Flow-mediated dilation and peripheral arterial tonometry are disturbed in preeclampsia and reflect different aspects of endothelial function
even decline at week 36 (16). Preeclamptic women are characterized by a significant reduction in FMD at the time of diagnosis (25). An alternative, user-independent technique in the assessment of endothelial function is peripheral arterial tonometry (PAT), measuring changes in digital pulse volume following reactive hyperemia. The resulting reactive hyperemia index (RHI) has prognostic value in the general and cardiovascular population compared with FMD (6). Data using PAT during pregnancy and PE are limited, based on small studies, showing conflicting results. Yinon et al. (24) described a reduced RHI in women with PE compared with controls. A study comparing RHI measurements at 16 and 28 gestational wk in normal and PE pregnancies showed a lower RHI at gestational week 28 compared with week 16 in both groups but no difference between PE and healthy pregnancies (3). However, because of measurement of peripheral microcirculatory function, PAT is less NO dependent than FMD. As such, FMD and PAT assess different aspects of vascular function (1, 8).

Arterial stiffness has been evaluated in pregnancy using applanation tonometry. When arterial wall stiffness is increased, the arterial pulse wave travels more rapidly away from the heart, and the reflected wave returns more rapidly, resulting in a significant augmentation of the systolic peak. This can be measured as a raised augmentation index (AIx) (11). During normal pregnancy, AIx falls during midpregnancy and rises at the end of pregnancy. In PE, AIx is increased significantly, and a significant role of first-trimester AIx in the early screening of PE has been proposed. Arterial stiffness is independently associated with cardiovascular risk and may, therefore, provide a potential marker to select women who will develop cardiovascular events later in life after PE (7, 11).

Normal pregnancy and PE are both known to exert inflammatory effects, apparent by a higher neutrophil-to-lymphocyte ratio (NLR) and a higher mean platelet volume (MPV) (5). An increase in NLR and MPV is described to be more prominent in PE, and these factors have been proposed as predictive biomarkers for PE (12, 22). This increased, systemic, low-grade inflammation in pregnancy possibly contributes to alterations in endothelial function.

In this study, we made a comprehensive evaluation of in vivo vascular function (including FMD, PAT, and arterial stiffness) in PE patients and compared them with normal pregnancies. In addition, we assessed the relation between vascular function and inflammation (NLR and MPV).

We hypothesize that PE is associated with a disturbed endothelial function (as determined by FMD and PAT) and increased arterial stiffness, probably even more so due to an exaggerated inflammatory response. During normal pregnancy, we expect a mild deterioration of endothelial function due to an augmented systemic inflammation at the end of pregnancy.

METHODS

Study Population

Fourteen preeclamptic patients (gestational age 29 ± 0–36 ± 5 wk, mean 31 wk), admitted to the Maternal Intensive Care Unit at the Antwerp University Hospital, were included between January and September 2016. We defined PE according to the revised International Society for the Study of Hypertension in Pregnancy definition (21). Exclusion criteria were (gestational) diabetes, multiple pregnancies, fetal malformations, hypercholesterolemia, kidney disease, autoimmune disorders, connective tissue diseases, or use of acetylsalicylic acid. Since the Antwerp University Hospital serves as a referral center, patients were already started on antihypertensive medication (Table 1) and MgSO4 at the moment of referral and inclusion. Fourteen age-, body mass index (BMI)-, and parity-matched healthy pregnant women served as controls. They were included in the study during their first trimester and were longitudinally followed throughout the entire pregnancy. They were free from medication and did not have a history of PE, pregnancy-induced hypertension, hypertension, cardiovascular disease, or other chronic conditions. The Research and Ethics Committee of the Antwerp University Hospital approved the study protocol (Belgian number B300201524783), and written, informed consent was obtained from all subjects.

