



Evaluation of sFlt-1/PlGF Ratio for Predicting and Improving Clinical Management of Pre-eclampsia: Experience in a Specialized Perinatal Care Center

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Background: Management of pregnant women at high risk of pre-eclampsia (PE) requires frequent monitoring, with referral to specialized perinatal care centers. Reliable tests are necessary to improve prediction of PE and related complications and to assess disease severity and progression. An imbalance in two biomarkers, soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF), is involved in PE pathogenesis. The sFlt-1 to PlGF ratio is increased in pregnant women before the onset of PE. An elevated ratio is highly predictive of PE, whereas the diagnosis of PE can be ruled out within one week for low ratios. The main objective of this study was to assess whether a low sFlt-1/PlGF ratio, below a cutoff of 38, can predict the absence of PE within one week.

Methods: We performed a prospective, monocentric, observational study to evaluate serum sFlt-1/PlGF ratio (Roche Diagnostics Cobas e411 system) for predicting PE in a group of 67 high-risk pregnant women (20–37 gestation weeks).

Results: Among the 67 patients included, 53 had a sFlt-1/PlGF ratio lower than 38; none developed subsequent PE leading to a negative predictive value of 100%. Eight patients developed clinical PE. The positive predictive value was 21% at one week and 18% at four weeks, in accordance with previous studies.

Conclusions: The serum sFlt-1/PlGF ratio showed highly predictive performances for ruling out PE. Using these biomarkers in routine management of PE may improve clinical care and avoid inappropriate hospitalization, which has a significant economic impact.

Key Words: Pre-eclampsia, Soluble fms-like tyrosine kinase 1/placental growth factor ratio, Performance

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INTRODUCTION

Pre-eclampsia (PE) is a heterogeneous disease defined by the onset of hypertension and proteinuria after 20 weeks' gestation, affecting 2–5% of pregnancies worldwide [1-3]. The clinical presentation and outcome of PE is variable, and early-onset severe and rapidly progressive PE with pre-term delivery to late-onset PE have been described [4]. PE remains an important cause of maternal and fetal/neonatal morbidity and mortality. Indeed, se-

vere and life-threatening complications such as intrauterine growth restriction (IUGR), eclampsia, and HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count) can occur [4]. Clinical management can be challenging because of the lack of effective treatment options other than delivery.

The pathogenesis of PE is multifactorial and not fully understood. However, abnormal remodeling of maternal spiral arteries is observed in women with subsequent PE, leading to placental hypoperfusion and systemic endothelial dysfunction [5]. Pla-

central dysfunction leads to altered circulating levels of pro-angiogenic or anti-angiogenic mediators, notably placental growth factor (PlGF), soluble endoglin and the soluble fms-like tyrosine kinase receptor-1 (sFlt-1), and a truncated and soluble form of vascular endothelial growth factor (VEGF) receptor, which neutralizes VEGF and PlGF. Hence, PE and its complications may occur because of an imbalance in circulating angiogenic factors [6].

Levine *et al* [7] showed that five weeks before development of clinical symptoms of PE, serum concentrations of s-Flt-1 are increased, whereas PlGF concentrations are decreased, resulting in an increased s-Flt-1/PlGF ratio. Increased sFlt-1 levels and reduced PlGF levels can be used to predict the subsequent development of PE.

While increasing data are becoming available and promising results have been observed for sFlt-1 and PlGF, soluble endoglin and VEGF have not been shown to be clinically effective for managing PE. Despite its pathophysiological implications, no specific increase in soluble endoglin in the context of PE or HELLP syndrome has been demonstrated [8]. The use of VEGF as a tool for managing PE appears to also be compromised. There are analytical difficulties in the measurement of VEGF because of the different assays available for total or free, biologically active VEGF measurement [9].

The potential relevance of determining s-Flt-1, PlGF, and the s-Flt-1/PlGF ratio for the diagnosis and prognosis of the disease has been investigated to help physicians identify patients at high risk of PE who require close monitoring. Interestingly, the sFlt-1/PlGF ratio appears to show better performance than single markers in predicting the risk of PE [10, 11]. Several strategies have been investigated for first trimester screening of women at high risk of PE to introduce, if applicable, low-dose acetylsalicylic acid and during the second trimester or later, to predict or rule out the diagnosis of PE [12].

