Clinical Performance of The sFlt-1/PlGF Test For The Prediction of Preeclampsia Among Asymptomatic and Symptomatic Pregnant Women in Estonia: A Nested Case-Control Study

Ele Hanson
University of Tartu
Kristiina Rull
University of Tartu
Kaspar Ratnik
University of Tartu
Pille Vaas
Tartu University Hospital
Pille Teesalu
Tartu University Hospital
Maris Laan (maris.laan@ut.ee)
University of Tartu

Research Article

Keywords: preeclampsia, hypertension in pregnancy, asymptomatic pregnant women, sFlt-1/PlGF test, predictive value

Posted Date: January 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-148900/v1

Abstract

Background: Pre-eclampsia (PE) is a pregnancy complication manifesting as new-onset hypertension and other maternal organ dysfunction after 20th gestational weeks. The study aimed to evaluate the applicability and limitations of the maternal serum soluble fms-like tyrosine kinase-1/placental growth factor (sFlt-1/PlGF) test in a clinical setting for the prediction of PE among symptomatic and asymptomatic pregnant women. There is limited knowledge on the performance of this test in asymptomatic women and thus, its value for screening purposes to predict PE is not confidently settled.

Methods: The study group comprised of 215 patients developing either PE (n=29) or gestational hypertension/proteinuria (n=22) or representing controls (n=164). Patients had been sampled within 180-291 gestational days in the presence (symptomatic, n=31) or absence (asymptomatic, n=216) of PE-alerting symptoms. Serum samples collected during prospective cohort study 'Happy Pregnancy' at the Women's Clinic, Tartu University Hospital, Estonia, and they were analyzed using the BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio test (Thermo Fisher Scientific, Henningdorf, Germany). The results were interpreted using recommendations by Stepan et al 2015 *Ultrasound Obstet Gynecol*.

Results: The assignment of 'Rule-out PE' (sFlt-1/PlGF ratio <38) had a negative predictive value >99% for four weeks for both asymptomatic and symptomatic women. Among 29 sera assigned to the 'Rule-in PE'(sFlt-1/PlGF >85/110), only 18 pregnancies (62.1%) eventually developed PE. For four weeks period, the overall PE detection rate was 83% for asymptomatic and 50% for symptomatic pregnancies. Pregnancies receiving false predictions of PE risk based on sFlt-1/PlGF ratio represented either cases with an isolated small-for-gestational age fetus or blood sampling after 34 gestational weeks.

Conclusions: The first study in Estonian patients confirmed high reliability of the proposed cut-off value sFlt-1/PlGF <38 as a "Rule-out PE" cut-off value for two weeks in both symptomatic and asymptomatic pregnancies. The test's limitation in our clinical setting appeared to be high false positive rate in pregnancies with other placental pathologies than PE or due to physiological increase in sFlt-1/PlGF in late gestation. For correct prediction of PE, more specific recommendations are urgently needed for the application and interpretation of the test in routine clinical practice.

Background

Pre-eclampsia (PE) is a pregnancy disorder defined by the new-onset of hypertension after 20 gestational weeks accompanied by proteinuria or other maternal organ dysfunction. In extreme cases it may rapidly progress to the form of life-threatening eclampsia. Additionally to severe damage to maternal health, preeclamptic pregnancies also cause fetal complications such as growth restriction or intrauterine death. PE affects 2 to 5% of pregnancies worldwide and is one of the major causes of maternal and perinatal morbidity and mortality.[1,2,3]

Current clinical predictive and diagnostic toolset for PE includes routine monitoring of blood pressure and urinary protein levels.[4] However, women with the developing PE may also miss any alerting symptoms. Extensive research has been devoted to identify maternal serum biomarkers for early PE prediction, facilitating timely intervention and preventive measures.

The pathogenesis of PE is associated with the imbalance of pro- and antiangiogenic factors such as sFlt-1 (soluble fms-like tyrosine kinase 1) and PlGF (placental growth factor). In symptomatic women the sFlt-1/PlGF test can help to distinguish between PE development and a transient elevation of blood pressure.[5] As increased sFlt-1/PlGF ratio in maternal serum can be detected up to 5 weeks before the onset of PE symptoms, these biomarkers represent potential screening tool among asymptomatic women.[6] Although currently no effective third trimester prevention for preeclampsia is available, early detection of the high risk for developing PE helps to select women who need more frequent follow-up visits for timely diagnosis and referral to specialized centres. The application of glucocorticoids and neuroprotection help to alleviate the problems caused by PE-induced preterm birth. Recently, manifestation of pravastatin since 36th g. week has attracted attention as a potential preventative or therapeutic candidate for late onset PE, supported by pilot clinical trials[7]. Commercially offered solutions for the detection of sFlt-1/PlGF biomarker ratios to predict PE development have been rapidly introduced and rule-in and rule-out thresholds for the PE prediction have been established.[5,8] However, international community has concluded that before use in daily clinical practice further studies on asymptomatic and symptomatic pregnant women are needed.[9]

This study aimed to retrospectively evaluate the applicability and limitations of the maternal serum sFlt-1/PlGF test in a routine clinical setting in Estonia to predict PE development during the second half of pregnancy. The potential of the sFlt-1/PlGF test as a PE screening

tool was evaluated by comparing the outcomes obtained for the asymptomatic women and patients presenting at least one PE symptom at sampling. The cases with false-positive, false-negative and inconclusive test results were critically assessed in the context of their detailed clinical history.

Methods

'Happy Pregnancy' cohort of pregnant women

The analyzed patients were recruited, and the respective clinical data and biomaterials were collected during a monocentric prospective 'Happy Pregnancy' project (full name 'Development of novel non-invasive biomarkers for fertility and healthy pregnancy': PI: M.L.). The project has been approved by the Ethics Committee of Human Research of the University Clinic of Tartu, Estonia (permissions no. 221/T-6, 17.12.2012 and 286/M-18, 15.10.2018) and was carried out in compliance with the Helsinki Declaration. A written informed consent to participate in the study was obtained from each individual prior to recruitment. All participants were of white European ancestry and living in Estonia.

During March 2013 – August 2015, in total 2,334 unselected pregnant women had been enrolled at their first antenatal visit at the Women´s Clinic, Tartu University Hospital, Estonia (clinical PI: K.R.). The pregnancy follow-up was based on the national guidelines approved at 2011 by the Estonian Gynaecologists` Society.[10] The collected clinical and epidemiological data, as well as applied clinical criteria are specified in Supplementary Methods S1, **Additional File 1**. Among the final cohort of 2,257 eligible women 61 (2.7%) had eventually developed preeclampsia (PE), 52 (2.3%) had been diagnosed with gestational hypertension (GH) and 17 (0.8%) with proteinuria (**Table 1**).

Table 1. Maternal and pregnancy characteristics of the study subjects

	Happy Pregnancy		Current study sample (subgroups)		
Parameter	Full study cohort	Current study	Preeclampsia	GH or Proteinuria	Control group
Study subjects	2,257	215	29 (13.5%)	22 (10.2%)	164 (76.3%)
Maternal age (years)	29 (18 – 43) 29 ± 5	28 (18 – 43) 28 ± 5	28 (20 – 40) 28 ± 5	28 (21 – 43) 31 ± 7	28 (18 – 40) 29 ± 5
Pre-pregnancy BMI (kg/m^2)	22.43 (18.29 – 31.51) 23.35 ± 4.33	23.9 (16.1 – 42.8) 25.3 ± 5.0	26.8 (18.0 – 42.8) 26.9 ± 6.0	28.5 (18.8 – 41.5)* 28.6 ± 5.8	23.5 (16.1 – 40.2) 24.6 ± 4.4
Primiparous	1,032 (45.5%)	120 (55.8%)	23 (79.3%)*	14 (63.6%)	83 (50.6%)
PE/GH in previous pregnancy	Not analyzed	31 (14.4%)	5 (17%)*	2 (9.1%)	24 (14.6%)
Pre-pregnancy diabetes	10 (0.4%)	2 (0.9%)	one T1D case (3.4%)	0	one T1D case (0.6%)
Pre-pregnancy HTN	18 (0.8%)	5 (2.3%)	1 (3.4%)	0	4 (2.4%)
IVF pregnancy	80 (3.4%)	8 (3.7%)	1 (3.4%)	2 (9.1%)	5 (3.0%)
Gestational diabetes	129 (5.5%)	19 (8.8%)	2 (6.9%)	5 (22.7%)*	12 (7.3%)
Gestational age at delivery (days)	280 (243 – 293) 272.44 ± 35.73	280 (199 – 295) 277 ± 13	263 (199 – 285)*† 260 ± 20	279 (262 – 293) 280 ± 9	282 (235 – 295) 280 ± 10
Preterm birth (n, %)	138 (5.9%)	15 (6.9%)	11 (37.9%)*†	0	4 (2.4%)
Cesarean section (n, %)	384 (17%)	54 (25.1%)	17 (58.6%)*	7 (31.8%)	30 (18.3%)
SGA newborns	186 (8.0%)	24 (11.2%)	5 (17.2%)*	1 (4.5%)	7 (4.3%)

