Preeclampsia is a devastating complication of pregnancy featuring proteinuria and hypertension that commences after 20 weeks of gestation. The condition affects 5% of pregnancies in the United States and Europe and represents a major cause of fetal and maternal morbidity and mortality. The cause of preeclampsia is unknown; however, evidence is accumulating that the disorder results from poor placentaion combined with underlying maternal constitutional factors, such as insulin resistance, obesity, and inflammation, that become accentuated during the physiological stress of pregnancy. Although the preeclampsia syndrome remits within days after delivery, women with a history of preeclampsia have a substantially higher cardiovascular risk later in life compared with women who experienced normal pregnancies.

Wallok et al showed that women with preeclampsia develop circulating angiotensin II type 1 (AT1) autoantibodies (AT1-AAs) that bind an amino acid sequence of the second extracellular loop of the angiotensin II type 1 receptor in a stimulatory fashion. AT1-AAs are detectable before preeclampsia develops and have biological effects that could contribute to many of the vascular and renal alterations in preeclampsia. AT1-AAs have also been demonstrated in pregnant rats transgenic for human renin and angiotensinogen genes and in pregnant rats subjected to reduced uterine perfusion pressure, animal models with parallels to human preeclampsia.

Although AT1-AA levels decline 50% by 1 week after delivery, it is not known whether they regress completely. The antibodies are analogous to activating antibodies in other conditions, such as those activating the thyroid-stimulating hormone receptor in Graves disease. Malignant hypertension has been associated with AT1-AA. Furthermore, agonistic antibodies against the adrenergic α1 receptor are...
more frequent among patients with essential hypertension (44%) than healthy control subjects (12%). Although the role of autoantibodies to α1 adrenoceptors is unclear, their agonistic activity suggests they may be capable of causing contraction of vascular smooth muscle cells in blood vessels, elevating peripheral vascular resistance, and promoting cardiac hypertrophy.

An imbalance between proangiogenic and antiangiogenic factors has been shown recently in preeclampsia. The soluble (s) fms-like tyrosine kinase (Flt-1), a secreted splice variant of Flt-1 that is capable of binding vascular endothelial growth factor (VEGF) and placental growth factor by preventing their binding to the target tissue receptors Flt-1 and kinase insert domain-containing receptor, is upregulated in placentas of women with preeclampsia. Increased sFlt-1 in maternal serum has been found 5 to 6 weeks before the development of preeclampsia and could serve as a preeclampsia risk marker. When infused into rats, sFlt-1 results in a condition reminiscent of preeclampsia in humans.

Hypoxia may be a driving force behind the increased placental production of Flt-1 and sFlt-1 during preeclampsia. AT1-AA and sFlt1 may be linked, because angiotensin II increases hypoxia-inducible factor-1 expression by nonhypoxic pathways and increases sFlt-1 production in human proximal tubule cells. Although decisive evidence that insulin resistance, AT1-AA, or sFlt-1 is causative for preeclampsia has not yet been provided, they are all increased weeks before development of the clinical syndrome, thus, the concerted interaction of these factors may be of importance.

Asymptomatic postpartum women who had recovered from preeclampsia 18±9 months earlier had higher sFlt-1 concentrations and insulin resistance homeostasis model assessment (HOMA) scores compared with women who had undergone normal pregnancies in the same timeframe. In this study, the women in the upper quartiles of both sFlt-1 and HOMA were the greatest odds of having had preeclampsia. Although postpartum sFlt-1 levels are 40-fold lower than intrapartum levels, the significant difference among postpartum groups suggests that women with previous preeclampsia are predisposed to overproduce this antiangiogenic factor.

Given these associations and the potential for contributing to cardiovascular risk, we tested the hypothesis that AT1-AA would be evident 18 months postpartum in women who have had a preeclamptic pregnancy. We also evaluated any correlation of AT1-AA, sFlt-1, and HOMA in these women.

