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The course of angiogenic factors in early- vs. late-onset preeclampsia and HELLP syndrome

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Abstract

Aims—Preeclampsia (PE) is considered a uniformly progressive disease, however, it shows a different pattern of clinical progression in patients with early (<34 weeks) or late (≥34 weeks) onset of the disease. Angiogenic factors such as soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) are closely related to the clinical course of PE. We evaluated sFlt-1 and PlGF levels in the clinical course of PE in women admitted with a diagnosis of PE at different gestational ages.

Methods—This retrospective study included 34 patients with PE, of which 11 patients had HELLP syndrome (over a period of 3 years). Serial measurements of sFlt-1 and PlGF were completed from admission until delivery. Values are presented as mean±standard deviation.

Results—Mean gestational age of admission among women with early onset PE was significantly lower, at 29±3 weeks compared to 37±1 weeks among patients with late onset disease. Mean prolongation of pregnancy was 6 days, which was similar within the two groups. Compared to women with late onset PE, women with early-onset PE had a greater increase in sFlt-1 (11% vs. 3% per day, $P<0.05$), greater decrease in PlGF levels (21% vs. 10% per day, $P=0.30$), resulting in a much higher increase in sFlt-1/PlGF ratio (23% vs. 8% per day, $P<0.05$). Patients with HELLP syndrome showed comparable progression patterns.

Conclusion—In a similar way to the progressively worsening clinical course observed in women with early onset PE, there were changes in the angiogenic profile that leads to a more anti-angiogenic state in these women with each passing day. These findings may have implications in identification of the women for appropriate patient management and possible future therapies based on the reduction of sFlt-1 levels.

Keywords

Angiogenic factors; biomarker; HELLP syndrome; preeclampsia; sFlt-1/PlGF ratio

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Introduction

Preeclampsia (PE) is a serious multisystem disorder in pregnancy and is a leading cause of maternal and fetal morbidity and mortality worldwide [7]. Occurring with an incidence of 2–5%, PE represents one of the major contributors of preterm birth, accounting for 15% of all preterm deliveries with subsequent neonatal morbidity [2, 3]. PE is defined as new onset of hypertension ($\geq 140/90$ mm Hg) and proteinuria (≥ 300 mg/d) in the second half of gestation [1].

Over the past decade there has been mounting evidence that an imbalance between angiogenic factors exists in women with PE, which plays a crucial role in the pathogenesis of PE. The leading hypothesis suggests that PE involves excess anti-angiogenic factors produced by the placenta which then circulate to distal organs and cause damage to the vasculature and peripheral organs [9–11]. Karumanchi et al. showed that excess placental derived soluble fms-like tyrosine kinase 1 (sFlt-1), an alternatively spliced variant of VEGF receptor 1, mediates the multiple symptoms of PE. Furthermore, elevated circulating levels are associated with clinical PE [11]. In women with very early preterm preeclampsia, circulating sFlt-1 levels are among the highest [19]. Circulating sFlt-1 inhibits local vascular endothelial growth factor (VEGF) signaling in target organs and placental growth factor (PlGF). Thus, circulating PlGF levels are decreased in preeclamptic women [10]. Similar to PE, hemolysis elevated liver enzymes and low platelets (HELLP syndrome), a form of severe PE, is also characterized by an “anti-angiogenic” state with an increased sFlt-1/PlGF ratio [21].

Numerous case-control [12, 14, 16, 17] as well as prospective studies [4, 8, 15] have highlighted the role of the measurement of plasma sFlt-1 and PlGF in pregnant women as a diagnostic and predictive test for PE. Our group recently showed that an elevated sFlt-1/PlGF ratio as assessed by automated Elecsys® assay allows the determination of PE in the clinical routine context [19]. The automated measurement of the sFlt-1/PlGF ratio is now widely accessible in Europe. In a recent study it was demonstrated that the sFlt-1/PlGF ratio is a rapid test for prediction of PE and is related to the clinical outcomes in women with an established diagnosis of PE [20].

Although PE is regarded as a progressive disease, the literature is sparse with respect to the course of angiogenic markers in women with a diagnosis of PE and HELLP syndrome. The aim of the work presented here is to characterize the behavior of the sFlt-1/PlGF ratio in women with PE and HELLP syndrome. We aimed to answer the question of whether the course of the sFlt-1/PlGF ratio differs between PE and HELLP syndrome and whether the dynamic of these factors depends on gestational age at clinical presentation and the clinical severity of the disease.

