

## OBSTETRICS

# The sFlt-1/PIGF ratio in different types of hypertensive pregnancy disorders and its prognostic potential in preeclamptic patients

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**OBJECTIVE:** The soluble fms-like tyrosine kinase (sFlt-1)/placental growth factor (PIGF) ratio is a reliable tool in the assessment of preeclampsia. We tested the hypothesis that the sFlt-1/PIGF ratio is able to identify women at risk for imminent delivery. We characterized the sFlt-1/PIGF ratio in different types of hypertensive pregnancy disorders.

**STUDY DESIGN:** We investigated 388 singleton pregnancies with normal pregnancy outcome, 164 with PE, 36 with gestational hypertension, and 42 with chronic hypertension. sFlt-1 and PIGF were measured in serum samples.

**RESULTS:** Patients with preeclampsia had a significantly increased sFlt-1/PIGF ratio as compared with controls and with patients with

chronic and gestational hypertension in <34 weeks and  $\geq 34$  weeks ( $P < .001$ ). Time to delivery was significantly reduced in women with preeclampsia in the highest quartile of the sFlt-1/PIGF ratio ( $P < .001$ ).

**CONCLUSION:** The sFlt-1/PIGF ratio allows the identification of women at risk for imminent delivery and is a reliable tool to discriminate between different types of pregnancy-related hypertensive disorders.

**Key words:** gestational hypertension, placental growth factor, PIGF, preeclampsia, singleton pregnancy, soluble fms-like tyrosine kinase, sFlt-1

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Preeclampsia (PE), a multisystem disorder in pregnancy, is still a leading cause of maternal and fetal morbidity and mortality.<sup>1</sup> Occurring with an incidence of 2-8% worldwide, PE represents one of the major contributors of preterm birth, accounting for 15% of all preterm deliveries.<sup>2,3</sup> PE is defined as the new onset of

hypertension ( $\geq 140/90$  mm Hg) and proteinuria ( $\geq 300$  mg per 24 hours) after 20 weeks of gestation.<sup>4</sup>

Other hypertensive disorders in pregnancy comprise gestational hypertension (GH) and chronic hypertension (chrHTN).<sup>4</sup> However, these definitions do not take into account the clinical variety of the

disease as well as its impact on mother and child. Moreover, the current classification does not consider the fact that the speed of the clinical disease progression can vary dramatically between patients with different subtypes of PE.<sup>5</sup>

Recently several modifications of this simple definition of a complex disease have tried to cope with the clinical diversity of the maternal syndrome.<sup>6-9</sup> Actual definition proposals try to implement early-onset PE as a severity criterion as well as include a definition of severe hypertension apart from mild hypertension. It is known that the perinatal risk and the maternal complications are the greater, the earlier PE occurs. However, the late preterm birth also carries an increased risk of perinatal morbidity, and maternal complications are also described in late PE.<sup>10</sup>

The reliable identification of high-risk PE patients is crucial because intensified monitoring and referral to specialized perinatal care centers substantially reduce maternal and fetal morbidity.<sup>11,12</sup>

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**TABLE 1**  
**Patient population containing all individuals and samples measured in this study**

Variable	<34 wks	≥34 wks	All
<b>PE/HELLP</b>			
Individuals			164
Visits for analysis <sup>a</sup>	69	95	164
<b>Controls</b>			
Individuals			388
Visits for analysis <sup>b</sup>	291	176	467
<b>PIH</b>			
Individuals			36
Visits for analysis <sup>b</sup>	11	26	37
<b>ChrHTN</b>			
Individuals			42
Visits for analysis <sup>b</sup>	28	26	54

In the PE/HELLP group, the last sample before delivery of every patient with PE/HELLP was measured. *chrHTN*, chronic hypertension; *GH*, gestational hypertension; *PE/HELLP*, preeclampsia/hemolysis, elevated liver enzymes, and low platelet count; *PIH*, pregnancy-induced hypertension.

<sup>a</sup> For every visit of the PE/HELLP group, there is 1 paired gestational age-matched control. For the subgroup controls, GH, and chrHTN, only 1 sample of an individual is measured per gestational age window; <sup>b</sup> Consequently, every patient contributes at most 1 visit per window but may contribute more than 1 sample in total.

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Quick and reliable detection of the disease allows expeditious intervention with steroids for fetal lung maturity,<sup>13</sup> magnesium sulfate for seizure prophylaxis,<sup>14</sup> and antihypertensive therapy.<sup>15,16</sup>

A multitude of case-control<sup>17-20</sup> as well as prospective studies<sup>21-24</sup> have highlighted the role of the measurement of soluble fms-like tyrosine kinase (sFlt-1) and placental growth factor (PlGF) in the peripheral blood of pregnant women as a diagnostic as well as a predictive test for PE. Other laboratory markers for prediction or diagnosis of PE, such as soluble endoglin or free fetal DNA, have been investigated.<sup>25,26</sup> We have shown recently that an elevated sFlt-1/PlGF ratio below the preliminary cutoff of 85 as assessed by the Elecsys assays allows the determination of PE in the clinical context.<sup>27</sup> However, the putative predictive ability of different levels of the sFlt-1/PlGF ratio in women with

clinical PE regarding pregnancy outcome is still unknown.

