Cardiovascular Biomarker Midregional Proatrial Natriuretic Peptide During and After Preeclamptic Pregnancies

Meryam Sugulle, Florian Herse, Lydia Hering, Martin Mockel, Ralf Dechend, Anne Cathrine Staff

Abstract—Preeclampsia is associated with increased risk of cardiovascular disease. Midregional proatrial natriuretic peptide (MR-proANP), a precursor of the atrial natriuretic peptide, is a biomarker for cardiovascular disease. We obtained plasma from 184 pregnant women in gestational weeks 24 to 42 (normotensive pregnancies: n=77, preeclampsia: n=107), from 25 of these women at 5 to 8 years after index pregnancy (normotensive pregnancies: n=11, preeclampsia: n=14), and from 49 normotensive, nonpregnant women and analyzed them by immunoassay for MR-proANP. To investigate potential sources, placental and decidual atrial natriuretic peptide mRNA expression levels were analyzed by quantitative real-time PCR in 21 normotensive and 23 preeclamptic pregnancies, as well as in human heart and kidney samples. For further confirmation, we measured circulating MR-proANP and performed expression studies in a transgenic rat model for preeclampsia. MR-proANP was significantly elevated in maternal plasma in preeclampsia compared with normotensive pregnancies (135 versus 56 pmol/L; \( P<0.001 \)). However, 5 to 8 years after pregnancy, there was no difference (formerly preeclamptic women versus formerly normotensive in pregnancy: 53 versus 49 pmol/L; \( P=0.5 \)). Our preeclamptic rat model confirmed the acute MR-proANP differences between preeclamptic and normotensive pregnancies (10.9±1.9 versus 4.3±0.3 pmol/mL; \( P=0.05 \)). Atrial natriuretic peptide expression was high in the heart but negligible in the uteroplacental unit in both normotensive humans and rats, whereas expression in maternal and fetal hearts in the preeclamptic rats was significantly increased, compared with controls. MR-proANP is a serviceable biomarker in preeclampsia, both in humans and a rat model, probably reflecting cardiovascular hemodynamic stress. (Hypertension. 2012;59:00-00.)

Key Words: biological marker cardiovascular disease midregional proatrial natriuretic peptide preeclampsia pregnancy postpartum transgenic rats

Preeclampsia, defined as the development of de novo hypertension and proteinuria after 20 weeks of gestation, affects 3% to 5% of all pregnancies.\(^1\) The pathophysiology of preeclampsia remains unknown; however, dysregulated immunologic factors are proposed to underlie a defective placentation and reduced remodeling of maternal uteroplacental spiral arteries by fetal trophoblasts.\(^2\) These mechanisms are suggested to result in an oxidatively stressed placenta that produces circulating factors causing an excessive systemic inflammatory response and generalized maternal endothelial dysfunction.\(^3,4\) Preeclampsia and cardiovascular disease share many risk factors.\(^5,6\) Women with preeclampsia have increased cardiovascular morbidity and mortality later in life.\(^7,8\)

Atrial natriuretic peptide (ANP) is a diuretic, natriuretic, and vasodilatory cardiac hormone. ANP is released into the circulation after atrial distension.\(^9,10\) Plasma ANP is considered a prognostic marker for acute heart failure, risk of cardiovascular events, and death.\(^11,12\) Previous studies of ANP in maternal circulation in pregnancy are controversial, with reports of increased,\(^13-16\) as well as similar,\(^17-19\) circulating ANP concentrations in preeclampsia compared with normotensive pregnancy. However, ANP is an unstable peptide, and it has been remarked that ANP assays used in previous studies may have underestimated the quantity of the circulating hormone because of an early degradation of crucial epitopes at the extreme ends of the precursor molecule.\(^20\) Midregional pro-ANP (MR-proANP), released in an equimolar ratio to ANP,\(^20\) is more stable than the N- or C-terminal part of the precursor.\(^21\) MR-proANP is associated with cardiovascular disease.\(^12\) Because women with pregnancies complicated by preeclampsia are at increased risk for developing cardiovascular disease later in life,\(^7,8\) we hypothesized that elevated circulating MR-proANP both during and after preeclamptic pregnancies might serve as a biomarker for cardiovascular stress.
Methods

