Performance of the sFLT-1 / PLGF Ratio in the Diagnosis of Preeclampsia in Four Hospitals in Brazzaville (Republic of Congo)

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Abstract: Preeclampsia is a serious obstetric situation, responsible for high maternal and perinatal morbidity and mortality. Its diagnosis is clinical and biological, with confirmation either by proteinuria on the urine dipstick or by 24-hour proteinuria constituting the gold standard. Current revisions to the definition of preeclampsia tend to free themselves from reliance on proteinuria for diagnosis. They are oriented towards a broader model where the involvement of a target organ concomitant with gestational hypertension is sufficient to make the diagnosis of preeclampsia. Late positivity of proteinuria after the onset of arterial hypertension (HTA) has been reported, contrasting with the precocity of angiogenic factors such as soluble Fms tyrosine kinase type 1 (sFlt-1) and placental growth factor (PlGF). *Objective.* To evaluate the performance of the sFLT-1/PLGF ratio in the diagnosis of preeclampsia. *Methods.* Cross-sectional multicenter analytical study conducted from October 17 to December 17, 2020 including hypertensive pregnant women, treated or not, with more than 20 weeks of amenorrhea. These all benefited from the Elisa assay of sFLT-1 and PlGF after carrying out the proteinuria on the urine dipstick during the consultation. Preeclampsia was defined by the double positivity of proteinuria on the urine dipstick associated with high blood pressure (Systolic blood pressure $\geq$ 140 mmHg and/or diastolic blood pressure $\geq$ 90 mmHg). SPSS software was used for statistical analysis and calculation of performance indices (Se; Sp; PPV; NPV; Youden "J" index, AUC). The p-value of the probability was considered significant for a value < 0.05. *Results.* Pregnant women were mostly multiparous with a median age of 31 years (24-35). Preeclampsia was noted in 69.2% of cases. sFLT-1 was significantly higher in preeclamptic pregnant women (409±18.9 vs 194.6±12.9; p<0.05). No significant difference was noted for PlGF (11.9 vs 13.6; p< 0.05). The sFlt-1/PlGF ratio was higher in case of preeclampsia (39.3 vs 14.6; p< 0.05). The threshold for the sFlt-/PlGF ratio retained was 18.5 (Se=86.4%; Sp=84.6%; PPV=90.5%; NPV=78.6%; J = 0.7; AUC =0.9). *Conclusion.* The sFlt-1/PlGF ratio was effective in the diagnosis of preeclampsia.

Keywords: Preeclampsia, sFLT-1/PLGF Ratio, Diagnostic, Brazzaville

1. Introduction

Still called toxaemia of pregnancy, preeclampsia (PE) is the association of arterial hypertension and significant proteinuria (300mg/24h) or a double positivity (++) of proteinuria in the urine dipstick occurring from 20 weeks of amenorrhea [1]. This is a serious obstetric situation, responsible for high maternal and perinatal morbidity and mortality. [2]. Therefore, its diagnosis in a population at risk during pregnancy remains a concern for the obstetrician.

The diagnosis of PE is clinical and biological, confirmed either by proteinuria on the urine dipstick or by 24-hour proteinuria, thus constituting the gold standard [3]. However, several authors have questioned the effectiveness of proteinuria using the urine dipstick due to the high rate of false positives in the event of urinary tract infection, the cumbersomeness of the procedure linked to the need to collect urine over 24 hours, and the long waiting time delaying optimal management [3]. Similarly, due to its syndromic and multisystemic nature, the initial presentation of preeclampsia is not necessarily dominated by renal involvement. Current revisions to the definition of preeclampsia tend to free themselves from reliance on proteinuria for diagnosis. They are moving towards a broader model where the involvement of a target organ concomitant with gestational hypertension is sufficient to make the diagnosis of preeclampsia. According to the 2013 American College of Obstetricians, Gynecologists (ACOG) guidelines, proteinuria is no longer mandatory for the diagnosis of preeclampsia. Thus, aproteinuric preeclampsia should no longer, stricto sensu, be considered as an atypical form. [4]. In addition, late positivity of proteinuria after the onset of arterial hypertension (HTA) has been reported [5], contrasting with the precocity of angiogenic factors such as soluble Fms tyrosine kinase type 1 (sFlt-1) and factor of placental growth (PlGF) [6]. Indeed, the imbalance of the sFlt-1/PlGF angiogenic balance plays a major pathophysiological role in the occurrence of PE, particularly in maternal endothelial dysfunction [7]. Although the predictive capacity of the sFlt-1/PlGF ratio during PE no longer needs to be demonstrated in the literature (PPV=99.1% at one week) [8], its contribution to diagnosis remains to be proven. Thus, the present study aimed to evaluate the performance of the sFlt-1/PlGF ratio in the diagnosis of preeclampsia in four hospitals in Brazzaville.