The aim of the work presented was to characterize the sFlt-1/PlGF ratio in different types of hypertensive pregnancy disorders including early- and late-onset preeclampsia. We tested the hypothesis that the level of the sFlt-1/PlGF ratio is predictive for a risk for imminent delivery and has thereby putative eligibility as a prognostic marker. Furthermore, we performed correlation analysis between the sFlt-1/PlGF ratio and clinical and laboratory markers of PE/HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome.

## MATERIALS AND METHODS

### Study population

Singleton pregnancies were enrolled at 7 European medical centers. An identical study protocol and data collection form were used at each center. The local ethics committees and institutional review boards approved the study, and all subjects gave their written informed consent before participation. A total of 630 individuals were enrolled in the study: 388 singleton pregnancies with normal pregnancy outcome, 164 singleton pregnancies with PE outcome, 36 subjects with GH, and 42 patients with chrHTN (Table 1).

PE was defined according to the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Briefly, hypertension was defined as the repeated measurement of systolic blood pressure of 140 mm Hg or greater (Korotkoff I) and diastolic blood pressure of 90 mm Hg or greater (Korotkoff V).

Proteinuria was defined as the excretion of 300 mg protein or greater per day in the 24 hour urine collection or a repeated dipstick of 1+ or greater.<sup>28</sup> PE cases were classified as early onset (<34 weeks of gestation) or late onset (≥34 weeks). Furthermore, PE was defined as severe in contrast to mild, when hypertension was 160/110 mm Hg or greater, proteinuria 5 g or greater per 24 hours, or the occurrence of organ failure (kidney, lung) or neurologic symptoms were observed.

HELLP syndrome, superimposed PE, chrHTN, and intrauterine growth restric-

tion were defined as previously published.<sup>27</sup> In this study, patients with any form of PE or HELLP were combined in the PE/HELLP group (PE/HELLP) for analysis.

We analyzed 2 gestational age windows: 24+0 to 33+6 weeks (<34 weeks) and 34+0 weeks or greater (≥34 weeks). In the PE/HELLP group, the sample obtained after diagnosis was used for allocation to the respective gestational age window and analysis. In case of repeated samples, the last sample before the delivery was analyzed to relate the sFlt-1/PlGF ratio in the sample closest to delivery of that PE patient. Patients of the PE/HELLP group were pairwise matched by gestational age to a healthy control (without GH, chrHTN, or intrauterine growth restriction of the fetus), resulting in the same sample size of PE/HELLP and control group.

Patients who had chrHTN or developed GH in pregnancy were included in the respective groups and appropriate gestational age windows. For the GH and chrHTN groups, only 1 visit was selected randomly per patient and gestational age window. Consequently, every patient contributed at most 1 visit per window. Patients with chrHTN or GH who developed superimposed PE in the later course of pregnancy were analyzed as part of the PE/HELLP group.

For time-to-delivery analysis, the correlation of the sFlt-1/PlGF ratio with the remaining pregnancy duration was performed. According to the standard operating procedures of the respective center, maternal and/or fetal indications for expeditious delivery (<48 hours) before 34 weeks were severe PE plus 1 additional criteria of clinical worsening.<sup>29</sup> Immediate delivery was also considered in severe late-onset PE, whereas in the milder forms, expectant management was recommended until 37 weeks. After 37 weeks of gestation, no prolongation of pregnancy was performed in PE/HELLP patients.

Investigators were blinded to sFlt-1 and PlGF levels, which excluded an influence of this information on decision making and the defining time point of delivery.

## Samples and immunoassays

Serum samples were collected according to a common standard operating procedure at each center. Maternal blood was collected by venipuncture in tubes without anticoagulant. After clotting, the samples were centrifuged with  $2000 \times g$  and serum was pipetted, and stored at  $-80^{\circ}\text{C}$  until testing. The sFlt-1 and PIGF concentrations of each sample were determined in parallel. For each sample sFlt-1/PIGF ratio was calculated. Single measurements were performed for sFlt-1 and PIGF on the fully automated Roche (Penzberg, Germany) Elecsys system (Elecsys PIGF, human PIGF, and Elecsys sFlt-1, sFlt-1) as described previously.<sup>30</sup>

## Statistics

Basic statistics (mean, median, SD, quartiles, and range) were performed for sFlt-1, PIGF, and the sFlt-1/PIGF ratio. To compare clinical subgroups, descriptive statistics (quartiles, mean  $\pm$  SEM) and according box plots were generated. For the statistical comparison of continuous variables (eg, marker levels in the respective subgroups), time-to-delivery and nonparametric tests (Wilcoxon rank-sum test) were applied.

To explore dependencies between marker values and the time until delivery, survival analysis was applied to the PE/HELLP group. Subgroups within the PE/HELLP group were built by dichotomizing at the third quartile (Q3) of sFlt-1/PIGF. Hazard ratios (adjusted for gestational age) by Cox regression, survival estimates (Kaplan-Meier), and *P* values of log-rank tests were shown for illustrating the differences of risk between high- and low-level marker groups.

In the PE/HELLP group, relations between laboratory parameters (aspartate aminotransferase [AST], aminotransferase [ALT], platelets) or clinical parameters (systolic blood pressure [BP], diastolic BP) and marker values are reported by Pearson correlation coefficients, if appropriate after transformation of values, and illustrated by scatter plots. All *P* values are 2 tailed.