Table 1. Antihypertensive medication and doses given to PE patients

<table>
<thead>
<tr>
<th>Medication</th>
<th>Dose/Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>No antihypertensive medication</td>
<td>4 patients</td>
</tr>
<tr>
<td>Labetalol, 100 mg 3×/day</td>
<td>4 patients</td>
</tr>
<tr>
<td>Labetalol, 6–8 ml/h iv</td>
<td>4 patients</td>
</tr>
<tr>
<td>Labetalol, 100 mg 3×/day + Felodipine, 10 mg 2×/day</td>
<td>1 patient</td>
</tr>
<tr>
<td>Felodipine, 5 mg 2×/day</td>
<td>1 patient</td>
</tr>
</tbody>
</table>

Vascular Function Measurements

Patients were asked, 24 h before examination, not to eat high-fat substances nor to drink caffeine or alcohol and to refrain from smoking at least 6 h before examination. Finger nails had to be short and no nail polish applied. Patients were studied in a quiet, temperature-controlled room (21–24°C), and stressful situations for the patient were avoided (people entering the room unexpectedly, telephone ringtones, etc.). The examinations were performed in a supine, lying position with the arm in a comfortable position for imaging the brachial artery. After 5 min of rest, one blood pressure measurement was taken using an automated blood pressure device (Intellisense; Omron Healthcare, Tokyo, Japan). This systolic blood pressure value was used to determine occlusion pressure for the FMD/RHI measurements.

FMD and RHI measurements were performed simultaneously. Repeat measurements in the control group were performed at the same arm and at approximately the same time of day.

Brachial artery flow-mediated dilatation. FMD was assessed by measuring changes in the brachial artery diameter in response to an increased shear stress during reactive hyperemia (20). An ultrasound diagnostic instrument (ProSound Alfa 6; Hitachi Aloka Medical, Tokyo, Japan), equipped with vascular software for two-dimensional imaging, color Doppler imaging, and ECG triggering, was used with a high-frequency linear-array transducer (5–13 MHz, UST-5413; Hitachi Aloka Medical). Patients were in a resting, supine position with the arm in a comfortable position for imaging the brachial artery. A blood pressure cuff was placed on the forearm, with the upper border of the cuff at a distance of 5–10 cm distal from the elbow (lateral epicondyle). The brachial artery was imaged above the ante-cubital fossa in a longitudinal plane with a clear delineation of both anterior and posterior intima-media interfaces. A special probe-holding device was used to ensure consistency of images during the measurement. The baseline artery diameter was automatically tracked, and the waveform of diameter changes over the cardiac cycle was displayed in real time using an automated edge-detection system (eTracking; Hitachi Aloka Medical). Arterial occlusion was created by cuff inflation to suprasystolic pressure at least 50 mmHg above systolic pressure (minimum value of 200 mmHg). After 5 min of occlusion, the cuff was deflated. A brachial diameter was recorded continuously (eTracking) from the time point of cuff inflation to 5 min after cuff deflation. FMD (in percent from baseline value) was expressed as (postischemical maximal diastolic diameter change – baseline diastolic diameter)/baseline diastolic diameter. All FMD measurements in the control group were performed at the same arm and at approximately the same time of day.
Peripheral arterial tonometry. PAC was recorded using the EndoPAT 2000 (software version 3.2.4; Itamar Medical, Caesarea, Israel) and the disposable fingertip probes (Itamar Medical), in accordance with the manufacturer’s recommendations. PAC is a less operator-dependent and more reproducible technique. The system uses pneumatic finger probes that assess digital volume changes accompanying pulse waves. Reactive hyperemia was induced, as described for FMD, and measurements were performed simultaneously to PAC. The ratio of the average amplitude of the PAC signal over a 1-min period, starting 1 min after cuff deflation (maximum pulse amplitude), divided by the average amplitude of the PAC signal over a 3.5-min period before cuff inflation (baseline pulse amplitude), was calculated. The control arm was used to correct for confounding factors (room temperature, systemic changes). The result is expressed as the RHI. All PAC recordings were performed by the same two experienced investigators (D. Mannaerts and E. Faes).

