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## Acute Pyelonephritis during Pregnancy Changes the Balance of Angiogenic and Anti-Angiogenic Factors in Maternal Plasma

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### Abstract

**Objective**—Angiogenic factors have been implicated in the pathophysiology of sepsis. In experimental models of sepsis (endotoxemia and/or cecal ligation puncture), there is increased expression of vascular endothelial growth factors (VEGF) and the administration of exogenous soluble VEGF receptor (sVEGFR)-1, an antagonist to VEGF, reduces morbidity and mortality. Moreover, a dramatic elevation in sVEGFR-1 has been demonstrated in human sepsis. Although a balance between angiogenic and anti-angiogenic factors is essential for fetoplacental development, the changes of angiogenic factors during pregnancy in the context of infection have never been explored. Angiogenic factors also play crucial roles in the pathophysiology of preeclampsia. This study was conducted to determine if maternal plasma concentrations of placental growth factor (PlGF), sVEGFR-2 and soluble endoglin (sEng) change in pregnancies complicated by acute pyelonephritis (AP) compared to normal pregnancy and preeclampsia (PE).

**Study Design**—A case-control study was conducted in patients with AP, normal pregnant women (NP) and patients with PE (n=36 for each group) matched for gestational age. AP was diagnosed in the presence of fever (temperature  $\geq 38^{\circ}\text{C}$ ), clinical signs of infection, and a positive urine culture for micro-organisms. Plasma concentrations of PlGF, sVEGFR-2 and sEng were determined by ELISA. The results of plasma sVEGFR-1 concentrations has previously been reported before, but were included in this study to provide a complete picture of the angiogenic/anti-angiogenic profiles. Serum concentrations of interleukin (IL)-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, interferon (IFN)- $\gamma$ , Granulocyte macrophage colony stimulating factor (GM-CSF), and tumor necrosis factor (TNF)- $\alpha$  were also determined using high sensitivity multiplexed immunoassays in patients with AP and NP.

**Results**—AP was associated with a lower median plasma concentration of PlGF and sVEGFR-2 than NP (both  $p < 0.001$ ). There were no significant differences in the median plasma concentrations of sEng and sVEGFR-1 between AP and NP ( $p = 0.7$  and  $0.5$  respectively). In contrast, there was a 5-fold decrease of the median plasma concentration of PlGF, and an 8-10 fold increase of the median plasma concentrations of sVEGFR-1 and sEng in PE compared to

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those in AP (all  $p < 0.001$ ). No significant difference in the median plasma concentration of sVEGFR-2 was observed between patients with PE and AP ( $p = 0.5$ ). Pregnant women with acute pyelonephritis had median plasma concentrations of IL-6, IL-7, IL-8, IL-10, IFN- $\gamma$ , and TNF- $\alpha$  significantly higher than those in normal pregnant women (all  $p < 0.001$ , except IL-7  $p = 0.004$ ).

**Conclusion**—AP is associated with changes in the profile of angiogenic and anti-angiogenic factors. Although some of these changes resemble those in PE (decreased PIGF and sVEGFR-2), the magnitude of the changes of PIGF is much higher in PE. We conclude that despite high plasma inflammatory cytokine concentrations, acute systemic inflammation in pregnancy has a different angiogenic/anti-angiogenic profile than that of preeclampsia.

### Keywords

preeclampsia; angiogenesis; angiogenic factor; acute pyelonephritis; cytokines; systemic inflammation; infection; inflammation

### Introduction

Pyelonephritis complicates 1–2% of pregnancies[1] and is one of the most common infectious causes of pregnancy-associated hospitalizations[2]. Despite the favorable outcomes of most patients, pregnant women with pyelonephritis are at risk of developing preterm labor [3], adult respiratory distress syndromes (ARDS)[4,5], sepsis[6] and even death [7]. Indeed, acute pyelonephritis is the most common causes of septic shock during pregnancy [7-9]. Sepsis is defined as a systemic inflammatory response triggered by bacteria or bacterial products. During an episode of sepsis, there is leukocyte activation, elevation of several pro- and anti-inflammatory cytokines/chemokines/ generation of reactive oxygen species, activation of coagulation/complement systems, and endothelial dysfunction leading to multi-organ failure. Recently, angiogenic factors have been implicated in the pathophysiology of sepsis[10-16]. In experimental models of sepsis (endotoxemia and/or cecal ligation puncture)[12-14] and observational studies in septic patients [10,11,15,16], an increase in the plasma concentrations of vascular endothelial growth factors (VEGF) [10-12,15], placental growth factors (PIGF)[14], and soluble VEGF receptor (sVEGFR)-1[13,16] was observed. The changes in sVEGFR-1 are considered an adaptive response to infection and to have survival value[13,14], since the administration of an adenovirus expressing for sVEGFR-1[12] or exogenous sVEGFR-1[13] attenuates the inflammatory response and reduces morbidity/mortality in mice[12,13]. It is unknown if these changes in angiogenic factors in response to infection are also present in pregnant women. Most research on angiogenic factors during pregnancy focus on preeclampsia, a pregnancy specific disorder, which has pathophysiologic changes that overlap with those of sepsis.

Preeclampsia is characterized by new-onset hypertension and proteinuria in the second half of pregnancy. A central feature in the pathophysiology of preeclampsia was originally thought to be endothelial dysfunction[17]. However, Redman and colleagues proposed that the maternal syndrome of preeclampsia is due to an excessive maternal intravascular inflammatory response to pregnancy[18]. The endothelial dysfunction in preeclampsia is part of a generalized intra-vascular inflammatory reaction involving circulating leukocytes as well as the coagulation and complement systems. Although the primary insult responsible for these abnormalities remains elusive[19-22], it is postulated that the poor placentation and reduced blood supply (or ischemic-reperfusion injury)[23] in the intervillous space of the placenta in early pregnancy leads to the release of factors[24] into the maternal circulation causing systemic endothelial cell dysfunction[17], intravascular inflammation[25,26] and multiple organ damage. Candidates for these unknown factors include oxidative stress metabolites[23], syncytiotrophoblast microparticles[27], cytokines[28], angiotensin II

receptor type 1 antibody[29], and sVEGFR-1, a powerful natural anti-angiogenic factor of VEGF[30,31].

