Angiopoietin-1/Angiopoietin-2 Ratio for Prediction of Preeclampsia

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Preeclampsia, characterized by new-onset of hypertension and proteinuria after 20-week gestation, is a multi-organ pregnancy-specific disorder that causes maternal and fetal morbidity and mortality.¹ The pathogenesis is unknown but current research has focused on inadequate placentalization due to abnormal trophoblast invasion of uterine blood vessels, as well as on genetic and epidemiological factors.² Inadequate trophoblast invasion has been proposed to induce placental hypoxia leading to an imbalance between different factors regulating vasculogenesis and angiogenesis.³ In preeclampsia, disturbed levels of soluble placenta-derived factors promoting angiogenesis, such as vascular endothelial growth factor (VEGF) or placental growth factor (PlGF),⁴,⁵ and factors antagonizing angiogenesis, such as soluble fms-like tyrosine kinase-1 or soluble endoglin⁶,⁷ have been observed. Disturbed levels of these factors are proposed as the cause of endothelial cell dysfunction and increased vascular permeability.⁸

A group of proteins called angiopoietins have been shown to be of importance during implantation and placental development.⁹,¹⁰ They are involved in the formation of the vasculature during vasulogenesis when endothelial progenitor cells, angioblasts, form a primitive vascular network. This process occurs mainly during fetal development.¹¹ Angiopoietins are also involved in angiogenesis when new blood vessels develop from pre-existing ones. Angiogenesis occurs during embryo implantation and placentalization.¹² Angiopoietin-1 (Ang-1) regulates vascular maturation by recruiting and stabilizing attachment of pericytes.¹³–¹⁵ Angiopoietin-2 (Ang-2) is a natural inhibitor of Ang-1 and loosens the attachment of pericytes and stimulates angiogenesis in the presence of VEGF.¹³ Ang-1 and Ang-2 are expressed in syncytiotrophoblasts in placenta.⁹,¹⁶ The angiopoietins act via vascular tyrosine kinase receptors called Tie-1 and Tie-2, which are expressed predominantly in endothelial cells.¹⁷,¹⁸ Factors related to the angiogenic balance could potentially be used as predictive biomarkers for preeclampsia, but none of the angiogenic or antiangiogenic factors studied so far have been shown to be reliable predictive markers for the disorder.

The aim of this prospective, longitudinal cohort study was to determine whether the ratio between Ang-1 and Ang-2 can be used to predict preeclampsia in a low-risk population.

METHODS
The study was approved by the regional Ethics Committee of the Medical Faculty of Uppsala University, and informed consent was obtained from each patient included in the study.

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**Study population.** A cohort of healthy pregnant women (n = 469) were enrolled in gestational week 8–12 at five participating prenatal centers in Värmland, Sweden during autumn 2004–spring 2007. Only women with singleton pregnancies were recruited. Women with a concurrent diagnosis such as chronic hypertension, episodes of high blood pressure before pregnancy, persistently elevated blood pressure before the 20th week of gestation, upper urinary tract infection, pre-existing renal disease, diabetes mellitus, and drug abuse were not included. Plasma samples were collected at gestational weeks 10, 25, 28, 33, and 37. In the cohort, 22 women developed preeclampsia, 19 of these women were included in this study. We had to exclude three women from the study due to an inadequate number of collected plasma samples. Preeclampsia was defined as new-onset hypertension, upper urinary tract infection, pre-existing renal disease, diabetes mellitus, and drug abuse were not included. Plasma samples were collected at gestational weeks 10, 25, 28, 33, and 37. In the cohort, 22 women developed preeclampsia, 19 of these women were included in this study. We had to exclude three women from the study due to an inadequate number of collected plasma samples. Preeclampsia was defined as new-onset hypertension (≥140/90 mm Hg) observed on at least two separate measurements ≥6 h apart, combined with proteinuria (≥2 g on a dipstick or in a 24-h urine sample showing ≥300 mg/24 h). In total, 302 women in the cohort had a normal healthy pregnancy and delivered at full term. From these, 43 women were randomly selected and included in the study as controls.