Data are given as median (minimum - maximum) and mean ± standard deviation or number (percentage) as appropriate. Parameter distributions between subgroups were compared using Chi^2 for categorical variables or Wilcoxon rank-sum test for continuous variables.

*P<0.05 compared to control group within current study sample

†P<0.05 in comparison between PE and GH/proteinuria in current study sample

Diagnosis of gestational hypertension for 18/22 patients and diagnosis of gestational proteinuria 4/22. Newborn was categorized as small-for-gestational-age (SGA) in case the Z-score for sex-and gestational age adjusted birthweight was lower than -1. To convert the newborn birth weight into gestational age and sex-adjusted z-scores the fetal growth calculator based on INTERGROWTH-21st Project was applied.[14]

PE, preeclampsia; GH, gestational hypertension; BMI, body mass index; IVF, in vitro fertilization; parity,presence of previous births

In total 138 pregnancies (5.9%) resulted in a preterm birth (PTB), a delivery before 37 gestational weeks (< 259 gestational days).

Serum sampling for research purposes was performed across gestation in parallel with regular clinical visits and blood-draw based tests according to routine pregnancy monitoring procedures (**Figure S1, Additional File 2**). At each blood draw, the symptoms alerting to PE were assessed and documented. The diagnosis of PE followed the international guidelines at the time of recruitment (ISSHP, 2014), simultanous co-occurence of a new-onset hypertension (HTN; ≥140/ ≥90 mmHg) after 20 gestational weeks and proteinuria or other signs of maternal organ dysfunction.[1] According to the absence or presence of the signs alerting to PE at blood draw or up to four days later, serum samples collected from the pregnant women were classified as asymptomatic or symptomatic (GH, proteinuria, PE).

The sample set and patient group analyzed in the current study

Prospectively drawn 252 serum samples from the 'Happy Pregnancy' cohort participants were retrospectively subjected to the commercially offered sFlt-1/PlGF test (Thermo Fisher Scientific). The samples had been collected from 215 pregnant women with different pregnancy outcome (PE, n=29; isolated GH or proteinuria, n=22; controls, n=164) 1-69 days before delivery and covered 180 - 291 gestational days (**Table 1**). The formation of the study group centered around the available serum samples drawn from PE cases (n=42 samples) and isolated GH/proteinuria cases (n=30 samples) after 25th gestational weeks until term. Respective gestational and maternal age matched control serum samples (n=180) representing uncomplicated pregnancies were selected from the cohort biobank, aiming to match two to three control serums per each included sample of a PE or isolated GH/proteinuria case (Figure S2, **Additional file 3;** Figure S3**, Additional file 4**). The study group included 35/215 pregnancies with the representation of several consecutive blood samples, enabling to assess also gestational dynamics of biomarkers (Table S1, **Additional file 5)**. From among 29 women, who eventually developed PE, 11 had been sampled twice and one patient thrice. Among 22 women with a later diagnosis of GH/proteinuria, six had been subjected to blood draw two and one patient three times. Control group included 16/164 pregnancies that had been sampled twice.

Implementation of sFlt-1/PlGF test for the cryopreserved serum samples

After blood draw, serum samples had been immediately separated and stored at -80°C for maximum 1.5 years (Supplementary Methods S2, **Additional file 6)**. After thawing the samples were aliquoted and sent on dry ice to the Synlab Germany service laboratory (Leinfelden, Germany) without no information regarding the pregnancy course and outcome. Freeze-thaw stability of serum sFlt-1 and PlGF concentrations has been previously reported.[11,12]

Concentrations of serum sFlt-1 and PlGF were analyzed using the BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio test (Thermo Fisher Scientific, Henningdorf, Germany). Reportable measurement values of both biomarkers were derived from the service provider for 247/252 (98%) serum samples (failure rate 2%; Table S2, **Additional file 7**). Failed samples were excluded from further analysis.

Prediction of the PE development was based on the estimated sFlt-1/PlGF ratio. As no validated cut-offs are published specially for Kryptor, Elecsys® assay validated cut-offs were used as recommended by Stepan et al.[13] Serum samples were classified as 'Rule-out PE at least within 1 week' when the calculated sFlt-1/PlGF ratio was < 38. 'Rule-in PE' decision alerting to the disease was applied for the samples exhibiting sFlt-1/PlGF ratio >85 when drawn before 34 gestational weeks; and > 110 when drawn from 34 gestational weeks onwards. Values of the sFlt-1/PlGF within these thresholds (38-85/110) were considered as inconclusive.[13]

Statistical analysis

Summary estimates of the data (median, minimum - maximum; mean ± standard deviation) were calculated and all statistical tests were implemented using the STATA software ver. 13.1 (StataCorp TX, USA). For the correct assignment of small-for-gestational-age (SGA) diagnosis, the fetal growth calculator based on INTERGROWTH-21st Project was applied to convert the newborn birth weight into gestational age and sex-adjusted z-scores.[14] A newborn was categorized as SGA in case the z-score for sex-and gestational age adjusted birthweight was lower than -1. To compare groups Wilcoxon rank-sum test was used for continuous variables and Chi^2 test for categorical variables. Statistical significance was defined as P<0.05.

Results

Characteristics of the sample set selected from the 'Happy Pregnancy' cohort

The study group comprised of 215 pregnancy cases (9.5%) recruited to the 'Happy Pregnancy' cohort, including 29 PE, 22 isolated GH/proteinuria and 164 uncomplicated gestations. To maximize the number of analyzed cases of each pregnancy scenario, the study group was enriched for the patients diagnosed with PE (13.5 *vs* 2.7% compared to the full cohort and GH/proteinuria during the index pregnancy (10.2% *vs* 3.1%; **Table 1**). In the analyzed sample set, compared to control group, PE subgroup included a higher proportion of women with the first pregnancy (79.3% versus 50.6%, $P = 3.0\times10^{-3}$) and multiparous women with PE/GH in their previous pregnancy (17% vs 14.6%, $P = 1.5\times10^{-2}$). Patients with GH/proteinuria exhibited higher BMI (mean 28.6 vs 24.6 kg/m^2, $P = 1.2\times10^{-3}$) and were diagnosed with gestational diabetes more often (22.7% vs 7.3% $P = 1.5\times10^{-2}$). There was no statististical in maternal age and the proportion of pregnancies concieved by *in vitro* fertilization procedure between the study subgroups.

The serum samples with reported sFlt-1/PlGF result (n=247) were categorized as either 'symptomatic' (n=31) or 'asymptomatic' (n=216) depending on the presence or absence of PE symptoms at the blood draw or up to 4 days (**Table 2**).

Table 2. Clinical characteristics of 247 serum samples from asymptomatic and symptomatic pregnant women stratified by sFlt-1/PlGF.