## RESULTS

### Patient population/baseline characteristics

A total of 630 individuals were included in the study: 388 singleton pregnancies with normal pregnancy outcome, 164 singleton pregnancies with PE outcome, 36 subjects with GH, and 42 patients with chrHTN. The PE/HELLP group consisted of 70 patients with mild PE, 70 with severe PE, 10 with superimposed PE, and 14 with HELLP (Table 1). No significant differences in age and ethnical origin were observed. Women with PE had a higher systolic and diastolic BP and had a lower mean birthweight of the neonate than normal pregnant women or women with GH or chrHTN. Women with PE/HELLP, GH, or chrHTN had a significantly higher weight and body mass index at booking as compared with healthy controls (Table 2).

### The sFlt-1/PIGF ratio in different hypertensive pregnancy disorders

To explore the sFlt-1/PIGF ratio in different hypertensive disorders, we analyzed sFlt-1/PIGF in patients with GH or chrHTN as well as in women with PE/HELLP and healthy controls  $<34$  weeks and  $\geq 34$  weeks. Patients with PE/HELLP had a significantly increased sFlt-1/PIGF ratio as compared with controls  $<34$  weeks ( $506.2 \pm 54.3$  vs  $9.1 \pm 2.1$  pair-wise comparison,  $P < .001$ ) as well as  $\geq 34$  weeks ( $168.5 \pm 17.7$  vs  $32.5 \pm 4.9$  pair-wise comparison,  $P < .001$ , Figure 1, A). Within these groups a shift in the value of the sFlt-1/PIGF ratio between  $<34$  weeks and  $\geq 34$  weeks was observed: in PE/HELLP, patients  $<34$  weeks had a significantly higher sFlt-1/PIGF ratio as compared with patients  $\geq 34$  weeks ( $506.2 \pm 54.3$  vs  $168.5 \pm 17.7$ ,  $P < .001$ ).

Control subjects had a significant increase in the sFlt-1/PIGF ratio in the group  $\geq 34$  weeks as compared with control subjects  $<34$  weeks ( $32.5 \pm 4.9$  vs  $9.1 \pm 2.1$ ,  $P < .001$ ).

In the window  $<34$  weeks, neither patients with GH ( $16.9 \pm 12.0$ ) nor chrHTN ( $16.3 \pm 8.4$ ) had a significantly increased sFlt-1/PIGF ratio as compared with controls ( $8.3 \pm 1.3$ ; GH vs controls,  $P = .88$ ; chrHTN vs controls,  $P = .44$ ). In contrast, these patients had a signifi-

cantly decreased sFlt-1/PIGF ratio as compared with patients with PE/HELLP ( $P < .001$ ).

In the window  $\geq 34$  weeks, patients with GH had a significantly increased sFlt-1/PIGF ratio as compared with controls ( $53.5 \pm 8.8$  vs  $26.5 \pm 3.0$ ,  $P < .001$ ). The same applied for the chrHTN group ( $31.6 \pm 4.8$  vs  $26.5 \pm 3.0$ ,  $P = .022$ ). When compared with patients with PE/HELLP, GH or chrHTN had a significantly lower sFlt-1/PIGF ratio ( $P < .001$ ). When the preliminary cutoff of 85 was applied, all groups except the PE/HELLP group had a mean sFlt-1/PIGF ratio below that cutoff (Figure 1, B).

### Correlation of clinical and laboratory markers with the sFlt-1/PIGF ratio in the PE/HELLP group

We next investigated the correlation of the sFlt-1/PIGF ratio with clinical (systolic BP, diastolic BP) and laboratory parameters (AST, ALT, platelets). There was a moderate correlation between the sFlt-1/PIGF ratio and systolic BP in  $<34$  weeks ( $r = 0.41$ ,  $P > .001$ ), whereas a weak and statistically not significant correlation was observed between systolic BP in  $\geq 34$  weeks ( $r = 0.19$ ,  $P = .06$ ). For diastolic BP, correlations were small and statistically not significant in both groups ( $r = 0.22$ ,  $P = .07$  for  $<34$  weeks and  $0.11$ ,  $P = .31$  for  $\geq 34$  weeks). The correlations between the sFlt-1/PIGF ratio and the laboratory markers were weak as well. In the  $<34$  weeks group, AST ( $r = 0.28$ ,  $P < .05$ ), ALT ( $r = 0.32$ ,  $P < .05$ ), and platelet count ( $r = -0.27$ ,  $P < .05$ ) yielded only a mild though significant correlation to the sFlt-1/PIGF ratio (Figure 2).