Materials and methods

Study population

In a retrospective, single center cohort study, we investigated 34 cases with PE, including 11 patients with HELLP syndrome.

PE was defined according to the National High Blood Pressure Education Program Working Group on high blood pressure in pregnancy [13]. Under this classification, PE is defined as blood pressure ≥ 140 mm Hg systolic or ≥ 90 mm Hg diastolic in women who were normotensive before 20 weeks of gestation on at least two measurements within a 3 day period. Significant proteinuria is defined as ≥ 0.3 g protein/24 h (or in emergency cases only,

a dipstick 1+ on more than one occasion or 30 mg/dl protein in a spot urine if a 24 h urine protein collection could not be obtained).

PE cases were sub-classified as early-onset (<34 weeks of gestation or late onset (≥34 weeks). HELLP syndrome was diagnosed if increased liver enzyme (AST, ALT) concentrations (more than two times the upper reference interval), reduced platelet counts (<100,000/μL) plus at least one of the following hemolysis criteria: increased LDH concentrations (more than two times the upper reference interval), increased indirect bilirubin serum concentrations (more than 1.2 mg/dL), or reduced haptoglobin serum concentrations (<0.3 g/L).

Within a period of 3 years (June 2009–May 2012) all patients with confirmed PE and HELLP syndrome who have been admitted to hospital were included in this study. We excluded patients with eclampsia and expected delivery within 24 h of admission. Samples were collected depending on the severity of the disease but they were collected at least once every 24 h. Each patient contributed at least two samples. All patients provided written informed consent.

Samples and immunoassays

Maternal blood was collected by venipuncture in tubes without anticoagulant. Serum was allowed to form and then the samples were centrifuged at 2000 g, pipetted, and stored at –80°C until testing. Single measurements were performed for sFlt-1 and PlGF on the fully automated Roche Elecsys® system (Elecsys® PlGF, human placental growth factor and Elecsys® sFlt-1, soluble fms-like tyrosine kinase-1; Roche Diagnostics). Both assays are sandwich immunoassays based on the electro-chemiluminescence technology. The total duration of the assays was 18 min and the sample volume was 20 μL for sFlt-1 and 50 μL for PlGF. The assays were calibrated with recombinant human sFlt-1 and PlGF and standardized against the R&D Systems (Roche Diagnostics, Mannheim, Germany) Quantikine VEGF-R1 and PlGF ELISAs. The technicians who performed the assays were blinded to any patient characteristic.

The sFlt-1/PlGF ratio was calculated for each sample. Basic statistics [mean, median, standard deviation (SD), quartiles, and range] was calculated for each of the markers (sFlt-1, PlGF, and sFlt-1/PlGF ratio). Levels were compared to previously published normative data [19].

Statistics

Data are expressed as mean±SD. Non-parametric tests (*U*-test) were applied. All *P*-values are two-tailed. *P*<0.05 was considered significant.

Results

A total of 34 individuals were included in the study, of which 11 patients had HELLP syndrome. For clinical parameters and demographic details see Table 1. From admission to delivery, an increase in sFlt-1 concentration with decrease in PlGF concentration was seen in all cases (Figures 1 and 2). Consequently, the sFlt-1/PlGF ratio showed an increase until delivery (Figure 3). The angiogenic factors showed a continuous shift of the angiogenic-anti-angiogenic balance towards an anti-angiogenic state during the clinical course of the disease.

The mean sFlt-1 plasma concentration at admission was 12095±4799 pg/mL in the early-onset PE group. The mean time to delivery was 6 days with an increase of the sFlt-1 to

18300±4577 pg/mL (+66%, $P<0.05$; Table 2). This refers to a mean sFlt-1 increase of 11% per each day (Table 2). This was paralleled by an increase of the sFlt-1/PlGF ratio from 446±240 to 981±576 (+137%, 23% per day, $P<0.05$; Table 2).

Likewise, in the early HELLP group, the sFlt-1 rose from 11464±3388 pg/mL to 17347±6053 pg/mL, equalizing an increase of 51% (13% increase per day, $P<0.05$; Table 2).

The late-onset PE group showed weaker sFlt-1 increase of 17% (3% per day, $P<0.05$) from 10340±3884 pg/mL to 11900±3910 pg/mL. This was paralleled by an increase of the sFlt-1/PlGF ratio of 39% (120±44 to 161±69), 8% per day (Table 2).

The late-onset HELLP subgroup showed an increase of only 1% sFlt-1 within the short time to delivery of 2 days, while the sFlt-1/PlGF ratio increased from 233±125 to 480±242 (+144%; Table 2).