Parameter	Full group	Rule-out subgroup sFlt-1/PlGF < 38	Inconclusive subgroup sFlt-1/PlGF 38 – 85 (<34 g.w.) sFlt-1/PlGF 38 – 110 (≥34 g.w.)	Rule-in subgroup sFlt-1/PlGF > 85 (<34 g.w.) sFlt-1/PlGF > 110 (≥34 g.w.)
Samples from asymptomatic pregnant women at blood draw				
Serum samples (n; % full group)	216 (100%)	171 (79%)	25 (12%)	20 (9%)
Gestational age at sampling (days)	230 (180 – 291) 233.5 ± 20.6	229 (182 – 280)* 230.4 ± 18.3	246 (215 – 282)* 247.7 ± 20.6	239.5 (180 – 291) 242.3 ± 29.3
Interval between sampling and delivery (days)	45 (1 – 104) 43.9 ± 21.6	50 (1 – 104)*† 49.3 ± 19.3	28 (1 – 65)*‡ 28.8 ± 18.6	15.5 (1 – 54) †‡ 17.4 ± 14.2
Gestational age at delivery (days)	280 (199 – 195) 277.4 ± 12.6	282 (247 – 295)† 279.7 ± 8.9	277 (258 – 294)‡ 276.5 ± 9.9	263.5 (199 – 292) †‡ 259.7 ± 24.4
Caesarean section (n, %)	46 (21.3%)	24 (14.0%)*†	8 (32.0%)*‡	14 (70.2%)†‡
PE diagnosis later (n, %)	27 (12.5%)	11 (6.4%)*†	5 (20.0%)*‡	11 (55.0%)†‡
Time to PE diagnosis if applicable (days)	29 (5 – 41) 30.5 ± 16.4	37 (16 – 68)† 38.8 ± 13.9	36.5 (16 – 60‡ 34.8 ± 15.6	15 (5 – 28)†‡ 15.9 ± 6.9
PE complicated by SGA (n, %)[b]	6 (2.8%)	1 (0.6%)	2 (8.0%)	3 (15.0%)
Isolated SGA	7 (3.2%)	3 (1.8%)†	1 (4%)	3 (15%)†
GH/proteinuria diagnosis later (n, %)	13 (6.0%)	8 (4.7%)*	5 (20.0%)*	0
Time to GH/proteinuria diagnosis if applicable (days)	34.6 (6-58) 34.6 ± 15.4	40 (17 – 58) 39.4 ± 11.8	28 (6 – 41) 24.2 ± 17.9	N/A
Samples from women with symptoms alerting to PE (HTN or proteinuria) or with clinical diagnosis of PE at blood draw				
Serum samples (n, %)	31 (100%)	13 (42%)	9 (29%), incl. 2 PE cases	9 (29%), incl. 4 PE cases
Gestational age at sampling (days)	251.5 (194 – 279) 247.9 ± 22.0	233 (194 – 276)† 235.8 ± 20.8	256 (206 – 277) 253.6 ± 22.6	262 (240 – 279)† 261.4 ± 12.6
Interval between sampling and delivery (days)	21 (0 – 82) 24.8 ± 20.8	42 (0 – 82)*† 39.6 ± 21.4	11 (1 – 35)* 16.7 ± 12.9	6.5 (1 – 21)† 9.9 ± 8.5
Gestational age at delivery (days)	274 (235 – 291) 272.7 ± 12.4	275 (256 – 291) 275.4 ± 11.2	274 (235 – 285) 270.2 ± 15.0	270.5 (254 – 286) 271.3 ± 11.8
Caesarean section (n, %)	12 (38.7%)	5 (38.5%)	3 (33.3%)	4 (33.3%)
Immediate or later diagnosis of PE (n, %)	15 (48.4%)	2 (15.4%)§*†	6 (66.7%)*	7 (77.8%)†
Time to PE diagnosis in GH/proteinuria cases (days)	27 (1 – 62) 23.1 ± 19.5	45 (28 – 62)† 45 ± 24.0	17.5 (1 – 29) 16.25 ± 13.9	15 (1 – 37)† 17.7 ± 18.1
GH/PTN diagnosis (n, %)	16 (51.6%)	11 (84.6%)†	3 (33.3%)‡	2 (22.2%)†‡
PE complicated by SGA (n, %)	3 (9.7%)	0	1 (11.1%)	2 (22.2%)

Data are given as either median (minimum - maximum)/mean ± standard deviation or number (%) for the continuous or categorical variables, respectively. Parameter distributions between subgroups were compared using Chi^2 for categorical variables or Wilcoxon rank-sum test for continuous variables.

'Rule-in PE' based on sFlt-1/PlGF >85 before 34 gestational weeks and sFlt-1/PlGF >110 after 34 gestational weeks; 'Rule-out PE' for 1 week, sFlt-1/PlGF <38; Inconclusive, neither 'rule-in' nor 'rule-out PE' based on the sFlt-1/PlGF value (38≤sFlt-1/PlGF≤85/110) (Stepan *et al*, 2015).[13]

Statistically significant differences (p<0.05) between the subgroups are indicated as following: *Rule-out vs Inconclusive; † Rule-out vs Rule-in; ‡ Inconclusive vs Rule-in.

§ Two consecutive serum samples from the same patient.

For the assignement of small-for-gestational-age (SGA) diagnosis, the fetal growth calculator based on INTERGROWTH-21st Project was applied to convert the newborn birth weight into gestational age and sex-adjusted z-scores.[14] Newborn was categorized as SGA in case the Z-score for sex-and gestational age adjusted birthweight was lower than -1.

BMI, Body mass index; GH, gestational hypertension; g.w., gestational week; HTN, hypertention; PE, preeclampsia; PlGF, placental growth factor; sFlt-1, soluble fmf-like tyrosine kinase-1.

Compared to the asymptomatic group, samples from symptomatic cases had been drawn later in gestation (247.9 ± 22.0 *vs* 233.5 ± 20.6 gestational days; $P = 5\times10^{-4}$) and exhibited a shorter interval between sampling and delivery (24.8 ± 20.8 *vs* 43.9 ± 21.6 gestational days; $P = 1.4\times10^{-5}$). Symptomatic cases presented a higher proportion of later diagnosis of PE (48.4 *vs* 12.5%; $P = 6.6\times10^{-7}$) and GH/proteinuria (51.6 vs 6.0%, $P = 1.1\times10^{-9}$). They also delivered at an earlier gestational age (272.7 ± 12.4 *vs* 277.4 ± 12.6 days; $P = 1.2\times10^{-2}$) and more frequently by C-section (38.7 *vs* 21.3%, $P = 2.8\times10^{-2}$).

Prognostic yield of sFlt-1/PlGF test in symptomatic and asymptomatic pregnancies

The profile of the sFlt-1/PlGF test outcome differed between the samples drawn from symptomatic and asymptomatic patients (**Figure 1, Table 2**). Although 'Rule-out PE' was the most prevalent sFlt-1/PlGF test outcome in both groups, it was far more frequent assignment among asymptomatic patients (79 vs 42%, $P = 8.7\times10^{-6}$), whereas outcome category 'Rule-in PE' was enriched among symptomatic cases (9% vs 29%, $P = 4.0\times10^{-3}$). The 'Rule-out' cut off sFlt-1/PlGF ratio <38 resulted in the estimated negative predictive value (NPV) >99% for up to four weeks for both, symptomatic and asymptomatic patients (**Table 3**).

Table 3. Value of sFlt-1/PlGF test in predicting of preeclampsia.