An imbalance between angiogenic (VEGF and PlGF) and anti-angiogenic factors (sVEGFR-1 and the soluble form of endoglin (sEng)) has been implicated in the pathophysiology of PE[31-41]. PlGF is a major member of the VEGF family, which can bind to VEGFR-1 and enhance the angiogenic response of VEGF on endothelial cells especially in pathological conditions such as limb ischemia or wound healing[42,43]. The soluble form of VEGFR-1 exerts its anti-angiogenic activity by binding to VEGF or PlGF and preventing VEGF from binding to its functional ligand, VEGFR-2 on endothelial cells[42,43]. sEng modulates the actions of transforming growth factors (TGF)- $\beta$ 1 as well as TGF- $\beta$ 3[44]. Indeed, patients with preeclampsia exhibit an angiogenic factor profile consistent with an “anti-angiogenic state” characterized by increased plasma concentrations of sVEGFR-1 and s-Eng; but decreased plasma concentrations of unbound VEGF and PlGF[31]. These changes have been observed prior to the clinical manifestation of the disease [36,38,40,41]. The role of sVEGFR-2 in human diseases is unclear and this protein has received less attention because of its less potency of binding to VEGF than sVEGFR-1[45]. However, plasma sVEGFR-2 concentration might be a surrogate marker of endothelial cell function in the maternal circulation, since VEGF signaling through the membranous isoform of this protein is essential for endothelial cell function and survival[46]. Similarly, a decreased plasma concentration of sVEGFR-2 in preeclampsia has been observed prior to the clinical diagnosis of this syndrome [47].

Although a balance between angiogenic and anti-angiogenic factors is essential for fetoplacental development, the changes of these factors during pregnancy in the context of infection have not been explored. The objectives of this study were 1) to examine if maternal plasma concentrations of PlGF, sEng and sVEGFR-2 change in pregnancies complicated by acute pyelonephritis; and 2) to determine if these changes differed from that seen in PE.

## Patients and Methods

### Study Design

This case-control study was conducted by searching the clinical database and bank of biologic samples of the Perinatology Research Branch. The following groups were included: 1) normal pregnant women; 2) pregnant women with pyelonephritis; and 3) patients with preeclampsia. Women with multiple pregnancies and fetal anomalies were excluded. Patients were considered to have a normal pregnancy outcome if they did not have any obstetrical, medical, or surgical complications of pregnancy, and delivered a term neonate of appropriate birth weight for gestational age without complications. Pyelonephritis was diagnosed in the presence of fever (temperature  $\geq 38^{\circ}\text{C}$ ), clinical signs of an upper urinary tract infection (e.g., flank pain, costovertebral angle tenderness), pyuria, and a positive urine culture for microorganisms. Blood cultures were also obtained in some patients. Preeclampsia was defined as hypertension (systolic blood pressure  $\geq 140$  mmHg or diastolic blood pressure  $\geq 90$  mmHg on at least two occasions, 4 hours to 1 week apart) and proteinuria ( $\geq 300$  milligrams in a 24-hour urine collection or one dipstick measurement  $\geq 2+$ ) [48]. Patients with chronic hypertension were excluded. Early-onset preeclampsia was defined as those who were diagnosed before 34 weeks of gestation [49]. The 3 studied groups were matched for gestational age at blood sampling (within 3 weeks).

All women provided written informed consent prior to the collection of plasma samples. The collection of samples and their utilization for research purposes was approved by the IRB of the Wayne State University and *The Eunice Kennedy Shriver* National Institute of Child

Health and Human Development (NICHD/NIH/DHHS). Many of these samples were used previously in studies of intravascular inflammation, angiogenesis and cytokine biology in normal and complicated pregnancies[26,50,51].

**Sample collection and angiogenic factors immunoassays**—Venipuncture was performed and the blood was collected into tubes containing EDTA. Samples were centrifuged and stored at  $-70^{\circ}\text{C}$ . 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 results of plasma sVEGFR-1 concentrations have previously been reported before [50], but included in this study for a complete picture of angiogenic profiles. 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.

**High sensitivity human cytokine multiplexed immunoassays**—Concentrations of interleukin (IL)-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, interferon (IFN)- $\gamma$ , granulocyte macrophage colony stimulating factor (GM-CSF), and tumor necrosis factor (TNF)- $\alpha$  in maternal serum of normal pregnant women and patients with acute pyelonephritis were determined simultaneously using microsphere-based high sensitivity human cytokine multiplex immunoassays obtained from Linco Research (St. Charles, MO). The microparticle beads were read using the Luminex100 analyzer (Luminex Corporation, Austin, TX). The instrument settings included measurement of 100 events per microparticle bead type. All standards and samples were assayed in duplicate wells. Five-parameter analysis was performed on the median (100 events) fluorescent units to derive the concentrations of each analyte using StatLIA (Brendan Scientific Corporation, Grosse Pointe Farms, MI). The sensitivities were 0.13 pg/ml. The inter- and intra-assay CV ranged from 7-10% and 5-9% respectively.

### Statistical analysis

Shapiro-Wilk was used to test the data for normal distribution. Kruskal Wallis with post-hoc Mann-Whitney U test was utilized to determine the differences of the median among and between groups. Contingency tables, Chi-square and Fischer's Exact test was employed for comparisons of proportions. Analysis was conducted with SPSS V.15 (SPSS Inc., Chicago, IL). A p value of  $<0.05$  was considered significant.

## Results

### Clinical characteristics of the study population

This study included 36 patients in each group. The preeclampsia group had a higher rate of nulliparous women than the normal pregnancy and acute pyelonephritis groups ( $p=0.009$  and  $p=0.02$  respectively; Table I). Among patients with pyelonephritis, 21% (7/34) delivered a small for gestational age neonate. The most common microorganism isolated from urine cultures was *Escherichia coli* [83% (30/36)]. Other microorganisms included *Klebsiella pneumoniae* ( $n=1$ ), *Proteus mirabilis* ( $n=1$ ), *Citrobacter koseri* ( $n=1$ ), *Pseudomonas aeruginosa* ( $n=1$ ), *Streptococcus agalactiae* ( $n=1$ ) and *Staphylococcus aureus* ( $n=1$ ).