Additionally, a major prospective clinical trial validated cut-offs for the sFlt-1/PlGF ratio for the management of PE in the PROGNOSIS study (Prediction of Short-Term Outcome in Pregnant Women with Suspected Preeclampsia Study), which clearly demonstrated that an sFlt-1/PlGF ratio of 38 or lower can be used to rule out the onset of PE within one week, independently of gestational age [13]. The negative predictive value was 99.3% [13]. Furthermore, a sFlt-1/PlGF ratio greater than 85 (for early-onset PE, <34 weeks of gestation) or 110 (for late-onset PE, ≥34 weeks) is indicative of a high risk of PE diagnosis or placenta-related disorders requiring close clinical and biological monitoring. For women with a sFlt-1/PlGF ratio between 38 and 85 or between 38 and 110, current PE can be ruled out; however, these women

carry a high risk of developing PE within four weeks [14].

Despite the simplicity of defining PE, which has recently been debated [15], several clinical situations are also relatively complex to assess in ruling out PE diagnosis: women with pre-existing nephropathy and presenting with chronic proteinuria, women with chronic pre-pregnancy hypertension and IUGR, development of hypertension without proteinuria or the opposite, and hypertension with thrombocytopenia or hepatic cytolysis without proteinuria. Because of current difficulties in diagnosing PE and conducting early identification of women at high risk of PE, other biological markers are necessary; sFlt-1 and PlGF appear to be excellent candidates in this context.

The objective of this study was to evaluate the routine use of sFlt-1, PlGF, and the sFlt-1/PlGF ratio in a specific population of high-risk patients, including multiple pregnancies, in clinical practice to improve care and prognosis for these patients.

METHODS

This mono-centric, prospective, non-interventional study was conducted from January to May 2014 in the specialized perinatal care center of the Department of Gynecology and Obstetrics in Nantes University Hospital, France. Patients did not object to taking part in the study. Written informed consent was obtained from each patient. The local ethics committee of the University Hospital of Nantes approved the study (No. RC13_0283).

1. Inclusion and exclusion criteria

All adult patients with ongoing pregnancy between 20 and 37 gestation weeks and with at least one risk factor were eligible (Table 1). Women with a confirmed diagnosis of PE at the specified gestation period were excluded from the study. During the enrollment period, 75 patients were included, and eight were excluded: seven did not meet the inclusion criteria (two for completing over 37 weeks of gestation, and five for already being diagnosed with PE) and one was excluded for medical termination of pregnancy. A total of 67 women were included in the study over 5 months.

2. Serum markers of PE

Once consent was obtained, an additional blood sample was collected during routine check-up to determine serum sFlt-1 and PlGF concentrations and calculate the sFlt-1/PlGF ratio. Blood was collected in a standard serum tube: samples were centrifuged and stored at -20°C until analysis. Serum samples were analyzed within six months of storage. sFlt-1 and PlGF con-

Table 1. Cohort distribution according to the inclusion criteria

Inclusion criteria	Repartition of the cohort
Abnormal uterine artery Doppler	20 (30%)
Multiple pregnancy	19 (28%)
BMI > 30 kg/m ²	14 (21%)
Previous pre-eclampsia	12 (18%)
Vascular intra-uterine growth restriction	12 (18%)
Thrombophilia	7 (10.5%)
Pre-existing isolated proteinuria	7 (10.5%)
Hepatic cytolysis	7 (10.5%)
Age > 40 years	6 (9%)
Nephropathy	6 (9%)
PAPP-A ≤ 0.4 MoM (1st trimester of pregnancy)	5 (7.5%)
Pre-existing hypertension	4 (6%)
Diabetes	4 (6%)
hCGβ ≥ 5 MoM (1st trimester of pregnancy)	2 (3%)
αFP or hCGβ ≥ 5 MoM (2nd trimester of pregnancy)	0

Results are shown as number and percentage.

Abbreviations: BMI, Body mass index; PAPP-A, pregnancy-associated plasma protein-A; hCGβ, human chorionic gonadotropin β; αFP, alpha-fetoprotein; MoM, multiple of the median.

centrations were measured in the Clinical Biochemistry Laboratory of Nantes University Hospital, using an electrochemiluminescence Elecsys immunoassay on a Roche Diagnostics Cobas e411 system (Roche Diagnostics, Mannheim, Germany). Each run was validated by measuring two levels of quality control material prior to starting the experiment. The reference range (5th–95th percentile) was defined by Roche Diagnostics in a cohort of 877 women ongoing normal single pregnancy from nine European centers (Germany, Spain, Austria, Czech Republic, and Switzerland) leading to 1,685 samples analyzed. PIGF and sFlt-1 assays previously demonstrated strong characteristics in terms of inter- and intra- assay precision with total imprecision CV below 5% [16].