	Diagnosis of preeclampsia among asymptomatic (n=216) and symptomatic (n=31) pregnant women*					
	Within 7 days		Within 4 weeks		Overall incidence until term	
Statistic	Asymptomatic	Symptomatic	Asymptomatic	Symptomatic	Asymptomatic	Symptomatic
sFlt-1/PlGF ratio at sampling >85/110						
DR %	1/1	5/8	10/12	6/12	11/27	7/15
	100 (5.4 – 100.0)	63 (24.5 – 91.5)	83 (50.1 – 97.1)	50 (21.1 – 78.9)	41 (23.0 – 61.0)	47 (21.3 – 73.4)
FPR %	19/215	4/23	10/204	3/19	9/189	2/16
	9 (5.5 – 13.7)	17 (5.0 – 38.8)	5 (2.6 – 9.1)	14(2.9 – 34.9)	5 (2.3 – 9.1)	13 (2.2 – 39.6)
PPV %	1/20	5/9	10/20	6/9	11/20	7/15
	5 (3.3 – 7.5)	56 (30.6 – 78.0)	50 (27.9 – 72.1)	67 (38.0 – 86.7)	55 (32.0 – 76.2)	78 (46.2 – 93.5)
NPV %	196/196	19/22	194/196	16/22	180/196	14/22
	100 (98.0 – 100.0)	86 (71.7 – 94.1)	98 (96.0 – 99.8)	73 (59.5 – 82.9)	92 (86.9 – 95.1)	63 (51.3 – 74.4)
sFlt-1/PlGF ratio at sampling ≥ 38						
DR %	1/1	9/9	11/12	12/12	16/27	13/15
	100 (5.5 - 100.0)	100 (62.9 – 100.0)	92 (59.8 – 99.6)	100 (73.5 – 100.0)	59 (39.0 – 77.0)	87 (59.5 – 98.3)
FPR %	44/215	9/22	34/204	6/19	29/189	5/16
	20 (15.5 – 27.0)	41 (21.5 – 63.3)	17 (12.7 – 23.0)	32 (13.6 – 56.5)	15 (10.7 – 21.5)	31 (12.1 – 58.5)
PPV %	1/45	9/18	11/45	12/18	16/45	13/18
	2 (0.1 – 13.2)	50 (26.8 – 73.2)	24 (13.4 – 39.9)	67 (50.8 – 79.5)	36 (22.3 – 51.3)	72 (55.0 – 84.7)
NPV %	171/171	13/13	170/171	13/13	160/171	11/13
	100 (97.2 – 100.0)	100 (71.7 – 100.0)	99 (96.3 – 100.0)	100 (71.7 – 100.0)	94 (88.5 – 96.6)	84 (59.2 – 95.4)

* 'Rule-in for PE' decision was assigned for the samples exhibiting sFlt-1/PlGF ratio >85 when drawn before 34 gestational week; and > 110 when drawn from 34 gestational week onwards. (Stepan *et al*, 2015).[13] sFlt-1/PlGF ratio ≥ 38, but ≤ 85/110 is considered inconclusive for both rule-in and rule-out PE. Symptomatic cases exhibited hypertension and/or proteinuria at sampling or up to 4 days. Asymptomatic cases had no PE-alerting symptoms at blood draw.

Detection rate (DR) was defined as the proportion of true positives among PE cases. False positive rate (FPR) was defined as ratio of false positives among controls. Positive predictive value (PPV) was calculated as ratio of true positives of all positives in the test outcome. Negative predictive value (NPV) was calculated as ratio of true negatives of all negatives in the test outcome. Data are given as n/N, % (95% CI). CI, confidence interval; PlGF, placental growth factor; sFlt-1, soluble fmf-like tyrosine kinase-1

However, one symptomatic and 10 asymptomatic patients (represented by 13 samples) with the sFlt-1/PlGF ratio <38 developed PE in 26-68 days after blood draw (**Table 2,** Table S3, **Additional file 8**). Among 29 sera assigned to the 'Rule-in PE' category (sFlt-1/PlGF >85/110), only 18 pregnancies (62.1%) eventually developed PE during the index gestation (**Figure 1**, **Table 2**). False positive 'Rule-in PE' was assigned to 11 cases, 2/31 (6.5%) and 9/216 (4.2%) samples from symptomatic and asymptomatic cases, respectively (**Table 3)**. In false-positive cases the serum samples were taken at 210–291 gestational days, and the patients delivered within 1–54 days after sampling either vaginally (n=7) or by C-section (n=4) (Table S4, **Additional file 9**). The estimated positive predictive value (PPV) for 'Rule-in PE' in next 28 days for asymptomatic cases was 50% and until term 55%, whereas for symptomatic cases it was 67% and 78%,

respectively. The overall detection rate (DR) to predict the onset PE during four weeks was 83% for asymptomatic and 50% for symptomatic pregnancies. When extending the period until delivery, the DRs were 41% and 47%, respectively (**Table 3**).

'Inconclusive' test results (38≤sFlt-1/PlGF≤85/110) were assigned to nine of 31 samples (29%) drawn from symptomatic patients compared to 12% (25/221) in asymptomatic cases ($P = 8.0\times10^{-3}$) (**Figure 1, Table 2**). Among the nine symptomatic patients, six cases (67%) either already had the clinical diagnosis (n=2) or developed PE 1-29 days later (n=4). Only 20% (25/221) of the 'Inconclusive' category samples representing asymptomatic cases had been drawn from pregnancies with a later onset of PE. When exploring the application of sFlt-1/PlGF ratio ≥38 to predict manifestation of PE for up to 4 weeks, the DR was nearly maximum, 100% for women with and 92% without PE-alerting symptoms (**Table 3**). However, introduction of this non-stringent cut-off would result in a high false positive rate (FPR). Among symptomatic pregnancies, the estimated FPR for the PE prediction during the next week was 41% and for the next month 32% (**Table 3**). Respective FPRs among asymptomatic women were 20% and 17%.

The test performance depends on the dynamics of sFlt-1 and PlGF across gestation

With advancing gestational age, a general trend towards increased serum sFlt-1, decreased PlGF and consequently, higher sFlt-1/PlGF estimates was observed in all investigated pregnancy outcomes (**Figure 2A-C)**. A rise in sFlt-1/PlGF ratio was detected in nearly all individual cases with two or more available consecutive serum samples, irrespective of the pregnancy scenario (n=35; **Figure 2D**). The most prominent increase in sFlt-1/PlGF ratio was detected in normal gestation near delivery (preterm *vs* term samples: 4.0 *vs* 37.8; $P = 2.9\times10^{-10}$; Table S5, **Additional file 10**). At term, there were no statistical differences in the distributions of sFlt-1 measurements between controls, preeclampsia and GH/proteinuric pregnancies. In total 11 of 38 (29%) term pregnancy samples exhibited sFlt-1/PlGF ratio >110, but only 4 of them developed PE (**Figure 2C**), underlying the limited value of this test near term.

Despite overall similar trends, the gestational dynamics of sFlt-1 and PlGF differed among the three studied pregnancy scenarios (**Table 4**). Already during 25-33rd gestational weeks, the future PE cases exhibited significantly higher sFlt-1 measurements compared to both, control pregnancies (median 2788 *vs* 1178 pg/ml; $P = 7.9\text{x}10^{-7}$) and those diagnosed later with GH/proteinuria (2788 *vs* 1290; $P = 2.9\text{x}10^{-3}$). However, there was no difference between the two non-PE groups. Interestingly, reduced serum PlGF levels were observed not only among future PE cases compared to controls (median PlGF 70 vs 311 pg/ml; $P = 1.9\text{x}10^{-8}$), but also in pregnancies developing isolated GH/proteinuria (156 *vs* 311 pg/ml; $P=9.7\text{x}10^{-3}$). It can be speculated that whereas low concentration of circulating PlGF represents a general hallmark of suboptimal pregnancy physiology, only increased sFlt-1 serum levels and consequently high sFlt-1/PlGF ratio drives the development of PE.