### Time-to-delivery analysis in the PE/HELLP group

We analyzed the time to delivery in all 164 patients with PE/HELLP within 2 days or less, 2-7 days, and later than 7 days. In the  $<34$  weeks group (69 of 164 patients), the mean sFlt-1/PIGF ratio in patients delivering within 2 days (38 of 69) was  $616.42 \pm 81.15$ . Patients delivering in 2-7 days (14 of 69) exhibited a mean sFlt-1/PIGF ratio of  $547.93 \pm 98.39$ . However, patients delivering later than 7 days (17 of 69) had a mean sFlt-1/PIGF ratio of  $225.55 \pm 60.59$  ( $P =$

**TABLE 2**  
**Baseline characteristics of the study population**

Characteristic	Group							
	PE/HELLP		Controls		GH		chrHTN	
	<34 wks	≥34 wks	<34 wks	≥34 wks	<34 wks	≥34 wks	<34 wks	≥34 wks
Age, y	31.3 ± 0.69	29.5 ± 0.68	30.6 ± 0.35	31.2 ± 0.41	31.1 ± 1.34	30 ± 1.28	32.5 ± 0.86	31.4 ± 1.13
Height, cm	164.6 ± 0.84	165.2 ± 0.68	165.4 ± 0.38	167 ± 0.48	168.3 ± 2.3	166.3 ± 1.42	164.2 ± 1.34	164.7 ± 1.57
Weight, kg	70.7 ± 2.22	71.7 ± 1.92 <sup>a</sup>	65.3 ± 0.8	65.2 ± 0.96	86.8 ± 8.25	77.3 ± 4.65	81.7 ± 4.48	91.2 ± 5.24
BMI, kg/m <sup>2</sup>	26.2 ± 0.85	26.2 ± 0.64 <sup>a</sup>	23.9 ± 0.3	23.4 ± 0.33	29.9 ± 2.77 <sup>b</sup>	27.8 ± 1.51 <sup>b</sup>	30.5 ± 1.75 <sup>b</sup>	33.7 ± 1.96 <sup>b</sup>
Birthweight, g	1372 ± 76.4 <sup>a</sup>	2834 ± 78.4 <sup>a</sup>	3024 ± 47.3	3370 ± 38.9	3230 ± 230.9 <sup>b</sup>	3324 ± 100.6 <sup>b</sup>	2881 ± 133.3 <sup>b</sup>	3309 ± 99.8 <sup>b</sup>
Gestational week of delivery	30.6 ± 0.38 <sup>a</sup>	37.4 ± 0.2 <sup>a</sup>	37.4 ± 0.21	39.2 ± 0.15	37.7 ± 0.87	38.6 ± 0.22 <sup>b</sup>	37.1 ± 0.4	38.7 ± 0.2 <sup>b</sup>
Diastolic BP, mmHg	100.3 ± 1.54 <sup>a</sup>	96.1 ± 1.12 <sup>a</sup>	67.9 ± 0.54	71.6 ± 0.71	80.3 ± 3.87 <sup>b</sup>	91.6 ± 1.63 <sup>b</sup>	82 ± 1.89 <sup>b</sup>	84.4 ± 2.8 <sup>b</sup>
Systolic BP, mmHg	160.2 ± 2.1 <sup>a</sup>	152 ± 1.67 <sup>a</sup>	113.5 ± 0.69	116.4 ± 0.99	134 ± 5.33 <sup>b</sup>	143.9 ± 2.96 <sup>b</sup>	127.8 ± 3.01 <sup>b</sup>	133.8 ± 3.43 <sup>b</sup>
Smoking status, n (%)								
Current	4 (5.8)	7 (7.4)	41 (14.1)	21 (11.9)	1 (9.1)	1 (3.8)	6 (21.4)	3 (11.5)
Past	8 (11.6)	12 (12.6)	45 (15.5)	23 (13.1)	3 (27.3)	7 (26.9)	4 (14.3)	6 (23.1)
Never	48 (69.6)	54 (56.8)	166 (57)	99 (56.2)	7 (63.6)	18 (69.2)	17 (60.7)	16 (61.5)
Unknown/NA	9 (13)	22 (23.2)	39 (13.4)	33 (18.8)	0 (0)	0 (0)	1 (3.6)	1 (3.8)
Race/ethnic group, n (%)								
White/Caucasian	58 (84.1)	81 (85.3)	249 (85.6)	152 (86.4)	9 (81.8)	21 (80.8)	23 (82.1)	25 (96.2)
Black/African American	3 (4.3)	3 (3.2)	5 (1.7)	2 (1.1)	1 (9.1)	2 (7.7)	0 (0)	0 (0)
Other	6 (8.7)	8 (8.4)	28 (9.6)	11 (6.2)	1 (9.1)	3 (11.5)	4 (14.3)	0 (0)
Unknown/NA	2 (2.9)	3 (3.2)	9 (3.1)	11 (6.2)	0 (0)	0 (0)	1 (3.6)	1 (3.8)
Family history of PE, n (%)								
Yes	4 (5.8)	6 (6.3)	4 (1.4)	4 (2.3)	0 (0)	0 (0)	1 (3.6)	1 (3.8)
No	56 (81.2)	72 (75.8)	254 (87.3)	156 (88.6)	9 (81.8)	17 (65.4)	21 (75)	22 (84.6)
Unknown/NA	9 (13)	17 (17.9)	33 (11.3)	16 (9.1)	2 (18.2)	9 (34.6)	6 (21.4)	3 (11.5)
Total, n	69	95	291	176	11	26	28	26

PE/HELLP patients were compared with gestational age-matched controls (<sup>a</sup>  $P < .05$ ). GH and chrHTN were compared with controls within the gestational age window <34 weeks to ≥34 weeks (<sup>b</sup>  $P < .05$ ). Data are expressed in mean ± SD.

BMI, body mass index; BP, blood pressure; chrHTN, chronic hypertension; GH, gestational hypertension; NA, not applicable; PE/HELLP, preeclampsia/hemolysis, elevated liver enzymes, and low platelet count.