Arterial stiffness. Systemic arterial stiffness was evaluated using pulse-wave analysis (PWA) and pulse-wave velocity (PWV) using the SphygmoCor system (AtCor Medical, West Ryde, Australia). For PWA, three measurements, at the level of the radial artery, were obtained with a quality operator index of at least 80%. The tonometer was placed at the area of interest, and its position was adjusted until a strong, accurate, and reproducible waveform was obtained. The AIX was calculated by analysis of the pressure waveform, expressed as the ratio of augmented pressure (attributed to wave reflection) to pulse pressure. Pulse pressure is defined as systolic pressure minus diastolic pressure. A rise in arterial stiffness causes earlier reflection (augmentation) of the pulse wave that reaches the heart in late systole and thus increases the cardiac workload. As AIX is affected by heart rate, it was standardized to a heart rate of 75 beats/min (AIX-75). For PWV, the distance between the site of maximal pulsation of the carotid artery and the femoral artery was measured using a tape measure in a straight line. Three measurements, at the level of the carotid artery and subsequently, the femoral artery, were obtained with an SD < 10%. As the arterial wall stiffens, the velocity of traveling waves in the lumen increases. In assessing the PWV, the aortic PWV [measured by carotid-femoral PWV (cfPWV)] is the gold standard (25). All PWA/PWV recordings were performed by the same two experienced investigators (D. Mannaerts and E. Faes).

Maternal Venous Blood

Maternal venous blood samples were taken for the quantification of MPV and NLR. Peripheral blood was collected by venepuncture at 9–11 and 24–28 wk and at term using a Vacutette tube. EDTA samples were analyzed using an ADVIA 120 Hematology System (Siemens Healthcare, Erlangen, Germany).

Statistical Analysis

Statistical analysis was performed using SPSS version 22.0 (IBM, Armonk, NY). Data are expressed as means ± SD. Normality of continuous variables was evaluated using Kolmogorov-Smirnov test. Groups were compared using independent t-test and paired sample t-test, as appropriate. Spearman correlation coefficient was used for univariate correlation analysis. A two-tailed P < 0.05 was considered significant.

RESULTS

Patient Characteristics

Characteristics of preeclamptic and normotensive patients are summarized in Table 2. Groups were comparable regarding age, parity, BMI, and cardiovascular risk. Blood pressure and birth weight were significantly different between groups. The differences in birth weight were due to differences in gestational age at birth. Patients with coexisting intrauterine growth restriction were excluded from the study.

Evolution of Vascular Function During Normal Pregnancy

Fourteen healthy pregnant controls underwent vascular assessment at the first (11 ± 6–13 ± 2 wk, mean 12 ± 4 wk) and third (34 ± 1–36 ± 3 wk, mean 35 ± 0 wk) trimester of pregnancy. FMD was comparable between the first and third trimester (8.95 ± 4.67 vs. 8.53 ± 4.09%, P = 0.78; Fig. 1A). The time to reach the peak diameter in the third trimester did not change compared with the first trimester (54.0 ± 17.5 vs. 47.1 ± 18.9 s, P = 0.31), but the change in artery diameter between baseline and end of occlusion was significantly higher in the third trimester (0.094 ± 0.112 vs. 0.003 ± 0.036 mm, P = 0.03). Baseline diameters were significantly different as well between the first and third trimester (2.9 ± 0.26 vs. 3.3 ± 0.4 mm, P = 0.002). To account for the influence of this augmented baseline diameter, we recalculated the FMD results of both the first and third trimester by dividing them with the baseline diastolic diameter of the first trimester. As a result, FMD measurements were even more comparable between the first and third trimester of pregnancy (3.14 ± 1.71 vs. 2.97 ± 1.48, P = 0.76). In contrast to FMD, RHI was significantly lower at gestational week 35 compared with week 12 (1.53 ± 0.33 vs. 2.30 ± 0.56, P = 0.001). This evolution was observed in all patients and suggests a deterioration in endothelial microvascular endothelial function with advanced pregnancy (Fig. 1B).

