Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health 3 (2013) 191–195

Contents lists available at SciVerse ScienceDirect

Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health

journal homepage: www.elsevier.com/locate/preghy

Original Article

sFlt-1/PlGF ratio as a prognostic marker of adverse outcomes in women with early-onset preeclampsia

Leandro De Oliveira [a,b,*], José C. Peraçoli [c], Maria T. Peraçoli [c], Henri Korkes [a,b], Giafranco Zampieri [d], Antonio F. Moron [a], Nelson Sass [a,b]

[a] Obstetrics Department, Universidade Federal de São Paulo, Brazil
[b] Laboratory of Clinical and Experimental Investigation, Hospital e Maternidade Vila Nova Cachoeirinha, São Paulo, Brazil
[c] Obstetrics Department, Universidade Estadual Paulista, Botucatu, Brazil
[d] Laboratory of Clinical Pathology Salomão & Zoppi, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 22 October 2012
Received in revised form 17 January 2013
Accepted 14 February 2013
Available online 23 April 2013

Keywords:
Preeclampsia
sFlt-1
PlGF
Inflammation

ABSTRACT

Soluble fms-like tyrosine kinase 1 (sFlt-1) is an anti-angiogenic factor released in higher amounts by preeclamptic placentas and it has been implicated in the endothelial dysfunction observed in the disease. In this study we evaluated if circulating sFlt-1/PlGF ratio is useful to predict adverse outcomes in women with early-onset preeclampsia. This is a cohort study of 88 preeclamptic women with singleton pregnancies at ⩽35 weeks of gestation. According to definitions used, adverse outcomes occurred in 46.5% ($N = 43$) of the patients. The median sFlt1/PlGF ratio (25th–75th centile) for all patients evaluated was of 42.26 (13.1–226.1). The median sFlt-1/PlGF ratio among women who had any adverse outcome ($N = 43$) versus no adverse outcomes ($N = 45$) was of 227.6 (80.3–346.1) versus 14.4 (3.35–30.0), ($P < 0.0001$). According to our analyses a sFlt-1/PlGF ratio cut-point of ⩾85 gave a sensitivity of 74.0% and specificity of 97.0%. The positive predictive value and the negative predictive value were 96.0% and 80.0%, respectively. The median sFlt-1/PlGF ratio (25th–75th centile) for patients who delivered within <7 days was 260.0 (127.7–404.7) as compared to 14.4 (3.35–34.97) for those patients who delivered within two weeks or more ($P < 0.0001$). Our results suggest that sFlt-1/PlGF ratio is a promising marker for adverse outcomes in women with early-onset preeclampsia.

Introduction

Preeclampsia occurs in about 5% of all pregnancies and it constitutes a major cause of maternal and fetal morbidity and mortality [1]. The pathogenesis of preeclampsia has its roots on deficient trophoblast invasion and on failure of spiral artery remodeling. This incomplete transformation of the spiral arteries leads to inadequate placental perfusion and consequently to placental oxidative stress [2]. The altered placenta then releases a great amount of microparticles, debris and anti-angiogenic factors into the maternal circulation [3,4].

Soluble fms-like tyrosine kinase 1 (sFlt-1) is an anti-angiogenic factor released in higher amounts by preeclamptic placentas and it has been implicated in the endothelial dysfunction observed in the disease. sFlt-1 acts as a soluble receptor for VEGF and PlGF, intercepting these angiogenic proteins before their binding to normal receptors at the cell membranes. The result of this process is a break of systemic endothelial homeostasis [5,6].

Current criteria for the diagnosis of preeclampsia involve blood pressure ⩾140/90 mmHg evaluated on two occasions 2 h apart after 20 weeks of gestation and

* Corresponding author. Address: Universidade Federal de São Paulo, Obstetrics Department, Rua Napoleão de Barros, 875 Vila Clementino, São Paulo 04024 – 002, Brazil. Tel.: +55 11 9949 8110; fax: +55 11 3815 6308.
E-mail address: Leminas@ig.com.br (L. De Oliveira).

