Angiogenic Factors and Preeclampsia

Predictive Value of Maternal Angiogenic Factors in Second Trimester Pregnancies With Abnormal Uterine Perfusion

Holger Stepan, Angela Unversucht, Niels Wessel, Renaldo Faber

Abstract—Angiogenic factors like placental growth factor and its antiangiogenic antagonist soluble fms-like tyrosine kinase 1 (sFlt1) are closely related to the pathogenesis of preeclampsia and intrauterine growth restriction. Because it is known that altered maternal sFlt1 and placental growth factor levels are detectable weeks before the onset of these pregnancy complications, it was the aim of the study to investigate the predictive value of these markers in high-risk second trimester pregnancies characterized by abnormal uterine perfusion. This prospective study includes 63 second trimester pregnant women with abnormal uterine perfusion. Twenty five of them developed a later complication (12 with preeclampsia, 11 with intrauterine growth restriction, and 2 with intrauterine death), whereas 38 had a normal course of pregnancy. Pregnancies with adverse pregnancy outcome showed in the second trimester significantly higher sFlt1 (1403.6±555 versus 451.8±42 pg/mL; P<0.05) and lower placental growth factor (139.6±24 versus 184.1±21 pg/mL) levels compared with those with normal outcome. These alterations were more pronounced in pregnancies with subsequent preeclampsia compared with intrauterine growth restriction and early onset diseases (delivery <34 weeks) compared with late-onset diseases. The combination of Doppler and sFlt1 increases the sensitivity of Doppler alone for iatrogenic preterm delivery from 64% up to 79% and the specificity from 63% up to 80%. Using both factors, sFlt1 and placental growth factor, early onset preeclampsia can be predicted with 83% sensitivity and 95% specificity. We conclude that the concurrent measurement of uterine perfusion and angiogenic factors allows an efficient prediction of early onset pregnancy complications, particularly preeclampsia. (Hypertension. 2007;49:818-824.)

Key Words: angiogenic factors ■ Doppler sonography ■ intrauterine growth restriction ■ preeclampsia ■ uterine perfusion

There is growing evidence that an imbalance between angiogenic factors, such as vascular endothelial growth factor or placental growth factor (PIGF), and factors inhibiting angiogenesis, such as soluble fms-like tyrosine kinase 1 (sFlt1), are closely related to the pathogenesis of preeclampsia. As a splice variant of the vascular endothelial growth factor receptor Flt1, this secreted form of Flt1 is a potent inhibitor of vascular endothelial growth factor and PIGF. As shown in animal models, sFlt1 is able to induce preeclamptic symptoms like hypertension, proteinuria, and renal damage and, therefore, seems to be an effective mediator of preeclampsia. In humans, sFlt1 is dramatically upregulated in the preeclamptic placenta, and there are numerous reports on elevated maternal sFlt1 concentrations in preeclampsia that normalize after delivery. Notably, clinical studies demonstrated that the marked increase of circulatory sFlt1 is observable weeks before the clinical manifestation of preeclampsia. In contrast to sFlt1, PIGF concentrations are reduced in preeclampsia, which results from binding with increased concentrations of circulating sFlt1. PIGF concentrations are reciprocal to the maternal sFlt1 levels and are lower in women who subsequently develop preeclampsia.

However, these markers are not totally specific for preeclampsia, because we and others demonstrated that also intrauterine growth restriction (IUGR) pregnancies are characterized by elevated sFlt1 concentrations. Similar to preeclampsia, PIGF concentrations seem to mirror the sFlt1 alterations in IUGR, because reduced maternal PIGF serum concentrations in normotensive IUGR pregnancies have also been reported.

Various studies have investigated sFlt1 and PIGF as possible predictors for preeclampsia (see Lam et al for details). These studies provided different odds ratios, sensitivities, and specificities for various sFlt1 and PIGF cutoff levels in different gestational periods. However, the clinical use of these markers as a screening test has not been fully clarified, also because of the relatively low incidence of preeclampsia and IUGR. Exactly these pregnancy complications, preeclampsia and IUGR, are mostly characterized by an abnormal perfusion of the uterine arteries, which can be...
detected by Doppler sonography. This finding reflects a high resistance in the uteroplacental circulation and is, therefore, an indirect sign of an inadequate trophoblast invasion that is thought to be the pathogenic key event for these pregnancy complications. For that reason, Doppler sonography is a useful tool to identify women at risk for these pregnancy complications. However, the positive predictive value of Doppler sonography is limited to \( \approx 30\% \), because women with an abnormal uterine perfusion only partly develop 1 of these pregnancy complications.\(^{13,14}\) Previous studies failed to identify an independent adjunct that is able to substantially improve the screening efficacy of Doppler sonography.\(^{15,16}\)