**Sample collection.** Plasma samples were collected in tubes containing lithium/heparin. After collection the samples were put immediately into a refrigerator, where they were kept for no longer than 30 min before they were centrifuged for 10 min at 1,500 g. The samples were then stored at −70 °C until analyzed. Clinical and laboratory routine parameters were registered.

**Measurement of Ang-1 and Ang-2 in plasma.** Plasma samples were analyzed for levels of Ang-1 and Ang-2 by sandwich enzyme-linked immunosorbent assay using commercially available enzyme-linked immunosorbent assay kits (DY923 and DY623; R&D Systems, Minneapolis, MN). The tests were performed according to the manufacturer’s recommendations and the total interassay coefficient of variation for the three assays was <7%. Lowest limit of the detection is 0.16 ng/ml for Ang-1 and 0.06 ng/ml for Ang-2.

**Statistics.** All statistical analysis was performed by the SPSS 15.0 for Windows software package (SPSS, Chicago, IL). Among the background variables in Table 1 a χ²-test was used for proportions. For comparisons of median values in Table 1 and Table 2, a Mann–Whitney U test was used for independent samples. All significance tests were two-tailed. P values ≤0.05 were considered as statistically significant. A receiver–operator characteristic curve was constructed to test arbitrarily chosen Ang-1/Ang-2 cutoff values for predicting preeclampsia. The Kaplan–Meier method was used to illustrate time to onset of preeclampsia in gestational week 25 by using a cutoff value of the ratio.

**RESULTS**

**Background characteristics**

Table 1 shows the baseline characteristics of the women who developed preeclampsia and the women with normal

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**Table 1 | Clinical characteristics and outcomes for the study population**

<table>
<thead>
<tr>
<th></th>
<th>Preeclampsia</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>19</td>
<td>43</td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>30 (23–35)</td>
<td>31 (22–41)</td>
</tr>
<tr>
<td>Primipara (%)</td>
<td>63</td>
<td>58</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 (18–41)</td>
<td>23.5 (19–34)</td>
</tr>
<tr>
<td>sCr (μg/l)</td>
<td>54 (44–66)</td>
<td>50 (36–66)</td>
</tr>
<tr>
<td>BP first trim (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>120 (105–130)*</td>
<td>110 (90–130)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>70 (55–80)</td>
<td>65 (50–80)</td>
</tr>
<tr>
<td>BP at delivery* (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>145 (125–192)*</td>
<td>120 (100–150)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>100 (85–110)*</td>
<td>70 (60–90)</td>
</tr>
<tr>
<td>BP medication (%)</td>
<td>53**</td>
<td>0</td>
</tr>
<tr>
<td>Gestational length (weeks)</td>
<td>38 (31–41)</td>
<td>40 (38–43)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3,330 (1,745–4,740)</td>
<td>3,715 (3,155–4,640)</td>
</tr>
</tbody>
</table>

Plus–minus values are median ± variation. Gestational length refers to length of gestation at delivery.
BMI, body mass index; BP, blood pressure; n, numbers of women; sCr, serum creatinine at enrolment.
*Two women developed severe preeclampsia defined as blood pressure ≥160/110 mm Hg. *P < 0.05. **P < 0.001.

**Table 2 | Plasma levels of Angiopoietin-1 and Angiopoietin-2**

<table>
<thead>
<tr>
<th></th>
<th>Week 10</th>
<th>Week 25</th>
<th>Week 28</th>
<th>Week 33</th>
<th>Week 37</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ang-1 normal</td>
<td>18.4</td>
<td>16.6</td>
<td>20.2</td>
<td>19.5</td>
<td>17.9</td>
</tr>
<tr>
<td>Ang-1 PE</td>
<td>15.2</td>
<td>15.7</td>
<td>17.8</td>
<td>16.1</td>
<td>18.4</td>
</tr>
<tr>
<td>Ang-2 normal</td>
<td>10.2</td>
<td>7.6</td>
<td>6.8</td>
<td>5.0</td>
<td>4.4</td>
</tr>
<tr>
<td>Ang-2 PE</td>
<td>13.8</td>
<td>7.4</td>
<td>9.0</td>
<td>8.1</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Values are presented as median levels of Ang-1 (ng/ml) and Ang-2 (ng/ml). PE, preeclampsia.

