Pregnancy Hypertension 16 (2019) 105–111

Contents lists available at ScienceDirect

Pregnancy Hypertension

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

Role of plasma PlGF, PDGF-AA, ANG-1, ANG-2, and the ANG-1/ANG-2 ratio as predictors of preeclampsia in a cohort of pregnant women

J.S.R. Machado[a], M.S.R. Machado[a], T.V. Bertagnolli[a], L.A.B. Martins[a], S.F. Freitas[a], P.P. Ovidio[b], V.C. Sandrim[c], V.C. Cardoso[d], H. Bettiol[d], M.A. Barbieri[d], R.C. Cavalli[a,*]

[a] Department of Gynecology and Obstetrics, Ribeirão Preto Medical School, University of São Paulo, 3900 Bandeirantes Avenue, 14049-900 Ribeirão Preto, SP, Brazil
[b] Department of Health Sciences, Ribeirão Preto Medical School, University of São Paulo, 3900 Bandeirantes Avenue, 14049-900 Ribeirão Preto, SP, Brazil
[c] Department of Pharmacology, Biosciences Institute, State University of São Paulo (UNESP), Botucatu 18618-970, São Paulo, Brazil
[d] Department of Childcare and Pediatrics, Ribeirão Preto Medical School, University of São Paulo, 3900 Bandeirantes Avenue, 14049-900 Ribeirão Preto, SP, Brazil

ARTICLE INFO

Keywords:
Preeclampsia
Predictors
PlGF
PDGF AA
ANG-1
ANG-2
ANG-1/ANG-2
Cohort

ABSTRACT

Introduction: Preeclampsia affects 3–5% of pregnancies worldwide and is the primary cause of maternal-fetal and neonatal mortality. Previous studies show that alterations in maternal concentrations of angiogenic factors, such as PlGF, PDGF AA, ANG-1, and ANG-2, may play fundamental roles in the pathophysiology of the disease.
Objective: Determine whether the PlGF, PDGF AA, ANG-1, and ANG-2 are predictors of preeclampsia occurrence in a prenatal cohort study.
Patients and methods: This is a case-control study associated with a prospective cohort of pregnant women, with gestational ages between 20 and 25 weeks, composed of 30 pregnant women with preeclampsia (PE) and 90 healthy pregnant women (HP). The plasma concentrations of the markers were determined using the ELISA method. The comparison between the case and control groups was performed using the *t* test on the SAS® 9.4 software. Also, ROC curves were constructed to evaluate the predictive potential of the biomarkers.
Results: Differences in the concentrations of PlGF, PDGF AA, ANG-1 and ANG-2, and the ANG-1/ANG-2 ratio were not observed between the PE and the HP groups. The predictive capacity of the biomarkers was assessed using ROC curves, in which the area under the curve for PlGF AUC = 0.55; PDGF AA AUC = 0.55; ANG-1 AUC = 0.47; ANG-2 AUC = 0.51, and the ANG-1/ANG-2 ratio AUC = 0.57.
Conclusion: In pregnant women, with gestational ages between 20 and 25 weeks significant differences in biomarker concentrations between groups PE and HP were not observed. The ROC curves showed that the biomarkers were ineffective as preeclampsia predictors in the analyzed cohort.

1. Introduction

Preeclampsia affects 3–5% of pregnancies worldwide and is the main cause of maternal-fetal and neonatal mortality, especially in middle- and low-income countries [1]. Research on markers capable of predicting the occurrence of the disease have become more numerous, given these tests would allow the identification of risk patients, thus enabling prophylactic interventions [2].

Angiogenic imbalance contributes to the development of preeclampsia, in addition to restricting fetal growth. Thus, pro-angiogenic molecules such as PlGF (placental growth factor), PDGF-AA (platelet-derived growth factor AA), ANG-1 (angiopoietin-1), and ANG-2 (angiopoietin-2) target biomarkers for early disease detection [3–5].

The PlGF is significantly expressed in the placenta at all stages of pregnancy, controlling trophoblast growth and differentiation, thus demonstrating the importance of this protein during trophoblast invasion in maternal decidua [6].

PDGF-AA is a potent growth factor for cells of mesenchymal origin and has the ability to stimulate cell circulation by chemotaxis. The protein is considered of extreme importance in angiogenesis, both during normal and abnormal processes, as in the case of embryonic development, cicatrization of tissue lesions, and tissue fibrosis [7–10].

ANG-1 promotes endothelial cell reorganization and maintains the structural integrity of blood vessels. It also inhibits vascular endothelial barrier activation and reduces leakage and migration of leukocytes to tissues induced by inflammatory agents [11,12].

* Corresponding author. at: Departamento de Ginecologia e Obstetrícia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, 3900, 14049-900 Ribeirão Preto, SP, Brazil.
E-mail address: rcavalli@fmrp.usp.br (R.C. Cavalli).

https://doi.org/10.1016/j.preghy.2019.03.011
Received 5 June 2018; Received in revised form 18 March 2019; Accepted 23 March 2019
Available online 23 March 2019

The essential function of ANG-2 is as an ANG-1 antagonist, as well as cleaving bonds between the endothelium and perivascular cells, promoting cellular death and vascular regression. In addition to fostering the development of vessels, angiopoietins also trigger stimuli for trophoblast growth and remodeling during placentation [12,13].