Our group showed in a retrospective pilot study that second trimester pregnancies with abnormal uterine perfusion and later adverse outcome show significantly elevated sFlt1 levels in maternal circulation.\(^{19}\) Consequently, the aim of this study was to investigate prospectively whether measurement of maternal sFlt1 and PlGF in second trimester pregnancies with abnormal uterine perfusion has a predictive power regarding pregnancy outcome. Secondly, we aimed at answering the question of whether angiogenic markers have useful predictive utility when combined with uterine Doppler.

**Methods**

The study was designed as a prospective cohort study and includes 63 pregnant women in the second trimester with abnormal uterine perfusion. All of the women gave informed consent. The Doppler investigations were performed using a LOGIQ 9 ultrasound machine (GE) with a 5.0-MHz convex transducer. To obtain data from both uterine arteries, the transducer was placed longitudinally in the right or left lower quadrant of the abdomen. Color Doppler imaging was used to identify the uterine artery at the point where it crossed the external iliac artery. The pulsed Doppler was then used to obtain flow velocity waveforms. An insonation angle of \( \leq 60\% \) was used to obtain waveforms acceptable for analysis. The pulsatility index (PI) and the presence or absence of a notch were noted. The procedure was then repeated on the opposite uterine artery.

The uterine perfusion was defined as pathological if there was bilateral notching and/or if the mean PI of both arteries was greater than the 90th percentile (mean PI: \( >1.45 \)) of its own reference group. Doppler investigations were performed by 2 operators (H.S. and R.F.) All of the pregnancies were singleton, and at the time of examination (range: 19 to 24 weeks of gestation), the women were healthy, normotensive, and without clinical signs of labor or cervical incompetence. The fetal growth was normal, and there were no obvious fetal defects. Patients with diabetes and pre-existing cardiovascular or renal diseases were excluded.

In the study group, 25 patients developed a later complication, whereas 38 had a normal course of pregnancy (“normal outcome”). In the group with a subsequent complication, 12 patients developed preeclampsia, and 13 patients developed IUGR. Two intrauterine deaths occurred in the IUGR group. Seventeen patients in the subgroup with complications required iatrogenic preterm delivery before 34 completed weeks of gestation, which was defined as primary cesarean section because of deteriorating maternal condition and/or signs of fetal distress. Preterm delivery because of preterm labor or premature rupture of the membranes did not occur in this study group.

Preeclampsia was defined as gestational proteinuric hypertension, which was developed antenatally, for the first time in labor or for the first time in the puerperium according to the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy.\(^{17}\) Under this classification, gestational hypertension is defined as a blood pressure \( >140 \text{ mm Hg systolic} \) or \( >90 \text{ mm Hg diastolic} \) in a woman who was normotensive before 20 weeks’ gestation. Significant proteinuria is defined as \( \geq 300 \text{ mg of total protein} \) in a 24-hour urine collection. This will usually correlate to \( \geq 1+ \) proteinuria by dipstick in a random urine determination with no evidence of urinary tract infection. All of the patients with preeclampsia in this study had appropriate-for-gestational-age fetuses.

IUGR was defined as a birth weight below the 5th percentile of a reference group. Women with IUGR pregnancies were normotensive and had no proteinuria. All of the IUGR pregnancies were characterized by \( \geq 1 \) sign of disturbed placental function, such as oligohydramnios and/or a PI of the umbilical artery greater than the 90th percentile (data not shown).

After the first Doppler examination, 1 venous blood sample (10 mL) was drawn from each woman into tubes containing EDTA. Immediately after sampling, plasma was separated by centrifugation at 4000g for 10 minutes and frozen at \(-80\^\circ\text{C}\). Maternal plasma sFlt1 and PlGF were measured using a commercial ELISA (R&D Systems).

The sFlt1/PlGF ratio was calculated as the quotient between both values. The clinical staff was not aware of the measurement results, and, thus, the sFlt1 and PlGF data could not influence clinical decisions. Group summaries are expressed as mean±SEM. Statistical analysis was performed via Mann–Whitney U test and Pearson’s correlation coefficients where appropriate using the program Statistical Package for Social Sciences (SPSS Inc). In all of the tests, the criterion for statistical significance is \( P<0.05 \).