We recruited 184 women with singleton pregnancies (gestational weeks 24–42) in 2001–2011 at Oslo University Hospital. 107 women with preeclampsia and 77 with normotensive pregnancies. We excluded women with preexisting hypertension, diabetes mellitus, heart or kidney disease, rupture of membranes, clinical signs of infection, or uterine contractions at the time of blood sampling. Preeclampsia was defined according to the American College of Obstetricians and Gynecologists criteria.2 Recruitment was conducted either before elective cesarean section (69 normotensive and 62 preeclampsia) or after admission to the high-risk obstetric ward regardless of the later delivery mode (8 normotensive and 45 preeclampsia). The newborn birth weight percentiles were calculated according to a Norwegian population-based, ultrasound-based growth percentile chart.22 Newborns with a birth weight percentile <10% were defined as small for gestational age (SGA). Umbilical artery Doppler velocimetry was defined as abnormal if the pulsatile resistance index was above the 95th percentile for gestational age or in the presence of abnormal waveforms.23

A subgroup of the women was recruited to a clinical follow-up study described in detail previously,24 with blood samples of 14 formerly preeclamptic women and 11 women with a previously normotensive pregnancy available for longitudinal comparison. Maternal EDTA plasma was obtained and stored as described previously.25 Mean arterial blood pressure (BP) was calculated according to the following formula: diastolic BP + (systolic BP – diastolic BP)/3. The Regional Committee for Medical Research Ethics in Eastern Norway approved the study, and informed written consent was obtained from each woman.

Our preeclampsia rat model is described elsewhere.26,27 Briefly, female Sprague-Dawley rats, transgenic for the human angiotensinogen gene, were crossed with male rats transgenic for the human renin gene. During pregnancy, this model shows preeclamptic phenotype, consisting of hypertension, proteinuria, and intrauterine growth restriction in the pregnant dams. Local authorities (Berlin, Germany) approved the animal protocol (permission No. 0268/06), which complied with criteria outlined by the American Physiological Society.

Total mRNA was isolated with a combined protocol of QIAzol lysis reagent and Qiagen RNeasy mini kit (including the RNase-Free DNase set; Qiagen) according to the manufacturer’s protocol from human decidua and placenta (normotensive controls: n=21; preeclampsia: n=23, from a subgroup of patients who underwent elective cesarean section) and rat kidney, heart, placenta, and mesometrial triangle. RNA quantity and quality were confirmed by RNA 6000 Chip in the 2100 Bioanalyzer (Agilent Technologies) and NanoDrop UV/VIS-Spectrometer (PegLab). Human RNA from heart and kidney was obtained from Human Total RNA Master Panel (BD Bioscience). RNA was reverse transcribed into cDNA by the Transcriptor First Strand cDNA synthesis kit from Roche Diagnostics and was analyzed by real-time quantitative PCR on ABI 7500 Fast sequence detection system (PE Biosystems). Primer and probes were designed with PrimerExpress 3.0 (Applied Biosystems): rat ANP (accession No. NM_021622.2) (forward) 5'-CCA TCA CCA AGG GCT TCT CT 3'; rat ANP (reverse) 5'-ACG GGA TTT GGT CCA ATA TG 3'; rat ANP (probe) 5'-FAM-TCC TGG CCT TTT GGC TCC CAG TAM-3' and human ANP (accession No. NG_012926.1) ANP (forward) 5'-TCAGACAGCAGCTATC-CCATG 3'; ANP (reverse) 5'-TCCAGCAATCTGTCCATCAA 3'; ANP (probe) 5'-FAM-CATAAGCCGTGTCACCA CAGACAGCAG TAM-3' and the endogenous-control Eukaryotic 18S rRNA (GenBank accession No. X03205; PE Biosystems).

Previously analyzed plasma concentrations of MR-proANP from 49 normotensive, nonpregnant, premenopausal women were provided by B.R.A.H.M.S Biomarkers (Clinical Diagnostics Division, Thermo Fisher Scientific, Hennigsdorf, Germany). The MR-proANP assay was performed according to the manufacturer’s instructions in duplicates and blinded for diagnosis at the laboratories of B.R.A.H.M.S Biomarkers, where it has been developed.20 The assay has a lower detection limit of 2.1 pmol/L. Within the concentration range of 20 to 1000 pmol/L, interassay and intra-assay coefficients of variation are <6.5% and <2.5%, respectively. Soluble fms-like tyrosine kinase receptor 1 (sFlt-1) concentrations in serum were analyzed by ELISA for human sFlt-1, according to the manufacturer’s instructions (R&D Systems).