Methods

Patients

The study population was derived from independent recruiting sites at Massachusetts General Hospital and the Magee-Womens Hospital (MWH; Pittsburgh, PA). The internal review boards approved the study, and written informed consent was obtained. The procedures followed were in accordance with institutional guidelines. Consecutive patients who developed preeclampsia during their first completed (nulliparous) pregnancy and participants with normotensive pregnancies within the same timeframe were enrolled. At both centers, preeclampsia was defined as hypertension (>140/90 mm Hg) and proteinuria (either ≥2+ by dipstick or >300 mg/24 hours) that first appeared after 20 weeks’ gestation and resolved within 12 weeks postpartum. All of the women in the normal pregnancy group delivered healthy, singleton infants at term (>38 weeks). Women with diabetes, a history of gestational diabetes, chronic hypertension, chronic proteinuria, or serum creatinine concentration >1.0 mg/dL were excluded.

We examined 29 normotensive women with a history of preeclampsia (18 from Massachusetts General Hospital and 11 from MWH) and 35 normotensive women with a history of normal pregnancy (18 from Massachusetts General Hospital and 17 from MWH) at an average of 18±9.7 (±SD) months postpartum in the general clinical research centers at the Massachusetts Institute of Technology and the MWH. Collecting samples in the window of 6 to 24 months postpartum was to allow normalization of pregnancy-induced cardiovascular changes while minimizing the loss of study participants to new pregnancies.

The postpartum characteristics of these women are summarized in Table 1. With the exception of 4 additional women with previous normal pregnancy from MWH and 1 with previous normal pregnancy from MWH that was not included in the present study because autoantibodies were not assayed, we reported previously the altered expression of angiogenesis-related sFlt-1 and free VEGF proteins and insulin resistance in these women. These were a broad cross-section of women recruited from private obstetric practices and university prenatal outpatient clinics that serve a mostly low-income, racially diverse population.

The study participants underwent a history, physical examination, and urine pregnancy test. Postpartum blood was collected on the morning after an overnight (>8 hours) fast. To confirm AT1-AA activity during pregnancy, we obtained predelivery serum samples from a subset of these same women (10 with preeclampsia and 7 with normal pregnancy). Blood samples were processed within 45 minutes of collection, and aliquots of the plasma and serum were stored at −70°C and subjected to only a single thaw before the assays were performed.

Cardiomyocyte Contraction Assay

The immunoglobulin fraction was isolated from 1- to 2-mL serum samples by ammonium sulfate precipitation at a saturation of 40% as outlined elsewhere. Antibodies were detected by the chronotropic responses to AT1 receptor-mediated stimulation of cultured neonatal rat cardiomyocytes coupled with receptor-specific antagonists. Isolation and cultivation of neonatal heart cells were performed as described previously. The basal contraction rate of spontaneously beating cardiomyocytes was determined. The mean (±SD) basal contraction rate among different preparations was 164±7 per minute. The immunoglobulin fractions from patients were added at a dilution of 1:40, and the beating rate was recounted after 60 minutes. To show specificity for AT1-AA or for adrenergic α1 receptor agonist antibodies, the AT1 receptor blocker losartan or the α-receptor blocker prazosin was added, and the contraction rate was counted again. The cutoff for antibody-positive bioassay was defined as ≥7.2 bpm. Increases were determined as nonspecific if the positive response was not reduced below the cutoff by either of the inhibitors or if the increase could not be blocked by just a single inhibitor.

Insulin, sFLT-1, and VEGF

Insulin concentrations were measured using the Linco’s radioimmunoassay (Linco Research), and commercial ELISA kits (R&D Systems) were used for sFlt-1 and free VEGF determinations, as described previously. HOMA scores were calculated ([insulin (microunits per milliliter)×glucose (millimoles per liter)]/22.5).

Statistics

Because of skewing of the distribution of changes in cardiomyocyte beat frequency from baseline and skewing of the VEGF and HOMA distributions, nonparametric Mann–Whitney U test was used compare these variables. Other univariate comparisons between the pregnancy outcome groups were performed using 2-sample t tests or χ2 test as appropriate. Correlations were by Spearman rank.
### Results

Subject demographics are summarized in Table 1. Systolic blood pressures were significantly increased in women with a history of preeclampsia. They were marginally more likely to have a family history of cardiovascular disease, defined as coronary artery disease, myocardial infarction, or cerebrovascular disease in any first-degree relative. We did not find significant differences for race, contraception use, smoking, or lactation status. As we reported previously, women with previous preeclampsia evidenced significantly higher serum sFlt-1 and HOMAIR scores.