Screening accuracy for later pregnancy complication and delivery \( <34 \) weeks was assessed by applying receiver–operating characteristics curve methodology. Overall accuracy was estimated with the area under the curve. Optimal cutoffs were calculated based on multifactorial receiver operator characteristic and predictive accuracy characteristics as described previously.\(^{18}\) The cutoff was set as optimal when the classification rate was optimal, that is, the sum of sensitivity and specificity was maximal.

**Results**

Clinical characteristics of the patients are given in Table 1. Patients with subsequent adverse pregnancy outcome were characterized by a significantly higher uterine PI (2.17 versus 1.76; \( P<0.05 \); Table 1), whereas this Doppler parameter alone has a sensitivity of only 68% and a specificity of 60% (Table 2). Maternal sFlt1 concentrations correlated positively with the uterine PI (Pearson 0.281; \( P<0.05 \)). In contrast, there was no significant correlation between PlGF and uterine resistance (Pearson –0.235; \( P \) value not significant).

The patients with a later complication showed significantly higher maternal sFlt1 concentrations compared with the group with a normal pregnancy outcome (1403.6±555 versus 451.8±42 pg/mL; \( P<0.05 \); Figure 1A). This elevation was more drastic in the subgroup with subsequent preeclampsia (1926.8±1084 pg/mL; \( P<0.05 \)) compared with the subgroup with subsequent IUGR (879.3±228 pg/mL; Figure 1A). When the sFlt1 cutoff was set at 500.5 pg/mL, the sensitivity to detect a later complication was 59%, and the specificity was 68% (Figure 1B). In contrast, there was a tendency to lower PlGF levels in the group with subsequent adverse outcome (139.6±24 versus 184.1±21 pg/mL; Figure 2A), which leads to an sFlt1/PlGF ratio of 120.9 in the group with adverse outcome versus 3.6 in the group with normal outcome. Again, the decrease of PlGF concentration was more pronounced in the patients with subsequent preeclampsia (118.7±32 pg/mL; IUGR: 155.5±33 pg/mL; Figure 2A). PlGF alone reaches a sensitivity of 54% and a specificity of 60% when the cutoff was set at 118.0 pg/mL (Figure 2B). Also, the combination of both markers in a ratio or the combined calculation of Doppler with either sFlt1 or PlGF...
did not improve the predictive value (Table 2). However, the separate analysis of patients with subsequent preeclampsia reaches a better prediction using sFlt1 and PlGF with a sensitivity of 77% and a specificity of 73% (Table 3).

The subgroup with iatrogenic early delivery before 34 weeks (n = 11005; 27%) showed significantly higher maternal sFlt1 concentration compared with the groups with a delivery ≥34 weeks of gestation (1969.5 ± 844 versus 445.1 ± 36 pg/mL; P < 0.001; Figure 3A). Likewise, this elevation was more drastic in the subgroup with subsequent early onset preeclampsia (4398.8 ± 2748 pg/mL; P = 0.05) compared with the subgroup with early onset IUGR (1376.6 ± 354 pg/mL; Figure 3A). When the sFlt1 cutoff was set at 631.3 pg/mL, the sensitivity was 71% and the specificity was 87% for any delivery <34 weeks (Figure 3B). PlGF concentrations of

### TABLE 1. Clinical Data of the Patient Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Outcome (n = 38)</th>
<th>All Patients With Subsequent Complication (n = 25)</th>
<th>Patients With Subsequent Complication and Delivery &lt;34 Weeks (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td>29 (18 to 37)</td>
<td>29 (19 to 36)</td>
<td>30 (23 to 35)</td>
</tr>
<tr>
<td>GA at measurement, wk</td>
<td>21 (19 to 24)</td>
<td>22 (19 to 24)</td>
<td>22 (20 to 24)</td>
</tr>
<tr>
<td>Primigravidae, No. (%)</td>
<td>15 (39.5)</td>
<td>5 (41.7)</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Primiparae, No. (%)</td>
<td>20 (52.6)</td>
<td>6 (50)</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Mean PI of both uterine arteries</td>
<td>1.76 ± 0.6</td>
<td>2.26 ± 0.6*</td>
<td>2.56 ± 1.2*</td>
</tr>
<tr>
<td>GA at delivery, wk</td>
<td>39 (35 to 42)</td>
<td>34 (26 to 40)*</td>
<td>30 (26 to 33)*</td>
</tr>
<tr>
<td>Prepregnancy BMI, kg/m²</td>
<td>24.3 ± 8.1</td>
<td>26.5 ± 9.3</td>
<td>20.9 ± 4.1</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>2902 ± 719</td>
<td>1890 ± 907*</td>
<td>1241 ± 362*</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>127 ± 15</td>
<td>179 ± 19*</td>
<td>171 ± 31*</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>80 ± 12</td>
<td>101 ± 10</td>
<td>109 ± 25*</td>
</tr>
<tr>
<td>Proteinuria, g/L</td>
<td>0</td>
<td>1.5*</td>
<td>3.0*</td>
</tr>
</tbody>
</table>

Clinical characteristics of the patient groups. Data are presented as mean ± SEM or mean (range). PE indicates preeclampsia; IUGR, intrauterine death; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; GA, gestational age.