Thirty-one (86%) patients had a blood culture for microorganisms performed and fourteen of them (45%) had positive blood culture results. *Escherichia coli* was the most common

microorganism isolated from blood cultures [64% (9/14)]. Other microorganisms included *Enterobacter aerogenes* (n=1), *Coagulase-negative staphylococcus* (n=2), *Staphylococcus aureus* (n=1) and *Streptococcus agalactiae* (n=1).

Clinical characteristics of patients with preeclampsia are displayed in Table II. Among patients with preeclampsia, 31 (86%) were diagnosed as severe preeclampsia, and 18 (50%) delivered neonates whose birthweights were below the 10<sup>th</sup> percentile for gestational age. There was no significant difference in the median gestational age at blood sampling among the three study groups (p>0.05).

All samples had sVEGFR-2, sEng and sVEGFR-1 concentrations above the detection limits. Whereas 12 (33%) patients with preeclampsia had plasma concentrations of PIGF below the detection limit, only one (2.8%) patient with acute pyelonephritis and one (2.8%) normal pregnant woman had plasma concentrations of PIGF below the detection limit.

### Angiogenic profiles of pregnant women with acute pyelonephritis

Pregnant women with acute pyelonephritis had median plasma concentrations of PIGF and sVEGFR-2 lower than those in normal pregnant women (PIGF: normal pregnancy: median 896 pg/ml, interquartile range (IQR) 567-1,200 pg/ml vs. acute pyelonephritis: median 366 pg/ml, IQR 154-547 pg/ml; and sVEGFR-2: normal pregnancy: median 12.9 ng/ml, IQR 10.1-15.7 ng/ml vs. acute pyelonephritis: median 8.7 ng/ml, IQR 6.9-10.3 ng/ml; both p<0.001, Figure 1 and 2 respectively).

The median plasma concentrations of both sEng and sVEGFR-1 in patients with pyelonephritis were not significantly different from those in normal pregnant women (sEng; normal pregnancy: median 6.6 ng/ml, IQR 5.1-8.3 ng/ml vs. acute pyelonephritis: median 6.7 ng/ml, IQR 5.2-8.9 ng/ml; p=0.7 Figure 3; and sVEGFR-1; normal pregnancy: median 1,052 pg/ml, IQR 644-1,539 pg/ml vs. acute pyelonephritis: median 903 pg/ml, IQR 540-1,355 pg/ml; p=0.5 Figure 4).

### Angiogenic profiles of patients with preeclampsia

The median concentration of PIGF in patients with PE was five-fold lower than that of patients with acute pyelonephritis. In contrast, the median plasma concentrations of sVEGFR-1 and sEng was 8-10 fold higher in patients with preeclampsia than in those with acute pyelonephritis (PIGF: acute pyelonephritis: median 366 pg/ml, IQR 154-547 pg/ml vs. preeclampsia: median 67 pg/ml, IQR 0-120.6 pg/ml; sEng: acute pyelonephritis: median 6.7 ng/ml, IQR 5.2-8.9 ng/ml vs. preeclampsia: median 62.3 ng/ml, IQR 34.6-100.9 ng/ml; and sVEGFR-1: acute pyelonephritis: median 903 pg/ml, IQR 540-1,355 pg/ml vs. preeclampsia: median 8,247 pg/ml, IQR 4,154-10,850 pg/ml; all p<0.001, Figure 1-4).

There was no significant difference in the median plasma concentration of sVEGFR-2 between patients with preeclampsia and those with acute pyelonephritis (acute pyelonephritis: median 8.7 ng/ml, IQR 6.9-10.3 ng/ml vs. preeclampsia: median 7.6 ng/ml, IQR 6.6-10.6 ng/ml; p=0.5; Figure 2).

Similar results were obtained when the comparisons were performed and included only patients with acute pyelonephritis who had a positive blood culture result for microorganisms and were matched for gestational age at blood sampling with normal pregnant women and patients with preeclampsia (Table III).

## Cytokine profiles of pregnant women with acute pyelonephritis

To confirm that patients with acute pyelonephritis had systemic inflammation, we determined serum concentrations of 12 cytokines in normal pregnant women and patients with acute pyelonephritis using multiplexed immunoassay. There were 31 cases matched for gestational age between the 2 groups and had serum samples available for cytokine determination. Patients with acute pyelonephritis had median serum concentrations of IL-6, IL-7, IL-8, IL-10, IFN- $\gamma$ , and TNF- $\alpha$  significantly higher than normal pregnant women (all  $p < 0.001$ , except IL-7  $p = 0.004$ ; Table IV).

## Discussion

### Principal findings

1) the angiogenic profile of pregnant women with acute pyelonephritis, regardless of the presence of bacteremia, was characterized by a lower plasma concentration of PIGF and sVEGFR-2, but no changes in plasma concentrations of sVEGFR-1 and sEng compared to that of normal pregnant women; 2) preeclampsia was associated with lower plasma concentrations of PIGF, but higher plasma concentrations of sVEGFR-1 and sEng than acute pyelonephritis in pregnancy; and 3) both preeclampsia and pregnant women with acute pyelonephritis had a similar decrease in plasma sVEGFR-2 concentrations from that of normal pregnant women.

### Angiogenic profiles in the septic non-pregnant state

In the current study, pregnant women with acute pyelonephritis were characterized by lower plasma concentrations of PIGF and sVEGFR-2, but no changes in plasma concentrations of sVEGFR-1 and sEng when compared to normal pregnant women. These findings are different from those in the septic non-pregnant state.

After the administration of lipopolysaccharides (LPS) or cecal ligation puncture in mice, there is an increase in plasma concentrations of VEGF, PIGF and sVEGFR-1 [12-14]. Observational studies in human sepsis also have reported similar results (healthy volunteer vs. sepsis; for PIGF: mean 0.18 pg/ml vs. 13 pg/ml; for sVEGFR-1: mean 369 pg/ml vs. 601 pg/ml) [12,16].