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.004 for ≤2 days vs 7 d,  $P = .0065$  for 2-7 days vs >7 days, and  $P = .89$  for ≤2 days vs 2-7 days). In the ≥34 weeks PE/HELLP group (95 of 164), patients delivering within 2 days (68 of 95) had a mean sFlt-1/PIGF ratio of  $190.02 \pm 23.11$ , whereas patients delivering within 2-7 days (17 of 95) had a mean sFlt-1/PIGF ratio of  $146.03 \pm 24.28$ . However, patients who were pregnant longer than 7 days (10 of 95) had a mean sFlt-1/PIGF ratio of  $60.47 \pm 21.89$  ( $P = .0026$  for ≤2 days vs >7 days,  $P = .037$  for 2-7 days vs >7 days,  $P = .66$  for ≤2 days and 2-7 days) (Figure 3, A).

We performed a Kaplan-Meier analysis to determine the hazard for imminent delivery of PE/HELLP pa-

tients with an sFlt-1/PIGF ratio in the upper quartile. In the <34 weeks PE/HELLP group, patients with an sFlt-1/PIGF ratio greater than 655.2 (Q3 for <34 weeks) were at highest risk for imminent delivery (Figure 3, B). After 48 hours, only 29.4% (95% confidence interval [CI], 14.1–61.4%,  $P = .016$ ) of patients remained pregnant, whereas 50% (95% CI, 38.3–65.6%) of patients with an sFlt-1/PIGF ratio below Q3 were still pregnant. After 7 days, only 5.9% (95% CI, 0.9–39.4%) of the PE/HELLP patients with an sFlt-1/PIGF ratio above Q3 were still pregnant as compared with 30.8% (95% CI, 20.5–46.3%) of the lower quartile group.

In the ≥34 weeks PE/HELLP group, only 16.7% (95% CI, 6.8–40.8%) of patients with an sFlt-1/PIGF ratio greater than 201 (Q3 for ≥34 weeks) were pregnant after 48 hours as compared with 32.4% (95% CI, 23.1–45.3%) of patients below Q3 ( $P = .001$ ). After 7 days, no patients with an sFlt-1/PIGF ratio greater than 201 remained pregnant as compared with 14.1% (95% CI, 7.9–25%) with an sFlt-1/PIGF ratio 201 or less ( $P = .001$ ).

For all PE/HELLP patients (<34 weeks as well as ≥34 weeks), an sFlt-1/PIGF ratio greater than 400 (Q3 for all PE/HELLP) is associated with a 3.35 increased risk for an immediate occur-

rence of delivery (95% CI, 2.04–5.51,  $P < .001$ ). In the  $<34$  weeks group, an sFlt-1/PlGF ratio above the third quartile ( $>655.2$ ) is associated with a 2.69 increased risk for an immediate occurrence of delivery (95% CI, 1.33–5.41;  $P = .006$ ). In the  $\geq 34$  weeks window, an sFlt-1/PlGF ratio greater than Q3 ( $>201$ ) is associated with a 3.5 times increased risk for immediate occurrence of delivery (95% CI, 2.06–5.94;  $P < .001$ ).

## COMMENT

The important findings of our study are that PE/HELLP patients with a high sFlt-1/PlGF ratio had a significantly increased risk for imminent delivery. Patients who delivered within 7 days had a significantly higher sFlt-1/PlGF ratio than patients who delivered later than 7 days. This holds true for the  $<34$  weeks and the  $\geq 34$  weeks groups.

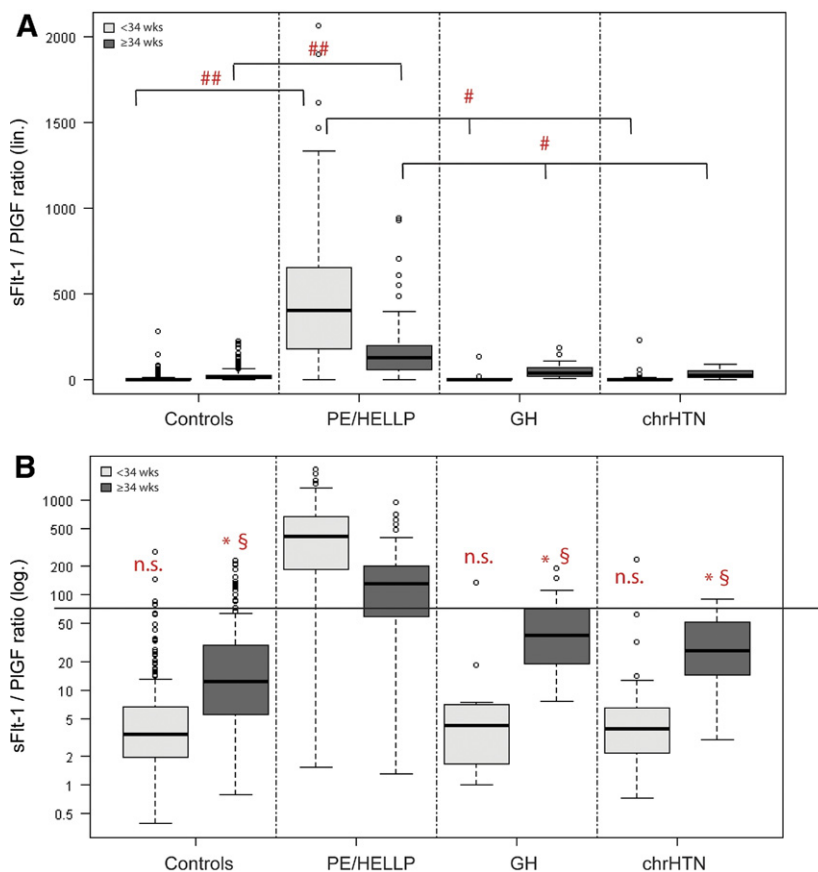
The Kaplan-Meier-analysis confirmed that an sFlt-1/PlGF ratio above the third quartile is associated with a significantly increased risk for immediate occurrence of delivery: In PE/HELLP patients  $<34$  weeks, only 29.4% of all patients with an sFlt-1/PlGF ratio above the third quartile remained pregnant after 48 hours. In the  $\geq 34$  weeks PE/HELLP group, only 16.7% of the patients with an sFlt-1/PlGF ratio greater than Q3 were still pregnant after 48 hours as compared with 32.4% with an sFlt-1/PlGF ratio below Q3. After 7 days no PE/HELLP patients  $\geq 34$  weeks with an sFlt-1/PlGF ratio greater than Q3 remained pregnant as compared with 14% of these patients below Q3.