Although the pathophysiology of preeclampsia is not fully understood, studies have shown the involvement of several biomarkers in this process, including PlGF, PDGF-AA, ANG-1, and ANG-2. Knowledge of how these possible biomarkers act is of paramount importance, especially at the beginning of gestation, since they can be used to predict and prevent hypertensive disorder in high-risk pregnant women. Moreover, they are useful for risk stratification to provide adequate care for pregnant women, especially if the results are extended to distant regions that lack adequate treatment centers.

Thus, the aim of the present study was to determine whether the pro-angiogenic serum factors PlGF, PDGF-AA, ANG-1, and ANG-2 are optimal predictors and possible biomarkers for the occurrence of preeclampsia in pregnant women in a prenatal cohort study.

2. Material and methods

2.1. Patients

The present assessment is linked to a prospective cohort conducted in two Brazilian cities: The Brazilian Ribeirão Preto and São Luís prenatal Cohort the Brazilian Ribeirão Preto and São Luís Birth Cohort (BRISA). However, only the data collected in the city of Ribeirão Preto were used.

In Ribeirão Preto, a total of 1400 pregnant women between 20 and 25 weeks of gestation were evaluated at the Clinical Hospital Ribeirão Preto Medical School, University of São Paulo. at the time of recruitment, 430 delivered in out-of-institution units and 30 pregnant women it was not possible to obtain the data of the childbirth. Of the remaining 940 pregnant women, 30 developed preeclampsia (cases) and 651 performed the delivery at a reference center of low-risk gestation, 90 healthy pregnant women were selected through random matching for the control group.

All participants signed an informed consent form, and the study was approved by the Ethics Committee of the Ribeirão Preto School of Medicine (Reference number 4116/2008), according to the Helsinki Declaration.

After the recruitment between 20 and 25 weeks of gestation, the first Doppler ultrasonography and the collection of blood were performed. The maternal venous blood samples were collected in Vacutainer® tubes (Becton-Dickinson, São Paulo, Brazil) containing EDTA. After collection, the samples were centrifuged at 3200 rpm for 10 min at room temperature, isolating the plasma, which was stored at −80 °C until further analysis.

Blood pressure, laboratory test results (proteinuria, creatinine, hemogram, ALT, and AST), ultrasounds, and information on the development of symptoms, such as the recent onset of cerebral or visual disorders and pulmonary edema were employed to confirm the diagnosis.

All information used to confirm the diagnosis of preeclampsia was obtained from the subsequent analysis of medical records of pregnant women. The gestational age of the preeclampsia diagnoses was on average 35.9 weeks or approximately 12 weeks after sample collection. Of the 30 pregnant women analyzed, 8 had a confirmed diagnosis before 34 weeks (average and standard deviation of gestational age 29.87 ± 2.59) and 22 after 34 weeks (average and standard deviation of gestational age 37.95 ± 1.50).

Preeclampsia was defined according to the guidelines of the International Society for the Study of Hypertension in Pregnancy (ISSHP) [14], and the severity of the disease was classified in accordance with the American College of Gynecology and Obstetrics [15], by the presence of one or more of the following factors: systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 110 mmHg, measured on two occasions within 4 h or more, while the patient is bedridden; thrombocytopenia (platelet count < 100,000/mL); hepatic insufficiency, indicated by abnormally high blood levels of liver enzymes (twice the normal concentration); progressive renal insufficiency (serum creatinine > 1.1 mg/dL or a 2x increase in concentration in the absence of another kidney disease); recent onset of cerebral or visual disturbances, and pulmonary edema.

2.2. Marker quantification

Biomarker quantification was performed on the human plasma using Quantikine® kits (R&D Systems, Minneapolis, MN – PlGF: cat. DPG00; PDGF AA: cat. DAA00B; ANG-1: cat. DANG10) by the ELISA (Enzyme-Linked Immunosorbent Assay) method, according to the manufacturer's instructions. Also, ANG-2 quantification was conducted using the Invitrogen™ kit (cat. KHC1641; Thermo Fisher Scientific).

The ultrasounds were performed according to the criteria described by Groom [16], using Voluson 730 Expert model (General Electric Healthcare, Zipf, Austria) and Philips HDI 11 model (Philips, California, USA) equipment, with a two-dimensional convex probe, at a frequency of 2–5 MHz. The images were documented and archived on their own hard drives.

2.3. Statistical analysis

Data are presented as means and standard deviations. The comparison between the case and control groups was performed considering the *t* test for independent samples and all the data presented the normal distribution. In all the tests, a probability value of $P < 0.05$ was considered significant. The SAS version 9.4 program was used in the statistical analyzes described above.

The ROC curve was used to verify whether biomarkers are good predictors of preeclampsia. The pROC package already implemented in program R version 3.3.3 was used to construct the prediction curves, to estimate the area on the curve, followed by the respective 95% confidence intervals and to estimate the posttest power of the prediction curves.