Table 4. Comparison of sFlt-1 and PlGF serum levels and sFlt-1/PlGF ratio within and between groups.

		sFlt-1 (pg/ml)			PlGF (pg/ml)			s-Flt-1/PlGF		
		Gestational age at sampling (weeks)								
Group	Number of samples*	25-33	34-42	*P*-value	25-33	34-42	*P*-value	25-33	34-42	*P*-value
Comparison within the diagnostic group according to gestational age										
Controls	113/63	1178 305 - 5286	1932 502 - 12030	**3.8×10^{-7}**	311 6 - 1775	146 34 - 1068	**1.0×10^{-4}**	3.8 0.4 - 535	15.9 1 – 250.6	**8.1×10^{-7}**
Preeclampsia	23/19	2788 704 - 8481	6420 617 - 11010	**2.2×10^{-2}**	70 12 - 319	56 38 - 225	9.0×10^{-1}	52 2.6 – 571.3	130.6 14.3 – 229.6	**3.1×10^{-2}**
GH/PTN	13/16	1290 454 - 6635	2640 157 - 6823	**1.4×10^{-2}**	156 53 - 474	80 8 - 380	**4.1×10^{-2}**	8.4 1 – 64.4	38.8 2.6 – 200.7	**8.5×10^{-3}**
Comparison between diagnostic groups according to gestational age (p-values)										
Preeclampsia *vs* control		**7.9×10^{-7}**	**2.0×10^{-4}**		**1.9×10^{-8}**	**2.0×10^{-4}**		**3.6×10^{-8}**	**2.4×10^{-6}**	
GH/PTN *vs* control		8.7×10^{-1}	1.9×10^{-1}		**9.7×10^{-3}**	**1.3×10^{-2}**		6.6×10^{-2}	**3.7×10^{-2}**	
Preeclampsia *vs* GH/PTN		**2.9×10^{-3}**	**8.5×10^{-3}**		**2.2×10^{-2}**	2.7×10^{-1}		**1.1×10^{-2}**	**1.7×10^{-2}**	

*Number of samples at 25+0 – 33+6 gestational week /at 34+0 – 42+0 gestational week

Data are given as median, minimum-maximum. Parameter distributions between groups were compared using Wilcoxon rank-sum test.

sFlt-1, soluble fmf-like tyrosine kinase-1; PlGF, placental growth factor; GH, gestational hypertension; PTN, proteinuria

Challenges in applying sFlt-1/PlGF test outcome in clinical decision-making

Although in several clinical cases the sFlt-1/PlGF test has a clear benefit in clinical decision making and prediction of PE (Cases A-C, **Table 5**), there is a challenge to handle inconclusive and false-positive test results in clinical routine. In one hand, an inconclusive test outcome may assist to identify the patients needing careful monitoring for early detection of PE (Case D), but it could also cause unreasonable anxiety and unnecessary visits (Case E). The most common causes behind false positive sFlt-1/PlGF test results in asymptomatic patients are isolated SGA (Case G) and closeness of delivery (Case H), especially when sampling occurred after 37th gestational weeks. Complications other than PE were noted in patients with sFlt-1/PlGF >110 near or at term such as uterine rupture (FP-4; Table S4, **Additional file 9**), fetal distress leading to emergency C-section (FP-7). High sFlt-1/PlGF ratio without development to PE could lead to earlier unnecessary interventions such as induced preterm delivery that may be not optimal for this pregnancy (Case I, **Table 5**).

Table 5. Example cases with beneficial, inconclusive or conflicting performance of sFlt-1/PlGF test in a clinical setting

	At recruitment			At sampling				Pregnancy outcome				
Case	Age (yrs)	BMI	Par	Time (g.d)	Sympt	sFlt-1/ PlGF	Result for PE	Type	PE (g.d.)	Delivery (g.d.)	BW (gram)	Comment
Beneficial performance in the clinical setting: test predicts pregnancy outcome, supportive in decision making												
A	23	19.4	primi	259	PE	167.0	Rule-in	PE, SGA	258	263	2438	Confirmed diagnosis
B	34	34.9	primi	228	HTN	3.6	Rule-out	GH, GDM	n.a.	274	4112	Correct and useful prediction
C	22	23.7	primi	180	No	571.3	Rule-in	ECL, SGA	199	199	816	Correct and useful prediction
Inconclusive performance in the clinical setting: close clinical surveillance and repeated testing, outcome may vary												
D	29	37.6	primi	206	HTN	65.8	Inconcl	PE	231	232	2044	Alert for careful monitoring
F	33	33.7	multi	240	No	65.3	Inconcl	none	n.a.	275	3614	Unreasonable anxiety to the patient
Conflicting performance in the clinical setting: rely on symptoms, not supprotive in clinical decision making												
G/ FP-1*	35	27.5	multi	210	No	535.0	Rule-in	SGA	n.a.	263	1,926	High sFlt-1/PlGF possibly due to uteroplacental dysfunction
H/ FP-9*	31	22.4	multi	291	No	250.6	Rule-in	none	n.a.	292	3,706	High sFlt-1/PlGF possibly due to overdue (41 weeks+4 days) and closeness of labour
I	33	32.0	primi	223 245	HTN HTN	121.2 157.1	Rule-in Rule-in	PE (mild sympt)	268	274	2,968	Clinical PE diagnosis only at term. sFlt-1/PlGF test alerted to the need for unnecessary earlier induction of labour

'Rule-in PE' based on sFlt-1/PlGF >85 before 34 gestational weeks and sFlt-1/PlGF >110 after 34 gestational weeks; 'Rule-out PE' for 1 week, sFlt-1/PlGF <38; Inconclusive, neither 'rule-in' nor 'rule-out PE' based on the sFlt-1/PlGF value (38≤sFlt-1/PlGF≤85/110) (Stepan *et al*, 2015).[13]

*code after slash refers to the same patient in **Table S4**

BMI, pre-pregnancy body mass index; BW, birthweight; g.d., gestational days; GH, gestational hypertension; GDM, gestational diabetes mellitus; HTN, hypertensio; Inconcl, inconclusive; ECL, eclampsia; n.a. not applicable; Par, parity = number of previous births; PE, preeclampsia; primi, primiparous; multi, multiparous; SGA, small-for-gestational-age newborn; symp, symptoms; yrs, years

Discussion

The study aimed to evaluate the applicability and limitations of the sFlt-1/PlGF test in a clinical setting in Estonia for the prediction of PE among symptomatic and asymptomatic women during the third trimester of pregnancy. The study analyzed 216 serum samples drawn from pregnant women without any signs alerting to PE after 26th g. week and compared the performance of the test with patients presenting newly onset hypertension or proteinuria. Additionally, analysis of longitudinal serum samples drawn from 2-3 time points during 180-291 g. days allowed to evaluate the natural gestational dynamics of maternal serum sFlt-1 and PlGF. The analyzed samples were taken during routine clinical setting, but the sFlt-1/PlGF testing was performed after the delivery. The availability of detailed clinical information on further pregnancy course allowed to assess putative errors in clinical decision making provided the test results would have been available during the pregnancy.

Consistent with previous reports, the study confirmed a high NPV (>99%) for the sFlt-1/PlGF<38 estimate to rule-out PE for up to two weeks in the presence or absence of PE symptoms.[5,15,16,17] In our study, 13 samples resulting sFlt-1/PlGF<38 but drawn from cases who later developed PE, the period between sampling and PE onset exceeded 16 days.

Elevated estimate of sFlt-1/PlGF often resulted in PE in symptomatic women, but at the expense of high FPR. Although cut-off ≥38 could identify correctly all cases with the PE onset in four weeks, almost every second or third woman with isolated HTN or proteinuria would be falsely classified as a possible PE pregnancy. However, when limiting PE diagnosis only with values exceeding the diagnostic cut-off (sFlt-1/PlGF>85/110), based on out data, FPR can be reduced to 17% and 14% in one and four weeks, respectively, but may fail to confirm the clinical diagnoses of PE in a third of the patients.