While VEGF is an amplifier of pro-inflammatory cytokine production [12], the increased plasma concentration of sVEGFR-1 is considered to have survival value since the administration of an adenovirus vector expressing sVEGFR-1 [12] or the exogenous administration of sVEGFR-1 [13] inhibits the recruitment of inflammatory cells into the peritoneal cavity and reduces morbidity / mortality in animal models of sepsis [13]. Similarly, the increased plasma PIGF concentrations after sepsis have been proposed to be an adaptive response to infection and has a protective role since PIGF knock-out mice or the administration of neutralizing PIGF antibody is associated with impaired cardiac function, increased vascular permeability and mortality [14]. The high plasma PIGF and VEGF concentrations in sepsis may be consequence of sepsis-induced inflammatory cytokines because the administration of LPS to triple mutant mice (null for IL-1 receptor, TNF receptor-1 and TNF receptor-2) results in a significantly lower induction of VEGF and PIGF [12]. Conflicting results have been reported regarding the mechanisms of action of sVEGFR-1 in sepsis. Yano et al demonstrated that the administration of an adenovirus vector for sVEGFR-1 gene before the endotoxin injection was able to block the increase of free VEGF and PIGF, but had no effect on the early increase of pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$  and IL-6). In contrast, Tsao et al found that the exogenous administration of sVEGFR-1 attenuated the increased plasma concentrations of TNF- $\alpha$  and IL-1 $\beta$  induced by intraperitoneal injection of endotoxin. They proposed that LPS-induced sVEGFR-1 release

occurs in a VEGF- and TNF- $\alpha$  independent manner since both VEGF- and TNF- $\alpha$  neutralizing antibodies failed to inhibit an LPS-induced sVEGFR-1 release[13]. Furthermore, the plasma concentrations of VEGF increased progressively from 2-24 hours after the administration of endotoxin, while plasma concentrations of sVEGFR-1 increased at 2 hours and decreased thereafter[13].

The sources of VEGF and sVEGFR-1 in the septic non-pregnant patients are partly from monocytes since LPS could stimulate monocytes to release VEGF and sVEGFR-1,[13] although several organs including the heart, liver, kidney, brain and spleen also show increased expression of VEGF and PlGF after experimental endotoxemia[12]. No information regarding the expression of sVEGFR-1 in organs affected by sepsis has been reported. On endothelial cells, another source of angiogenic factors, there is evidence that TNF- $\alpha$  could stimulate endothelial cells to release sVEGFR-1 and sEng[52]. There was, however, no information regarding plasma concentrations of sEng and sVEGFR-2 in septic patients. Collectively, the major sources of angiogenic factors during sepsis in non-pregnant women are monocytes and endothelial cells.

### Angiogenic profiles of normal pregnant women

During pregnancy, the uterine decidua and placenta are rich sources of angiogenic factors. Plasma concentrations of estrogen increase substantially during pregnancy. There is evidence that estrogen up regulates VEGFR-2, but not VEGFR-1, and promotes VEGF-mediated endothelial cell proliferation[53]. The increased plasma concentration of sVEGFR-1, a natural inhibitor of VEGF, during pregnancy is thought to counter-balance the VEGF-induced vasodilatation effect[46]. A recent study examining the concentrations of PlGF and sVEGFR-1 in maternal serum, coelomic fluid and amniotic fluid in pregnant women at 7-9 weeks of gestation suggested that the placenta is the main source of PlGF and sVEGFR-1 in pregnancy[55].

Another important source of angiogenic factors during pregnancy is leukocytes. Pregnancy is considered a unique immunological state characterized by activation of the innate immune system and suppression of the adaptive limb of the immune response in order to promote tolerance to the fetus, as well as protect the mother against infection[56]. In contrast to leukocytes of the non-pregnant women, the granulocytes and monocytes, two principal immune cells of the innate immune system, show evidence of phenotypic and metabolic changes, consistent with increased activation in the circulation of normal pregnant women[25,57]. The increased plasma soluble adhesion molecule concentration during pregnancy is consistent with increased activation of leukocytes and platelets[58]. Furthermore, peripheral blood mononuclear cells of pregnant women have been demonstrated to be able to release sVEGFR-1[59].

Since the placenta, in addition to leukocytes and endothelial cells, is a major source of angiogenic factors in the maternal circulation, angiogenic profiles in normal pregnancy are different from that in the non-pregnant state. In non-pregnant women, plasma concentrations of PlGF and sVEGFR-1 are usually below the detection limits of the assays (or detectable at very low concentrations)[12,14,16,35,50] and those of sEng are generally detectable at low concentrations (median 4.5 ng/ml: unpublished data, Chaiworapongsa, T). In contrast, during pregnancy, plasma PlGF and sVEGFR-1 concentrations increase substantially (PlGF: median 896 pg/ml; sVEGFR-1: median 1,052 pg/ml). Plasma sEng concentrations have a modest increase (median 6.6 ng/ml), while no significant change in plasma sVEGFR-2 concentrations has been observed during pregnancy[46].

## Angiogenic profiles of pregnant women with acute pyelonephritis

Why were the changes in angiogenic profiles different between pregnant women with acute pyelonephritis and septic non-pregnant women? We propose that the differences in angiogenic profiles between pregnant women exposed to bacterial infection and non-pregnant septic patients could be explained, at least in part, by much higher plasma concentrations of pro and anti-angiogenic factors in normal pregnant women in the basal state and the different sources of these angiogenic factors between the pregnant and the non-pregnant state.

The increased plasma concentration of PIGF during normal pregnancy is attributable mainly to the growing placenta[35]. Both Toll-like Receptor (TLR)-4[60,61], a pattern recognition receptor for Gram negative bacteria, and TNF receptor type 1 are expressed on trophoblasts[62,63]. There is evidence that either LPS or TNF- $\alpha$  could induce apoptosis (programmed cell death) of both cytotrophoblast and syncytiotrophoblasts[63-65]. Moreover, the administration of LPS or inflammatory cytokines (TNF- $\alpha$  or IL-1  $\beta$ ) to first trimester trophoblast cell lines decreased PIGF protein expressions[66]. Consistent with these observations, in the current study, pregnant women exposed to bacterial infection (acute pyelonephritis) had a median plasma concentration of PIGF lower than normal pregnant women, but higher than patients with preeclampsia. If the infection is chronic or repetitive, this could result in a further decrease of plasma PIGF concentrations. These findings may explain, at least in part, the association between urinary tract infection, periodontal disease and preeclampsia reported in a recent meta-analysis[67].