3. Results

The present study evaluated 30 pregnant women with preeclampsia (PE) and 90 healthy pregnant women (HP). Regarding ethnicity, in the HP group, 47% were white, 36% brown, and 17% black; and in the PE group, 56.7% were white, 36.7% brown, and 6.6% black. When analyzing the type of delivery performed, in the HP group, 72% had normal parturition, 19% underwent a cesarean section, and 9% required the use of forceps; and in the PE group, 33.3% were normal, 60% cesarean section, and 6.7% forceps.

Some of the clinical and laboratory characteristics of the pregnant women at the time when PE was diagnosed are shown in Table 1.

A summary of the clinical characteristics of the 120 pregnant women selected for this study is shown in Table 2, of which 90 consisted of healthy pregnant women, and 30 had preeclampsia.

When comparing the HP and PE groups, differences in age, Gestational Age at 1st ultrasonography (GA 1st US), BMI and pulse were not observed. Although the blood pressure levels were related to the time of recruitment, that is, before the women developed the disease, the mean systolic arterial pressure (MSAP), mean diastolic arterial pressure (MDAP) and mean arterial pressure (MAP) in the PE group were higher when compared to the HP group ($P = 0.001$, $P < 0.001$ and $P < 0.001$ respectively).

The plasma concentrations of PlGF, PDGF-AA, ANG-1, ANG-2, and the ANG-1/ANG-2 ratio in the HP and PE groups are listed in Table 3. Differences between the concentrations of the assessed biomarkers in the HP and the PE group were not verified ($P = 0.907$; $P = 0.174$;

Table 1
Clinical and laboratory characteristics of the pregnant women at the time of preeclampsia diagnosis.

Parameters	Group PE (n = 30)
Age (years)	28.0 ± 5.7
Smoker (%)	16.7
BMI (Kg/m^2)	30.8 ± 5.84
MSAP (mmHg)	141.1 ± 16.7
MDAP (mmHg)	82.69 ± 15.2
MAP (mmHg)	111.9 ± 14.9
HR (beats/min)	81.3 ± 17.7
Glycemia (mg/dL)	98.38 ± 36.38
Hb (g/dL)	11.5 ± 1.5
Hct (%)	35.5 ± 4.3
GPT (U/L)	26.4 ± 38.2
GOT (U/L)	19.6 ± 10.5
Platelets (mm^3)	220.6 ± 65.3
Creatinine (mmol/L)	0.73 ± 0.17
Proteinuria (mg/24 h)	288.4 ± 559.7
GA (weeks)	35.9 ± 3.8

PE, preeclampsia; BMI, Body mass index (kg/m^2); MSAP, Mean Systolic Arterial Pressure (mmHg); MDAP, Mean Diastolic Arterial Pressure (mmHg); MAP, Mean Arterial Pressure (mmHg); HR, Heart Rate (beats/minute); Hb, hemoglobin (g/dL); Hct, hematocrit (%); GPT, glutamic-pyruvic transaminase (U/L); GOT, glutamic-oxaloacetic transaminase (U/L); GA, gestational age of childbirth (weeks). Values expressed as means ± MSD.

Table 2
Clinical characteristics at time of recruitment of the healthy pregnant women and those that developed preeclampsia.

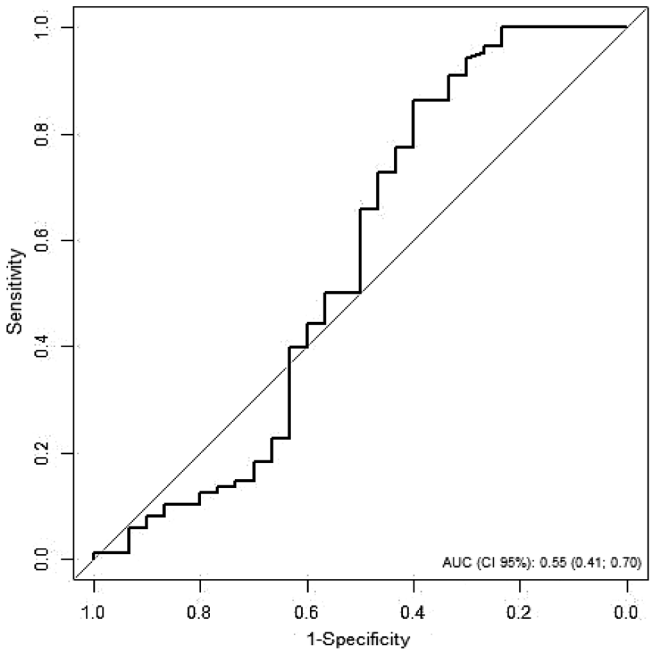
Parameters	HP (n = 90)	PE (n = 30)	P-value
Age (years)	26.17 ± 5.88	28.43 ± 5.79	0.069
GA 1st USR (Weeks)	23.47 ± 11.47	23.42 ± 9.01	0.888
BMI	28.25 ± 5.29	30.53 ± 5.85	0.052
MSAP	108.41 ± 11.93	116.87 ± 11.67	0.001
MDAP	65.94 ± 7.73	73.75 ± 8.80	< 0.001
MAP	87.30 ± 9.15	95.31 ± 9.66	< 0.001
PULSE	80.45 ± 10.42	78.57 ± 9.79	0.428
RUAPI	0.82 ± 0.33	1.03 ± 0.55	0.012
LUAPI	0.84 ± 0.42	1.06 ± 0.44	0.015
MPIUA	0.83 ± 0.31	1.05 ± 0.4	0.003

