Chapter 3

Microparticle Orientated Risk Evaluation for Preeclampsia Prediction Among Risk graviDas -the MORE PrePARd Study

Justine S. Fitzgerald, Claudia Göhner, Heike Hoyer, Ulrike Schumacher, Ekkehard Schleussner
Abstract

The pregnancy-complication preeclampsia (PE) affects 2-8 % of pregnancies and causes approximately 16 % of maternal death worldwide. A reliable prediction of PE is limited by suitable markers and currently based on abnormal uterine artery Doppler ultrasound due to an impaired remodeling of maternal spiral arteries during placentation in PE compared to normal pregnancy. During pregnancy, the placenta secretes a plethora of factors into the maternal circulation. Especially the release of syncytiotrophoblast extracellular vesicles (STB EV) by the placenta has been associated to the pathophysiology of PE and it has been described that the concentration of STB EV in the maternal plasma is increased in late pregnancy in PE compared to normal pregnancy. In this study, we aimed to evaluate the usability of the peripheral plasma STB EV concentration as an accessory tool to uterine artery Doppler velocimetry for the prediction of PE. We identified women at high-risk for PE by abnormal uterine artery Doppler ultrasound and included 78 patients of which 16 developed PE or the associated hemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome. Women in the high-risk group who did not develop PE served as control group. We compared the STB EV concentration primarily at the 20th gestational week and additionally at the 28th and 36th gestational weeks. Secondary interest was on the association between STB EV and severity of the symptoms of PE and associated complications (HELLP, intrauterine growth retardation, intrauterine fetal demise, placental abruption, preterm delivery). We found a slight increase of the STB EV concentration over the time of pregnancy, but no significant difference between PE cases and control patients. Therefore, we have to conclude that the peripheral plasma STB EV concentration is not suitable as an accessory tool to uterine artery Doppler velocimetry for the prediction of PE.
Introduction

Preeclampsia (PE) is a placenta-related complication that occurs in 2–8% of all pregnancies, and is a potentially mortal syndrome accounting for approximately 16% of maternal deaths worldwide.[1,2] With termination of pregnancy the only known causal therapy, PE is also a leading motive for induced preterm birth, making PE a major contributor also to neonatal mortality and morbidity.[3,4]

Although the overall cause of PE remains elusive, for over a decade a predominantly accepted hypothesis describes that abnormal placentation secondary to inadequate trophoblast invasion leads to impaired remodeling of maternal deep spiral arteries of the uterus (reviewed in [2,5,6]). Deep trophoblast invasion into the myometrial arteries is believed to guarantee a decrease in uterine vascular resistance thus improving uteroplacental circulation.[7,8] Failure to accomplish this would lead to suboptimal perfusion of the placental bed, resulting in a cascade often described as follows: (a) oxidative stress to parts of the placenta [9] and thus (b) shedding of placental, syncytiotrophoblast extracellular vesicles (STB EV) into the maternal circulation [10], eventually (c) harming maternal endothelium through inflammatory processes that ultimately lead to endothelial dysfunction [8]. Endothelial dysfunction is thought to cause hypertension, but also either proteinuria, liver swelling, thrombocytopenia, and neurological symptoms or a combination of these problems depending upon which endothelial bed is the mother’s point of weakness (kidney, liver, vessels, brain).[5] These PE symptoms present during the 2nd half of pregnancy (mostly at or near term), during which the concentration of STB EV in the maternal circulation rise due to the size of the growing placenta.[8] This corresponds with the general definition of PE as the development of hypertension and proteinuria after the 20th gestational week in a previously normotensive woman.[11]

When all fails, the reduced distribution of nutrition and oxygen to the fetus can lead to, a much feared complication that often accompanies severe cases of PE.[5] Interestingly, intrauterine growth retardation is also an independent syndrome that can develop without the presence of PE.[12,13] The idea of monitoring the impedance of the uterine arteries with ultrasound, which would reflect the flow of blood through these vessels, originates from the acceptance of the above mentioned hypothesis.[14,15] Uterine artery Doppler velocimetry at midgestation is a readily available, non-invasive, broadly utilized and accepted technique that detects pregnancies at risk for developing PE and related adverse pregnancy outcomes.[14–17] Several reviews have reported somewhat conflicting evidence toward the accuracy of this method.[15,18–21] All in all, it is recognized that uterine artery Doppler velocimetry measurements, as well as most other diagnostic tests, reach rather high specificity, but low sensitivity.[22]