Statistical analyses were performed using SPSS (version 15.0, SPSS Inc, Chicago, IL). MR-proANP and sFlt-1 concentrations are presented as median values and 95% CIs, with clinical data as mean±SD. Parametric Student t test and the nonparametric Mann-Whitney U test were used when appropriate. MR-proANP concentrations during and after pregnancy were compared by Wilcoxon signed-ranks test. Correlations were investigated using the Spearman correlation coefficient. Linear regression analyses were conducted to adjust for potential confounders. A P level of <0.05 was considered statistically significant. Receiver operating characteristic (ROC) curve was constructed for maternal circulating MR-proANP and sFlt-1 as the discriminator between preeclampsia and normotensive pregnancy.

Results

Demographic information is given in the Table. Of the 107 included preeclamptic patients, 54 had severe preeclampsia, as defined by ACOG criteria.1 In the preeclampsia group, 67 (63%) of 107 women were prematurely delivered, that is, before gestational week 37, and 42 of these before gestational week 34. Nearly all of the normotensive women delivered after week 37 (74 of 77 [96%]); of the 3 prematurely delivered patients, 2 delivered before gestational week 34. Labetalol, methyldopa, nifedipine, or combinations of the 3 were administered in 38 preeclamptic patients (36%) at blood sampling time. In 104 of the 107 patients with preeclampsia, Doppler measurements from the fetal umbilical artery were documented in the patient’s medical chart. Of these, 17 patients had an abnormal blood flow. All of the preeclamptic patients had creatinine values within the normal range (50–90 µmol/L; median: 61 µmol/L). The majority of women in both the normotensive and the preeclampsia groups were white (88% and 80%).

In preeclampsia, median plasma MR-proANP concentration was significantly higher than in normotensive pregnant women and normotensive nonpregnant women (Figure 1A). In the preeclampsia group, MR-proANP concentration correlated inversely to gestational age at sampling time (Spearman r = −0.3; P=0.001), reflected by higher median MR-proANP in preeclamptic women with a gestational age <37 weeks at blood sampling (76) compared with those at term (n=31; 148 versus 114 pmol/L; P=0.05). However, after correcting for gestational age, the difference in median MR-proANP concentration between preeclampsia and normotensive pregnancies remained significant (P<0.001).

There was no significant difference in median MR-proANP between severe (n=54) and nonsevere (n=53) preeclampsia cases (149 versus 116 pmol/L; P=0.09). In the preeclampsia group, MR-proANP correlated inversely with newborn weight percentile (Spearman correlation: −0.35; P<0.001). Median plasma MR-proANP was significantly higher in the preeclamptic women delivering SGA infants (n=51) as compared with the preeclamptic women delivering non-SGA infants (n=56; 177 versus 114 pmol/L; P<0.001). This difference persisted also when defining SGA as birth weight percentile <5.0% or <2.5% (data not shown).

Placenta weight did not correlate with maternal MR-proANP concentration (data not shown) in any of the study groups. In the preeclamptic patients with pathological fetal
umbilical artery Doppler measurements (n = 17), median MR-proANP concentration was not different than in those with normal measurements (n=87; 152 versus 133 nmol/L; P=0.3). There was no difference in median MR-proANP concentration between preeclamptic patients who had received corticosteroids for fetal lung maturation within a week before blood sampling (n=41) compared with those who had not received this treatment (n=5), nor between preeclamptic patients receiving antihypertensive medication (n=38) or not receiving medication (n=69).

MR-proANP concentration correlated positively with serum creatinine in the preeclampsia group (Spearman correlation: 0.5; P=0.001). Prepregnancy body mass index, body mass index at sampling time, diastolic BP in week before 20 weeks’ gestation, systolic and diastolic BPs, and mean arterial BP at blood sampling were all not correlated with MR-proANP concentrations, neither in the normotensive nor in the preeclampsia group.

Among the women restudied 5 to 8 years after delivery, only 1 of the formerly preeclamptic women received antihypertensive medication. These findings suggest the usefulness of MR-proANP measurements in preeclampsia care and for study of women’s long-term health, as well as the additional information provided by the measure's correlation with serum creatinine.