HP, healthy pregnant women; PE, preeclampsia; BMI, body mass index (kg/m^2); MSAP, mean systolic arterial pressure (mmHg); MDAP, mean diastolic arterial pressure (mmHg); MAP, mean arterial pressure; GA 1st US R, gestational age at the first ultrasound at recruitment; RUAPI, right uterine artery pulsatility index; LUAPI, left uterine artery pulsatility index; MPIUA, mean pulsatility index of the uterine arteries (right and left). Values expressed as means ± MSD. P-value according to the *t* test for independent samples.

Table 3
Plasma concentrations of PlGF, PDGF-AA, ANG-1, ANG-2 and ANG-1/ANG-2 Ratio in HP × PE.

Biomarkers	HP (n = 90)	PE (n = 30)	P-value
PLGF (pg/ml)	526.86 ± 302.58 451.6 (298.4; 632.2)	518.55 ± 419.62 413.9 (201.9; 805.4)	0.907
PDGF-AA (pg/ml)	337.8 ± 305.1 243.3 (154.9; 393.2)	257.6 ± 167.7 413.9 (201.9; 805.4)	0.174
ANG-1 (pg/ml)	4929.3 ± 4831.9 3395.2 (2028.2; 5415.2)	3700.5 ± 2441.4 3634.0 (1456.7; 5159.3)	0.185
ANG-2 (pg/ml)	9570.4 ± 5227.1 8596.9 (6017.0;12130.1)	10197.6 ± 5888.8 8799.1 (6821.8; 12176.4)	0.583
ANG-1/ANG-2 Ratio	0.71 ± 0.83 0.38 (0.21; 0.88)	0.45 ± 0.43 0.33 (0.19; 0.49)	0.107

HP, healthy pregnant women; PE, preeclampsia. Values expressed as means ± standard deviation, and median (1st quartile; 3rd quartile). P-value according to the *t* test for independent samples.

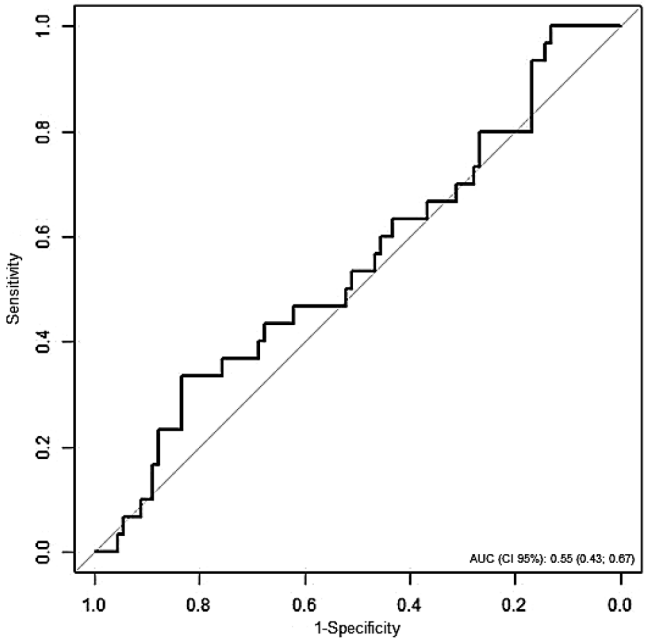


Graph 1. PlGF ROC curve.

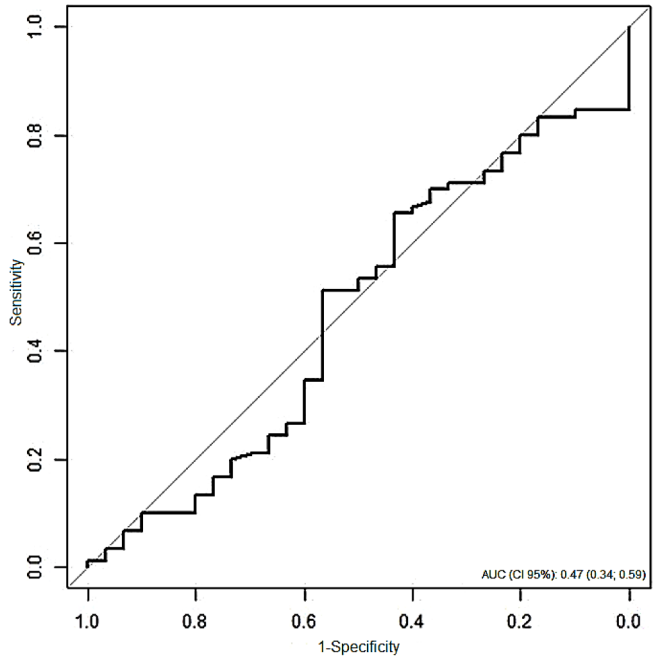
P = 0.185; P = 0.583, and P = 0.107, respectively).