From these findings we conclude that the sFlt-1/PlGF ratio correlates with the risk for an immediate occurrence of delivery. Therefore, the determination of the sFlt-1/PlGF ratio in patients with clinical PE might have value for clinical management, counseling, and risk anticipation.

## The use of sFlt-1/PlGF ratio in the differential diagnosis of hypertensive disorders in pregnancy

Others reported the use of the sFlt-1/PlGF ratio in the differential diagnosis of PE.<sup>24,25,31</sup> We have shown that  $<34$  weeks, patients with neither GH nor chrHTN had signifi-

**FIGURE 1**  
sFlt-1/PlGF ratio in PE/HELLP, GH, chrHTN, and healthy controls



Box-and-whisker-plots displaying the sFlt-1/PlGF ratio in patients with PE/HELLP, GH, and chrHTN, and healthy controls. Light gray boxes show patients  $<34$  weeks, and dark gray boxes show patients  $\geq 34$  weeks. Boxes indicate interquartile range; whiskers indicate range; error bars indicate median. **A**, The linear distribution of the sFlt-1/PlGF ratio in patients with PE/HELLP, GH, and chrHTN and controls. For visualization, individual P values by 2-sided Wilcoxon rank sum statistics are shown: in PE vs controls  $<34$  weeks ( $n = 69$ ) and  $\geq 34$  weeks ( $n = 95$ ), a pairwise comparison was conducted (double number signs indicate all  $P < .001$ ). PE/HELLP  $<34$  weeks ( $n = 69$ ) was compared with GH ( $n = 11$ ; number sign indicates  $P < .001$ ) and chrHTN ( $n = 28$ ; number sign indicates  $P < .001$ ). PE/HELLP  $\geq 34$  weeks ( $n = 95$ ) was compared with GH ( $n = 26$ ; number sign indicates  $P < .001$ , and  $n = 26$ ; number sign indicates  $P < .001$ ). **B**, The logarithmic distribution of the sFlt-1/PlGF ratio in patients with PE/HELLP, GH, and chrHTN and controls. For visualization, the preliminary cutoff of 85 is drawn into the graph as a solid line. For visualization, individual P values by 2-sided Wilcoxon rank sum statistics are shown: controls  $<34$  weeks ( $n = 291$ ) were compared with GH ( $n = 11$ , not significant,  $P = .88$ ) and chrHTN ( $n = 28$ , not significant,  $P = .44$ ). Controls  $\geq 34$  weeks ( $n = 176$ ) were compared with GH ( $n = 26$ ; section mark indicates  $P < .001$ ) and chrHTN ( $n = 26$ ; section mark indicates  $P < .001$ ). Furthermore, controls, GH, and chrHTN  $\geq 34$  weeks had a higher sFlt-1/PlGF ratio as compared with  $<34$  weeks (asterisk indicates  $P < .05$ ).

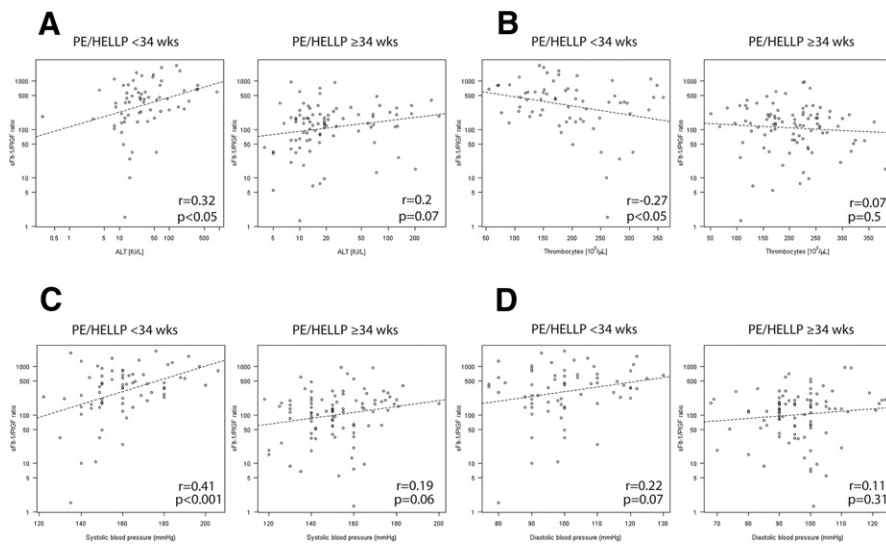
chrHTN, chronic hypertension; GH, gestational hypertension; PE/HELLP, preeclampsia/hemolysis, elevated liver enzymes, and low platelet count; sFlt-1/PlGF, soluble fms-like tyrosine kinase/placental growth factor.