There is a paucity of information regarding the sources of sVEGFR-2 during pregnancy. The soluble form of VEGFR-2 can be detected in conditioned media obtained from human endothelial cells[45], suggesting that endothelial cells are one of the sources of plasma sVEGFR-2[68]. The membranous isoform of VEGFR-2 is expressed mainly on endothelial cells, a fraction of hematopoietic cells (also known as circulating endothelial progenitor cells) and basophils, but not monocytes[45,69]. A low level of VEGFR-2 expression is also observed in neurons, osteoblasts, pancreatic duct cells, and retinal progenitor cells[45]. In term human placentas, VEGFR-2 mRNA and protein expression are localized almost exclusively within vascular endothelial cells, but not trophoblasts[70,71]. These observations suggest that the predominant sources of sVEGFR-2 in pregnancy are vascular endothelium and hematopoietic cells similar to those in the non-pregnant state. The findings that the plasma sVEGFR-2 concentrations decreased in pregnant women with acute infection and in patients with preeclampsia would be consistent with the view that both preeclampsia and systemic infection are disorders that involved vascular endothelial cell function[46]. It is likely that plasma concentrations of sVEGFR-2 in non-pregnant women with acute infection would also decrease.

While several lines of evidence indicate that the major source of plasma sVEGFR-1 is the placenta, the source(s) of sEng in maternal circulation is (are) unclear. There is an increased expression of both mRNA and protein of sEng and sVEGFR-1 in the placenta, and the plasma concentrations of these proteins decrease after delivery[31,39,72-75]. Other potential sources include maternal endothelial cells and peripheral blood mononuclear cells[42,44,45].

Amniotic fluid contains several angiogenic factors, however, it is difficult to reconcile how these angiogenic factors gain access into maternal blood. In normal pregnancy, the concentrations of sVEGFR-1 were much higher in amniotic fluid than in maternal plasma both in the mid- and in the third -trimester of pregnancy (mid-trimester: 10,236 pg/ml vs. 441 pg/ml[76]; third trimester: 33,490 pg/ml vs. 3,417 pg/ml)[77]. In contrast, the concentrations of sEng were much higher in maternal circulation than in amniotic fluid



(median 15.1 ng/ml vs. 0.6 ng/ml)[78]. These observations suggest that sVEGFR-1 and sEng in maternal plasma might originate from different sources. Plasma concentrations of sVEGFR-1 and sEng are already elevated during pregnancy and our findings indicate that the exposure of trophoblasts, monocytes and endothelial cells to bacterial products or high concentrations of pro-inflammatory cytokines is not able to stimulate or release more sVEGFR-1 or sEng into the maternal circulation. One possible explanation for these findings could be that peripheral blood mononuclear cells are already activated and the exposure to microbial products or pro-inflammatory cytokines could not further increase the release of sVEGFR-1.

### **Angiogenic profiles of patients with preeclampsia**

The changes in angiogenic/anti-angiogenic factors in preeclampsia are more severe than those observed in acute pyelonephritis. There was a 5-fold decrease in the median plasma concentration of PIGF, while the median plasma concentrations of sVEGFR-1 and sEng increased 8 to 10 fold in preeclampsia compared to women with acute pyelonephritis. These findings indicate that acute systemic inflammation during pregnancy could not reduce the concentrations of angiogenic factors or increase the concentration of anti-angiogenic factors in maternal plasma to the extent observed in preeclampsia, and that preeclampsia is a much more severe “anti-angiogenic state” than pregnancy with acute systemic inflammation (acute pyelonephritis). There was, however, no significant difference in plasma concentrations of sVEGFR-2, a surrogate marker of endothelial cell function, between patients with preeclampsia and pregnant women with acute pyelonephritis.

### **Strength and limitation of the study**

This study is the first to examine the changes in plasma concentrations of pro- and anti-angiogenic factors in pregnant women with acute infection. The finding that the changes of angiogenic profiles in pregnant women with acute pyelonephritis are different from those observed in the septic non-pregnant patients is novel and deserve further investigation. Moreover, we confirmed that women with acute pyelonephritis in this study had high serum cytokine concentrations, which were consistent with systemic inflammation. The limitation of this study is that it did not include pregnant women with more severe clinical manifestations of sepsis (eg: septic shock or multi-organ failure). However, we have previously reported that most patients (90%) with pyelonephritis fulfilled the criteria of systemic inflammatory response syndrome (SIRS) for non-pregnant subjects at admission[79]. Of note, the criteria for SIRS from the 2001 International Sepsis Definition conference[80] do not consider the physiologic changes of normal pregnancy, and there is no modified definition of SIRS for pregnant women[80].

In conclusion, the changes of angiogenic profiles in pregnant women with acute pyelonephritis from normal pregnant women are different from those reported in septic non-pregnant patients. Although some of these changes resemble the observations reported in preeclampsia (decreased PIGF), the magnitude of the changes is much more severe in preeclampsia. These findings lead us to conclude that despite high plasma inflammatory cytokine concentrations, acute systemic inflammation in pregnancy has different angiogenic profiles from those of preeclampsia.