All false positive samples (sFlt-1/PlGF >85/110) from asymptomatic patients observed in our study were taken after 33 g. weeks, most of them at term. The study data showed that a normal rise in the sFlt-1/PlGF ratio with advancing gestation limits the diagnostic accuracy of the test already after 33-34 gestational weeks and especially close to delivery. Increasing sFlt-1/PlGF ratio towards the end of pregnancy has been also reported before.[6,18] In our study, only four of 11 term pregnancy samples exhibiting sFlt-1/PlGF ratio >85/110, developed further to PE. As most PE cases manifest at term, a clearly interpretable diagnostic/predictive test for this time window is especially warranted.[19] As a solution, inclusion of additional biomarkers has been suggested to improve the prediction accuracy, especially close to term.[20,21]

Another scenario behind increased sFlt-1/PlGF ratio in asymptomatic women is fetal growth restriction (FGR) and other placental syndromes, such as premature birth, intrauterine fetal death, abruption of placenta etc.[22,23] Remarkable proportion of adverse maternal and neonatal outcomes among patients with false positive test for PE taken at mid-pregnancy has also been pointed out by Black et al.[24] In concordance, more than third of false positive cases in our study suffered from SGA or fetal distress.

Apart from PE prediction sFlt/PlGF ratio test could be also useful pointing out the pregnant women increased risk for other placenta related adverse outcome. However, the main challenge for sFlt/PlGF ratio test as a predictive tool remains the timing of the test since the prediction time for PE is limited up to 4 weeks and the usefulness of the test is starting to decrease after 34 weeks due to physiological increase in sFlt/PlGF ratio.

It has been proposed that sFlt-1 alters normal endothelial cells by hindering the angiogenetic effect of PlGF.[6,25] We observed low PlGF both among patients with isolated GH/proteinuria and PE, but the level of sFlt-1 before 34 gestational weeks was higher only in cases destined to develop PE (**Table 4).** It can be speculated that lower sFlt-1 level prevents from generalized endothelial damage leading to manifestation of PE. Our finding is in line with several other studies.[26,27,28] However, opposite results have been demonstrated.[29,30] The physiological increase of sFlt-1 from 34 g. weeks onwards but constantly low PlGF due to placental stress shift the sFlt-1/PlGF ratio among patients with isolated GH/proteinuria and the discrimination these cases from PE may be more complicated.

Our study had also some limitations. The applied methodological approach is restricted by the the lack of platform-specific validated clinical cut-off values for the BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio test. The currently applied cut-off values have been validated for the Elecsys® assay (Roche Diagnostics, Mannheim, Germany). Although several studies have shown highly comparable results in clinical performance of Kryptor and Elescys®, a recent study has raised a concern that the Kryptor test measures PlGF concentrations ~20% lower that Elescys® assay.[31,32,33] Consequently, this results in a higher sFlt-1/PlGF estimates and, therefore may increase FPR for PE prediction when applying the Elescys®-platform based cut-off values.

Conclusions

Despite a relatively small number of samples analysed our study, we could replicate the major findings from larger studies.[5,15,16,17] sFlt-1/PlGF ratio estimated for the third trimester serum measurements is highly reliable for the exclusion of PE for at least two weeks. However, elevated sFlt-1/PlGF level could not be currently considered as an essential part for the diagnosis nor prediction of PE. Although the recommended diagnostic cut-off often predicted PE development, the presence of PE alerting symptom(s), SGA or additional placental pathologies as well as advanced gestational age at sampling must be considered in clinical decision making. Clearer guidelines for the clinical practice, e.g. timing of the test, further management, clear cut-offs for different platforms, are needed.

Abbreviations

FPR – false positive rate

GH – gestational hypertension

gw – gestational weeks

PE – preeclampsia

PlGF – placental growth factor

sFlt-1 – soluble fms-like tyrosine kinase

SGA – small for gestational age

Declarations

Ethics approval and consent to participate: The project has been approved by the Ethics Committee of Human Research of the University Clinic of Tartu, Estonia (permissions no. 221/T-6, 17.12.2012 and 286/M-18, 15.10.2018) and was carried out in compliance with the Helsinki Declaration. A written informed consent to participate in the study was obtained from each individual prior to recruitment.

Consent for publication: not applicable

Availability of data and materials: All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests: The authors declare no competing interest.

Funding: This work was supported by the Estonian Research Council grants (IUT34-12 and PRG1021; ML) and EU European Regional Development Fund (project 3.2.0701.12-0047; ML, KR).

Authors' contributions: EH, KrR, PV and PT contributed to the recruitment of the patients and collection of medical data. KaR was involved in handling the serum samples. EH, KrR and ML analysed the data and drafted the final manuscript. All authors have read and approved the final version to be published.

Acknowledgements: We thank the HAPPY PREGNANCY study participants. The clinical and laboratory personnel at the Women's Clinic and United Laboratories of Tartu University Hospital and Human Genetics Research group, University of Tartu are acknowledged for the assistance in recruitment, phenotyping, sample logistics and aliquoting.

References

1. Tranquilli AL, Dekker G, Magee L, Roberts J, Sibai BM, Steyn W et al. The classification, diagnosis and management of the hypertensive disorders of pregnancy: a revised statement from the ISSHP. *Pregnancy Hypertens* 2014; 4: 97-104
2. Poon LC, Shennan A, Hyett JA, Kapur A, Hadar E, DivakarH, et al. The International Federation of Gynecology and Obstetrics (FIGO) initiative on pre-eclampsia: A pragmatic guide for first-trimester screening and prevention. *Int J Gynecol Obstet* 2019; 145: 1-33
3. American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet Gynecol* 2013; 122: 1122-1131
4. National Institute for Health and Care Excellence. Hypertension in pregnancy: diagnosis and management. NICE guideline [NG133]. Published date: June 2019. Available from: https://www.nice.org.uk/guidance/ng133. Date of access 23.11.2019.
5. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennström M, et al. Predictive Value of the sFlt-1:PlGF Ratio in Women with Suspected Preeclampsia. *N Engl J Med* 2016; 374: 13-22
6. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* 2004; 350: 672-683
7. Kumasawa K, Iriyama T, Nagamatsu T, Osuga Y, Fujii T. Pravastatin for preeclampsia: From animal to human. *J Obstet Gynaecol Res*; 2020 Aug; 46(8):1255-1262