3. Judgment criteria

The main endpoint was the occurrence of PE with hypertension (≥140/90 mmHg) and proteinuria (≥0.30 g/24 hours) and/or related complications such as HELLP syndrome or eclampsia. The secondary objective of this study was to evaluate the sensitivity, specificity, and negative and positive predictive values of sFlt-1/PIGF ratio of 38, as determined in the PROGNOSIS study, in the specific population of high-risk pregnancies.

In this high-risk population, a prevalence of 15% of PE was expected. Considering a specificity of 95% for the sFlt-1/PIGF

ratio with an accuracy of 5%, 86 inclusions were necessary. Taking into account that 2% of the biomarker results would not be analyzable, our goal was to include 88 patients to ensure the desired accuracy.

4. Statistical analysis

Data from patients' clinical histories and serum markers of PE were collected in a database using Microsoft Excel 2010 software (Microsoft Corporation, Redmond, WA, United States). All the data in this study are presented as mean ± SD. Sensitivity, specificity, and positive and negative predictive values were determined, and a contingency table was prepared. Statistical analysis according to PE status was performed using MedCalc software for Windows, version 15.0 (MedCalc Software, Ostend, Belgium). Kruskal-Wallis test was conducted to analyze continuous variables and Fisher's exact test for categorical variables. A *P* value below 0.05 was considered statistically significant.

RESULTS

1. Description of the population

A total of 67 women were included in the study over five months. All women had at least one risk factor. An abnormal uterine artery Doppler (notch or/and high pulsatility index) was the most common risk factor (N=20; 30%). Seven patients (10.5%) with pre-existing isolated proteinuria and four patients (6%) with pre-existing chronic hypertension were included. The mean age was 32 years (±5 years). Inclusion criteria and cohort distribution according to these criteria are shown in Table 1. Among the cohort of 67 patients, eight developed clinical PE: five before 34 gestation weeks, one between 34 and 37 weeks, and two after 37 weeks. The demographic and clinical characteristics of the cohort, shown in Table 2, did not significantly differ between the two groups, except for arterial blood pressure.

2. Serum markers of PE

sFlt-1 and PIGF concentrations and sFlt-1/PIGF ratio assessments are shown in Table 2. Twenty-two patients (33%) had a sFlt-1 concentration higher than the 95th percentile, with five (62%) in the pre-eclamptic group. For PIGF, eight patients (12%) had a concentration lower than the 5th percentile, with one in the PE group (12%). The mean sFlt-1/PIGF ratio was 36.5 ± 12 for the entire cohort, which was significantly higher (69 ± 13) for women who developed PE than for women without PE (32 ± 25) (*P*=0.01). Individual results for women with PE are shown in Table 3.

Table 2. Demographical data, clinical characteristics, serum sFlt-1, PIGF concentrations, and sFlt-1/PIGF ratios of patients

	Total cohort	Women with pre-eclampsia	Women without pre-eclampsia	P value
Number	67	8	59	
Age (year)	32 (±5)	32 (±3)	32 (±6)	NS
BMI (kg/m ²)	25 (±6)	24 (±5)	26 (±7)	NS
Pregnancy term on inclusion				NS
20–28 weeks' gestation	15 (22%)	4 (50%)	11 (19%)	
29–33 weeks' gestation	33 (50%)	2 (25%)	31 (52%)	
34–37 weeks' gestation	19 (28%)	2 (25%)	17 (29%)	
Smoking	14 (21%)	2 (25%)	12 (20%)	NS
Aspirin treatment	13 (19.5%)	1 (12.5%)	12 (20%)	NS
Arterial pressure on inclusion				
Systolic (mmHg)	111 (±15)	130 (±10)	115 (±14)	<0.01
Diastolic (mmHg)	72 (±12)	84 (±7)	71 (±12)	<0.01
sFlt-1 (pg/mL)	4,434 ± 499	7,044 ± 509	4,079 ± 1,652	NS
sFlt-1 >95th percentile	22 (33%)	5 (62%)	17 (29%)	NS
PIGF (pg/mL)	405 ± 41	153 ± 45	440 ± 30	NS
PIGF <5th percentile	8 (12%)	1 (12%)	7 (12%)	NS
sFlt-1/PIGF ratio	36.5 ± 12	69 ± 13	32 ± 25	0.01

Results are shown as number (percentage) or mean ± standard deviation when appropriate. Abbreviations: BMI, Body mass index; NS, Not Significant.