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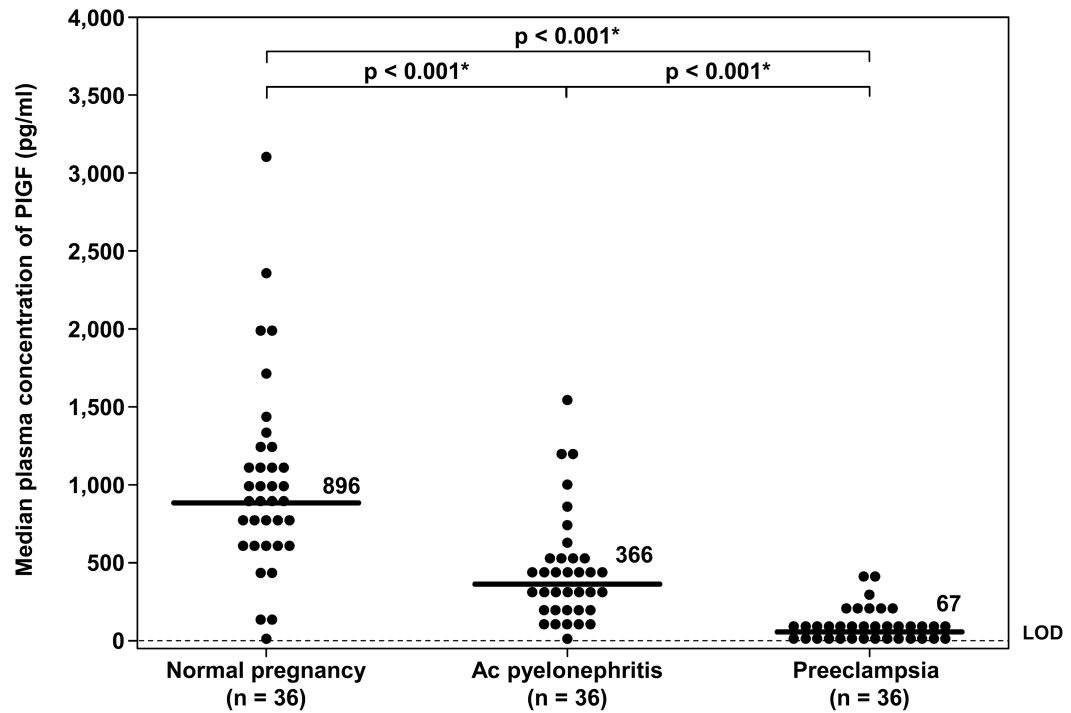
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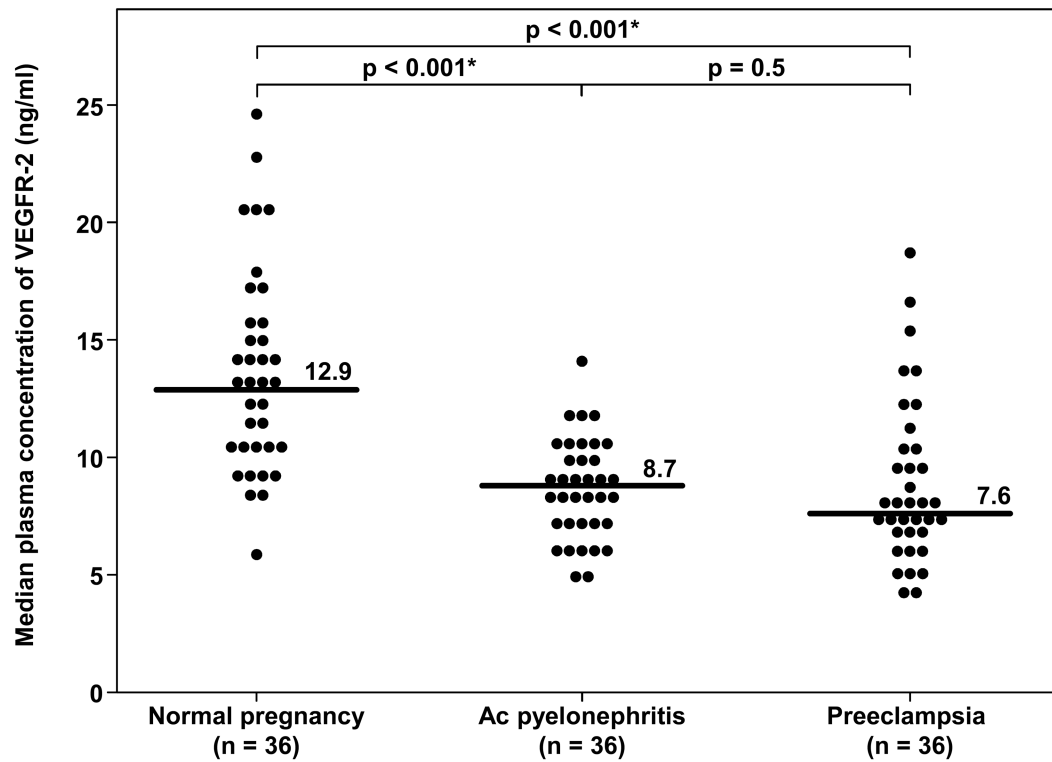
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**Figure 1.**

