Angiotensin II Type 1 Receptor Agonistic Antibodies Reflect Fundamental Alterations in the Uteroplacental Vasculature

Thomas Walther, Gerd Wallukat, Alexander Jank, Sabine Bartel, Heinz-Peter Schultheiss, Renaldo Faber, Holger Stepan

Abstract—Abnormal uterine perfusion detected by Doppler sonography reflects impaired trophoblast invasion, a factor involved in the pathogenesis of pregnancy complications such as preeclampsia or intrauterine growth retardation. Recent studies have demonstrated an autoantibody against the angiotensin type 1 (AT1) receptor in pregnant women with preeclampsia. Our aim was to determine whether the AT1 autoantibody precedes the clinical symptoms and is thus predictive of preeclampsia. We therefore detected this antibody in serum from second trimester pregnancies with abnormal uterine perfusion because these women show an indirect sign of inadequate trophoblast invasion. Then the AT1 autoantibody distribution/concentration was compared with that of women at term with or without pregnancy pathology. The AT1 autoantibody was already detectable in second trimester pregnant women with abnormal uterine perfusion who later developed intrauterine growth retardation (60%) or even had a normal course of pregnancy (62%). In the third trimester, the AT1 autoantibody was demonstrated in 89% of patients with manifest preeclampsia, 86% of those with manifest intrauterine growth retardation, and even in healthy pregnant women at term with a history of abnormal uterine perfusion in the second trimester. We conclude that the AT1 autoantibody is an early but nonspecific marker for preeclampsia. The generation of this antibody seems to be associated with distinct types of pregnancy disorders resulting from impaired placental development. The AT1 autoantibody may thus be causative for pathological uteroplacental perfusion. (Hypertension. 2005;46:1275-1279.)

Key Words: angiotensin II ■ antibodies ■ hypertension, gestational ■ preeclampsia ■ pregnancy ■ receptors, angiotensin
and could therefore mediate the vascular alterations in preeclampsia. The hypothesis was further supported by Dechend et al, who demonstrated increased activation of NADPH oxidase. This could contribute to oxidative stress and an inflammatory response, which are pathophysiological factors in preeclampsia. Thus, the AT1-AA is discussed as the “missing link” between the still unknown origin of the disease and the variety of maternal symptoms associated with clinically manifest preeclampsia, including hypertension, endothelial dysfunction, and renal damage. The fact that the AT1-AA is an interesting candidate for mediating or even causing the disease was further supported by the finding that a transgenic rat model of preeclampsia is also characterized by the presence of the AT1-AA. However, decisive evidence has not yet been provided.

Attention was focused on determining whether AT1-AA precedes the clinical symptoms of preeclampsia or is only a secondary effect of clinically evident preeclampsia and therefore not the primary cause of the disease. To examine this question, we detected the AT1-AA in serum from second trimester pregnancies with abnormal uterine perfusion as an indirect sign of inadequate trophoblast invasion because these women are likely to develop preeclampsia but have no symptoms of the disease in this phase of the pathophysiological cascade.

### Patients and Methods

All patients gave written informed consent, and the study was approved by the institutional ethics committee and adhered to the principles of the Declaration of Helsinki. We collected blood from 31 pregnancies with pathological uterine perfusion (18th to 22nd week of gestation) and 21 with normal perfusion. Thirty-six pregnancies in the third trimester with a normal outcome or manifest disease and the variety of maternal symptoms associated with preeclampsia. Preeclampsia was defined according to the guidelines of the International Society for the Study of Hypertension in Pregnancy. Gestational hypertension is defined here as 1 diastolic blood pressure reading of 110 mm Hg on any occasion or 2 consecutive diastolic blood pressure readings of 90 mm Hg ≥4 hours apart. Significant proteinuria is defined as 300 mg of total protein in a 24-hour urine collection or, if these data are not available, 1+ proteinuria by dipstick on 2 consecutive occasions ≥4 hours apart. IUGR was defined as a birth weight below the fifth percentile of our reference group.

The Doppler investigations were performed using a LOGIQ 9 ultrasound device (GE) with a 5.0-MHz convex transducer. Color Doppler imaging was used to identify the uterine artery at the point where it crossed the external iliac artery. The pulsatility index (PI) and the presence or absence of a notch were noted. Uterine perfusion was defined as pathological if there was bilateral notching or if the mean PI of both arteries was greater than the 90th percentile (mean PI >1.45) of our reference group. All pregnancies were singleton, and the women were healthy and normotensive at the time of examination. The persistence of pathological uterine perfusion was confirmed in the 24th week of gestation. 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 4000 g for 10 minutes and frozen at −80°C. The AT1-AA was measured by the same reference laboratory as described previously, where mainly the beating rates of focal contractile neonatal rat cardiomyocytes were counted before and after treatment with the purified IgG fraction. The protocol has been modified as follows. The basal contraction rate of spontaneously beating cardiomyocytes was determined. The immunoglobulin fractions from patients were added at a concentration of 10⁻⁵ M, and the contraction rate was recounted after 5 minutes.