In order to assess the acuity of PlGF, PDGF-AA, ANG-1, ANG-2, and the ANG1/ANG2 ratio as predictors for the development of preeclampsia, ROC curves were constructed. According to the ROC curve, a marker is considered a good predictor when the area under the curve is close to 1, indicating that it is highly sensitive and specific.

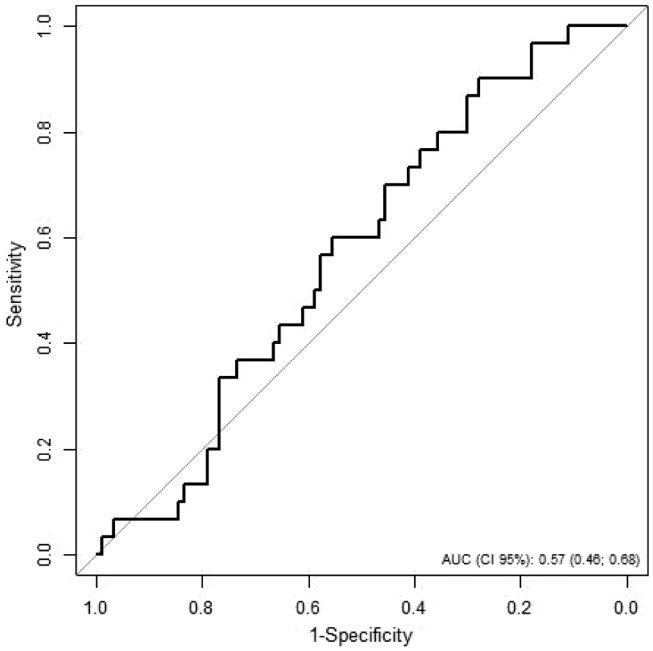
In this study, the area under the ROC curve of PlGF was 0.55 (0.41:0.70) (Graph 1). The same was observed regarding the area under curves of PDGF-AA AUC (0.55; 0.43:0.67) (Graph 2), the ANG-1 AUC (0.47; 0.34:0.59) (Graph 3), the ANG-2 AUC (0.51; 0.39:0.63) (Graph 4), and the ANG-1/ANG-2 ratio (0.57; 0.46:0.68) (Graph 5). These results suggest that none of the analyzed biomarkers have the power to predict preeclampsia development in our samples.



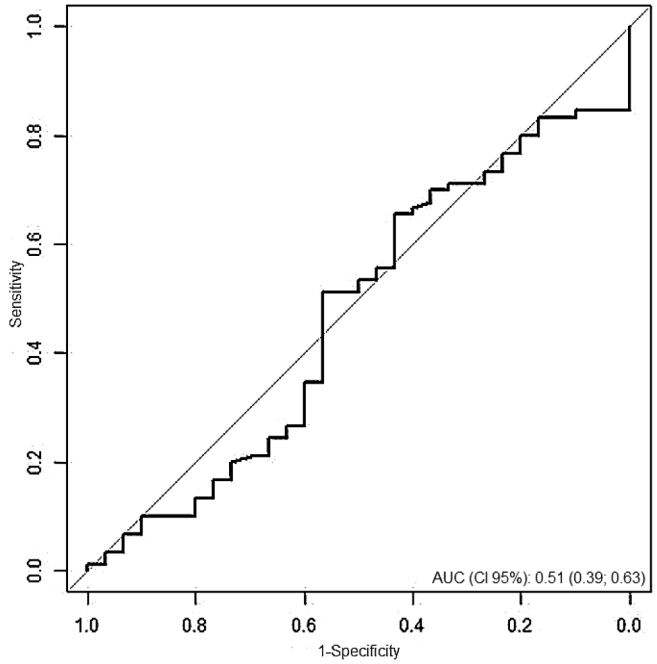
Graph 2. PDGF AA ROC curve.



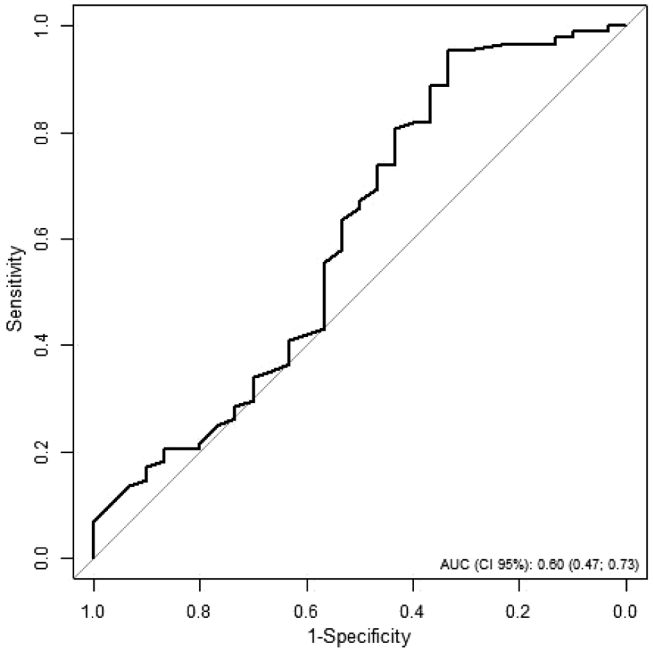
Graph 3. ANG-1 ROC curve.