After preparation of the IgG fractions, we investigated their effect in the cardiac contraction assay. During pregnancy, IgG from women with preeclampsia increased the beat number (median change in bpm: 21.2 [interquartile range: 17 to 21.4]; n = 10), which was different from IgG from women with normal pregnancies (median change in bpm: 0.8 [interquartile range: 0.58 to 14.7]; n = 7; P < 0.01). All but 2 women with preeclampsia were clearly AT1-AA positive; these 2 were likely AT1-AA positive but counted as nonspecific, because the increase was reduced by losartan and by prazosin. In contrast, all of the women with uncomplicated pregnancy had negative AT1-AA status, but 2 also had adrenergic α1 receptor antibodies. Eighteen months after delivery, serum from former preeclamptic women still induced a significant, but variable, increase in the overall cardiomyocyte beating rate compared with former control (median change in bpm: previous preeclampsia, 2.0 [interquartile range: 1.3 to 17.5] versus control, 1.3 [interquartile range: 0.3 to 2.5]; P < 0.05). AT1-AAs were found in 17.2% of former preeclamptic women compared with 2.9% (P < 0.05) of former normal pregnant women (Table 2). No significant difference was observed for α-receptor antibodies and unspecific increase in bioassay (Table 2).

The correlation between activating autoantibodies and markers of angiogenesis postpartum is shown in the Figure. Women with activating autoantibodies show significantly increased serum sFlt-1 concentrations compared with women with negative bioassay results (Figure, panel A). Concentrations of free VEGF were significantly reduced (Figure, panel B).

### Table 1. Postpartum Characteristics According to Pregnancy Outcome

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Previous Preeclampsia (n=29)</th>
<th>Control (n=35)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>33.7±5.8</td>
<td>30.8±6.7</td>
<td>0.08</td>
</tr>
<tr>
<td>Months postpartum</td>
<td>18±10</td>
<td>18±9</td>
<td>0.8</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>111±10</td>
<td>106±8</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>73±10</td>
<td>69±8</td>
<td>0.09</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>29.2±7.8</td>
<td>25.9±6.5</td>
<td>0.06</td>
</tr>
<tr>
<td>Race, % white</td>
<td>86</td>
<td>86</td>
<td>1.0</td>
</tr>
<tr>
<td>Smoking, % current</td>
<td>7</td>
<td>3</td>
<td>0.3</td>
</tr>
<tr>
<td>Oral or subcutaneous contraception, %</td>
<td>31</td>
<td>35</td>
<td>0.8</td>
</tr>
<tr>
<td>Lactation, % current</td>
<td>3</td>
<td>6</td>
<td>0.6</td>
</tr>
<tr>
<td>sFlt-1, pg/mL</td>
<td>41.6±6.7</td>
<td>30.9±10.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Free VEGF, pg/mL</td>
<td>424 (184 to 628)</td>
<td>216 (145 to 431)</td>
<td>0.07</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>4.53±0.37</td>
<td>4.46±0.36</td>
<td>0.45</td>
</tr>
<tr>
<td>HOMAIR</td>
<td>2.8 (1.7 to 4.0)</td>
<td>1.8 (1.5 to 2.9)</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Family history of CVD, %*</td>
<td>45</td>
<td>18</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Continuous variables are reported as mean±SD or median (interquartile range) as appropriate. Family history of CVD is defined as coronary artery disease, myocardial infarction, or cerebrovascular disease in any first-degree relative.

*Family history data were available in 48 of 64 women.