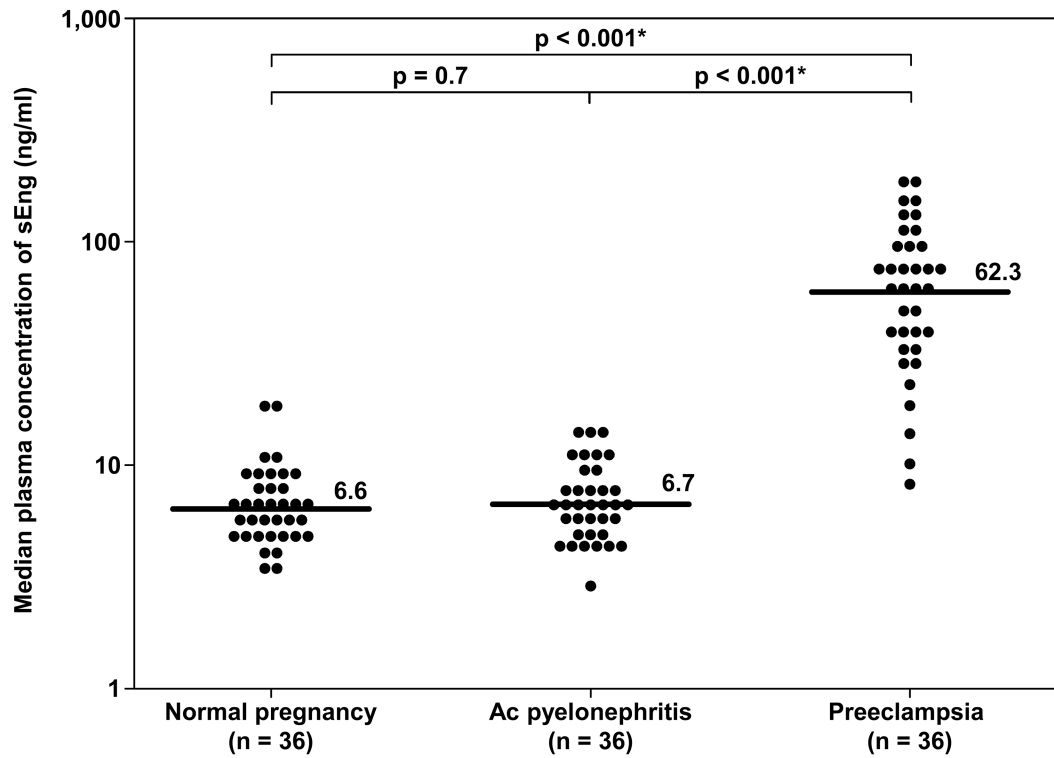
Plasma concentrations of PIGF in normal pregnant women, pregnant women with acute pyelonephritis, and patients with preeclampsia. Pregnant women with acute pyelonephritis and patients with preeclampsia had median plasma concentrations of PIGF lower than normal pregnant women (normal pregnancy: median 896 pg/ml, inter-quartile range (IQR) 567-1,200 pg/ml; acute pyelonephritis: median 366 pg/ml, IQR 154-547 pg/ml; preeclampsia: median 67 pg/ml, IQR 0-120.6 pg/ml; both  $p < 0.001$ ). However, patients with preeclampsia had a median plasma concentration of PIGF lower than those with acute pyelonephritis ( $p < 0.001$ ). \*:  $p < 0.05$ .



**Figure 2.**

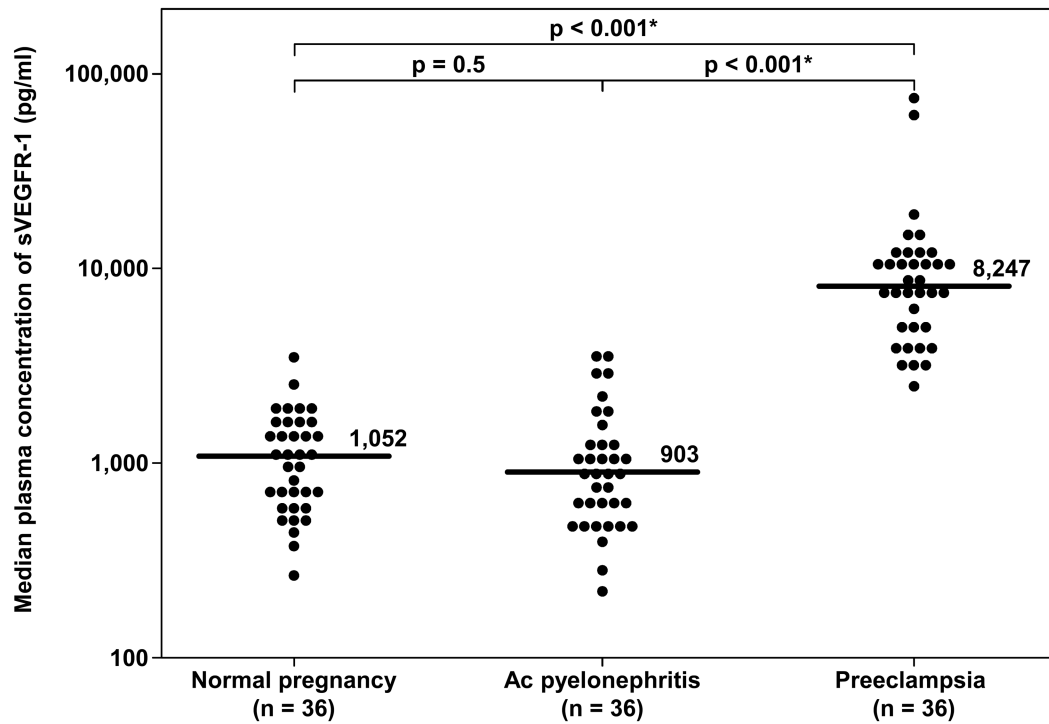
Plasma concentrations of sVEGFR-2 in normal pregnant women, pregnant women with acute pyelonephritis, and patients with preeclampsia. Pregnant women with acute pyelonephritis and patients with preeclampsia had median plasma concentrations of sVEGFR-2 lower than normal pregnant women (normal pregnancy: median 12.9 ng/ml, IQR 10.1-15.7 ng/ml; acute pyelonephritis: median 8.7 ng/ml, IQR 6.9-10.3 ng/ml; preeclampsia: median 7.6 ng/ml, IQR 6.6-10.6 ng/ml; both  $p < 0.001$ ). There was no significant difference in the median plasma concentrations of sVEGFR-2 between patients with preeclampsia and those with acute pyelonephritis ( $p = 0.5$ ). \*:  $p < 0.05$ .





**Figure 3.**

Plasma concentrations of sEng in normal pregnant women, pregnant women with acute pyelonephritis, and patients with preeclampsia. Patients with preeclampsia had a median plasma concentration of sEng higher than normal pregnant women and higher than patients with acute pyelonephritis (normal pregnancy: median 6.6 ng/ml, IQR 5.1-8.3 ng/ml; acute pyelonephritis: median 6.7 ng/ml, IQR 5.2-8.9 ng/ml; preeclampsia: median 62.3 ng/ml, IQR 34.6-100.9 ng/ml; both  $p < 0.001$ ). There was no significant difference in the median plasma concentrations of sEng between patients with acute pyelonephritis and normal pregnant women ( $p = 0.7$ ). \*:  $p < 0.05$ .