Graph 5. ROC curve of the ANG-1/ANG-2 ratio.



Graph 4. ANG-2 ROC curve.



Graph 6. RUAPI ROC curve.

The Doppler was not the primary objective of the present study, however, these data were collected and used as secondary results.

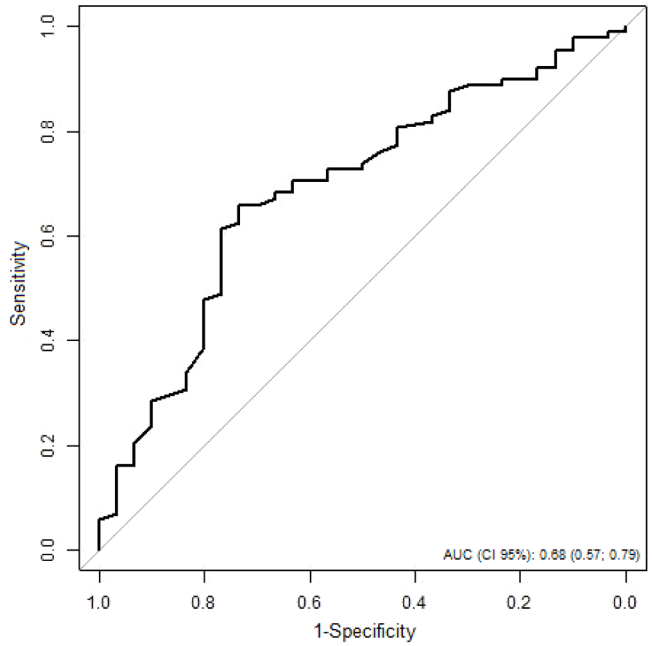
The women with preeclampsia had a more significant Right Uterine Artery Pulsatility Index (RUAPI), Left Uterine Artery Pulsatility Index (LUAPI) and Mean Pulsatility Index of the Uterine Arteries (right and left) (MPIUA) (P = 0.012, P = 0.015 and P = 0.003) when compared to health pregnant women, data are shown in Table 2.

ROC curve analyses were also conducted to evaluate the predictive power of the right and left uterine artery pulsatility indices (RUAPI and LUAPI) (Graph 6 and 7, respectively) and the mean pulsatility index of the uterine arteries (right and left) (MPIUA) (Graph 8).

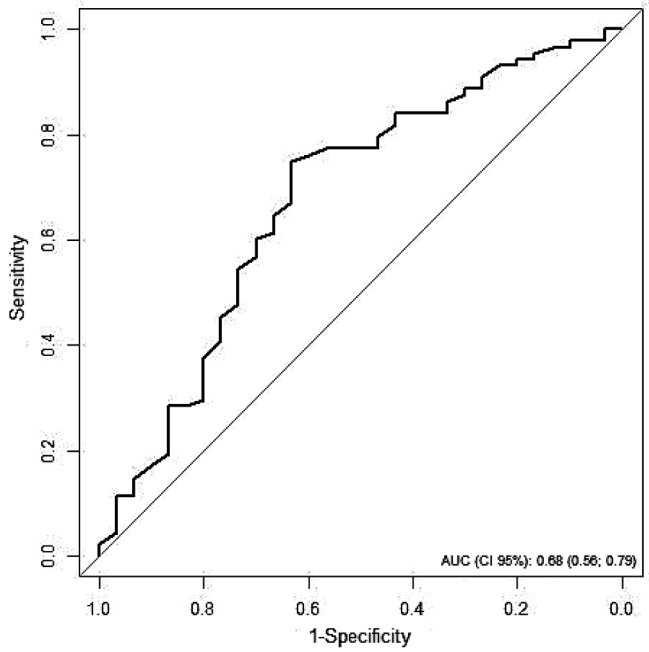
4. Discussion

The identification of biomarkers capable of predicting preeclampsia is critical, given it allows the stratification of high-risk pregnant women and their referral to appropriate treatment centers, thus reducing neonatal morbidity and maternal and fetal mortality [17–19].

In the present study, the pregnant women who developed preeclampsia exhibited higher systolic, diastolic, and mean arterial pressure at the first prenatal visit, where the mean gestational age was 23 weeks, that is, before developing the disease. As expected, during gestation, blood pressure levels continued to rise in pregnant women who developed preeclampsia, corroborating the study by Rana et al. (2007), in which the women who developed PE showed more elevated blood pressure levels when compared with healthy pregnant women



Graph 7. LUAPI ROC curve.



Graph 8. MPIUA ROC curve.

when analyzed between 11 and 13 weeks and 17–20 weeks of gestation [20].