Table 3. Individual results of the eight patients who developed pre-eclampsia

	sFlt-1 (pg/mL)	PIGF (pg/mL)	sFlt-1/PIGF ratio	Delay of pre-eclampsia	Term of pre-eclampsia (gestation weeks+days)
Patient 1	14,011	126	111	2 days	32
Patient 2	7,256	34	213	8 days	29+6
Patient 3	10,298	175	59	8 days	32+6
Patient 4	6,806	115	59	31 days	38+5
Patient 5	4,528	335	14	55 days	30+3
Patient 6	866	136	6	86 days	36+2
Patient 7	1,396	151	9	93 days	33+2
Patient 8	11,189	150	75	10 days	37+4

3. Predictive performances

For a sFlt-1/PIGF ratio threshold of 38, 14 women exhibited a high sFlt-1/PIGF ratio (Table 4). Among them, three developed PE within less than one week (two days for one patient, eight days for two patients). The negative predictive value of the sFlt-1/PIGF ratio at one week with a threshold at 38 was 100%; the positive predictive value was 21%. Sensitivity and specificity were 100% and 83%, respectively (Table 4).

Concerning the risk of PE occurring within four weeks at the

Table 4. Predictive performance of s-Flt1/PIGF ratio

	Women with pre-eclampsia	Women without pre-eclampsia	Total
At 1 week			
s-Flt1/PIGF ratio <38	0	53	53
s-Flt1/PIGF ratio ≥38	3	11	14
Total	3	64	67
Negative predictive value		100%	
Positive predictive value		21%	
At 4 weeks			
s-Flt1/PIGF ratio <38	0	53 (3 pre-eclampsia at 55, 86, and 93 days)	53
s-Flt1/PIGF ratio ≥38	2	9	11
Total	2	62	64
Negative predictive value		100%	
Positive predictive value		18%	

Results are shown as number or percentage when appropriate.

same threshold and after excluding the three patients who presented with PE before one week, two of the remaining patients developed PE (one patient at 10 days and the other at 31 days). The negative predictive value of the ratio for the risk of PE occurring at four weeks was 100%; positive predictive value was

18%. Sensitivity was 100%, and specificity was 85% (Table 4).

4. Discrepancies

Among the patients who did not develop PE despite a sFlt-1/PIGF ratio greater than 38, three presented with PE but in atypical form: one patient exhibited symptoms of PE with HELLP syndrome but without hypertension, while two patients had PE without proteinuria.

Three patients with a ratio below 38 developed PE at more than four weeks (55, 86, and 93 days).

DISCUSSION

Our prospective study, conducted in a specialized perinatal care center, demonstrated that the sFlt-1/PIGF ratio can be used to rule out PE in a specific population of high-risk patients with excellent negative predictive value. Our negative predictive value for ruling out PE within one week was 100%, in accordance with that from the PROGNOSIS study (99.3%) [13]. The aim of using the sFlt-1 and PIGF biomarkers is to rule out PE onset, avoiding unnecessary hospitalization and identifying high-risk pregnant women who require intensive monitoring. Use of the two serum biomarkers and their ratio appears to be essential for pregnant women at high-risk of PE or when diagnosis of PE is difficult because of the absence of symptoms or pre-existing diseases.

In fact, hypertensive disorders other than PE such as gestational hypertension or chronic hypertension can occur during pregnancy. The onset of hypertension during pregnancy or worsening of pre-existing chronic hypertension without proteinuria represents a complex situation, in which it is essential to rule out PE. Verlohren *et al* [17] showed that the sFlt-1/PIGF ratio in patients with PE is significantly higher compared with that in patients with chronic and gestational hypertension. These data, recently confirmed in a Spanish cohort [17], demonstrated the role of the sFlt-1/PIGF ratio in differentiating PE and other pregnancy-related hypertensive disorders [18].

Thus, because both PE and chronic kidney disease (CKD) are characterized by proteinuria, hypertension, and progressive renal impairment, differential diagnosis may be difficult during pregnancy. Rolfo *et al* [19] evaluated the potential value of using sFlt-1 and PIGF to differentiate between CKD and PE. They demonstrated that women with CKD exhibited normal levels of sFlt-1, PIGF, and sFlt-1/PIGF ratio. The sFlt-1/PIGF ratio was generally significantly higher in subjects with PE than in those with CKD, suggesting that serum biomarkers can be used to distin-

guish these two diagnoses [19]. Ghosh *et al* [20] also demonstrated that serum PIGF measured in the early second trimester of pregnancy may be an effective predictor of early-onset PE in another group of high-risk pregnant women who are overweight or obese rather than in normal or underweight women.