2. Methods

This was an analytical cross-sectional study, conducted from October 17 to December 17, 2020 in the maternity wards of four hospitals in Brazzaville (Brazzaville University Hospital Center, Makelekele Base Hospital, Talangaï Reference Hospital, Specialized Mother Hospital child Blanche Gomes) and in the laboratory of the Faculty of Health Sciences of Marien Ngouabi University). Were included the hypertensive pregnant women, of more than 20 weeks of amenorrhea (SA), seen in hospitalization or in consultation, beneficiary or not of an anti-hypertensive treatment. High blood pressure was defined as systolic blood pressure (SBP) $\geq$ 140 mmHg and/or diastolic blood pressure (DBP) $\geq$ 90 mmHg. The association with arterial hypertension of a ++ proteinuria on the urine dipstick defined preeclampsia.

The variables studied were: age, parity, history of diabetes, chronic hypertension and/or preeclampsia, proteinuria on the urine dipstick, sFlt-1 and PlGF assay.

The equipment used consisted of an ELISA Microplate Photometer device (model: Phomo from Autobio Labtec, serial number: 301 100 3811), a VWR MEGA STAR 3.0R centrifuge, a BioSystems BTS-350 spectrophotometer and consumables.

Urine dipstick proteinuria

After brief immersion (5 seconds) of the urine strip in the urine collected in a sterile pot, the strip was drained by passing the slice against the edge of the container. The test was then read after one minute by comparing the color of the reactive area with that of the colorimetric range of the label. The reactive zone, a colored indicator buffered at acidic pH, is yellow in the absence of proteins. At this same pH and in the presence of proteins, it takes on a green tint of varying intensity. This test was particularly sensitive to albumin. Albuminuria > 300 mg/l corresponded to ++, i.e., a dark green tint.

Assay of sFlt-1 (soluble fms-like tyrosine kinase 1) and PlGF (placenta growth factor)

After a 5ml blood sample taken at the bend of the elbow in dry tubes, the serum was decanted after centrifugation and then frozen at -30°C in cryotubes during the period of inclusion of pregnant women. The analysis was then carried out using the sandwich ELISA method. The Micro-Elisa stripplate provided by the kit has been pre-coated with an antibody specific for sFlt-1 or PlGF. The standards or samples were added to the appropriate Micro-Elisa stripplate wells and combined with the specific antibody. Then, a horseradish peroxidase (HRP)-conjugated antibody specific for sFlt-1 or PlGF was added to each incubated Micro-Elisa stripplate. Loose components were taken away. TMB, a substrate solution, was added to each well. Only wells containing sFlt-1, HRP, or PlGF and HRP appeared blue and then turned yellow after the addition of Stop Solution. The optical density (OD) was measured by spectrophotometry at a wavelength of 450 nm. The OD value was proportional to the concentration of sFlt-1 or PlGF.

SPSS version 25 software was used for data analysis. The qualitative variables were represented as a proportion, and the quantitative variables as the mean ± standard deviation or the median with its quartiles (q1 - q3). Fischer's exact test was used to compare proportions and the t-Student test and Mann Whitney's test to compare means and medians respectively. The p-value of the probability was considered significant for a value < 0.05. The performance indices (sensitivity "Se", specificity "Sp", positive predictive value "PPV", negative predictive value "NPV", Youden index "J") were used to establish the ROC curve, to visualize

synthetically the different performances according to the selected thresholds and to determine the area under the curve (AUC).

3. Results

The median age of the patients was 31 years (24-35) with extremes of 16 and 38 years.