In the present study, the concentrations of PlGF in the second gestational trimester (20–25 weeks) were analyzed. When comparing the healthy pregnant women with those who developed preeclampsia, differences in PlGF concentrations were not observed in our sample. Additionally, when analyzing the ROC curve for PlGF, the biomarker was verified as not being a good predictor of the disease in the studied groups.

When assessing the accuracy of PlGF in predicting preeclampsia in the second gestational trimester (17 weeks), Lim et al. (2008) described results that contrasted with those in this study. Their data showed that the concentration of PlGF was lower in the PE group when compared with healthy pregnant women [26]. The ROC curve analysis shows that, in the present sample, this marker does not retain good predictive power. In the study by Lim et al. (2008), the ROC curve for the sFlit-1/PlGF ratio was analyzed, and it was verified that the ratio has substantial predictive power for preeclampsia. Nevertheless, the ROC curve for PlGF was not analyzed separately [21].

Villa et al. (2013) evaluated the concentrations of sFlt-1, PlGF, and the sFlt-1/PlGF ratio for the prediction of early- and late-onset preeclampsia in a high-risk cohort. In the assessment, serum samples were evaluated at 12, 14, 18, 20, 26, and 28 weeks of gestation. Their results showed that low concentrations of PlGF and the highest sFlt-1/PlGF ratio between 26 and 28 weeks of pregnancy may predict early-onset preeclampsia [22].

When analyzing the plasma concentrations of PDGF-AA, differences between the studied groups were not observed. Few studies have analyzed PDGF-AA concentrations in pregnant women with preeclampsia, and those who did so utilized other methodologies and in women with distinct gestational ages than those evaluated in the present study.

Using immunohistochemistry, Zhang et al. (2009) and Gurski et al. (1999) analyzed pregnant women with preeclampsia in the third trimester of gestation. The authors verified high PDGF-AA expression in placental tissue when compared to the placentas of normotensive pregnant women [23,24].

The increase in PDGF-AA concentrations can be justified by the fact that, in preeclampsia, placental tissue undergoes reduced perfusion and hypoxia, thus recruiting PDGF-AA into the physiological mechanism of tissue repair [25–29].

Given ANG-1, ANG-2, and the ANG-1/ANG-2 ratio exert essential roles in the growth and development of the placental vascular system, their concentrations were evaluated in pregnant women that were healthy and who exhibited preeclampsia. When comparing the biomarker concentrations in the groups, statistical differences were not verified between them in our samples.

Different results were observed in a study conducted by Schneuer et al. (2014), in which ANG-2 concentrations were lower in patients with preeclampsia when compared with healthy pregnant women. Note should be given since their study was performed on a larger sample, the assessed populations were comprised of distinct ethnicities, and the analysis was conducted in serum, in contrast with the present study, which evaluated the plasma. Regardless of the different aspects of both assessments, the researchers also observed that ANG-1 and ANG-2 were not good predictors of the disease [30].

In a study by Hirokoshi et al. (2005), in which only ANG-2 was analyzed, the authors observed that its concentrations were significantly lower in pregnant women with preeclampsia than in healthy women. The material used for biomarker dosage was serum, in a population quite distinct from Brazilians [31].

When analyzing the ratio between ANG-1/ANG-2 in the present study, statistical differences between the groups were not observed. Nonetheless, in the prospective study conducted by Bolin et al. (2009), the authors verified that the median of the ANG-1/ANG-2 ratio, at 25 and 28 weeks of gestation, was higher in the HP group than in the PE group. These results indicate that the ANG-1/ANG-2 ratio might be a good biomarker for the prediction of late-onset preeclampsia [32].

In the study carried out by Han et al. (2012), the authors evaluated ANG-1 and ANG-2, comparing normotensive pregnant women with pregnant women with severe preeclampsia, in the third trimester of gestation. The researchers verified that ANG-2 concentrations were higher in the PE group than in the HP group. When analyzing ANG-1 concentrations, statistical differences were not observed between the groups [33].

In another study conducted by Leinonen et al. (2010), the authors monitored ANG-1 and ANG-2 levels between the 12th and 20th week of gestation. They noted that the concentrations decreased gradually throughout pregnancy, both in healthy pregnant women and those with preeclampsia. However, ANG-2 levels were higher in pregnant women with preeclampsia, which was possibly due to the action of angiogenic

factors, such as VEGF (induces the release of Tie-2 into the bloodstream), which binds circulating ANG-2, regulating its activity [34].

Many of the studies that analyzed possible markers for the prediction of preeclampsia present different results, and in all of them a very important point that must be considered is the gestational age at which these biomarkers were analyzed, our data were collected from pregnant women between 20 and 25 weeks of gestation who subsequently developed pre-eclampsia. The interval between collection of samples and development of the disease was approximately 12 weeks and although the literature shows that some markers may present altered several weeks before the clinical onset of the disease, they also show that changes in angiogenic factor levels with advancing gestation may be more predictive of preeclampsia than levels at any single timepoint [35].

When we consider gestational age as an important factor in the study of biomarkers in the prediction of preeclampsia, we can infer that in our sample (pregnant women between 20 and 25 weeks gestation) these markers did not prove to be good predictors for the disease, however, the literature shows that when they are analyzed at different gestational ages they are reliable tools for the prediction of preeclampsia.