### Table 2. Prevalence of Agonistic AT1 Receptor and Adrenergic α1 Receptor Autoantibodies by Postpartum Group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Previous Preeclampsia (n=29)</th>
<th>Control (n=35)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT1-AA</td>
<td>5 (17.2%)</td>
<td>1 (2.9%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Antibody against adrenergic α1 receptor</td>
<td>3 (10.3%)</td>
<td>5 (14.2%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Nonspecific increase in bioassay</td>
<td>1 (3.4%)</td>
<td>2 (5.7%)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Absolute numbers (percentages) of postpartum women with previous preeclamptic or normal pregnancy (control) are listed according to type of bioassay-positive response as described in Methods. Bioassay responses were determined as nonspecific if the positive response was not reduced below the cutoff by either of the inhibitors (AT1 receptor blocker losartan or the α1-receptor blocker prazosin) or if the response was blocked by losartan and by prazosin. n.s. indicates not significant.

*Agonistic autoantibody bioassay based on chronotropic response of neonatal rat cardiomyocytes.
B), whereas HOMA\textsubscript{IR} scores were significantly higher (Figure, panel C) in women with positive autoantibody status. Postpartum women within the upper quartile (75th percentile) of the fasting insulin and sFlt-1 distributions had significantly higher increases in the beating rate compared with the women with values below the 75th percentile (Figure, panel D). Overall, 44% (8 of 18) of patients with insulin levels at or above the 75th percentile were autoantibody positive compared with 14% in the group with insulin levels below the 75th percentile ($P<0.02$). Among women with a history of preeclampsia, those with positive autoantibody status had higher diastolic blood pressures than those with a history of normal pregnancy ($80.3\pm3.9$ mm Hg versus $70.9\pm2.0$ mm Hg; $P<0.04$). We did not find any association of autoantibody status with age, current body mass index, body mass index before pregnancy, systolic blood pressure, resting heart rate, use of oral contraceptives, positive family history, or stage of menstrual cycle.

Discussion

Preeclampsia is associated with a significantly increased risk for long-term cardiovascular disease.\cite{3,5,6} Several cardiovascular risk factors are elevated, and endothelium-dependent, flow-mediated vasodilator function is reduced among women who have had a previous preeclamptic versus a previous normotensive pregnancy.\cite{2,3} Taken together with the evidence linking preconception and intrapartum cardiovascular risk to preeclampsia, this suggests that pre-existing (but often subclinical) risk, under the stress of pregnancy, is manifest as preeclampsia. That same risk later in life is revealed as cardiovascular morbidity.\cite{2}

Our main finding is that the AT1-AA persists (or reappears) to a perceptible degree in patients who have had a preeclamptic pregnancy $>1$ year postpartum. In contrast to autoantibodies against the adrenergic $\alpha_1$ receptor, AT1-AAs are more common in women with a history of preeclampsia than women with a history of normal pregnancy.

The possibility that AT1-AA could contribute to disease was first suggested by a study of renal transplant patients with severe vascular rejection and malignant hypertension but without anti-human leukocyte antigen antibodies. These patients harbored circulating AT1 receptor autoantibodies directed against the same epitope as preeclampsia patients, as well as a closely related epitope also involving the receptor’s second extracellular loop. One of the transplant recipients with humoral rejection had had preeclampsia many years before.\cite{19} These findings underscore the possibility that AT1-AA may contribute to cardiovascular disease.

AT1-AA obtained from women with preeclampsia stimulate reduced nicotinamide-adenine dinucleotide phosphate oxidase in vascular smooth muscle and trophoblast cells. The oxidase is profoundly activated in placentas of women with preeclampsia.\cite{11} AT1-AA also induces these cells to activate AP-1 and nuclear factor $\kappa B$ and to produce excess tissue factor.\cite{10} All of these effects are prevented by AT1 receptor blockers but not AT2-R or adrenergic receptor blockers.\cite{9} Xia et al\cite{12} independently verified the AT1-AA in patients with preeclampsia and showed that it induces plasminogen activator inhibitor-1 production and shallow invasion by trophoblast cells in matrigel assay. They also showed that AT1-AA mobilizes intracellular calcium, activates the nuclear factor of activated T cells in AT1 receptor–transfected Chinese hamster ovary cells,\cite{13} and increases interleukin-6 and plasmino-
gen activator inhibitor-1 secretion from mesangial cells.\textsuperscript{14} These data suggest that the AT1-AA is a candidate mediator of the vascular and renal alterations in preeclampsia.