**Figure 1 | Median values of the Ang-1/Ang-2 ratio in different gestational weeks. The P-values are <0.05 in gestational weeks 25 and 28. Ang, Angiopoietin.**

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pregnancies. The groups did not differ from each other according to maternal age, parity, body mass index, and serum creatinine levels at enrolment. Women who developed preeclampsia had a significantly shorter gestational age at delivery (38 compared to 40 gestational weeks, \( P < 0.01 \)). All women had normal blood pressure in the first trimester, although women who later developed preeclampsia had a significantly higher systolic blood pressure at recruitment in gestational week 10 (118 mm Hg compared to 112 mm Hg, \( P < 0.05 \)). Women who developed preeclampsia had a significantly shorter gestational age at delivery (38 compared to 40 gestational weeks, \( P < 0.01 \)).

All women had normal blood pressure in the first trimester, although women who later developed preeclampsia had a significantly higher systolic blood pressure at recruitment in gestational week 10 (118 mm Hg compared to 112 mm Hg, \( P < 0.05 \)). Women with preeclampsia had a significantly higher blood pressure at delivery and 53% of these women were treated during pregnancy with some kind of antihypertensive medication. Blood samples analyzed for a number of different clinical and laboratory routine parameters indicated that hemoglobin, leukocytes, platelets, sodium, potassium, creatinine, aspartate aminotransferase, alanine aminotransferase, and urate were normal in both groups at gestational week 10 (data not shown). Levels of urate, potassium, and leukocytes were, however, significantly higher in the group who developed preeclampsia compared to controls in week 10 (\( P < 0.05 \), \( P < 0.05 \), and \( P < 0.05 \)).

**Differences in plasma levels of Ang-1 and Ang-2**

Median levels of Ang-1 and Ang-2 in plasma during pregnancy are presented in Table 2. The levels of Ang-1 and Ang-2 varied during pregnancy. The median Ang-1/Ang-2 ratio increased during pregnancy in all women, but the ratios were significantly lower at gestational week 25 and 28 in women who later developed preeclampsia than in normal pregnant women (1.48 compared to 2.19 and 2.12 compared to 3.54, \( P < 0.05 \) and \( P < 0.05 \), Figure 1 and Table 3). After gestational week 28 the Ang-1/Ang-2 ratio was still lower in women who developed preeclampsia compared to women with normal pregnancy, but the difference was not statistically significant. The variability of the ratios in gestational weeks 25 and 28 are shown in Figure 2.

**Cutoff value for prediction of preeclampsia**

Receiver–operator characteristic curves regarding the prediction of preeclampsia at arbitrarily chosen Ang-1/Ang-2 cutoff values were constructed for gestational week 25 (Figure 3a) and week 28 (Figure 3b). A cutoff value of 1.41 for the Ang-1/Ang-2 ratio at gestational week 25 showed a sensitivity of 47% and a specificity of 87% to predict preeclampsia later in pregnancy with an accuracy of 76% and a likelihood ratio of 3.6. A cutoff value of 1.84 in gestational week 28 for the Ang-1/Ang-2 ratio resulted in a sensitivity of 50% and a specificity of 80% with an accuracy of 82% and a positive likelihood ratio of 2.6.

To provide an overview of the time to clinical signs of preeclampsia in women with an Ang-1/Ang-2 ratio <1.4 in gestational week 25, a Kaplan–Meier curve was constructed. As shown in Figure 4, there was a delay of at least 9 weeks before clinical signs of preeclampsia appeared.