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cantly higher sFlt-1/PlGF ratios than healthy controls, whereas in  $\geq 34$  weeks, both groups had a significantly elevated sFlt-1/PlGF ratios as compared with controls. However, at all

instances, the values were below the preliminary cutoff of 85. Patients with PE/HELLP had significantly elevated sFlt-1/PlGF ratios as compared with all other groups.

**FIGURE 2**  
**Correlation of sFlt-1/PlGF to diagnostic criteria of HELLP and PE in patients with PE/HELLP**



Line and scatter plots displaying the correlation of the sFlt-1/PlGF ratio to diagnostic criteria of HELLP syndrome (A, ALT; B, thrombocytes) and PE (C, systolic BP; D, diastolic BP) in PE/HELLP patients. Correlation coefficients (Pearson) and significance levels (*P* values) between the respective parameter and logarithmized sFlt-1/PlGF ratios are shown for <34 weeks and ≥34 weeks. Dots indicate individual sFlt-1/PlGF ratios; line indicates regression line.

ALT, aminotransferase; BP, blood pressure; chrHTN, chronic hypertension; GH, gestational hypertension; PE/HELLP, preeclampsia/hemolysis, elevated liver enzymes, and low platelet count; sFlt-1/PlGF, soluble fms-like tyrosine kinase/placental growth factor.

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Our reports are in line with Levine et al,<sup>25</sup> who found higher sFlt-1/PlGF ratios in patients with GH as compared with healthy controls only after 33 weeks of gestation. Others also found increased levels of the sFlt-1/PlGF ratio<sup>32</sup> or the single markers<sup>33</sup> in GH. In a prospective study, the sFlt-1/PlGF ratio in GH was similar to term PE.<sup>34</sup> In contrast to our findings regarding chrHTN, Salahuddin et al<sup>31</sup> found no difference in sFlt-1 as compared with controls.

Only a few studies have addressed the role of the sFlt-1/PlGF ratio in chrHTN.<sup>35,36</sup> Our findings are of clinical importance because they show the utility of the automated measurement of the sFlt-1/PlGF ratio in differentiating PE/HELLP from other hypertensive pregnancy disorders. The pathophysiology of GH and chrHTN is putatively different than that of PE/HELLP as reflected by the sFlt-1/PlGF ratio with potential relevance to the prediction of clinical outcome of a patient with a hypertensive pregnancy disorder. However, a major limitation refers to the sample size in the <34 weeks group. Although statistically significant, patient num-

bers are low for both GH and chrHTN. Larger studies must follow to confirm these results.

### Correlation of clinical and laboratory markers with the sFlt-1/PlGF ratio in the PE/HELLP group

We have shown that there is an absence of significant correlations between the sFlt-1/PlGF ratio and clinical and laboratory markers of PE/HELLP. Solely the systolic blood pressure in the <34 weeks PE/HELLP group showed a moderate correlation with the sFlt-1/PlGF ratio. This is in line with other reports showing the absence of a correlation of angiogenic and antiangiogenic factors, hypertension, and proteinuria.<sup>37</sup> Interestingly, it has been shown that there is a correlation between blood pressure and sFlt-1/PlGF ratio in normotensive pregnancies but not in preeclamptic pregnancies.<sup>38</sup> However, others found a correlation of sFlt-1/PlGF ratio with blood pressure.<sup>39</sup> Given the clinical heterogeneity of PE/HELLP, the sFlt-1/PlGF ratio might be of

value in unclear situations. The sFlt-1/PlGF ratio putatively constitutes an additive and continuative diagnostic tool for PE that is independent from blood pressure or laboratory markers of HELLP syndrome.