2210-7789/$ - see front matter © 2013 International Society for the Study of Hypertension in Pregnancy. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.preghy.2013.02.003

proteinuria of ⩾300 mg/24 h [7]. However, growing evidence has supported the use of anti-angiogenic and angiogenic proteins in the diagnosis and prediction of preeclampsia. Initial publications suggested that an imbalance between angiogenic and anti-angiogenic factors occurs about 4–5 weeks before clinical manifestations of preeclampsia [8,9]. In addition, a prospective work developed by Sunderji et al. demonstrated that sVEGFR1/PlGF ratio is superior to aid in the diagnosis of preeclampsia instead of any of these analytes alone, with 3% of false positive rate [10]. Recently, Rana et al. demonstrated that circulating sFlt-1/PlGF ratio is also useful to predict adverse outcomes in women with suspected preeclampsia if presented at <34 weeks. The accuracy of this test was substantially better than other measured parameters such as proteinuria, ALT and platelet count [11].

In this work we evaluated the sFlt-1/PlGF ratio as a prognostic marker of adverse outcomes in pregnant women with a standard diagnosis of preeclampsia at 35 weeks of gestation or less.

Methods

Study design

This is a cohort study of 88 women with singleton pregnancies who were present at The Obstetrics Department of the School Maternity Vila Nova Cachoeirinha, São Paulo, Brazil from March 2011 through March 2012. These patients were either referred from first-care units because of increased blood pressure or self-presented with signs or symptoms of preeclampsia (headache, edema). All patients included in this cohort study were at 35 gestational weeks or less at the time of the first evaluation and were admitted to the hospital with the diagnosis of preeclampsia or superimposed preeclampsia. Preeclampsia was defined as a blood pressure ⩾140/90 mmHg evaluated on two occasions 2 h apart after 20 weeks of gestation and proteinuria of ⩾300 mg/24 h [7]. Superimposed preeclampsia was defined as the appearance of proteinuria in patients with known chronic hypertension or with blood pressure ⩾140/90 mmHg diagnosed before 20 gestational weeks.

To evaluate the utility of sFlt-1/PlGF as a prognostic marker for adverse outcomes in preeclampsia, blood samples were collected just after the patient's admission and sera were stored at −80 °C until required for analysis. All patients were followed according to the institutional protocol until delivery and adverse outcomes were adjudicated by two or more staffs without assay results. The study was approved by the ethics committee of our institution and all patients provided informed consent.

sFlt-1 and PlGF assays

Serum concentrations of sFlt-1 and PlGF were measured by automated electrochemiluminescence immunoassay according to manufacturer's instructions (Roche Diagnostics, IN). Samples were analyzed in duplicate as a joint group analysis after all deliveries.

Diagnoses and outcomes

Adverse outcomes were defined as the necessity of proceeding to delivery because of any of the following conditions: uncontrolled hypertension after the use of two antihypertensive drugs (methyldopa plus nifedipine or pindolol), clinical signs of severe preeclampsia (headache, flashes and/or abdominal pain), abruption (clinical and/or pathological), seizure (in a woman without underlying seizure disorder), altered laboratory parameters as alanine aminotransferase (ALT) (>70 U/L), Platelet count $<100 \times 10^9$/L/uL, acute renal failure (creatinine >106.08 μmol/L) and/or alteration on fetal wellbeing surveillance defined as abnormal umbilical artery Doppler (absent or reverse flow). Fetal growth restriction was defined as a fetus at or below the tenth percentile in weight for its gestational age.

Statistical analyses

The statistical analyses were performed using Prism software (version 4.02, GraphPad Software Inc., San Diego, CA) and PASW statistic software (version 18.0). Differences between measured parameters were evaluated using the Mann–Whitney *U* test. The Wilcoxon rank sum test and the Chi-squared test were used when appropriate in the analyses of maternal and neonatal characteristics.

Receiver operating characteristic (ROC) analysis was used to determine the predictive value of each measured parameter to identify women at risk for adverse outcomes: sFlt-1/PlGF ratio, proteinuria, platelet count, ALT and creatinine. Sensitivity, specificity, positive and negative predictive values for the sFlt-1/PlGF ratio cut-point of ⩾85 were calculated.

The Pearson product correlation was used to assess the relationship between sFlt-1/PlGF ratios and time elapsed between presentation to hospital and delivery. Differences were considered statistically significant at $P < 0.05$.