The PlGF dropped in the early-onset PE group from 31.9±15.7 pg/mL to 26.1±13.8 pg/mL (21% per day, $P<0.05$), whereas the PlGF reduction was less pronounced in the late-onset PE group (from 92.5±33.6 pg/mL to 84±43 pg/mL, 10% per day; Table 2). The strongest decrease of PlGF was observed in the late-onset HELLP group; PlGF decreased from 76±49.8 pg/mL to 35.1±19.4 pg/mL (−37%; Table 2).

Discussion

In this small retrospective study we found that angiogenic factors correlate with the clinical course of disease progression in women admitted with PE. We found that the sFlt-1 concentration and consequently the sFlt-1/PlGF ratio increased from admission to delivery in all cases of PE and HELLP syndrome. We also observed that the slope of the increase was much greater in the early-onset cases. The maternal sFlt-1 increased by 3.4% per day in the late-onset cases, the daily increase was much higher in the early-onset cases (11%). This observation indicates that PE presenting before 34 weeks has a more aggressive course. Notably, the speed of the sFlt-1 increase is greater. Furthermore, the absolute levels exceed those of the late-onset cases. As it has been shown that there is an increase in sFlt-1 in both normal and preeclamptic patients with advancing gestational age [11], the exceeding sFlt-1 levels in the early-onset group demonstrate that these patients present the most rapid and strongest acceleration of the anti-angiogenic state.

The mean time to delivery in the early-onset group is 6 days. This is in agreement with Haddad et al., who reported a time to delivery of 4 to 6 days in their cohort [5]. The relation between a high sFlt-1/PlGF ratio in the very early preterm subgroup and a short remaining interval until delivery is also in line with the report of Verlohren et al., who showed an inverse correlation between sFlt-1/PlGF ratio and time to delivery [20]. The time to delivery of 5 days in the late-onset group results from a different clinical management. In these patients, iatrogenic delivery rather than expectant management lead to a comparable time to delivery. It could be argued that an observation of a further increase of sFlt-1 and the sFlt-1/PlGF ratio is because of this limitation respecting time. Yet we are able to show that the absolute values as well as the average daily changes are lower in that group.

Early- as well as late-onset HELLP patients showed an elevated sFlt-1/PlGF ratio which increased until delivery (+55% vs. +144%). While the early-onset group obtains this status through increasing sFlt-1 levels (+51%), the elevated ratio results from decreasing PlGF (−37%) within the late-onset group. Thus, the mechanism of HELLP syndrome seems to be different depending on the point of manifestation.

Notably, the pattern between PE and HELLP does not differ. Therefore, our results confirm that patients with HELLP syndrome have a continuous rise of the maternal sFlt-1 concentrations despite the undulated clinical presentation [6]. We are, however, unable to clarify whether HELLP syndrome is a particular clinical presentation of PE or an independent entity as both forms show the same anti-angiogenic dynamics.

Our study has certain limitations. The sample size was limited especially when subdividing into categories. We also did not study women with other complications of severe preeclampsia such as eclampsia, pulmonary edema, small for gestational age or neonatal death. Nonetheless, the trends we saw were convincing. Our analysis included complete datasets of well characterized pregnancies with clear-cut clinical presentation. Moreover, all samples were collected and processed within a short period of time in a blinded fashion on an automated platform. This provides quick accurate assessment of angiogenic factors. To our knowledge, this is the first study that uses serial samples to document a worsening angiogenic state in women with diagnosed PE. It also provides a biochemical basis of the observation that early onset PE has a more aggressive phenotype.

Even though patients after 34 weeks did not show rapid progression of an anti-angiogenic state, they had a similar duration of pregnancy. This results from different clinical management; the indication for delivery is easier beyond 34 weeks of gestation as the risk of prematurity is lower. Nevertheless, measuring these levels in the range of 34+0 weeks to 37+0 weeks will still have clinical benefit with the potential of reducing iatrogenic preterm delivery. This could improve both neonatal and maternal outcome.

Currently there is no treatment of PE. In the absence of therapy, all patients showed a similar trend with rising sFlt-1 levels. However, in experimental sFlt-1-based treatment studies for PE [18], sFlt-1/PlGF ratio was used as a surrogate for disease progression and successful intervention. In future, therapy might be directed to the group of patients with a worsening angiogenic profile to obtain maximum benefit.