*P < 0.05 vs normal outcome; Mann–Whitney U test for intergroup differences.

### TABLE 2. Prediction of Subsequent Pregnancy Complication

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All Complications</th>
<th>Preeclampsia + IUGR</th>
<th>Delivery &lt;34 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutoff</td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>PPA</td>
</tr>
<tr>
<td>Doppler (PI)</td>
<td>1.78</td>
<td>0.68</td>
<td>0.60</td>
</tr>
<tr>
<td>sFlt1</td>
<td>500.5 pg/mL</td>
<td>0.59</td>
<td>0.68</td>
</tr>
<tr>
<td>PlGF</td>
<td>118.0 pg/mL</td>
<td>0.54</td>
<td>0.60</td>
</tr>
<tr>
<td>sFlt1/PlGF ratio</td>
<td>3.15</td>
<td>0.63</td>
<td>0.50</td>
</tr>
<tr>
<td>sFlt1 + PlGF</td>
<td>631.3±81.8 pg/mL</td>
<td>0.68</td>
<td>0.71</td>
</tr>
<tr>
<td>Doppler + sFlt1</td>
<td>2.26/567.2 pg/mL</td>
<td>0.68</td>
<td>0.68</td>
</tr>
<tr>
<td>Doppler + PlGF</td>
<td>1.78/332.1 pg/mL</td>
<td>0.68</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Results of tests for detection/prediction of adverse pregnancy outcome (preeclampsia/IUGR) and the subgroup requiring iatrogenic preterm delivery at <34 weeks. PPA indicates positive predictive accuracy.

Figure 1. A, Maternal plasma concentration of sFlt1 in pregnancies with abnormal uterine perfusion (mean gestational age: 21 weeks) and normal pregnancy outcome vs later pregnancy complication (all complications, preeclampsia [PE], and IUGR). Data are presented as mean ± SEM. *P < 0.05 vs normal outcome. B, Receiver–operating characteristics curves for maternal plasma sFlt1 in the prediction of later pregnancy complication.
patients with an early complication were significantly decreased (127.1 ± 33 versus 180.4 ± 18 pg/mL; *P < 0.05), with the lowest level in the subgroup with early onset preeclampsia (Figure 4A). Notably, the combination of Doppler and sFlt1 increases the sensitivity of Doppler alone from 64% up to 79% and the specificity from 63% up to 80% (Table 2). The predictive value of the angiogenic factors increases in the patients with iatrogenic delivery at 34 weeks when a diagnosis-specific analysis is performed (Tables 3 and 4). For instance, the combined analysis of sFlt1 and PlGF is able to predict early onset preeclampsia with a sensitivity of 83% and a specificity of 95% (Table 3).

Discussion

Based on the observation that the maternal sFlt1 concentration is elevated weeks before the manifestation of preeclampsia and IUGR,4,5,10 it was the logical progression to raise the question of whether this elevation is already detectable in second trimester pregnancies with abnormal uterine perfusion and a later complication. This approach seems to be rational, because pregnant women with abnormal Doppler findings are a preselected group of women at risk for preeclampsia and also other pregnancy complications, such as IUGR. In a retrospective pilot study, our group could demonstrate that,
indeed, second trimester pregnancies with abnormal uterine perfusion and adverse outcomes have higher sFlt1 concentrations compared with those with normal pregnancy outcomes despite increased uteroplacental resistance. This observation could have a significant clinical value, as one could use sFlt1 to identify patients at risk for adverse outcomes among those patients with abnormal uterine perfusion. Previous studies indicated that the predictive potential of sFlt1 can be improved when the relation between sFlt1 and PlGF is analyzed. Thus, the measurement of both proteins in a preselected risk group was to be evaluated for clinical use.