Considering this, a further and more sensitive test marker would be highly valuable to the clinician, since the patient who is likely to develop symptoms
could thus be distinguished early and opportune managed and monitored. Presently, the identification of such an accessory biomarker is the focus of highly volatile research. Some of the most promising candidates include angiogenic factors (e.g. soluble vascular endothelial growth factor receptor-1, placental growth factor as reviewed in [23,24]). Aside from the sometimes conflicting evidence, these markers also harbor disadvantages, since these are usually identifiable only weeks before symptoms occur, because these markers are produced only after or while damage transpires, plus these markers often overlook a large percentage of women who develop PE (as reviewed in [23–25]).

A newer hypothesis indicates that alterations of trophoblast differentiation, and not hypoxia, may lead to STB EV-shedding, while failure of trophoblast cells in transforming maternal spiral arteries may lead to intrauterine growth retardation.[25,26] And indeed, we showed that preeclamptic placentae show an altered expression pattern of stem cell and differentiation factors compared to normal or intrauterine growth retardation placentae.(Weber et al., accepted for publication in Cell Adhesion & Migration, see publication list of the PhD candidate) Accepting this hypothesis, evidence of excess STB EV-shedding would serve as a precursor to the PE syndrome. And indeed, STB EV are found in large amounts in women suffering from especially early-onset PE, but measured in concentrations comparable to healthy controls in pregnancies with pure intrauterine growth retardation.[27]

In light of this background, the aim of this project was to elucidate whether STB EV are a suitable candidate as an accessory marker to midgestational uterine artery Doppler velocimetry in predicting the development of PE, especially among a high risk population. Our secondary interest is centered on the association between STB EV and severity of the symptoms and complications associated with PE: syndrome of hemolysis, elevated liver enzymes and low platelet count (HELLP); intrauterine growth retardation; intrauterine fetal demise; placental abruption; and necessity of preterm delivery. Therefore, we measured the peripheral plasma STB EV concentration in high risk women for PE, who have been identified by uterine artery Doppler velocimetry, primarily at the 20th (+/- 1) gestational week and additionally at the 28th (+/-1) and 36th (+/-1) gestational week. Next, we compared the STB EV concentration of women within the risk group who did not develop PE with that of women who developed PE and further looked for associated complications in the PE group.
Methods

Objectives and Study Design

The trial is a multi-center, prospective, blinded, prognostic marker study (ISRCTN 92923842). The primary objective was to elucidate whether maternal plasma STB EV concentrations within a group of pregnant women at high risk of PE as identified via abnormal uterine perfusion measured at the 20th (+/-1) gestational week can discriminate between women who will develop PE (case group) and those who will not (control group).

The secondary objectives were (1) to compare intra-individual changes in plasma STB EV concentrations in controls and cases in which PE developed after 28 (+/-1) or 36 (+/-1) gestational weeks, respectively, (2) to determine a cut-off point and estimation of sensitivity and specificity for risk-assessment of PE, provided that STB EV concentrations differ significantly and (3) to examine maternal STB EV concentrations in further subgroups with PE-associated complications (HELLP syndrome, intrauterine growth retardation, intrauterine fetal demise, preterm birth). The study protocol was approved by the local ethics committees of the participating study sites.

Patient Recruitment

Pregnant females between the 19th and 21st gestational week were referred on a regular basis to all study centers for the purpose of prenatal ultrasound screening. All study centers are tertiary referral hospitals fulfilling Level III Perinatal Standards. The recruiting process was accomplished consecutively. Pregnant women fulfilling inclusion criteria were offered participation in this diagnostic study. After receiving patient information brochures and counselling by a physician, signed patient consent forms were collected when applicable.