There was no significant difference in PWA (5.14 ± 9.47 vs. 9.00 ± 9.74, P = 0.21) nor in cfPWV (6.18 ± 0.67 vs. 6.03 ± 0.78, P = 0.31) between the first and third trimester of pregnancy (Fig. 1, C and D).

Vascular Function in Preeclamptic vs. Normotensive Pregnancy

Results of the vascular function assessments that were performed simultaneously in 14 preeclamptic patients (mean gestational week: 30.6 ± 3.67) and in 14 age-matched control patients (mean gestational week: 34.6 ± 0.75) are shown in Table 3 and Fig. 2. FMD and RHI measurements were performed simultaneously to reduce diurnal and biological variation. FMD was severely reduced in preeclamptic patients compared with control patients (P = 0.014). Totally opposite to this observation, there was a higher RHI in preeclamptic

### Table 2. Patient characteristics

<table>
<thead>
<tr>
<th>Preeclampsia, n = 14</th>
<th>Normotensive, n = 14</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yr)</strong></td>
<td>29 (22–37)</td>
<td>31 (25–36)</td>
</tr>
<tr>
<td><strong>BMI 3rd trimester, kg/m²</strong></td>
<td>26.7 ± 3.4</td>
<td>28.0 ± 3.6</td>
</tr>
<tr>
<td><strong>SBP 3rd trimester, mmHg</strong></td>
<td>155.7 ± 15.1</td>
<td>128.8 ± 11.2</td>
</tr>
<tr>
<td><strong>DBP 3rd trimester, mmHg</strong></td>
<td>92.5 ± 8.5</td>
<td>74.6 ± 7.6</td>
</tr>
<tr>
<td><strong>MAP 3rd trimester</strong></td>
<td>113.6 ± 10.0</td>
<td>92.7 ± 7.9</td>
</tr>
<tr>
<td><strong>Nulliparous, n</strong></td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td><strong>Gestation at delivery (wk)</strong></td>
<td>30.7 (25–37)</td>
<td>38.9 (37–40)</td>
</tr>
<tr>
<td><strong>Birth weight, g</strong></td>
<td>1,435 ± 699</td>
<td>3,457 ± 418</td>
</tr>
<tr>
<td><strong>Smoking, n</strong></td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are means ± SD, as median (range) or as number of total (n). SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; NA, not applicable.
versus control patients ($P = 0.0008$). Arterial stiffness, as measured by AIx, PWA, and cfPWV, was significantly higher in preeclamptic patients compared with normotensive patients (all $P < 0.001$).

MPV and NLR Measurements

Gestational age-specific longitudinal changes for MPV and NLR are presented in Fig. 3. There is a significant increase in MPV during pregnancy (overall significance, $P = 0.0002$; first trimester $7.94 \pm 1.05$; second trimester $8.70 \pm 1.05$; third trimester $9.42 \pm 1.50$). NLR was significantly increased between the first trimester ($3.62 \pm 0.99$) and the second trimester ($5.63 \pm 2.10$) but decreased significantly between the second and third trimester ($4.58 \pm 1.47$). We found no significant difference in MPV or NLR between PE patients (MPV $8.96 \pm 1.35$; NLR $6.67 \pm 3.62$) and healthy pregnant controls ($P = 0.42$ and $P = 0.06$, respectively). Neutrophils were significantly higher in the PE group compared with normal pregnancy ($10.86 \pm 4.25$ vs. $8.14 \pm 2.45$, $P = 0.05$; Fig. 4).