### Results

All women with normal uterine perfusion had a normal course of pregnancy with a normal delivery at term and no symptoms of hypertension or IUGR (Table). None of these women harbored an AT1-AA at the 20th week of pregnancy, whereas 4 of 5 pregnancies with subsequent development of preeclampsia already tested positive at this time point. However, 3 of 5 women who later developed IUGR but showed no

### Clinical Data of the Patient Groups

<table>
<thead>
<tr>
<th>Pregnancies at 18th to 22nd Week</th>
<th>Normal Perfusion</th>
<th>Abnormal Perfusion/Normal Course</th>
<th>Abnormal Perfusion/PE</th>
<th>Abnormal Perfusion/IUGR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>28 (23–34)</td>
<td>30 (26–33)</td>
<td>27 (23–31)</td>
<td>26 (22–30)</td>
</tr>
<tr>
<td>Gestational age at sampling (weeks)</td>
<td>19 (18–22)</td>
<td>20 (19–22)</td>
<td>20 (20–21)</td>
<td>21 (19–22)</td>
</tr>
<tr>
<td>Mean uterine PI</td>
<td>0.84±0.23</td>
<td>1.59±0.34*</td>
<td>1.68±0.21*</td>
<td>1.72±0.33*</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3440±307</td>
<td>3210±410</td>
<td>1940±188*</td>
<td>1180±322*</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>39 (38–41)</td>
<td>40 (39–42)</td>
<td>33 (27–35)*</td>
<td>34 (29–38)*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pregnancies at delivery</th>
<th>Normal Outcome/Normal Perfusion</th>
<th>Normal Outcome/Abnormal Perfusion</th>
<th>PE</th>
<th>IUGR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>32 (27–35)</td>
<td>30 (28–33)</td>
<td>24 (19–28)</td>
<td>27 (22–32)</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>39 (38–41)</td>
<td>39 (37–40)</td>
<td>33 (26–37)*</td>
<td>34 (28–35)*</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>121±21</td>
<td>116±24</td>
<td>168±10*</td>
<td>123±9</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>72±8</td>
<td>60±7</td>
<td>96±8*</td>
<td>72±9</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3530±250</td>
<td>3190±300</td>
<td>1700±208*</td>
<td>1420±105*</td>
</tr>
</tbody>
</table>

Mean±range/SEM; *P<0.05 vs normal perfusion (normal outcome/nomral perfusion); Mann–Whitney U test for intergroup differences. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; PE, preeclampsia.
sign of a hypertensive disorder also had the agonistic AT1 antibody. More interestingly, test results were positive even in 13 of the 21 women with an uneventful pregnancy and normal delivery despite the pathological uterine perfusion (Figure, A). To exclude a gestational age–dependent autoimmune antibody regulation, we performed a second study for AT1-AA detection in 10 pregnancies with a normal and 26 with an adverse outcome at delivery, also including patients from the first group. Eighty-nine percent of all preeclamptic pregnancies were AT1-AA positive. As already suggested by positive tests at the 20th week in women who later developed IUGR, the antibody was detectable in 6 of 7 tested pregnancies with IUGR at delivery (Figure, B). Even more important was the fact that 5 of the 10 women without pathology harbored the antibody. A longitudinal investigation of these 5 women disclosed abnormal uterine perfusion at the 20th week of gestation. Finally, by comparing the increase in beat frequency between the groups, we investigated an AT1-AA concentration-dependent effect on the pathophysiological outcome because only the highest concentrations may complicate a pregnancy by preeclampsia. Noteworthy is the absence of significant differences between the AT1-AA–positive groups, which excludes a clinically relevant threshold (Figure, C).

**Discussion**

The syndrome of preeclampsia has been ascribed previously to generalized maternal endothelial dysfunction, poor placentalation, and excessive maternal inflammatory response. Several reports have indicated that increased levels of antiphospholipid antibodies, anti-DNA antibodies, or autoantibodies against G-protein–coupled receptors as the AT1 receptor may be associated with preeclampsia. Our investigations aimed at answering the question of whether the AT1-AA described as specific for preeclamptic patients is already detectable before the onset of the disease. They were designed to clarify whether the AT1-AA antedates the clinical manifestation of the disease and could therefore have pathogenetic relevance.

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Percentage and concentration of the AT1-AA in pregnancies at the early second trimester and at term. Distribution and subdivision of pregnancies at the early second trimester (18th to 22nd week of gestation (51 women; A) and of a reference group at term (36 women) in the presence of an AT1-AA (B). Numbers: negative for AT1-AA/positive for AT1-AA; in parenthesis: percentage of positive tested women within the group. C, Contractility increase in beats per 15 s (mean ± SEM) reflecting the AT1-AA concentrations in the autoantibody-positive pregnancies of the different groups. P indicates perfusion; PE, preeclampsia.
or is only a phenomenon secondary to preeclampsia. Although our study is the first to show that the $\text{AT}_1$-$\text{AA}$ is already detectable in second trimester women with abnormal uterine perfusion and subsequent clinical manifestation of preeclampsia, the antibody was also found in women who later developed other pregnancy diseases such as IUGR and even in those with abnormal uterine perfusion but without later clinical pathology.