In conclusion, the angiogenic factors, particularly the sFlt-1/PlGF ratio, characterize PE and HELLP syndrome as continuously progressive diseases with a uniform development towards an anti-angiogenic state as pregnancy continues until intervened by delivery. Early-onset subtypes show more aggressive progression with faster sFlt-1 increase. Regarding this change of the angiogenic balance, PE and HELLP show comparable progression patterns. Further studies are needed to validate these findings in large prospective studies including more patients and different PE phenotypes.

Acknowledgments

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Figure 1.

Course of the maternal soluble fms-like tyrosine kinase 1 (sFlt-1) plasma concentration in pg/mL from admission until delivery. Each line represents one patient. The dark gray line represents the 95th percentile of sFlt-1 at 24 – 28 weeks for < 34 weeks and 34 – 36 weeks for ≥ 34 weeks. The light gray line represents the 95th percentile of sFlt-1 at 29 – 33 weeks for < 34 weeks and 37 weeks delivery for ≥ 34 weeks.

Figure 2.

Course of the maternal placental growth factor (PIGF) plasma concentration in pg/mL from admission until delivery. Each line represents one patient. The dark gray line represents the 5th percentile of PIGF at 24 – 28 weeks for < 34 weeks and 34 – 36 weeks for ≥ 34 weeks. The light gray line represents the 5th percentile of PIGF at 29 – 33 weeks for < 34 weeks and 37 weeks delivery for ≥ 34 weeks.

Figure 3.

Course of the maternal soluble fms-like tyrosine kinase 1 (sFlt-1)/placental growth factor (PlGF) ratio from admission until delivery. Each line represents one patient. The dark gray line represents the 95th percentile of sFlt-1/PlGF ratio at 24 – 28 weeks for < 34 weeks and 34 – 36 weeks for ≥ 34 weeks. The light gray line represents the 95th percentile of sFlt-1/PlGF ratio at 29 – 33 weeks for < 34 weeks and 37 weeks delivery for ≥ 34 weeks.

Table 1

Patient characteristics.

Patient characteristics	PE			HELLP syndrome		
	< 34 weeks (n = 13)	34 weeks (n = 10)	P-value ^a	< 34 weeks (n = 7)	34 weeks (n = 4)	P-value ^b
Maternal age (years)	28 ± 6	30 ± 4	0.29	27 ± 3	32 ± 4	< 0.05
BMI (kg/m ²)	30 ± 6	34 ± 7	0.13	29 ± 10	31 ± 5	0.74
GA on admission (weeks)	29 ± 3	37 ± 1	< 0.05	28 ± 3	36 ± 1	< 0.05
GA at delivery (weeks)	30 ± 3	37 ± 2	< 0.05	29 ± 3	37 ± 2	< 0.05
Highest systolic BP (mm Hg)	160 ± 24	163 ± 27	0.78	156 ± 16	143 ± 5	0.15
Highest diastolic BP (mm Hg)	99 ± 14	105 ± 15	0.40	99 ± 13	98 ± 10	0.81
Birth weight (g)	1027 ± 441	2612 ± 433	< 0.05	934 ± 399	2537 ± 846	< 0.05
Cesarean section (%)	100	50	< 0.05	100	25	< 0.05
Duration of pregnancy (days)	6 ± 5	5 ± 4	0.46	4 ± 2	2 ± 1	0.09

PE = preeclampsia, BMI = body mass index, GA = gestational age, BP = blood pressure, C-section = cesarean section.

^aP comparing PE < 34 weeks vs. PE 34 weeks.^bP comparing HELLP < 34 weeks vs. HELLP 34 weeks; significant at P < 0.05.

Table 2

Course of angiogenic factors in PE and HELLP patients.

Angiogenic factors	PE			HELLP syndrome		
	< 34 weeks (n = 13)	34 weeks (n = 10)	P-value ^a	< 34 weeks (n = 7)	34 weeks (n = 4)	P-value ^b
sFlt-1 increase (%) (% per day)	66 ^a (11.0)	17 ^a (3.0)	< 0.05	51 ^a (13.0)	1 (0.5)	< 0.05
PIGF decrease (%) (% per day)	21 ^a (4.0)	10 (2.0)	0.30	1.3 (0.3)	37 (18.5)	0.13
sFlt-1/PIGF ratio (%) (% per day)	137 ^a (23.0)	39 (8.0)	< 0.05	55 ^a (14.0)	144 (72.0)	0.14

PE = preeclampsia, sFlt-1 = soluble fms-like tyrosine kinase 1, PIGF = placental growth factor.

^aP comparing PE < 34 weeks vs. PE 34 weeks.^bP comparing HELLP < 34 weeks vs. HELLP 34 weeks; significant at P < 0.05.