%)	96 (44.9%)
	Other	86 (10.9%)	28 (13.1%)
			0.23
Cigarette smoking during pregnancy			
	No	587 (74.3%)	171 (79.9%)
	Discontinued during pregnancy	66 (8.4%)	13 (6.1%)
	Yes	137 (17.3%)	30 (14.0%)
Gestational age (weeks of first prenatal visit)			
	Median	10	10
	Range	(1-25)	(4-24)
Gestational age (weeks) at delivery (mean and SD)	37.1 (2.8)	36.4 (2.8)	<0.0001
Birth weight (gm) (mean and SD)	2917 (805)	2864 (862)	0.29

TABLE III

Plasma concentration of sTNF-R1 and sTNF-R2 in patients who did and did not develop preeclampsia according to first or second sample.

	Sample 1 (13–26 weeks)		Sample 2 (24–28 weeks)	
	No Preeclampsia (n=790)	Preeclampsia (n=214)	No Preeclampsia (n=790)	Preeclampsia (n=214)
sTNF-R1				
Median (pg/ml)	913.4	936.2	1008.7	1039.8
Range	359.0–5060.1	448.50–3239.3	313.0–4758.0	422.1–4071.4
Interquartile Range	756.9–1080.2	775.4–1139.4	845.3–1208.5	853.9–1318.1
P-value	0.19		0.14	
sTNF-R2				
Median (pg/ml)	2534.9	2677.5	2673.5	2872.7
Range	1021.5–13000.0	933.6–7835.4	786.9–11740.0	1237.5–11100.0
Interquartile Range	2128.1–3051.0	2217.4–3254.5	2222.7–3269.0	2291.0–3513.3
P-value	0.02		0.01	

TABLE IV

Odds ratios (OR) and 95% confidence intervals (CI) for preeclampsia by quartile of plasma concentrations of sTNF-R2 by Sample 1 (13–26 weeks) and Sample 2 (24–28 weeks).

Quartiles of sTNF-R2 (pg/ml)	Preeclampsia (n=214)	No Preeclampsia (n=790)	Unadjusted OR	95% CI	Adjusted OR*	95% CI
Sample 1						
< 2128.1	46 (18.9%)	197 (81.1%)	1.00	(reference)	1.00	(reference)
2128.1–2534.8	45 (18.5%)	198 (81.5%)	0.97	0.62–1.54	1.02	0.64–1.61
2534.9–3051.0	56 (22.1%)	197 (77.9%)	1.22	0.79–1.89	1.29	0.83–2.02
> 3051.0	67 (25.3%)	198 (74.7%)	1.45	0.95–2.21	1.50	0.97–2.31
Sample 2						
< 2222.7	51 (20.6%)	197 (79.4%)	1.00	(reference)	1.00	(reference)
2222.7–2673.5	38 (16.1%)	198 (83.9%)	0.74	0.476–1.189	0.77	0.48–1.23
2673.6–3269.0	49 (19.9%)	197 (80.1%)	0.96	0.62–1.49	1.00	0.64–1.57
> 3269.0	76 (27.7%)	198 (72.3%)	1.48	0.99–2.23	1.55	1.02–2.35

* Adjusted for body mass index, black race and chronic hypertension at baseline.

Odds ratios (OR) and 95% confidence intervals (CI) for preeclampsia by quartile of plasma concentrations of sTNF-R2 by Sample 1 (13–26 weeks) and Sample 2 (24–28 weeks) and by aspirin or placebo allocation.

TABLE V

		Aspirin		Placebo	
Quartiles of sTNF-R2 (pg/ml)		Adjusted OR*	95% CI	Adjusted OR*	95% CI
Sample 1					
< 2128.1		1.00	(reference)	1.00	(reference)
2128.1–2534.8		1.01	0.52–1.97	1.01	0.53–1.94
2534.9–3051.0		1.58	0.85–2.99	1.09	0.58–2.08
> 3051.0		1.21	0.64–2.29	1.82	1.01–3.36
Sample 2					
< 2222.7		1.00	(reference)	1.00	(reference)
2222.7–2673.5		0.49	0.24–0.95	1.24	0.63–2.45
2673.6–3269.0		0.53	0.27–1.00	1.89	1.00–3.67
> 3269.0		0.98	0.54–1.75	2.54	1.39–4.77

* Adjusted for body mass index, black race and chronic hypertension at baseline.