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**Table.** Demographic Information

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>C (n=77)</th>
<th>PE (n=107)</th>
<th>Nonpregnant (n=49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age at blood sampling, y</td>
<td>32.3±4.8</td>
<td>31.1±4.5</td>
<td>31.2±9.2</td>
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<tr>
<td>Prepregnancy BMI, kg/m²</td>
<td>22.9±3.7</td>
<td>25.2±5.5</td>
<td>P=0.001</td>
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<tr>
<td>BMI at blood sampling, kg/m²</td>
<td>27.9±3.7</td>
<td>31.2±5.9</td>
<td>P&lt;0.001*</td>
</tr>
<tr>
<td>Parity</td>
<td>0.7±0.9</td>
<td>0.4±0.6</td>
<td>P=0.02*</td>
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<tr>
<td>Gestational age at blood sampling, wk</td>
<td>37.5±3.7</td>
<td>34.3±3.8</td>
<td>P&lt;0.001*</td>
</tr>
<tr>
<td>Gestational age at delivery, wk</td>
<td>38.4±2.0</td>
<td>34.5±3.8</td>
<td>P&lt;0.001*</td>
</tr>
<tr>
<td>Neonatal weight, g</td>
<td>3489±534</td>
<td>2328±992</td>
<td>P&lt;0.001*</td>
</tr>
<tr>
<td>Neonatal weight percentile°</td>
<td>65.6±25.9</td>
<td>28.5±31.6</td>
<td>P&lt;0.001*</td>
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<td>Systolic BP &lt;wk 20, mm Hg</td>
<td>111±10</td>
<td>115±11</td>
<td>P=0.005*</td>
</tr>
<tr>
<td>Diastolic BP &lt;wk 20, mm Hg</td>
<td>67±6</td>
<td>71±7</td>
<td>P&lt;0.001*</td>
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<tr>
<td>Systolic BP at blood sampling, mm Hg</td>
<td>120±11</td>
<td>162±16</td>
<td>P&lt;0.001*</td>
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<tr>
<td>Diastolic BP at blood sampling, mm Hg</td>
<td>69±9</td>
<td>98±7</td>
<td>P&lt;0.001*</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>85±7</td>
<td>118±9</td>
<td>P&lt;0.001*</td>
</tr>
</tbody>
</table>

C indicates normotensive pregnancies, named controls; PE, preeclampsia; BMI, body mass index; BP, blood pressure; MAP, mean arterial pressure. Values are presented as mean±SD. All P values are as compared with the control and preeclampsia group, respectively, by Student t test. *P<0.05.
preeclampsia and those who had been normotensive during their pregnancy, were normotensive at follow-up. None had a diagnosis of cardiovascular disease. There were no residual differences in plasma MR-proANP concentrations, comparing formerly preeclamptic to formerly normotensive pregnant women (53 versus 49 pmol/L; \(P=0.5\); Figure 1B). Formerly preeclamptic women had significantly lower median plasma MR-proANP concentrations compared with concentrations during their pregnancy (53 versus 135 pmol/L; \(P=0.001\)), whereas the median value for the formerly normotensive women was unaltered (49 versus 56 pmol/L; \(P=0.2\)). There were no significant correlations within any of the study groups for circulating MR-proANP values between the pregnancy and postpartum concentrations. Furthermore, 5 to 8 years after pregnancy, median MR-proANP concentrations in formerly preeclamptic and formerly normotensive women did not differ from median MR-proANP concentrations in non-pregnant women (53 and 49 versus 46 pmol/L; \(P=0.7\) and \(P=0.2\)).

The sensitivity and specificity of plasma MR-proANP in discriminating between preeclamptic and normotensive pregnant women at delivery were high, with an area under the curve (AUC) of 0.85 (95% CI: 0.79–0.90; \(P<0.0001\); Figure 2A). For comparison, we constructed the ROC curve of serum sFlt-1 in the same study population for a subgroup of our pregnant population in which this marker was available (controls: \(n=61\); preeclampsia: \(n=100\)). sFlt-1 performed slightly better than MR-proANP as a discriminator between preeclampsia and normotensive pregnancy, with an AUC of 0.94 (95% CI: 0.91–0.98; \(P<0.0001\); Figure 2B). The combination of a MR-proANP and sFlt-1 ROC curve resulted only in a slight, but not statistically significant, improved AUC compared with the sFlt-1-ROC curve alone (data not shown).