Results

Subject characteristics and clinical outcomes

Eighty-eight patients were enrolled in this study. According to previous definitions, adverse outcomes occurred in 46.5% ($N = 43$) of the patients. Table 1 shows the characteristics of all patients, measured parameters at the time of hospital presentation and adverse outcomes.

sFlt-1/PlGF ratios as predictive markers

The median sFlt-1/PlGF ratio (25th–75th centile) for all patients evaluated was of 42.26 (13.1–226.1). The median sFlt-1/PlGF ratio among women who had any adverse outcome ($N = 43$) versus no adverse outcomes ($N = 45$) was of 227.6 (80.3–346.1) versus 14.4 (3.35–30.0), ($P < 0.0001$).

The ROC analysis for each laboratory parameter evaluated at the time of hospital presentation is shown in Table 2. Fig. 1 depicts ROC curves for prediction of adverse outcomes using the sFlt-1/PlGF ratio, 24 h proteinuria,

Table 1
Subject characteristics, measured parameters and clinical outcomes.

Variable	Adverse outcomes ($N = 43$) Median (25^{th}–75th centile) or N°% or Mean	No adverse outcomes ($N = 45$) Median (25th–75th centile) or N°% or Mean	P value
Age (years)	25.7	26.8	0.265
Nulliparous	31 (72.0%)	21 (46.6%)	0.0018
Gestational age at presentation	35 (33–35)	35 (34–35)	0.098
Race White/Caucasian Black/African American	32 (74.4%) 11 (25.6%)	27 (60.0%) 18 (40.0%)	
Chronic hypertension	5 (11.6%)	11 (24.4%)	0.031
Preexisting diabetes	0	2 (2.2%)	N/A
Proteinuria	4.5 (2.1–7.2)	0.45 (0.37–0.70)	<0.0001
Systolic blood pressure, mmHg	147 (9.39)	143.6 (5.29)	0.034
Diastolic blood pressure. mmHg	94.4 (7.17)	91.67 (4.88)	0.014
ALT (U/L)	39 (29–72)	24 (22–29)	<0.0001
Creatinine (μmol/L)	70.72 (70.72–79.56)	(61.88–70.72)	0.0004
Platelet count ($\times 10^9$/L)	154 (127–175)	190 (180–210)	<0.0001
Abnormal umbilical artery Doppler	25 (28.4%)	0	
Gestational age at delivery	34 (33–35)	37 (36.4–37)	<0.0001
Preterm delivery	41 (95.3%)	0	N/A
Birthweight (g)	1720 (1380–1920)	2800 (2625–3015)	<0.0001
Fetal growth restriction (<10th centile)	33 (76.7)	4 (8.8%)	<0.0001
Cesarean section	38 (88.3%)	27 (60%)	<0.0001

Table 2
ROC analysis for each measured parameter.

Variable	AUC	Std error	95% CI
sFlt1/PlGF	0.954	0.019	0.917–0.991
24 h Proteinuria	0.926	0.032	0.865–0.988
ALT	0.843	0.046	0.753–0.825
Platelets	0.811	0.048	0.717–0.905
Creatinine	0.719	0.054	0.613–0.825

ALT and creatinine. The results suggest that sFlt-1/PlGF ratio had superior performance to other parameters measured at the time of hospital presentation. According to our analyses a sFlt-1/PlGF ratio cut-point of $\geqslant 85$ gave a sensitivity of 74.0% and specificity of 97.0%. The positive predictive value and the negative predictive value were 96.0% and 80.0%, respectively.

The sFlt-1/PlGF ratio was inversely correlated with the time elapsed between presentation and delivery