8. Verlohren S, Herraiz I, Lapaire O, Schlembach D, Zeisler H, Calda P, et al. New gestational phase–specific cutoff values for the use of the soluble fms-like tyrosine kinase-1/placental growth factor ratio as a diagnostic test for preeclampsia. *Hypertension* 2014; 63(2): 346–352
9. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al on behalf of ISSHP. Hypertensive Disorders of Pregnancy. ISSHP Classification, Diagnosis, and Management Recommendations for International Practice. *Hypertension* 2018; 72: 24-43
10. Vaas P, Rull K, Põllumaa S, Kirss A, Meigas D. Guideline for antenatal care (Raseduse jälgimise juhend). Estonian Gynaecologists Society 2011;v4. Estonian. Available from: https://www.ens.ee/ravijuhendid. Date of access 23.06.2019.
11. Cowans NJ, Alfthan H, Stenman UH, Spencer K. Stability of first trimester placental growth factor in serum and whole blood.*Prenat Diagn* 2011; 31: 1193-1197
12. Wa Law L, Sahota DS, Wai Chan L, Chen M, Kin Lau T, Leung TY. Effect of long-term storage on placental growth factor and fms-like tyrosine kinase 1 measurements in samples from pregnant women. *J Matern Fetal Neonatal Med* 2010; 23: 1475-80
13. Stepan H, Herraiz I, Schlembach D, Verlohren S, Brennecke S, Chantraine F, et al. Implementation of the sFlt-1/PlGF ratio for prediction and diagnosis of pre-eclampsia in singleton pregnancy: implications for clinical practice. *Ultrasound Obstet Gynecol*2015; 45: 241-246
14. Villar J, Papageorghiou AT, Pang R, Ohuma EO, Ismail LC, Barros, F. The likeness of fetal growth and new-born size across non-isolated populations in the INTERGROWTH-21st Project: The Fetal Growth Longitudinal Study and Newborn Cross-Sectional Study. *Lancet* 2014; 2(10): 781-792 Available from: https://intergrowth21.tghn.org/fetal-growth/#fg1. Date of access 1.11.2018.
15. Zeisler H, Llurba E, Chantraine F. J, Vatish M, Staff A. C, Sennström et al. Soluble fms-like tyrosine kinase-1 to placental growth factor ratio: ruling out pre-eclampsia for up to 4 weeks and value of retesting. *Ultrasound Obstet Gynecol*2019; 53: 367-375
16. Dragan I, Georgiou T, Prodan N, Akolekar R, Nicolaides KH. Screening for pre-eclampsia using sFlt-1/PlGF ratio cut-off of 38 at 30-37 weeks' gestation. *Ultrasound Obstet Gynecol* 2017; 49: 73-77
17. Sovio U, Gaccioli F, Cook E, Hund M, Charnock-Jones DS, Smith GC. Prediction of preeclampsia using the soluble fms-like tyrosine kinase 1 to placental growth factor ratio. A prospective cohort study of unselected nulliparous women. *Hypertension* 2017; 69: 731-738
18. Dragan I, Wright D, Fiolna M, Leipold G, Nicolaides K. H. Development of pre-eclampsia within 4 weeks of sFlt-1/PlGF ratio>38: comparison of performance at 31–34 vs 35–37 weeks' gestation. *Ultrasound Obstet Gynecol* 2017; 49: 209-212
19. Kenny LC, Black MA, Poston L, Taylor, Myers RT, Baker PN, et al. Early pregnancy prediction of preeclampsia in nulliparous women, combining clinical risk and biomarkers. *Hypertension* 2014; 64: 644-652
20. Andrietti S, Silva M, Wright A, Wright D, Nicolaides KH. Competing-risks model in screening for pre-eclampsia by maternal factors and biomarkers at 35-37 weeks' gestation. *Ultrasound Obstet Gynecol* 2016; 48: 72-79.
21. Ratnik K, Rull K, Hanson E, Kisand K, Laan M. Single-tube multimarker 6PLEX assay for estimating the risk to develop preeclampsia. *J Appl Lab Med*, (in press)
22. Herraiz I, Quezada MS, Rodriguez-Calvo J, Gómez-Montes E, Villalaín, Galindo A. Longitudinal change of sFlt-1/PlGF ratio in singleton pregnancy with early-onset fetal growth restriction. Ultrasound Obstet Gynecol 2018; 52: 631-638
23. Staff AC. The two-stage placental model of preeclampsia: An update. *J Reprod Immunol* 2019; 134-135: 1-10
24. Black C, Al-Amin A, Rolnik DL, Kane SC, Stolarek C, White A et al. Midpregnancy testing for soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF): An inter-assay comparison of three automated immunoassay platforms. *Placenta* 2019; 86: 11-14
25. Redman CW, Staff AC. Preeclampsia, biomarkers, syncytiotrophoblast stress, and placental capacity. *Am J Obstet Gynecol* 2015; 213: S9.e1, S9–11
26. Holston AM, Qian C, Yu KF, Epstein FH, Karumanchi SA, Levine RJ. Circulating angiogenic factors in gestational proteinuria without hypertension. *Am J Obstet Gynecol* 2009; 200: 392.e1-10
27. Noori M, Donald AE, Angelakopoulou A, Hingorani AD, Williams DJ. Prospective study of placental angiogenic factors and maternal vascular function before and after preeclampsia and gestational hypertension. *Circulation* 2010; 122: 478-487
28. Verlohren S, Herraiz I, Lapaire O, Schlembach D, Moertl M, et al. The sFlt-1/PlGF ratio in different types of hypertensive pregnancy disorders and its prognostic potential in preeclamptic patients. *Am J Obstet Gynecol*2012; 206: 58.e1-8

29. Salahuddin S, Lee Y, Vadnais M, Sachs BP, Karumanchi SA, Lim KH. Diagnostic utility of soluble fms-like tyrosine kinase 1 and soluble endoglin in hypertensive diseases of pregnancy. *Am J Obstet Gynecol* 2007; 197: 28.e1-6
30. Leaños-Miranda A1, Méndez-Aguilar F, Ramírez-Valenzuela KL, Serrano-Rodríguez M, Berumen-Lechuga G, Molina-Pérez CJ, et al. Circulating angiogenic factors are related to the severity of gestational hypertension and preeclampsia, and their adverse outcomes. *Medicine (Baltimore)*.2017; 96: e6005.
31. Van Helden J, Weiskirchen R. Analytical evaluation of the novel soluble fms-like tyrosine kinase 1 and placenta growth factor assays for the diagnosis of preeclampsia. *Clin Biochem* 2015; 48: 1113-1119
32. Dröge LA, Höller A, Ehrlich L, Verlohren S, Heinrich W, Perschel FH. Diagnosis of preeclampsia and fetal frowth restriction with sFlt-1/PlGF ratio: Diagnostic accuracy of the automated immunoassay Kryptor. *Pregnancy Hypertens* 2017; 8: 31–36
33. Stepan H, Hund M, Dilba P, Sillman J, Schlembach D. Elecsys® and Kryptor immunoassays for the measurement of sFlt-1 and PlGF to aid preeclampsia diagnosis: are they comparable? *Clin Chem Lab Med* 2019; 57:1339-1348