We tested the relation between concentrations of MR-proANP and sFlt-1 concentrations in both normotensive controls, as well as patients with preeclampsia (Figure 2C). Although for both groups there is a significant positive correlation (preeclampsia: Spearman correlation: 0.4, \(P<0.0001\); normotensive pregnancy: Spearman correlation: 0.3, \(P<0.01\)), the increase in MR-proANP per unit sFlt-1 was higher in the preeclamptic than in the normotensive group.

Because ANP is expressed in various tissues, including the uteroplacental unit, we explored the source of MR-proANP in pregnancy (Figure 3A). ANP expression in human heart tissue was \(\approx 20\) 000 fold higher than in kidney and decidual tissue and \(\approx 30\) 000 fold higher than in placenta, indicating that the source of the observed upregulation in circulating MR-proANP in preeclamptic patients is not the uteroplacental unit.

To further explore the origin for the increase in circulating MR-proANP in preeclampsia, we also investigated MR-proANP expression in the circulation and in different tissues (heart, kidney, placenta, and mesometrial triangle) of our transgenic preeclamptic rat model. The preeclamptic females had elevated (\(P=0.05\)) circulating MR-proANP values (Figure 3B). Similar to the humans, ANP expression was highest in the heart (Figure 3C). Preeclamptic female rats had higher values (\(P<0.001\)) than normotensive pregnant Sprague-Dawley controls (Figure 3D); the increase occurred 2 days after BP began to climb in the transgenic pregnant females. ANP gene expression was not differently regulated in the kidney but was slightly upregulated in the preeclamptic uteroplacental unit (placenta 2.0-fold and mesometrial triangle 2.4-fold compared with the normotensive rat). ANP expression was also increased in the fetal hearts of the preeclamptic rats (\(P<0.001\)) compared with fetal hearts from normotensive rats (Figure 3E).

**Discussion**

Our important finding is that preeclamptic women have significantly elevated circulating MR-proANP values, most likely reflecting substantial cardiovascular hemodynamic stress. We found that, 5 to 8 years later, women who had developed preeclampsia in their index pregnancy no longer had elevated MR-proANP values compared with women who had not had preeclampsia. To our knowledge, this report is the first to provide these observations and their follow-up. The MR-proANP values that we observed in preeclampsia were similar to those reported in acute ischemic stroke but lower than in patients with acute unstable heart failure. In preeclampsia, median plasma MR-proANP concentrations were \(\approx 2.5\)-fold increased and \(>3\)-fold increased compared with nonpregnant, normotensive women and to normotensive, pregnant women. The MR-proANP ROC showed a high...
discrimination between preeclamptic and normotensive pregnancies and was only marginally inferior to the established “preeclampsia biomarker” sFlt-1.

Our MR-proANP findings are largely in accord with earlier reports looking at ANP concentrations in preeclampsia. However, other studies were conflicting, possibly because of differences in the assay methodology. A strength of our study lies in the fact that the assay that we used detects more stable parts of the precursors of ANP. MR-proANP is produced in equimolar amounts to the mature hormone. The increased MR-proANP concentration in our study, therefore, reflects an increased concentration of the mature ANP protein in the preeclamptic circulation.

An increased circulating MR-proANP could originate from the preeclamptic placenta, from the heart, or from both organs. Our finding of ANP placental gene expression in our patients and in our rat model convincingly showed that the ureteroplacental unit could not be the main source of circulating MR-proANP in pregnancy. We conclude that the heart is the source. Both normal pregnancy and preeclampsia are characterized by marked alterations in hemodynamics. Global diastolic dysfunction and an adaptive left-ventricular remodeling have been observed in preeclampsia. As opposed to previous reports, we found no correlation between MR-proANP concentration and systolic BP or mean arterial BP in our preeclamptic patients. In contrast to previous reports, we found a positive correlation between MR-proANP concentrations and creatinine in preeclamptic patients. Decreased renal clearance has been suggested previously as a cause of increased circulating ANP; however, all of the preeclamptic women in our study had creatinine values within a normal range. As proxies of placental function and severity of preeclampsia, we used birth weight of an SGA infant, pathological blood flow in the umbilical artery, and placenta weight in relation to MR-proANP concentration. A previous longitudinal study of circulating ANP concentrations in formerly preeclamptic women had a follow-up period limited to a few weeks postpartum. A significant decrease of ANP concentration in the second week postpartum has been reported. At that time point, there were