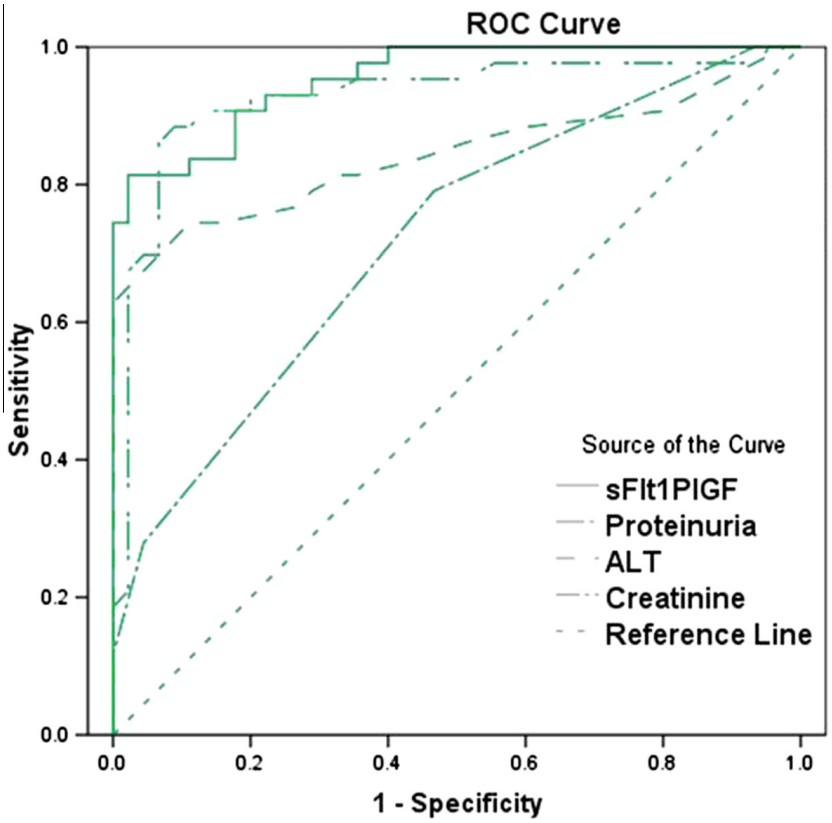

Fig. 1. ROC curves for prediction of adverse outcomes using the sFlt1/PlGF ratio, proteinuria, ALT and creatinine.

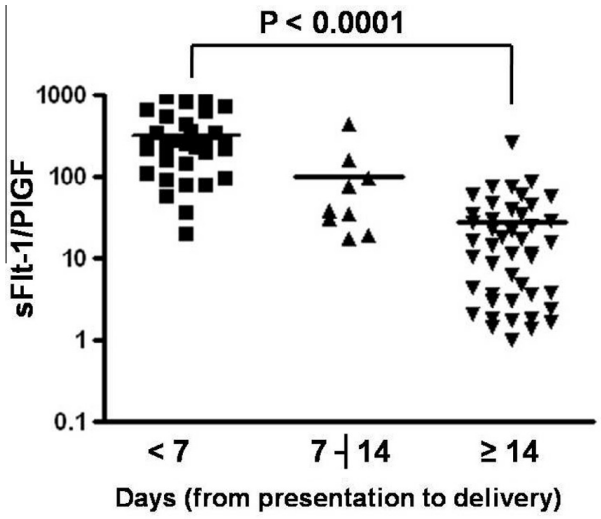

Fig. 2. Elapsed time between admission to hospital and delivery, according to sFlt-1/PlGF ratio at admission.

($r = -0.65$, $P < 0.0001$). Fig. 2 depicts elapsed time between hospital admission and delivery according to sFlt-1/PlGF ratios measured in sera collected at the time of presentation to hospital. The median sFlt-1/PlGF ratio (25th–75th centile) for patients who delivered within <7 days was 260.0 (127.7–404.7) as compared to 14.4 (3.35–34.97) for those patients who delivered within 2 weeks or more ($P < 0.0001$). The median sFlt-1/PlGF ratio for patients who delivered between 1–2 weeks was 38.2 (24.64–125.3).

Discussion

To find specific parameters to stratify patients according to the severity of preeclampsia has been a challenge all over the world. Apart from those patients who present to hospital with clinical signs of severe preeclampsia and/or imminent eclampsia (headache, abdominal pain, uncontrolled blood pressure), it is very difficult to correctly evaluate the severity of the disease at first investigation. Therefore, most cases require hospital admissions for more than 48 h and a full panel of laboratory parameters is needed to help doctors take any decision. However, it is not clear if this current approach really brings crucial information to deal with preeclamptic patients [13]. In addition, the stratification based on current laboratory parameters provides limited information and only classifies patients as mild or severe preeclampsia. Although clinicians may have an idea that patients stratified as severe preeclampsia are at higher risk than those with mild preeclampsia, women and fetuses involved in the diagnosis of mild preeclampsia are not completely free of complications [12,14].