The patient fulfilled the inclusion criteria when an abnormal uterine artery Doppler ultrasound was measured in a 19+0 to 21+0 gestational week singleton pregnancy with an appropriate-for-gestational-age fetus in an otherwise healthy and normotensive gravida between 18-40 years of age. Abnormal uterine artery Doppler ultrasound was defined as the presence of bilateral notching, mean RI > 0.7 (or > 95th percentile of reference group) or unilateral notch and average RI > 0.65 (or > 90th percentile of reference group). Appropriate-for-gestational age was defined as a fetus measured within the 10th and 90th percentile according to the standardized values of Hadlock.[28]

Experienced sonographers performed Doppler ultrasound of uterine arteries using real-time ultrasound equipment with a 3.5 MHz or a MHz curvilinear probe. The right and left uterine arteries are identified in an oblique plane of the pelvis at the crossover with the external iliac arteries, and the Doppler signals are
sampled. When three similar consecutive waveforms are obtained, the pulsatility and resistance indexes of the two vessels and their means are calculated. The presence of an early diastolic notch was determined according to the criteria proposed by Bower et al.[29,30]

Exclusion criteria were defined when the patient was participating in an interventional clinical study or when the pregnancy fulfilled any of the following criteria at inclusion: multiple pregnancy, premature rupture of membrane (PROM), suspected/diagnosed amnion infection syndrome (AIS), signs of pre-term labor/cervical incompetence, fetal genopathia, suspected major fetal defects, pre-existing maternal disease, e.g. diabetes mellitus, cardiovascular or renal disease and expected non-compliance (e.g. drug or substance abuse, residence of patient).

**Determination of outcomes**

Diagnosis of PE was based on the guidelines as recommended by the German Society of Obstetrics and Gynecology (DGGG): hypertension, as measured by systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg measured twice at an interval between 6 hours and 1 week, or singular measurement ≥ 150 / 100 mmHg; and proteinuria as measured by a urine dipstick test ≥ 1+ or ≥ 300 mg total protein in 24 hour urine collection.

Severe PE has occurred when any of the following parameters apply: systolic blood pressure > 160 mmHg or diastolic blood pressure > 110 mmHg on any two occasions measured > 6 hours apart, proteinuria as measured by a urine dipstick test of 3+ or ≥ 5 g total protein in 24 hour, oliguria < 500 cc / 24 hours, cerebral or visual symptoms, epigastric or right upper quadrant pain, pulmonary edema or cyanosis, occurrence of HELLP-syndrome, early onset PE < 32 gestational weeks.

Mild PE is diagnosed as any PE that is not considered severe (according to criteria listed above) or late onset PE > 34 gestational weeks.

Several laboratory parameters were monitored during the study visits to identify the HELLP syndrome, which was defined as the simultaneous occurrence of pathological values for haptoglobin (< 0.3 g/l); for the liver enzymes aspartat-aminotransferase (ASAT > 0.52 µmol/l*s), alanine aminotransferase (ALAT > 0.56 µmol/l*s) and lactate dehydrogenase (LDH > 4.12 µmol/l*s); and thrombocytopenia < 100 Gpt/l.

Intrauterine growth retardation was defined as a birthweight of below the 10th percentile for gestational age at birth according to the national birthweight distribution.

Intrauterine fetal demise was diagnosed when no fetal heart rate could be measured through conventional cardiotocography (CTG) or ultrasound.

Preterm birth was defined as birth before 36 + 0 gestational weeks.

All necessary criteria for the diagnosis of PE are visible either in the “Mutterpass”, which is a German “pregnancy passport” that is issued by the attending physician and which contains all results of pregnancy-relevant tests, or in hospital
documentation. Definitive diagnosis was made on the basis of the four following options: (1) a physician during a study visit; (2) a physician outside of the study center e.g. the patients’ regular gynecologist, who advised the patient into the study center hospital; (3) within a peripheral hospital setting if immediate medical attention was necessary; or (4) if the patient experienced a healthy pregnancy and birth took place at term (control group). In the last two settings, standardized outcome reports, which were miniaturized versions of the study center outcome reports and which were placed in the “Mutterpass” of all patients were filled out by the respective physician and delivered to study centers.

All diagnosis have been reviewed by one of the authors, JSF, to guarantee compliance with the before mentioned diagnosis criteria. In one instance, diagnosis was divergent which resulted in an equivocal case-control status and led to exclusion of that specimen.