Correlation Between Vascular Function and Inflammatory Markers

In the case-control study, we found no correlation between RHI and FMD ($P = 0.42$) or between endothelial function and inflammatory parameters [RHI vs. MPV ($P = 0.54$), FMD vs. MPV ($P = 0.72$), FMD vs. NLR ($P = 0.34$), and RHI vs. NLR ($P = 0.16$)].

DISCUSSION

Vascular dysfunction plays a key role in the pathogenesis of PE. However, differences in assessment techniques have generated apparently paradoxical results when it comes to the presence of endothelial dysfunction and arterial stiffness in PE. This is the first study to compare simultaneously two methods for assessing endothelial function, namely FMD and RHI, and to perform a thorough assessment of different aspects of in vivo vascular function in PE and in normotensive pregnant women.

We found that RHI is decreased at the end of pregnancy compared with the first trimester in a normal, uncomplicated pregnancy, whereas no changes are seen when FMD is measured.

In literature, FMD is known to increase during normal pregnancy until 32 wk and to stabilize or to decline at 36 wk (16). Most papers that describe a higher FMD at the end of gestation take their last measurement between 30 and 32 wk (17). In contrast with our hypothesis, we were not able to detect a deterioration in FMD between the first and last trimester of pregnancy. There was, however, a significant increase in baseline diameter of the brachial artery during normal pregnancy,

Table 3. Vascular function in preeclamptic vs. normotensive pregnancy

<table>
<thead>
<tr>
<th></th>
<th>Preeclamptic Patients</th>
<th>Normotensive Patients</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD, %</td>
<td>4.83 ± 3.15</td>
<td>8.53 ± 4.09</td>
<td>0.014</td>
</tr>
<tr>
<td>Time-to-peak diameter, s</td>
<td>61.3 ± 44.3</td>
<td>54.0 ± 17.5</td>
<td>0.58</td>
</tr>
<tr>
<td>Change in artery diameter between baseline and end of occlusion, mm</td>
<td>0.049 ± 0.166</td>
<td>0.094 ± 0.0112</td>
<td>0.45</td>
</tr>
<tr>
<td>RHI</td>
<td>2.08 ± 0.38</td>
<td>1.53 ± 0.33</td>
<td>0.0008</td>
</tr>
<tr>
<td>AIx-75</td>
<td>23.00 ± 8.80</td>
<td>9.00 ± 9.74</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>cfPWV</td>
<td>7.66 ± 0.93</td>
<td>6.03 ± 0.78</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

Values are means ± SD. $P$ values represent between-group comparison (t-test). FMD, flow-mediated dilation; RHI, reactive hyperemia index; AIx-75, augmentation index standardized to a heart rate of 75 beats/min; cfPWV, carotid-femoral pulse-wave velocity.
which makes it difficult to interpret our results. Recalculation of the FMD measurements to minimize for the influence of an augmented baseline diameter showed FMD measurements that were even more comparable between the first and third trimester of pregnancy. Since vascular stiffness is directly proportional to vascular diameter, a possible explanation can be found in the inability to vasodilate further from baseline in advanced pregnancy, due to the overt vasodilatation at the end of pregnancy. Regarding the execution of FMD measurements, in particular, the methodological approach can critically impact the magnitude of the FMD response; therefore, the paradoxical results between the present study and previous studies could also be explained by differences in technical aspects, such as cuff placement (20).

The RHI, on the contrary, is decreased at the end of pregnancy compared with the first trimester in our study population.
tion, as we stated in our hypothesis. In the third trimester of normal pregnancy, microvascular endothelial dysfunction seems to arise. It is uncertain whether this is caused by a higher level of systemic inflammation (higher neutrophils), a mild form of disseminated intravascular coagulation (higher MPV), or a chronic volume overload at the end of normal pregnancy. We found an increase in NRL and MPV during pregnancy, supporting this theory of increased inflammatory response at the end of pregnancy. A recent paper by Melchiorre et al. (14) describes an increase in total vascular resistance and end-systolic wall stress at term as a consequence of the persistent overload of volume during pregnancy. During normal pregnancy, there is an overt systemic vasodilatation at all levels of the vascular tree, resulting in a higher shear stress, possibly compromising microvascular endothelial function. An alternative explanation is that the lower RHI results are not a sign of endothelial dysfunction but rather, a consequence of normal pregnancy. Since the RHI is the ratio of the postocclusion pulse amplitude over the baseline pulse amplitude, the RHI decreases when the vessel is in a vasodilated state (3). This can explain why the PAT results are lower at the end of normal pregnancy without the presence of endothelial dysfunction.