**DISCUSSION**

An imbalance between angiogenic and antiangiogenic factors has been suggested to cause preeclampsia.\(^{19}\) Our results support this theory because we can show that the ratio between Ang-1 and Ang-2 in plasma is significantly lower in pregnant women who later develop preeclampsia. The women in this

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**Table 3 | Plasma levels of the Angiopoietin-1/Angiopoietin-2 ratio**

<table>
<thead>
<tr>
<th>Week 10</th>
<th>Week 25</th>
<th>Week 28</th>
<th>Week 33</th>
<th>Week 37</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ang-1/Ang-2 Normal</td>
<td>1.45 (0.28–11.17)</td>
<td>2.19* (0.49–44.10)</td>
<td>3.54* (0.35–31.95)</td>
<td>4.40 (0.65–14.18)</td>
</tr>
<tr>
<td>Ang-1/Ang-2 PE</td>
<td>1.31 (0.25–26.65)</td>
<td>1.48 (0.47–4.31)</td>
<td>2.12 (0.94–3.97)</td>
<td>2.17 (0.39–12.38)</td>
</tr>
</tbody>
</table>

Values are presented as median levels ± variation of the Ang-1/Ang-2 ratio.

PE, preeclampsia.

*\( P < 0.05 \).
study are prospectively and longitudinally included and this is, to our knowledge, the first project where the Ang-1/Ang-2 ratio has been studied during pregnancy.

Reduced levels of PlGF in women developing preeclampsia compared with controls have been shown earlier and we used PlGF as a positive control in our study. The result from our investigation concurs with earlier observations (data not shown). Hirokoshi et al. have studied Ang-2 levels and the soluble fms-like tyrosine kinase-1/Ang-2 ratio in serum in women with preeclampsia. They concluded that the ratio was increased in women with clinical signs of preeclampsia compared with healthy controls. The Ang-2 level in their material was significantly lower in women with preeclampsia, which is in disagreement with our results. Both their studies were cross-sectional and blood samples were collected at the onset of clinical signs of preeclampsia and therefore it is difficult to compare the results to ours, as our study is longitudinal.

On the basis of expected changes during normal gestation it has been hypothesized that the expression of hypoxia inducible factor and VEGF would be normal before mid-gestation and at term. In preclinical and clinical stages of preeclampsia, a placental dysfunction and an increased vascular permeability has been proposed leading to inadequate oxygen saturation and subclinical placental hypoxia. A reduction of pO2 has been shown to upregulate Ang-2 and VEGF mRNA but not the expression of Ang-1 or the Tie-2 receptor, which is in agreement with our findings of increased levels of Ang-2 and a decreased Ang-1/Ang-2 ratio in women developing preeclampsia.

VEGF has been shown to be of importance in vasculogenesis, angiogenesis, and the development of placenta. Recently, a number of publications have focused on the importance of the angiopoietins, which act together with VEGF. Geva et al. have shown that levels of both VEGF and Ang-1 mRNA are increased in placenta during a normal pregnancy, but the level of Ang-2 mRNA is decreased. It has furthermore been reported from Seval et al. that the angiopoietins and their receptors are crucial in the development of the early placenta and the authors identified both Ang-1 and Ang-2 in endothelial cells as well as in syncytiotrophoblasts. We have results in agreement with these publications where we have identified a placental localization of Ang-1, Ang-2, Tie-1, and Tie-2 by immunohistochemical stainings (data not shown).

During the past decade, many biophysical and biochemical markers have been proposed as predictors of which women will develop preeclampsia. Factors involved in the angiogenic balance are suggested as candidate biochemical markers. Lately, the ratio between the antiangiogenic factor soluble fms-like tyrosine kinase-1 and the proangiogenic factor PlGF has been investigated as a predictor for preeclampsia, but there is still not sufficient data to recommend the soluble fms-like tyrosine kinase-1/PlGF ratio as a screening test. The angiopoietins also have the potential to be biochemical markers for preeclampsia as they are of great importance during implantation and placental development. In this study, we have shown that the ratio between Ang-1 and Ang-2 in the second
trimester has a moderate ability to discriminate between women who will develop preeclampsia and those who will not. One limitation of our study is that the number of women included was relatively small and larger prospective studies are needed to determine whether this ratio can be used as a clinically relevant predictor.

In conclusion, our data indicate that in a low-risk population of pregnant women the Ang-1/Ang-2 ratio in plasma constitutes a possible biomarker for prediction of later onset of preeclampsia. Its suitability might be improved when combined with uterine artery Doppler measurements or in combination with other markers.

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