**Work schedule**

Patients were recruited as described above upon which the baseline exam was taken, in which a basic physical examination, as well family, patient and case history was recorded (baseline visit). The patient was followed at two visits taking place at (1) 26 (+/-1) gestational weeks and (2) 36 (+/-1) gestational weeks. During all visits, uterine artery Doppler velocimetry, fetal growth scan, blood samples for STB EV quantification and routine measurements (e.g. measurement of HELLP syndrome parameters), blood pressure and urine dipstick test measurements were registered.

All physicians attending the patient and the patient herself were instructed which line of action should be taken when PE-relevant symptoms occur (please refer to Figure 1), namely that the patient should preferentially be admitted to a study center hospital or, if physically not possible, to any hospital after informing the study center. This might take place either before (via regular pregnancy exams accomplished by the patients’ regular gynecologist) or during study visits (by a study center physician). Definitive diagnosis of PE was made again at the hospital. If the patient was within a study center hospital, then a further blood sample for STB EV measurement was collected either before or at the latest 24 hours after delivery. When no symptoms occurred, the patient proceeded to accept regular pregnancy exams through her usual health care provider until the next visit, at which all study measurements were made at the study center.

A standardized outcome report was filled out by the delivery room doctor (often a study center physician) and submitted to the study center. The study participation for patients ended upon report of delivery of infant. All necessary subsequent treatment(s) remained in the hands of the responsible physician.
To obtain plasma samples for STB EV measurement, venous blood was withdrawn from a cubital vein using a 20 G cannula and minimal tourniquet. The first 3 ml were discarded and the next 9 ml were collected in a monovette (Sarstedt AG & Co., Nümbrecht, Germany) containing 1 ml of 0.106 mol/l sodium citrate solution. The blood sample was centrifuged within 30 min for 5 min at 5,000 x g. The plasma supernatant was carefully removed by a pipette and stored at -80 °C until further analysis. Specimens were collected at each study center and labeled according to registration and center ID number. Specimens were transported to the reference laboratory immersed in dry ice and re-stored at -80 °C immediately.

Specimens were unfrozen carefully over night at 4 °C, pre-diluted in PBS (Dulbecco’s phosphate buffered saline; Sigma-Aldrich Chemie GmbH, Hamburg, Germany), STB EV were pelleted by ultra-centrifugation at 100,000 x g for 45 min

Figure 1: Flow chart of patient characteristics
GW – gestational week

**STB EV measurement**
at 4 °C and STB EV were re-suspended in 1 % BSA in 0,05 % Tween20/PBS. STB EV were quantified using the enzyme-linked sorbent assay described by Göhner et al.[31]. Briefly, 200 µL/well 3 µg/mL Annexin V in sodium carbonate coating buffer (15 mM Na2CO3, 28.5 mM NaHCO3, both Merck KGaA, Darmstadt, Germany; pH = 9.6) were immobilized in microtiter plates (Immuno 96 MicroWell™ Solid Plates, MaxiSorp, flat bottom, Nunc, Thermo Fisher Scientific Inc., Waltham, MA, USA) for 20 h at 4 °C. Wells were washed once with 380 µL Tris-buffered saline (TBS, 50 mM Tris-HCl, Sigma-Aldrich Chemie GmbH; 150 mM NaCl, Carl Roth GmbH + Co.KG, Karlsruhe, Germany; pH = 7.5) at RT. Free binding positions were blocked by incubation of 380 µL/well 4 % milk (powdered milk, blotting grade, low fat, Carl Roth GmbH + Co.KG) in TBS for 2 h at RT. Wells were washed three times at RT with 380 µL/well 0.05 % Tween®20 in TBS. 100 µL sample/standard was incubated for 1 h at RT. Standards represented concentrations from 6000 STB EV/mL to 93.75 STB EV/mL and 0 STB EV/mL as blank. Wells were washed at RT two times with 400 µL/well 0.05 % Tween®20 in TBS and afterwards two times with 400 µL/well TBS. STB EV were detected due to their membranous human placental alkaline phosphatase using the kit ELISA amplification system (Invitrogen Corporation, Carlsbad, CA, USA) with incubation of substrate for 1 h at 25 °C and amplifier for 15 min at 25 °C. Detection was performed at 495 nm using a SPECTROstar Omega UV/Vis absorption spectrometer (BMG LABTECH GmbH, Ortenberg, Germany). All measurements were accomplished in duplicates and averaged for the analysis.