As stated in our hypothesis, we found an increased arterial stiffness in PE. This can be explained by the inflammation and oxidative stress caused by circulating toxic factors produced by the ischemic placenta attacking the vessel, resulting in widespread endothelial dysfunction (13). Our results are in line with published literature on arterial stiffness in PE. Arterial stiffness is independently associated with cardiovascular risk and may, therefore, provide a potential marker to select women who will develop cardiovascular events later in life after PE (7, 11). We found no difference in arterial stiffness between the first and last trimester of pregnancy, suggesting that normal pregnancy has no influence on the stiffness of maternal vessels.

Supporting our initial stated hypothesis, FMD is decreased in preeclamptic pregnancies compared with normal controls in our study population. This is in line with literature, suggesting endothelial dysfunction in PE (19). Women with PE show a significant reduction in brachial artery diameter compared with normotensive women.

When we compare RHI among preeclamptic patients and healthy controls, we surprisingly find a higher RHI in PE. This finding is not in accord with our hypothesis, stating that overall endothelial dysfunction is present in PE. In the literature, less research exists concerning the use of PAT during preeclamptic pregnancy (compared with FMD), and small studies show conflicting results. In a study by Yinon et al. (24), the control group had an average gestational age of 29 wk, whereas the preeclamptic patients were, on average, 32 wk pregnant. Control patients in our study were, on average, 35 wk pregnant, which makes it less reliable to compare results. Our finding suggests that PAT and FMD are not interchangeable and that PAT should not be used as a substitute for FMD to measure endothelial function, according recent literature (1, 8).

We hypothesize that measurements with FMD and PAT reflect different aspects of endothelial function. This can be explained by the different measuring targets in the arterial tree. FMD measures endothelial regulation of vascular reactivity at the conduit arteries (brachial artery), whereas PAT measures the transient increase in blood flow that occurs following a brief period of ischemia in resistance arteries of the finger. There are important differences between the microvasculature in the finger and the brachial artery, including vessel size, number of capillaries, and arteriovenous anastomoses, and they may all show a different response to ischemia. During PE, there is a vasoconstriction at the level of the arteries and arterioles; this in combination with an increased blood volume during pregnancy and results in augmented shear stress (at the
level of the arteries). The constricted arterioles protect the microvascular system, which could explain the increased RHI. Another explanation can be found in a paper by Beinder and Lang (2). They describe changes in microcirculatory reactivity in patients with PE and found that in PE, there is a higher vasodilatory reserve during reactive hyperemia compared with healthy pregnancy, indicating an increased resting vasomotor tone in the microcirculation. They used local cooling as a vasoconstrictor stimulus and found that vascular reactivity was significantly greater in preeclamptic patients than in controls. These findings are in line with our findings, suggesting vasoconstriction in the microcirculation during PE with the ability to vasodilate after stimulation. Therefore, we hypothesize that not only do FMD and PAT assess different aspects of endothelial function in pregnancy and cannot be used interchangeably, but also, we suggest that the arterial vasculature and microcirculation undergo distinct changes during preeclamptic pregnancies.