Interestingly, all women who tested positive shared the 1 common feature of abnormal uterine perfusion, whereas none of those with normal uterine perfusion harbored the antibody. Testing 15 women at both time points showed that the antibody remained detectable with advancing gestational age, and even the concentrations remained stable. This also excludes alteration of the antibody concentration as a possible tool for predicting whether a woman with abnormal uterine perfusion will develop preeclampsia or IUGR or will have a normal outcome. Thus, the recent postulate that $\text{AT}_1$-$\text{AA}$ is specific and possibly causative for preeclampsia has to be revised. This is strongly supported by the observation that a bioactive $\text{AT}_1$-$\text{AA}$ with pathological properties is also detectable in nonpregnant patients with renal allograft rejection.

Moreover, the presence of the $\text{AT}_1$-$\text{AA}$ correlates with abnormal uterine perfusion regardless of the later course and outcome of the pregnancy. This seems all the more important because abnormal uterine perfusion is caused by elevated uteroplacental impedance attributable to impaired placental development and maturation. Precisely this phenomenon with probable impairment of trophoblast invasion into the maternal compartment causing persistence of a high-pressure system is thought to be the key event in the etiology and pathogenesis of proteinuric hypertensive disorders in pregnancy and IUGR. Therefore, early occurrence of the $\text{AT}_1$-$\text{AA}$ in pregnancy suggests a crucial role of $\text{AT}_1$-$\text{AA}$ in all types of placental vascularization disorders. Because there are first attempts to detect an abnormal uterine perfusion in the first trimester as an early screening for preeclampsia, it would be interesting to investigate in upcoming studies, whether the $\text{AT}_1$-$\text{AA}$ is detectable even in the first trimester of gestation, because the critical phase of trophoblast invasion and development takes place in this stage.

Because an $\text{AT}_1$ receptor–mediated pathway has also been discussed in kidney-transplanted patients with refractory vascular rejection, this autoantibody may even be a key factor in the development of different types of vasculopathy. However, it still remains to be clarified what triggers the generation of the $\text{AT}_1$-$\text{AA}$ early in pregnancy or in nonpregnancy-related vasculopathies and to what antigen the antibody is originally directed.

Because our data confirm that late complications occur in only some pregnancies with abnormal uterine perfusion, additional factors must be involved in the development of preeclampsia or IUGR. Although we identified $\text{AT}_1$-$\text{AA}$–positive patients with abnormal uterine perfusion but a normal outcome, the agonistic antibody clearly interacts with the $\text{AT}_1$ receptor to cause in vitro effects characteristic of preeclampsia such as increased reactive oxygen species generation and angiotensin II responsiveness. Thus, the antibody in combination with $\geq 1$ other factors could still be a relevant pathogenetic factor for pregnancy pathology, as also indicated by recent findings of Dechend et al in transgenic rats.

Our study has 2 major implications. The clinical importance of the $\text{AT}_1$-$\text{AA}$ has changed in the light of evidence characterizing it as an early but not entirely specific marker for preeclampsia. Interestingly, the $\text{AT}_1$-$\text{AA}$ seems to be associated with distinct types of pregnancy disorders that result from impaired placental development, and the process that leads to impaired development of the uteroplacental vasculature is linked to a hitherto unknown antigen presentation or trigger for $\text{AT}_1$-$\text{AA}$ generation. Therefore, additional studies are needed to identify the antigen or the $\text{AT}_1$ receptor modification initiating a direct autoimmune response against the receptor. Additional aims are to discover the key event that triggers a disorder in pregnancies with abnormal uterine perfusion and to find out what determines the type of disease that will develop in a particular case.

Perspectives
It is well recognized that autoantibodies such as the $\text{AT}_1$-$\text{AA}$ receptor or an anti–$\beta_1$-adrenergceptor autoantibody are involved in the pathogenesis of a variety of cardiovascular complications. This had led to first therapeutic implications such as specific or nonspecific removal of these autoantibodies from circulation. Clinical trials are needed to prove whether methods such as therapeutic immunoadsorption are applicable in pregnancy. This would of course not be a causal treatment of hypertensive pregnancy disorders. However, if this therapy could achieve a prolongation of pregnancy, perinatal and maternal morbidity may be already significantly improved.

It would be of great interest to study the presence of detectable levels of $\text{AT}_1$-$\text{AA}$, in combination with impaired perfusion, at the first trimester. The determined time course of $\text{AT}_1$-$\text{AA}$ generation could identify the antibody as a cause or consequence of abnormal perfusion.

Finally, it is still unclear which cofactors are decisive in determining the clinical course of pregnancies with abnormal uterine perfusion in presence of the $\text{AT}_1$-$\text{AA}$. Thus, to clarify the pathophysiological sequence of disturbed placental development, the identification of these cofactors and their interaction with $\text{AT}_1$-$\text{AA}$ is the objective of further studies.

References


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