The median parity was 2 (1-3) with extremes of 0 and 5. The multiparous were the most representative (16/35 or 45.7%). The nulliparous and the primiparous represented respectively 28.6% (10/35) and 25.7% (9/35). The history of preeclampsia was noted in 20% of the cases (7/35), chronic arterial hypertension and diabetes in 11.4% (4/35) each. Proteinuria on the urine dipstick was double positive in 62.8% (22/35) of cases, indicating the presence of preeclampsia, which occurred early before 34 WA in 63.3% (14/22).

In the case of preeclampsia, there was a significant increase in the mean sFlt-1 (409±18.9 vs 194.6±12.9; $p<0.001$) and the median sFlt-1/PlGF ratio (39.3 vs 14. 6; $p<0.001$). No significant difference was noted for PlGF (11.9 vs 13.5; $p<0.3$).

Tables 1 and 2 report the thresholds of the sFlt-1/PlGF ratio and their performance indices.

The threshold of 22.62 was the one with the right compromise between sensitivity, specificity, positive predictive value and Youden's index (figure 1).

Table 1. *Performance indices.*

sFlt-1/PlGF ratio thresholds	Se	Sp	1 - Sp	J
3.52	1	0	1	0
6.77	1	0.07	0.92	0.07
9.68	1	0.15	0.84	0.15
11.06	0.95	0.15	0.84	0.11
11.93	0.95	0.23	0.76	0.18
12.71	0.95	0.31	0.69	0.26
13.40	0.95	0.38	0.61	0.34
13.63	0.91	0.38	0.61	0.29
13.96	0.91	0.46	0.53	0.37
14.12	0.91	0.54	0.46	0.44
14.14	0.91	0.61	0.38	0.52
14.64	0.91	0.69	0.31	0.60
16.33	0.86	0.69	0.31	0.55
17.97	0.86	0.77	0.23	0.63
18.53	0.86	0.84	0.15	0.71
19.58	0.82	0.84	0.15	0.66
20.67	0.77	0.84	0.15	0.62
21.70	0.73	0.84	0.15	0.57
22.62	0.73	0.92	0.07	0.65
23.62	0.68	0.92	0.07	0.60
25.38	0.68	1	0	0.68
29.58	0.64	1	0	0.63
35.15	0.59	1	0	0.59
38.90	0.54	1	0	0.54
41.01	0.50	1	0	0.50
42.04	0.45	1	0	0.45
43.33	0.41	1	0	0.41
44.98	0.36	1	0	0.36
46.51	0.32	1	0	0.31
49.36	0.27	1	0	0.27
52.94	0.23	1	0	0.22
54.64	0.18	1	0	0.18

Table 2. *Flt-1/PlGF ratio at the threshold of 22.62 and proteinuria on the urine dipstick.*

	Urine dipstick proteinuria				Total
	Positive		**Negative**		
	n	**%**	**n**	**%**	
Flt-1/PlGF ratio threshold					
≥22.62	16[(1)]	72.7	1[(3)]	7.7	17
<22.62	6[(2)]	27.3	12[(4)]	92.3	18
Total	22	100	13	100	35

[(1)] True positive [(2)] False negative [(3)] False positive [(4)] True negative.
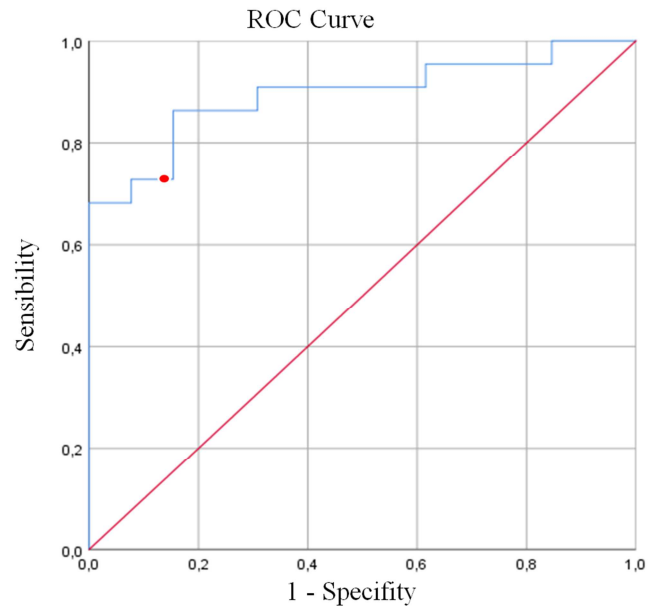

Figure 1. *ROC Curve.*

For a threshold of the sFlt-1/PlGF ratio retained at 22.62; the area under the curve (AUC) was 0.9 with CI (95%) ranging from 0.8 to 1.0 revealing a very good performance. Also, at this same threshold, 16 cases of preeclampsia confirmed by urinary dipstick were noted, including 11 before 34 WA, i.e. 68.7%.