Our prospective study confirms that second trimester pregnancies with a later preeclampsia but also IUGR have increased sFlt1 levels. Moreover, we find reduced PlGF concentration in women with subsequent disease. These results indicate that sFlt1 and PlGF are relevant pathogenic factors for preeclampsia and IUGR, because their changes in maternal plasma are observable weeks before the onset of the disease. Our results demonstrate also that the antenatal changes of these factors are more pronounced in preeclampsia when compared with normotensive IUGR pregnancies. This finding allows 2 interpretations: sFlt1 and PlGF are pathogenic factors for preeclampsia rather than for IUGR or IUGR pregnancies may have a different sequence of the symptoms with early signs of fetal alterations and a delayed maternal reaction.

However, when any type of pregnancy complication is defined as the end point criterion regardless of the type of diagnosis and gestational age of the onset of the disease, angiogenic factors do not improve the test efficiency. In contrast, the combined analysis of uterine Doppler and angiogenic factors substantially improves the sensitivity and specificity for an iatrogenic preterm delivery. Our result that the measurement of sFlt1 in the second trimester improves sensitivity for a pregnancy complication and iatrogenic delivery up to 79% and specificity up to 87% has clinical impact. From a clinical point of view, it is not desirable to predict a pregnancy complication, per se. In contrast, pregnancies with early onset diseases leading to preterm delivery with subsequent neonatal morbidity and mortality have to be identified and predicted. We conclude that the measurement of angiogenic factors has a useful predictive power when used in a risk group and focused to severe and early presenting forms of preeclampsia and IUGR. This finding is in accordance with the observation that early and late-onset preeclampsias are associated with different alterations of angiogenic and other biochemical markers. Furthermore, early and late-onset preeclampsias are characterized by different morphometric villous and vascular abnormalities, which confirms the existence of 2 subsets of preeclampsia. Because placental changes in late-onset preeclampsia are minimal, this condition has to be regarded as a distinct clinical entity. Consequently, the application of angiogenic factors for diagnosis and prediction has to be focused on early onset diseases. Moreover, the predictive value is substantially improved when only pregnancies with preeclampsia are included in the analysis.

Secondly, our results show that the observed changes of the angiogenic factors are not confined to preeclampsia, because...
in the study group, the same number of patients develops IUGR and no hypertension. The question of whether IUGR and no hypertension. The question of whether IUGR is also preceded and associated with high sFlt1 and low PlGF concentration has been answered controversially. We and others have demonstrated that IUGR pregnancies are characterized by significantly elevated sFlt1 concentrations. The reason that other studies came to conflicting results might be the different definitions of IUGR. Studies reporting unchanged sFlt1 levels define IUGR as a fetus below a certain weight percentile, which also includes healthy fetuses with a low genetic growth potential. These pregnancies do not have a placental problem and have presumably normal sFlt1 levels. IUGR in a narrow meaning includes only fetuses that do not reach their growth potential because of placental insufficiency. The IUGR pregnancies enrolled in this study are all characterized by increased placental impedance as a consequence of an inadequate trophoblast invasion. As mentioned above, angiogenic factors are also altered in IUGR, but the changes are moderate when compared with preeclampsia. The predictive value is also improved when these patients are analyzed as an independent subgroup. How can the predictive performance be further improved? A previous study showed that the measurement of angiogenic factors in the first trimester identifies pregnant woman with later preeclampsia, gestational hypertension, and IUGR. There are also investigations demonstrating the feasibility of assessing utero-placental blood flow by Doppler sonography in the first trimester. Consequently, a future study has to prove whether a concurrent measurement of uterine perfusion and angiogenic factors in weeks 11 to 14 may improve the power to identify women at risk for early preeclampsia or IUGR. Our study has 2 important implications: in pregnancies that subsequently develop preeclampsia or IUGR, maternal sFlt1 concentrations are increased and PlGF concentrations are already decreased in the second trimester where only an abnormal uterine perfusion indicates a placental alteration. This underlines the pathogenic significance of angiogenic factors for these diseases. In a selected group of high-risk pregnancies characterized by abnormal uterine Doppler, angiogenic factors have a predictive value regarding early onset pregnancy complications higher than previously reported clinical tests.

Perspectives

The research on circulating angiogenic factors has forwarded the understanding of the pathobiology of preeclampsia and other related placental disorders like IUGR. After proper evaluation, proteins like sFlt1 and PlGF have the potency to become diagnostic markers in clinical routine for the individual patient. Moreover, addressing the altered angiogenic—antiangiogenic balance is a promising approach to overcome the stagnation in the management of hypertensive pregnancy disorders and IUGR. With the identification of new and additional angiogenic factors like soluble endoglin, we have the optimistic and realistic perspective of an improved situation in terms of early detection of placental diseases like preeclampsia and IUGR and a possible therapeutic intervention.

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Disclosures

None.

References


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