Our finding that 1 of the 35 women with previous normal pregnancy was AT1-AA bioassay positive is consistent with the recent report that AT1-AA was already detectable during the second trimester in most women with abnormal uterine perfusion (uterine artery Doppler assessment), not only in women who subsequently developed preeclampsia or growth restricted infants, but also in 13 of 21 women with an uneventful pregnancy and normal delivery despite pathological uterine perfusion.\textsuperscript{9} The generation of the AT1-AA, thus, seems to be closely associated with reduced uteroplacental perfusion in which pregnancy disorders frequently, but not always, develop. An important issue concerns factors modulating the impact of reduced perfusion in a specific pregnancy.

Concentrations of free VEGF were significantly lower, whereas sFlt-1 was higher, among autoantibody positive women compared with women without detectable autoantibodies, although free VEGF was marginally higher overall in women with previous preeclampsia compared with control subjects. We do not yet fully understand these associations. After a preeclamptic pregnancy, the presence of autoantibodies might indicate ongoing endothelial dysfunction leading to diminished VEGF expression and secretion. As we have discussed previously,\textsuperscript{32} the molar concentrations of sFlt-1 in both groups of postpartum women were \( \geq 20\)-fold lower on average than VEGF, suggesting that sFlt-1 does not substantially influence circulating levels of free VEGF in the basal postpartum state. However, sFlt-1 is elevated after the onset of acute coronary syndromes.\textsuperscript{36} Therefore, it is plausible that sFlt-1 could be induced to pathogenic levels in disease states, such as atherosclerosis, especially in those patients with higher baseline levels. The sFlt1 protein is upregulated by hypoxia via the hypoxia-inducible transcription factors.\textsuperscript{27,27} Angiotensin II increases ongoing hypoxia-inducible factor-1\( \alpha \) translation by a reactive oxygen species--dependent activation of the phosphatidylinositol 3-kinase pathway.\textsuperscript{28,29} Thus, the nonhypoxic induction of HIF-1\( \alpha \) via AT1 receptor pathways might provide a link with sFlt-1.

**Perspectives**

It is well recognized that autoantibodies such as the AT1-AA or an anti-\( \alpha_1 \)-adrenocereceptor autoantibody are involved in the pathogenesis of a variety of cardiovascular complications. Various novel pathways have been elucidated recently in the area of preeclampsia pathogenesis, including AT1 receptor activation.\textsuperscript{38} AT1-AA and sFlt-1 offer potential therapeutic targets in terms of potential binders that could be infused early in the course of preeclampsia or in the short interval available before the condition develops. Such agents could open a new avenue of treatment, as suggested for insulin resistance and sFlt-1 previously.\textsuperscript{32} AT1-AA may also have significance in terms of predisposing to and/or predicting subsequent cardiovascular risk.

**Acknowledgments**

We thank the general clinical research center staffs at the respective institutions for their invaluable assistance.

**Sources of Funding**

This work was supported by grants M01RR01066 (Massachusetts Institute of Technology General Clinical Research Center), M01RR00056 (Mage-Womens Hospital Satellite General Clinical Research Center), RR017376 (M.W.), HD049453 (C.A.H.), HD30367 (J.M.R.), and HD39223 (R.T.) from the National Institutes of Health and the American Heart Association (M.W.).

**Disclosures**

R.T. has a consulting relationship with Abbott Diagnostics, Beckman Coulter, Ortho-Clinical Diagnostics, and Roche Diagnostics and has patents filed in preeclampsia diagnostics and licensed to diagnostic companies. The remaining authors report no conflicts.

**References**


Agonistic Angiotensin II Type 1 Receptor Autoantibodies in Postpartum Women With a History of Preeclampsia

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Hypertension. 2007;49:612-617; originally published online January 8, 2007; doi: 10.1161/01.HYP.0000256565.20983.d4

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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