Figures

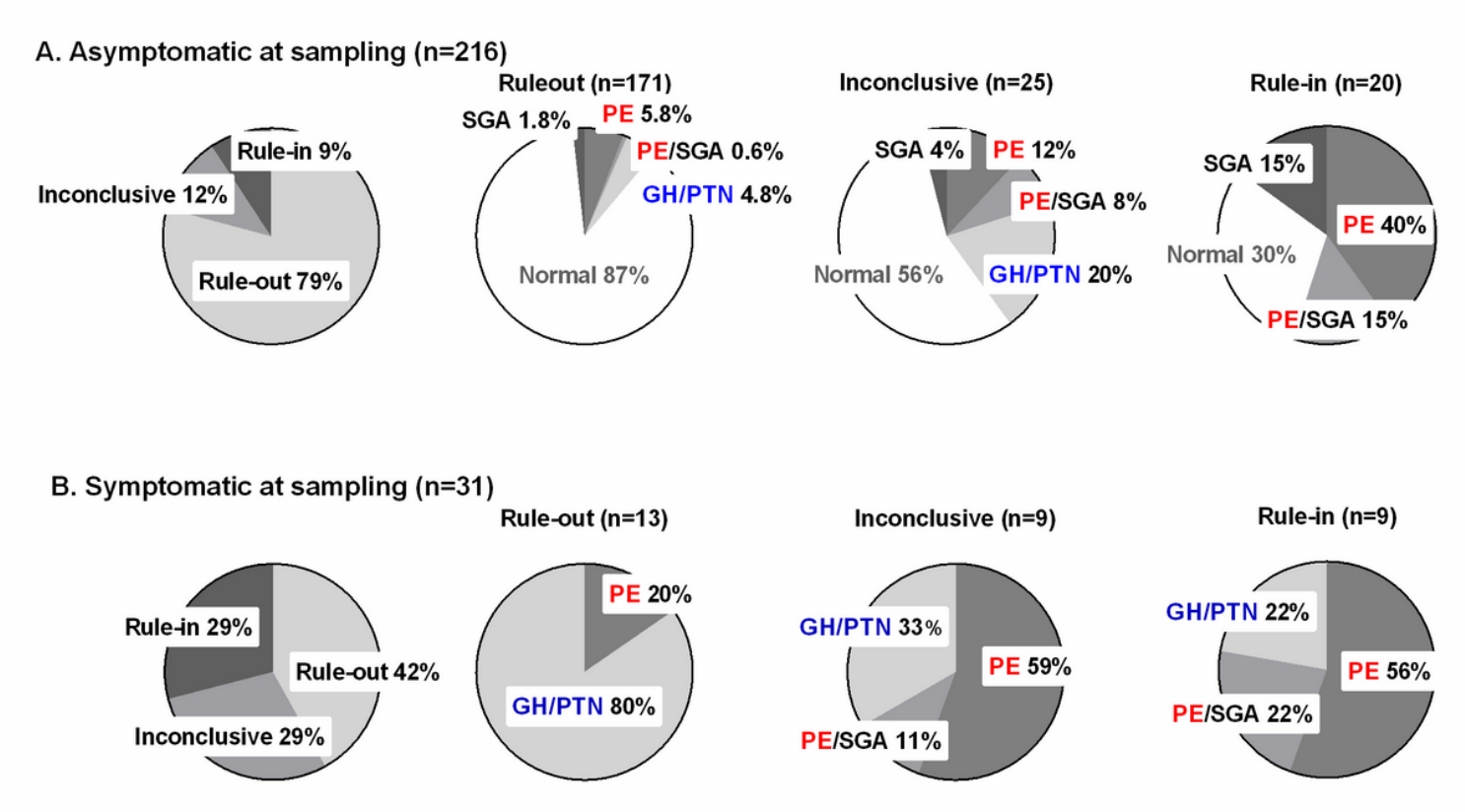

Figure 1

Spectrum of the outcomes of sFlt-1/PlGF test and pregnancy courses in the study group. Serum samples stratified based on the sFlt-1/PlGF test result and pregnancy outcome for the (A) asymptomatic pregnant women (n= 216 samples) at sampling and (B) patients with symptoms alerting to preeclampsia (PE; n=31) at or within 4 days of blood draw. Alerting symptoms of PE were considered either hypertension (HTN) or proteinuria. SGA refers to the pregnancy delivering a small-for-gestational-age newborn categorized as SGA in case the Z-score for sex-and gestational age adjusted birthweight was lower than -1 according to fetal growth calculator based on INTERGROWTH 21st Project.14 The applied PE prediction criteria were: 'Rule-out PE' (sFlt-1/PlGF <38), 'Rule-in PE' (sFlt-1/PlGF >85 before 34 gestational weeks and >110 after 34 gestational weeks) and inconclusive (sFlt-1/PlGF ratio 38-85 before 34 gestational weeks and ≤110 after 34 gestational weeks) as recommended by Stepan et al, 2015).13

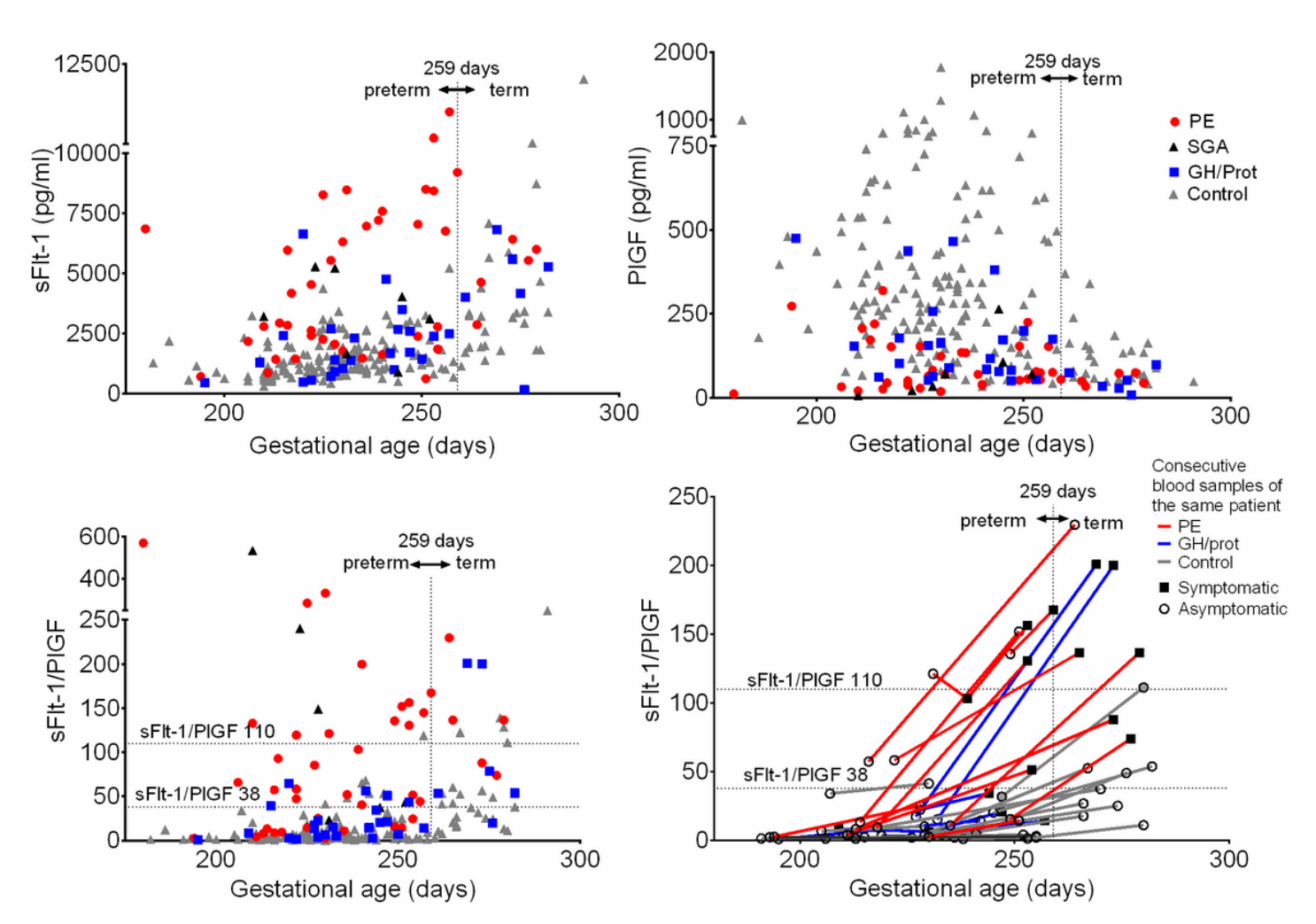

Figure 2

sFlt-1/PlGF test outcome in the study group plotted against the gestational age at sampling. Serum sample measurements of (A) sFlt-1 and (B) PlGF, and (C) the estimated sFlt-1/PlGF ratio in cases, who eventually developed preeclampsia (PE) are shown in red circles, pregnancies with the later diagnosis of gestational hypertension/proteinuria (GH/PTN) in blue squares. Gestations without maternal complications but ending with the delivery of a small-for-gestational-age newborn (SGA) are marked with black triangel. Data representing control samples from healthy pregnancies are presented with grey triangles. The dynamics of the sFlt-1/PlGF ratio across 35 pregnancies with serum samples available from multiple consecutive timepoints are highlighted comparatively for the cases reported as symptomatic or asymptomatic at the blood draw (D). Dashed horizontal lines represent sFlt-1/PlGF ratio cut-off thresholds for the decision of 'Rule-out' (<38) and 'Rule-in' (>110 when drawn from 34 gestational weeks onwards) for PE.13 GH diagnoses was assigned when hypertension (HTN; blood pressure ≥140/90 mmHg) was documented at more than one consecutive clinical visit. Proteinuria represents cases with protein measurements in urine exceeding 0.3 g/24h. PE was diagnosed upon simultaneous detection of HTN and proteinuria. For the assignment of small-for-gestational-age (SGA) diagnosis, the fetal growth calculator on INTERGROWTH-21st Project was applied to convert the newborn birth weight into gestational age and sex-adjusted Z-scores.14 Newborn was categorized as SGA in case the Z-score for sex-and gestational age adjusted birthweight was lower than -1. Preterm pregnancy refers to the delivery before 259 gestational days.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.docx
- Additionalfile2.pptx

- Additionalfile3.tif
- Additionalfile4.tif
- Additionalfile5.docx
- Additionalfile6.docx
- Additionalfile7.docx
- Additionalfile8.docx
- additionalfile9.docx
- Additionalfile10.docx