In our work, a ROC analysis demonstrated that the sFlt-1/PlGF ratio was useful to identify patients at risk of having adverse outcomes with better performance than other commonly used parameters. An inverse correlation between sFlt-1/PlGF ratio and remaining duration of pregnancy was also identified. These results are in agreement with other publications addressing this issue [11]. Recently, Verlohen et al. also evaluated the potential role of sFlt-1/PlGF ratio as a prognostic marker for women with preeclampsia and additionally demonstrated its function as a reliable tool to discriminate between different types of pregnancy-related hypertensive disorders [15].

All current information about the participation of angiogenic and anti-angiogenic factors in the diagnosis and prognosis of preeclampsia highlights the importance of these proteins in the pathogenesis of the disease and encourages new researches on the development of new strategies for the treatment of preeclampsia.

Conclusion

The use of reliable markers to correctly classify women with preeclampsia in terms of severity of the disease could contribute to make the management of preeclampsia more uniform among clinicians and reduce unnecessary admissions to hospitals. The use of such markers could also contribute to the reduction of iatrogenic preterm deliveries related to preeclampsia. Recently, sFlt-1 and PlGF have been presented as promising markers with this purpose. Therefore, the potential role of these proteins for risk stratification in preeclampsia should be tested by clinicians in multi-centric projects to determine the impact of clinical decisions based on the sFlt-1/PlGF ratio on maternal and perinatal outcomes.

References

[1] Wallis AB, Saftlas AF, Hsia J, Atrash HK. Secular trends in the rates of preeclampsia, eclampsia, and gestational hypertension, United States, 1987–2004. Am J Hypertens 2008;21:521–6.
[2] Pijnenborg R, Dixon G, Robertson WB, Brosens I. Trophoblastic invasion of human decidua from 8 to 18 weeks of pregnancy. Placenta 1980;1:3–19.
[3] Burton GJ, Charnock-Jones DS, Jauniaux E. Regulation of vascular growth and function in the human placenta. Reproduction 2009;138(6):895–902.
[4] Redman CW, Sargent IL. Circulating microparticles in normal pregnancy and pre-eclampsia. Placenta 2008;29(Suppl. A):S73–7.
[5] Wang A, Rana S, Karumanchi SA. The role of angiogenic factors in its pathogenesis. Physiology 2009;24:147–58.
[6] Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. Proc Natl Acad Sci USA 1993;90:10705–9.
[7] ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. Obstet Gynecol 2002;99:159–67.
[8] 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;350:672–83.
[9] Chaiworapongsa T, Romero R, Kim YM, Kim GJ, Kim MR, Espinoza J, et al. Plasma soluble vascular endothelial growth factor receptor-1 concentration is elevated prior to the clinical diagnosis of pre-eclampsia. J Matern Fetal Neonatal Med 2005;17:3–18.
[10] Sunderji S, Gaziano E, Wothe D, Rogers LC, Sibai B, Karumanchi SA, et al. Automated assays for sVEGF R1 and PlGF as an aid in the diagnosis of preterm preeclampsia: a prospective clinical study. Am J Obstet Gynecol 2010;202:40 [e41–e47].
[11] Rana S, Powe CE, Salahuddin S, Verlohren S, Perschel FH, Levine RJ, et al. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. Circulation 2012;125(7):911–9.
[12] von Dadelszen P, Menzies JM, Payne B, Magee LA. PIERS (Pre-eclampsia Integrated Estimate of RiSk) study group. Predicting adverse outcomes in women with severe pre-eclampsia. Semin Perinatol 2009;33(3):152–7.
[13] von Dadelszen P, Payne B, Li J, Ansermino JM, Broughton Pipkin F, Cote AM, et al. Prediction of adverse maternal outcomes in preeclampsia: development and validation of the fullPIERS model. Lancet 2011;377:219–27.

[14] Koopmans CM, Zwart JJ, Groen H, Bloemenkamp KW, Mol BW, Van Pampus MG, et al. Risk indicators for eclampsia in gestational hypertension or mild preeclampsia at term. Hypertens Pregnancy 2011;30(4):433–46.

[15] Verlohren S, Herraiz I, Lapaire O, Schlembach D, Moertl M, Zeisler H, 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(1) [58.e1–58.e8].