4. Discussion

In the cohort of 35 pregnant women, the frequency of preeclampsia is significantly higher than that observed in the general population, thus reflecting a high risk of preeclampsia within the cohort. The observed values of sensitivity and specificity, at the more specific than sensitive threshold of 22.62; are superimposable on the 78% and 84% respectively of sensitivity and specificity reported in Liu's meta-analysis [9] based on 20 studies of different methodology using different assay methods for angiogenic markers. Indeed, like the Elisa method preferentially carried out in our laboratories, several Western pharmaceutical companies have developed sFlt-1 or PlGF assays on an automaton with a great heterogeneity of immunoassay kits; delocalized "bed-side test" type assays and simultaneous assay of the two markers PlGF and sFlt-1 offering an sFlt-1/PlGF ratio like the company Roche on Elecsys [10].

Although not statistically different, a downward trend in PlGF values was noted in case of preeclampsia in the present study, corroborating the findings of Viellefosse [11]. Similarly, like Aksas in Algeria [12], sFlt-1 values were higher in preeclampsia. Indeed, it is observed during preeclampsia, an anomaly of trophoblastic invasion, responsible for a defect in the remodeling of the spiral arteries at the uterine level. This results in placental hypoperfusion responsible for hypoxia and oxidative stress of the placenta. The placenta releases an increased amount of anti-angiogenic factors such as sFlt-1 and endoglin [13]. sFlt-1 is a receptor for pro-angiogenic factors like PlGF. Placental overexpression of sFlt-1 in preeclampsia leads to a relative deficiency of VEGF on its endothelial receptor responsible for the production of nitric oxide and vasoconstrictor prostacyclins. This results in the occurrence of arterial hypertension and an imbalance between pro-angiogenic factors and anti-angiogenic factors. As a result, an increase in the blood level of sFlt-1 and a decrease in that of PlGF are observed, resulting in an increase in the sFlt-1/PlGF ratio detectable from the second half of pregnancy [13]. Thus, the ratio observed in our series was significantly high in the case of preeclampsia and close to that of the Algerian series using the same assay method [12]. The area under the curve performed very well and made it possible to use the sFlt-1/PlGF ratio as a diagnostic test for PE, as reported by Veuberg [14] and Liu [9]. Although using different immunoassay techniques, several authors have reported higher thresholds of the sFlt-1/PlGF ratio, proportional to gestational age and severity of preeclampsia [8, 9, 13-16]. Conversely, in nearly two-thirds of cases, preeclampsia was early and of varying severity, which may have contributed to our threshold. Furthermore, in order to minimize false positives, which are more significant in the case of a screening test (more sensitive than specific), the choice of the threshold has been oriented towards a more specific and precise value, useful for diagnostic confirmation. Thus, the specificity was close to that reported by Liu [9] but far lower than that observed in the series by Verlohren [16] and Zeisler [8] in a population of preeclamptic pregnant women. However, only one case of false positive was noted, suggesting the possibility of aproteinuric preeclampsia as reported by ACOG [4].

Despite the small sample size, the results of the present study confirm the effectiveness of the sFlt-1/PlGF ratio in the early diagnosis of preeclampsia in a population at risk. Also, although not having assessed the severity of preeclampsia, the modifications observed in the literature of the sFlt-1/PlGF ratio according to the severity of preeclampsia, could be used as determinants in the decision-making of fetal extraction.

5. Conclusion

Preeclampsia remains frequent despite the efforts undertaken in terms of prevention. Its seriousness for the mother-child couple requires more precise diagnostic means. The ratio of sFlt-1/PlGF angiogenic factors is very effective in the diagnosis of preeclampsia. In the event of severe pregnancy-induced hypertension in the face of difficulties in achieving proteinuria, the dosage of angiogenic factors should be considered.

Conflicts of Interest

The authors declare no conflict of interest.

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