**Statistical analysis**

Considering the pilot nature of the study a sample size of 80 to 100 was planned. Assuming an incidence of PE of 20% to 25%, about 16 to 25 cases were expected with this sample size. In a balanced design, a sample size of 20 cases and 20 controls would have 80% power to detect a probability of 0.76 that STB EV concentration in controls is less than in cases using a Wilcoxon-Mann-Whitney test with a two-sided significance level of 5 %. The final analysis set consisting of 16 cases and 56 controls had 91 % power to detect the defined effect in an unbalanced design (SAS Macro ROCPOWER Single Reader, www.bio.ri.ccf.org/doc/rocpower.sas; download 09.02.2010).

Characteristics of patients were described by adequate statistical measures. STB EV measures were presented with descriptive statistics by visit. The Receiver Operating Characteristics (ROC) for baseline STB EV was displayed and Area Under Curve (AUC) was estimated including 95 % confidence levels. Group difference was analyzed by means of two sided Wilcoxon-Mann-Whitney test on significance level of 5 %.
Results

78 patients were recruited in three centers between June 2009 and May 2011. The analysis population consisted of 73 patients with valid inclusion- and exclusion criteria and appropriate outcome data (please refer to Figure 1). 16 patients with PE or HELLP were identified as cases, of which 9 had PE, 2 had HELLP, and 5 had PE and HELLP. One patient with inconsistent PE evaluation was assigned neither to controls nor to cases.

The demographic characteristics of the control versus the case group can be seen in Table I. Compared to controls, women of the case group were older (mean 30 vs. 26.8 years) and more obese (mean 75.1 kg vs. 67.0 kg). They had higher systolic (mean 123.3 vs. 108.2 mmHg) and diastolic (80 vs. 63.3 mmHg) blood pressure. The relative frequency of PE, intrauterine growth retardation and intrauterine fetal demise during an earlier pregnancy was higher in cases than in controls. Furthermore, the pregnancy length was shorter, the birth weight was lower and the percentage of intrauterine growth retardation and premature placental abruption were higher in the cases compared to controls.

In Figure 2, the distribution of maternal STB EV concentration was displayed by case-control status and gestational week. Quartiles of STB EV which define the boxes in Figure 2 are presented in Table II. Except for cases at gestational week 36 (+/-1) (n=7) there was low variation in medians and interquartile ranges between cases and controls. However, the spread of data above the third quartile deserves further exploration. Due to technical problems during the measurement, several samples had to be excluded from the analysis. This effects controls and cases as well as all three times of sample collection (gestational week 20 (+/-1), 28 (+/-1) and 36 (+/-1)) to the same extend

**Maternal STB EV concentration at the 20th gestational week does not predict PE or is associated with complications**

Concerning the primary endpoint of this study, the STB EV concentration at the 20th (+/-1) gestational week, displayed by median (1st - 3rd quartile), was not statistically different (p=0.588) between cases (2.50 (2.17–2.81) STB EV/ml) and controls (2.44 (2.12–2.76) STB EV/ml). An AUC of 0.54 with 95% confidence interval of (0.35, 0.73) was estimated based on a comparison of 12 cases and 47 controls after exclusion of the mentioned cases.

Similar findings were seen in the secondary endpoints: HELLP (7 patients; 2.26 versus 2.52 STB EV/ml), intrauterine growth retardation (16 patients, 2.67 versus 2.39 STB EV/ml), placental abruption (2 patients; 2.34 versus 2.52 STB EV/ml) and preterm delivery (20 patients; 2.39 versus 2.52 STB EV/ml). No cases of intrauterine fetal demise were reported. Also, there was no significant difference of the median (1st - 3rd quartile) plasma STB EV concentration between cases and controls.
at the 28th (+/-1) gestational week (2.83 (2.33–2.94) STB E/ml in cases versus 2.51 (2.18–2.70) STB EV/ml in controls) and at the 36th (+/-1) gestational week (2.63 (2.61–3.2) STB E/ml in cases versus 2.52 (2.15–3.00) STB EV/ml in controls).