In PE, the mechanism that causes endothelial dysfunction is not yet fully established. Toxic factors that are released from the ischemic placenta injure the endothelial wall (glycocalyx), compromising the endothelial function and in particular, NO release. Endothelial cells release NO, and the main stimulus for this is shear stress caused by increased blood flow. Evidence exists that PAT is a less NO-dependent technique in relation to FMD (8). Similar to NO, prostacyclin [prostaglandin I$_2$ (PGI$_2$)] is secreted by endothelial cells and acts as a potent vasodilator. In healthy arteries, NO has an inhibitory effect on PGI$_2$ secretion. It is postulated that in contrast to normotensive, young subjects, hypertensive patients produce significant amounts of vasoactive PGI$_2$. Since PAT is a less NO-dependent technique, it is possible that it encloses a larger role for PGI$_2$, and this may be another explanation for the better RHI results in preeclamptic pregnancies (18). An alternative explanation can be that vasodilatation in resistance arteries is less NO dependent than in the conduit arteries, possibly because of a higher amount of vascular smooth muscle in the conduit arteries sensitive to NO.

In contradiction with our initial hypothesis, we found no significant difference in MPV and NLR, comparing normal pregnant patients in their third trimester of pregnancy with preeclamptic patients. Since the majority of patients in the control group were in the first stage of labor when blood samples were collected, this could have influenced our results (4). Still, there is a trend toward significance regarding the NLR. NLR, in normal pregnancy, was 4.58 ± 1.47 vs. NLR in PE 6.67 ± 3.62 (P = 0.06). Furthermore, neutrophils were significantly higher in the PE group compared with normal pregnancy (10.86 ± 4.25 vs. 8.14 ± 2.45, P = 0.05). A larger sample size is mandatory to examine whether NLR and thus systemic inflammation are indeed augmented in preeclamptic pregnancies.

Despite these novel findings, our study has limitations. First, in the normal pregnancy group, there is a large gap between the vascular measurements (12 and 35 wk). In this manner, we do not know how vascular stiffness, FMD, and PAT fluctuate during the course of normal pregnancy. Second, we only studied vascular function in PE at the moment of diagnosis; we cannot compare these results with previous measurements in the same patient. Third, the PE patients were already on medication—started at the moment of their vascular assess-

ment and blood sample—which could have influenced our results. Last, our blood samples in the control group were taken during the first stage of labor, possibly influencing our markers of inflammation.

The main strength of our study is the longitudinal design in normal pregnancy. This way, we were able to investigate and understand the physiological changes in systemic inflammation and endothelial function related to pregnancy itself before comparing them with preeclamptic pregnancies. To our knowledge, serial changes in maternal endothelial function have not been evaluated previously in normal pregnancy using two different methods, i.e., FMD and RHI simultaneously, nor have FMD and RHI been investigated in preeclamptic pregnancies simultaneously. Furthermore, we have studied the association between endothelial function and arterial stiffness on the one hand and between endothelial function and maternal inflammatory response on the other hand in the same population.

**Perspectives and Significance**

The results of this study allow us to conclude that there is higher arterial stiffness and lower FMD in preeclamptic pregnancies compared with healthy, pregnant controls. Controversially, the RHI is higher in PE compared with normal pregnancy. Together, these findings support the assertion that measurements with FMD and PAT reflect different aspects of endothelial function and that PAT should not be used as a substitute for FMD as a measure of endothelial function in pregnancy. We hypothesize, however, that arterial vasculature and microcirculation undergo distinct changes during preeclamptic pregnancies. Future research is imperative to improve our understanding of the longitudinal evolution of vascular function during and after preeclamptic pregnancies to apprehend better the pathophysiology of this severe pregnancy complication.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

D.M., J.C., W.G., M.S., E.M.V.C., and Y.J. conceived and designed the research; D.M., E.F., I.G., and T.S. performed experiments; D.M. analyzed data; D.M. interpreted results of experiments; D.M. and E.M.V.C. prepared figures; D.M. drafted manuscript; E.F., J.C., W.G., E.M.V.C., and Y.J. edited and revised manuscript; M.S., E.M.V.C., and Y.J. approved final version of manuscript.

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