### Time-to-delivery analysis and use of the sFlt-1/PlGF ratio as a potential prospective marker of PE/HELLP

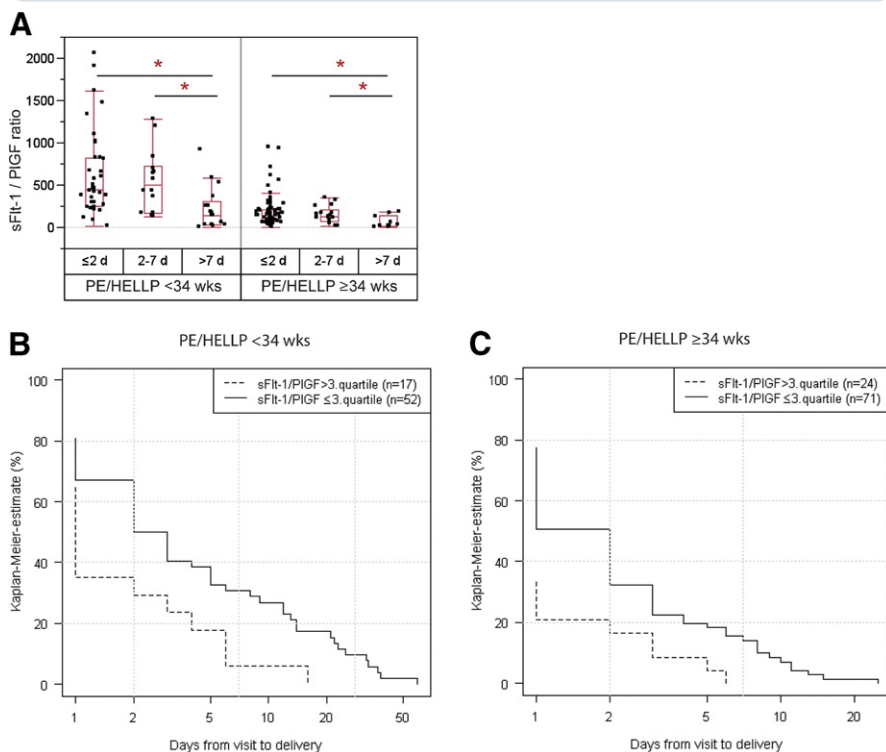
The diagnosis of PE is established by measuring blood pressure and proteinuria. It is known that this definition is not providing any information about the severity of the disease, its clinical course, or the impact on maternal and fetal morbidity and mortality.<sup>40</sup> Here we are able to show for the first time that the sFlt-1/PlGF ratio is correlated to the remaining pregnancy duration.

Patients with a sFlt-1/PlGF ratio in the upper quartile have a significantly reduced duration of pregnancy. This allows individualized risk stratification for an imminent delivery of patients with diagnosed PE. Especially in the <34 weeks PE/HELLP group, the early identification of patients with a high risk for delivery is of relevance for maternal and fetal morbidity and mortality because the timely referral to a perinatal care center alone is able to reduce perinatal morbidity and mortality by 20%. Thus, the consideration of the sFlt-1/PlGF ratio aids to individually adapt clinical monitoring and management and by that might contribute to a reduction in morbidity and mortality. However, our analysis is limited by a small sample size. Prospective data with larger numbers and an analysis of the predictive accuracy of the sFlt-1/PlGF ratio as a prognostic marker are needed.

### Conclusion and outlook

This study shows an important clinical implication for the use of the sFlt-1/PlGF ratio for diagnosis, differential diagnosis, and risk stratification in PE/HELLP patients. In our previous article, we reported that the sFlt-1/PlGF ratio as determined by the automated measurement on the Elecsys platform is able to assess PE with a high sensitivity and specificity. Here we were able to show that a high sFlt-1/PlGF ratio is associated with a significantly increased

**FIGURE 3**  
**sFlt-1/PIGF ratio and time of delivery in PE/HELLP-patients**



**A**, Box-and-whisker plots showing the distribution of the sFlt-1/PIGF ratio in PE/HELLP patients delivering within 2 days, within 2-7 days, and later than 7 days from blood sampling in <34 weeks and ≥34 weeks. Boxes indicate interquartile range; whiskers indicate range; error bars indicate median. Asterisk indicates  $P < .05$  where appropriate. **B, C**, Kaplan-Meier graphs showing the time to delivery in patients with PE/HELLP. The y-axis shows the Kaplan-Meier estimate, in percentage, the x-axis days from visit to delivery. **B**, Patients with PE/HELLP <34 weeks with the sFlt-1/PIGF ratio greater than the third quartile (dotted line,  $n = 17$ ) are compared with patients with an sFlt-1/PIGF ratio below the third quartile (solid line,  $n = 52$ ). **C**, Patients with PE/HELLP ≥34 weeks with an sFlt-1/PIGF ratio above the third quartile (dotted line,  $n = 24$ ) are compared with patients with an sFlt-1/PIGF ratio below the third quartile (solid line,  $n = 71$ ).

PE/HELLP, preeclampsia/hemolysis, elevated liver enzymes, and low platelet count; sFlt-1/PIGF, soluble fms-like tyrosine kinase/placental growth factor.

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risk for an immediate occurrence of delivery. Furthermore, the measurement of the sFlt-1/PIGF ratio can differentiate between different forms of hypertensive disorders.

We propose that the sFlt-1/PIGF ratio has potential relevance as a prognostic parameter for imminent delivery in patients with diagnosed PE/HELLP in the clinical setting. In addition to established clinical examination and laboratory testing in the diagnostic process of PE/HELLP, determination of the sFlt-1/PIGF ratio might add valuable information about the clinical course and progression speed of the disease. Quick and reliable individualized risk

stratification can be performed in a patient with clinical preeclampsia, and clinical management can be adapted accordingly.

Although the complete puzzle of the pathogenesis of PE is not yet fully elucidated, we took a big step toward a better understanding of its clinical course. Further research, especially prospective data, is needed to clarify the potential role of the sFlt-1/PIGF ratio in the diagnostic algorithm of PE. ■

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