Figure 2: Boxplot of the maternal plasma syncytiotrophoblast extracellular vesicle concentration (STB EV/ml) by case-control status and gestational age (for cases only measurements before preeclampsia/HELLP were included)

GW - gestational week

Figure 3: Receiver operating characteristics (ROC) of the syncytiotrophoblast extracellular vesicle concentration
### Table I: Characteristics of study population at baseline (gestational week 19-21)

<table>
<thead>
<tr>
<th></th>
<th>Cases (n=16)</th>
<th>Controls (n=56)</th>
<th>Total (n=73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age [years], mean (SD)</td>
<td>30.0 (3.8)</td>
<td>26.8 (6.4)</td>
<td>27.5 (6.0)</td>
</tr>
<tr>
<td>Maternal weight [kg], mean (SD)</td>
<td>75.1 (11.6)</td>
<td>67.0 (13.2)</td>
<td>69.5 (14.6)</td>
</tr>
<tr>
<td>Systolic blood pressure [mmHg], mean (SD)</td>
<td>123.3 (10.2)</td>
<td>108.2 (11.6)</td>
<td>112.0 (13.2)</td>
</tr>
<tr>
<td>Diastolic blood pressure [mmHg], mean (SD)</td>
<td>80.0 (11.4)</td>
<td>63.3 (10.7)</td>
<td>67.2 (12.9)</td>
</tr>
<tr>
<td>Number of earlier pregnancies, mode (min-max)</td>
<td>1 (0 - 6)</td>
<td>1 (0 - 5)</td>
<td>1 (0 - 6)</td>
</tr>
<tr>
<td>Number of children, mode (min-max)</td>
<td>0 (0 - 2)</td>
<td>0 (0 - 4)</td>
<td>0 (0 - 4)</td>
</tr>
<tr>
<td>Having at least one earlier pregnancy, n (%)</td>
<td>15 (94)</td>
<td>51 (91)</td>
<td>67 (92)</td>
</tr>
<tr>
<td>History of preeclampsia, n (% of ever pregnant)</td>
<td>1 (7)</td>
<td>2 (4)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>History of IUGR, n (% of ever pregnant)</td>
<td>3 (20)</td>
<td>3 (6)</td>
<td>6 (8)</td>
</tr>
<tr>
<td>History of IUFD, n (% of ever pregnant)</td>
<td>5 (33)</td>
<td>3 (6)</td>
<td>8 (11)</td>
</tr>
<tr>
<td>Alcohol consumption during pregnancy, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>16 (100)</td>
<td>55 (98)</td>
<td>72 (99)</td>
</tr>
<tr>
<td>Occasional</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Smoking during pregnancy, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>16 (100)</td>
<td>46 (82)</td>
<td>62 (85)</td>
</tr>
<tr>
<td>≤15 cigarettes / day</td>
<td>0 (0)</td>
<td>10 (18)</td>
<td>10 (14)</td>
</tr>
<tr>
<td>&gt;15 cigarettes / day</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

* Including one patient with equivocal case-control-status

IUGR – intrauterine growth retardation, IUFD – intrauterine fetal demise

### Table II: Summary statistics of maternal STB EV concentration (STB EV/ml) by case-control status and gestational age

<table>
<thead>
<tr>
<th>Gestational Week</th>
<th>Cases* (n)</th>
<th>STB EV/ml Median (IQR)</th>
<th>Controls (n)</th>
<th>STB EV/ml Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19-21</td>
<td>15</td>
<td>2.50 (2.17–2.81)</td>
<td>56</td>
<td>2.44 (2.12–2.76)</td>
</tr>
<tr>
<td>27-29</td>
<td>14</td>
<td>2.83 (2.33–2.94)</td>
<td>49</td>
<td>2.51 (2.18–2.70)</td>
</tr>
<tr>
<td>35-37</td>
<td>7</td>
<td>2.63 (2.61–3.2)</td>
<td>39</td>
<td>2.52 (2.15–3.00)</td>
</tr>
</tbody>
</table>