Serum sFlt-1 and PIGF PE markers and their ratio show highly predictive performance for clinical care. In addition to the many advantages described above, they are easier to use than uterine artery Doppler and represent an additional method of management in clinical practice. In fact, establishing velocimetric parameters is relatively straightforward. However, uterine artery Doppler has several weaknesses: reproducibility is limited, the resulting indices vary with the anatomical site of insonation, and uterine artery blood flow can be difficult to measure accurately by Doppler sonography [21]. Recent studies have demonstrated the poor sensitivity of using only second-trimester Doppler ultrasound measurements for general prediction of PE in a well-characterized, low-risk, nulliparous population [22]. However, some studies suggested that combining the uterine artery pulsatility index with PIGF and sFlt-1 may be useful for predicting PE [23, 24].

The actual impact and clinical usefulness of the sFlt-1 and PIGF biomarkers in pregnant women with suspected PE was evaluated in the pre-eclampsia open study (PreOS). sFlt-1/PIGF ratio results led to a change in the decision to hospitalize women for 16.9% of the cohort (20/118). These results demonstrated the usefulness of the sFlt-1/PIGF ratio for clinical decision-making of hospitalization in a considerable proportion of women with suspected PE. In addition to improving clinical care, assessment of the sFlt-1/PIGF ratio helps avoid unnecessary stress and anxiety for the patient. Reducing unnecessary hospitalizations is a major consideration in the current context of a heavy financial burden in many healthcare institutions [25]. The economic impact of introducing sFlt-1 and PIGF for managing PE appears to be positive. In the PROGNOSIS study by Vatish *et al* [26], hospitalizations were reduced by more than a half, generating cost-savings of £344 per patient. The expected annual cost savings for the UK National Health Service would be approximately £24 million per annum, based on a cohort of 68,900 women presenting annually with hypertensive disorders including suspected PE [26].

Based on this data, the National Institute for Health and Care Excellence (NICE) guidance recommends sFlt-1/PIGF ratio testing to rule out PE in women presenting with suspected PE between 20 and 34+6 gestation weeks. However, there is currently insufficient evidence and some contradictory recent data pre-

venting their routine use in diagnosing PE [27, 28]. An intermediate and rational method of introducing the sFlt-1/PIGF ratio into clinical practice as a first step, and with respect to the currently relatively high cost, would be to reserve these markers for high-risk populations with suspected PE or for differential diagnosis.

Our study has some limitations, such as the size of the cohort, heterogeneity of the patients included, the mean age of the patients, and influence of multiple pregnancies. Nevertheless, it represents a practical population. The mean age of the patients was 32 ± 5 years with 34 patients out of 67 (50%) aged 30–35 years. Indeed, hypertensive disorders and HELLP syndromes are known to be associated with increasing maternal age during gestation. Concerning multiple pregnancies, 19 patients (28%) in our population had at least a twin pregnancy. Dröge *et al* [29] reported that women with twin pregnancy who did not develop PE had a higher median serum sFlt-1 concentration and sFlt-1/PIGF ratio than women with singleton pregnancies; however, the median serum PIGF concentration was unchanged. For patients with twin pregnancy and PE, the median sFlt-1/PIGF ratio was not significantly different compared with singleton pregnancies with PE. Current cut-offs for the diagnosis of PE were established using data from singleton pregnancies alone, which, based on our results, appears to be inappropriate: Dröge *et al* [29] suggested a threshold of 53 as an optimal cut-off for this patient category.

Broader use of the sFlt-1/PIGF ratio in maternity care may support targeted clinical care by helping to identify women who are at high risk of developing PE and require close monitoring and management from women who are at a low risk of developing PE and can simply be reassured, thus avoiding unnecessary hospitalization [25].

This study demonstrated that a cut-off of 38 for the sFlt-1/PIGF ratio is appropriate for ruling out PE in a representative cohort of high-risk patients. Use of the sFlt-1/PIGF ratio in clinical practice will improve management of the disease and reduce health expenditures, while ensuring safety.

Authors' Disclosure of Potential Conflict of Interest

All authors declare that they have no conflicts of interest.

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