Figure 3. A, Atrial natriuretic peptide (ANP) expression in human tissue of heart, kidney, placenta, and decidua is shown (*P<0.0001 vs all other tissues). B, ANP expression in rat tissue of heart, kidney, placenta, and mesometrial triangle is shown (*P<0.0001 vs all other tissues). C, Circulating midregional pro-ANP (MR-proANP) levels in preeclamptic rats (PE) and normotensive control rats (SD). D, ANP expression in heart tissue of PE and SD rats is shown at “not pregnant status,” day 15 and day 21 of pregnancy (*P<0.05 and *P<0.001, respectively). E, ANP expression in heart tissue of fetuses of PE and SD rats at day 21 of pregnancy (*P<0.001).
no differences between the preeclamptic patients and normotenive control women postpartum. The natriuretic peptides ANP and brain natriuretic peptide are released according to atrial and ventricular wall tensions. Brain natriuretic peptide has also been found elevated in preeclampsia, and elevated circulating concentrations of MR-proANP and brain natriuretic peptide seem to be equally useful in the diagnosis of heart failure. Women after preeclamptic pregnancies are at increased risk for cardiovascular disease later in life, and recent findings reveal that a high proportion of formerly preeclamptic women meets echocardiographic criteria of asymptomatic heart failure at 1 year postpartum. Therefore, we were interested to determine whether MR-proANP was still elevated in this group of fertile women several years after a preeclamptic pregnancy, which was fortunately not the case. Further longitudinal studies are, however, needed to explore whether the women with the highest circulating MR-proANP concentrations in pregnancy also are in the highest risk group of developing cardiovascular disease later in life, when reaching older, postmenopausal age.

A strength of our study is the fact that biobank samples are taken before labor. Therefore, differences in labor duration and delivery mode (vaginal versus cesarean) did not confound our laboratory analyses. Such uterine contraction differences may cause variations in placental oxidative stress, with unknown effects on circulating MR-proANP. The lack of matching for gestational age of preeclamptic and normotensive subjects may be viewed as a limitation. Women with preeclampsia are often delivered prematurely to reduce maternal and fetal morbidity and mortality. A previous study found elevated ANP in preeclampsia as compared with healthy pregnancy and a significant increase in ANP in both groups of pregnant women, with third trimester levels exceeding first trimester levels. ANP concentrations in the second trimester in women who later developed preeclampsia were also high. Thus, the lack of matching for gestational age in this study may not be crucial, because patients defined as healthy, normotensive controls in the third trimester may be assumed to have had lower concentrations of the biomarker compared with the ones who later would become preeclamptic and those already overtly preeclamptic. Therefore, if the patient groups had been gestationally matched in pregnancy, we would have expected a larger difference in MR-proANP between the study groups. Furthermore, MR-proANP and gestational age at sampling time were inversely correlated. Also, adjusting for gestational age by means of linear regression analyses did not alter our conclusions regarding MR-proANP in preeclampsia. Our data of increased circulating MR-proANP, a marker of heart failure, add to the increasing evidence of impaired cardiovascular function in preeclamptic women.

**Perspectives**

MR-proANP represents an interesting and novel biomarker in preeclampsia, likely to mirror the hemodynamic and cardiovascular alterations and endothelial dysfunction in preeclampsia. MR-proANP could represent a supplement to the well-established biomarkers of preeclampsia of predominantly placental origin, such as sFlt-1. Follow-up studies of women after preeclamptic pregnancy could elucidate whether high concentrations of MR-proANP in pregnancy concentrations represent early predictors of susceptibility for later cardiovascular events, not only in the mother, but also in the offspring of such pregnancies. If such was the case, women at significant risk for cardiovascular diseases could be identified and subjected to individualized follow-up and intervention programs to prevent or postpone development of cardiovascular disease.

**Sources of Funding**

The Deutsche Forschungsgemeinschaft (DE631/1) and the Helmholtz Foundation support R.D. and F.H. (DFG; HE 6249/1-1). B.R.A.H.M.S. Biomarkers helped with MR-proANP measurements, Clinical Diagnostics Division, Thermo Fisher Scientific, 16761 Henningsdorf, Germany.

**Disclosures**

None.

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