* Considering only STB EV measurements before preeclampsia/HELLP

IQR – Interquartile range (1st quartile – 3rd Quartile)
Discussion

In our study, we show a slight, but not significant, slope elevation in maternal plasma STB EV-concentrations among high-risk pregnancies, regardless of whether they developed PE or associated complications. In contrast, it has been demonstrated in the literature that maternal STB EV-concentrations rise during normal, healthy, low-risk pregnancies.[27,32] Additionally, we did not find a significant difference of the plasma STB EV concentration between women who developed PE or associated complications and women who did not – neither at the primary measuring point in the 20th gestational week nor later in the 28th or 36th gestational week – although other studies have shown a significant increase of the plasma STB EV concentration in cases of early onset PE.[10,27] This difference may result from the differences in study design between our study and the other studies. We aimed to clarify whether the plasma STB EV concentration may assist PE prediction before development of the actual symptoms. Therefore, we included measurements only when specimens were collected before diagnosis of PE or associated complications. In the other studies, blood sampling was performed after diagnosis of the complication and not earlier than the 24th gestational week. [10,27] Since we analyzed specimens only before PE symptoms or associated symptoms appeared, it may be possible that the plasma STB EV concentration was not elevated yet in the PE cases. That suggests that an increase of the plasma STB EV concentration in PE only accompanies the appearance of PE symptoms and not precedes them. As discussed in the following chapters of this thesis, it might not be an elevated plasma STB EV concentration which causes PE. Rather it might be an altered functionality of STB EV from preeclamptic compared to STB EV from normal placentae which is involved in the development of preeclamptic symptoms.

Our findings are in line with the findings of a similar study by Salomon et al. [33], in which a secondary analysis of prospectively collected sera from pregnant women in the 24th gestational week was conducted. Here, the concentration of all general microparticles, regardless of origin (e.g. platelets, endothelium, lymphocytes, etc.), was measured by flow cytometry. Trophoblastic microparticles were not specifically measured; instead the non-platelet and non-endothelial microparticles were loosely hypothesized to stem from the trophoblast. None of the microparticles measured in that study could predict the development of pregnancy complications, including PE and its associated complications. Although many components of Salomon’s and our study are differing, such as gestational week, main outcome measurement and measurement method, the main conclusions are similar. For this reason, the results of both studies reciprocally reinforce the conclusion that maternal plasma STB EV concentrations may not predict PE at midgestation or later.

The possibility that our enzyme-linked sorbent assay method is not yet sensitive enough to pick up the very low concentration of STB EV during mid-gestation should be considered. Although we regard our method to be more exact and more
specific than all methods introduced in the past, there is as yet no gold standard available to validate our measurements. Especially the group around Sargent and Redman at the University of Oxford, but also others, are involved in the promotion of a nanoparticle tracking analysis method (NanoSight) capable of measuring small particles in the nano area in fluids.[34,35] This method tracks light which is scattered from particles crossing a laser beam and follows the Brownian motion of the particles, which allows calculation of size and concentration of these particles.[36] Progress in immune-labeling of particles based on e.g. origin-specific markers even hold out the prospect of a reliable specific detection of particles from certain origin, such as the placenta, in the near future.[34]

In conclusion, we showed that the peripheral plasma concentration of STB EV slightly increases over the time of pregnancy, but found no significant difference between PE cases and controls. Thus, the peripheral plasma concentration of STB EV does not seem to be a suitable accessory tool for early PE prediction. Furthermore, we hypothesize that an increase in STB EV plasma concentration potentially does not precede PE symptoms but accompanies them.
Acknowledgement

This study was funded by the German Research Foundation (Deutsche Forschungsgemeinschaft). C. Göhner was partly financed by the German Research Foundation and the Thuringian Ministry for Education, Science and Arts. C. Göhner was further financed by a fellowship of the Abel Tasman Talent Program of the University of Groningen.

References

Microparticle Orientated Risk Evaluation for Preeclampsia Prediction Among Risk graviDas - the MORE PrePARd Study