**Figure 4.**

Plasma concentrations of sVEGFR-1 in normal pregnant women, pregnant women with acute pyelonephritis, and patients with preeclampsia. Patients with preeclampsia had a median plasma concentration of sVEGFR-1 higher than normal pregnant women and higher than those with acute pyelonephritis (normal pregnancy: median 1,052 pg/ml, IQR 644-1,539 pg/ml; acute pyelonephritis: median 903 pg/ml, IQR 540-1,355 pg/ml; preeclampsia: median 8,247 pg/ml, IQR 4,154-10,850 pg/ml; both  $p < 0.001$ ). There was no significant difference in the median plasma concentration of sVEGFR-1 between patients with acute pyelonephritis and normal pregnant women ( $p = 0.5$ ). \*:  $p < 0.05$ .

Table 1

## Clinical characteristics of the study population

	Normal pregnancy (n = 36)	p	Acute pyelonephritis (n=36)	p <sup>1</sup>	Preeclampsia (n = 36)	p <sup>2</sup>
Age (y)	23 (21-28)	0.7	22 (19-29)	0.8	23 (19-29)	0.9
Race: African American	32 (88.9%)	0.2	27 (75%)	0.7	28 (78%)	0.6
Caucasian	2 (5.6%)		6 (17%)		5 (14%)	
Hispanic	1 (2.8%)		3 (8%)		2 (6%)	
Other	1 (2.8%)		--		1 (2%)	
Nulliparity	11 (30.6%)	0.8	12 (33.3%)	0.02*	22 (61.1%)	0.009*
Smoking	3 (8.3%)	0.7	5 (13.9%)	0.7	6 (16.7%)	0.3
GA at blood sampling (weeks)	31.6 (26.2-36.0)	0.9	31.6 (25.5-36.1)	0.5	31.0 (28.9-35.3)	0.5
GA at delivery (weeks)	39.0 (38-40.1)	0.5	39.3 (38.3-40.4)	<0.001*	31.9 (29.2-35.6)	<0.001*
Birthweight (grams)	3337 (3015-3672)	0.05	3175 <sup>a</sup> (2697-3558)	<0.001*	1320 (1030-2227)	<0.001*
BW<10%	0	0.004*	7 <sup>a</sup> (20.6%)		18 (50%)	<0.001*

Value expressed as median (inter-quartile range) or number (percent); GA: gestational age; <sup>a</sup> = 2 patients had no birthweight information. p<sup>1</sup> : comparisons between acute pyelonephritis and preeclampsia;

p<sup>2</sup> : comparisons between normal pregnancy and preeclampsia

\* p<0.05

**Table II**  
**Clinical characteristics of patients with preeclampsia**

<b>Blood pressure (mmHg)</b>	
Systolic	173 ± 18
Diastolic	106 ± 13
Mean arterial pressure	129 ± 13
Aspartate aminotransferase (SGOT) (U/L)	95 ± 171
Platelet count ( $\times 10^3$ ) ( $\mu$ /L)	163 ± 52
Severe preeclampsia	31 (86.1%)
Early-onset preeclampsia	25 (69%)

Value expressed as mean  $\pm$  SD or number (percent)

**Table III**  
**Plasma concentrations of PIGF, sVEGFR-2, sEng and sVEGFR-1 in pregnant women with acute pyelonephritis who had a positive blood culture result and patients with preeclampsia matched for gestational age at blood sampling**

	Normal pregnancy (n = 14)	p	Acute pyelonephritis (n=14)	p <sup>1</sup>	Preeclampsia (n = 14)	p <sup>2</sup>
GA at blood sampling (weeks)	32.3 (25.9-35.0)	0.9	32.4 (25.1-35.0)	0.7	32.4 (25.6 - 35.2)	0.8
PIGF (pg/ml)	919.4 (661-1,404)	0.001*	343 (128-474)	0.003*	80 (0-150)	<0.001*
sVEGFR-2 (ng/ml)	11.2 (8.5-24.7)	0.01*	9.1 (5.1-14.2)	0.3	7.7 (4.5-13.9)	0.001*
sEng (ng/ml)	6.9 (4.6-9.5)	0.3	7.8 (6.2-10.5)	<0.001*	63.0 (29.3-100.4)	<0.001*
sVEGFR-1 (pg/ml)	1,207 (665-1,519)	0.6	1,213 (606-2,286)	<0.001*	8,247 (4,557-11,825)	<0.001*

Values expressed as median (inter-quartile range); GA: gestational age

p<sup>1</sup> : comparisons between acute pyelonephritis and preeclampsia; p<sup>2</sup> : comparisons between normal pregnancy and preeclampsia

\* p<0.05

**Table IV**  
**Serum concentrations of 13 cytokines (pg/ml) evaluated by multiplexed immunoassay in normal pregnant women, pregnant women with acute pyelonephritis and patients with preeclampsia**

	Normal pregnancy (n = 31)	Acute pyelonephritis (n=31)	p
GA at blood sampling (weeks)	31.4 (26.3-36.1)	31.9 (26.7-36.4)	0.9
IL-1 $\beta$	0.7 (0.3-3.4)	0.9 (0.3-2.9)	0.9
IL-2	2.3 (0.5-4.3)	2.1 (0.1-4.7)	0.9
IL-4	7.4 (0-32.3)	3.7 (0.5-24.9)	0.8
IL-5	0 (0-0.7)	0.1 (0-0.9)	0.8
IL-6	3.5 (1.6-11.8)	40.0 (22.6-117.5)	<0.001*
IL-7	3.8 (2.8-5.2)	6.3 (4.3-9.8)	0.004*
IL-8	2.5 (1.9-4.3)	9.2 (4.9-18.9)	<0.001*
IL-10	3.5 (2.5-7.1)	20.3 (7.9-53.8)	<0.001*
IL-12p70	0.3 (0-2.6)	0.5 (0-1.7)	0.8
IL-13	0 (0-2.7)	0.3 (0-3.9)	0.3
IFN- $\gamma$	0 (0-6.5)	7.7 (3.3-14.3)	0.001*
GM-CSF	0.9 (0-3.3)	1.1 (0.3-2.6)	0.7
TNF- $\alpha$	2.5 (1.7-2.8)	5.3 (4.1-9.3)	<0.001*

Values expressed as median (inter-quartile range);

\*  
p<0.05