The patients who developed preeclampsia exhibited alterations in the right uterine artery pulsatility index, left uterine artery pulsatility index and this has remained on mean pulsatility index of the uterine arteries when comparing HP *vs.* PE, indicating greater resistance to the uterine flow at the beginning of gestation and before the establishment of the disease.

Espinoza et al. (2007) evaluated the relationship between the Doppler of the uterine arteries and the concentrations of PlGF and sFlt-1. The authors noted that the combination of abnormal Doppler indices and plasma concentrations of PlGF between 22 and 26 weeks indicated high risks of developing preeclampsia and severe preeclampsia [36].

The literature contains several prospective studies that investigate the role of angiogenic factors in high-risk pregnant women identified by abnormal Doppler of the uterine artery. The combination between the use of ultrasound at the beginning of the second trimester (including measures of pulsatility index) and the determination of pro-angiogenic and anti-angiogenic factors considerably improved the predictive value of the ultrasound alone [36–39].

Research on biomarkers capable of predicting preeclampsia are of paramount importance, given they allow not only the identification of high-risk pregnant women but also the early referral of patients to appropriate treatment centers, thus reducing maternal and fetal morbidity and mortality. In a country such as Brazil, which has continental dimensions and where great discrepancies between small localities and large centers of reference are notable, this early and directed diagnosis would be decisive for treatment success.

This study presents some limitations, the fact that Cohort (BRISA) was designed to evaluate prematurity and performed a single sample collection of pregnant women between 20 and 25 weeks of gestation. Thus, the assessment of the predictive accuracy of biomarkers to predictive preeclampsia was restricted to this gestational age.

Another limitation was the difficulty of access to the medical records of the cohort participants, which comprised the determining factor for the selection of cases only of pregnant women who gave birth at the Clinical Hospital of Ribeirão Preto, the regional reference center in high-risk gestation, and the controls at the Maternity Center of the Women's Health Reference Center of Ribeirão Preto - Mater.

Moreover, after the statistics analyze was observed that the test power of the prediction curves of all biomarkers were less than 30%.

The present study is noteworthy given it constitutes the first in Brazil to evaluate PlGF, PDGF-AA, ANG-1, and ANG-2 regarding their power in predicting preeclampsia. It also comprises an indention from a prospective cohort with 1417 Brazilian women.

5. Conclusion

The present study did not show a significant difference in the plasma concentrations of the analyzed biomarkers between the HP and PE groups. The same was observed with ANG-1/ANG-2 ratio. The ROC curves indicated that the assessed biomarkers were not effective as predictors of preeclampsia in the analyzed sample which was composed of pregnant women between 20 and 25 weeks of gestation who subsequently developed preeclampsia.

The present study was supported by the ‘Coordination for the Improvement of Higher Education Personnel’ (CAPES), the Research Support Foundation of the State of São Paulo (FAPESP), and the National Council for Scientific and Technological Development (CNPq).

Acknowledgements

This study was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES), the Research Support Foundation of the State of São Paulo (FAPESP), and the National Council for Scientific and Technological Development (CNPq).

Conflicts of interest

All authors declare that there are no competing financial interests regarding the present study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2019.03.011.

References

[1] B.W.J. Mol, et al., Pre-eclampsia, Lancet (2016).
[2] F. Lyall, M. Belfort, Achados no sistema nervoso central na pré-eclâmpsia e na eclâmpsia, Cap. 28, in: F. Lyall, M. Belfort (Eds.), Pré-eclâmpsia: etiologia e prática clínica, McGraw-Hill Interamericana do Brasil Ltda., 2009, pp. 424–436.
[3] H. Hagmann, et al., The promise of angiogenic markers for the early diagnosis and prediction of preeclampsia, Clin. Chem. 58 (5) (2012) 837–845.
[4] N. Bardin, P. Murthi, N. Alfaidy, Normal and pathological placental angiogenesis, Biomed. Res Int. (2015).
[5] S.G.E. Sunderji, 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) Janeiro.
[6] S. De Falco, The discovery of placenta growth factor and its biological activity, Exp. Mol. Med. (2012) 1–9.
[7] H. Morita, et al., Abundant expression of platelet-derived growth factor in spiral arteries in decidua associated with pregnancy-induced hypertension and its relevance to atherosis, Eur. J. Endocrinol. (2001).
[8] M. Uzumcu, K.A. Dirks, M.K. Skinner, Inhibition of platelet-derived growth factor actions in the embryonic testis influences normal cord development and morphology, Biol. Reprod. (2002).
[9] J. Andrae, R. Gallini, C. Betsholtz, Role of platelet-derived growth factors in physiology and medicine, Genes Dev. (2008).
[10] M. Shao, et al., PDGF induced microRNA alterations in cancer cells, Nucl. Acids Res. (2011).
[11] D. Kappou, et al., Role of the angiopoietin/Tie System in Pregnancy (Review), Exp. Therapeutic Med. (2015).
[12] H.G. Augustin, et al., Control of vascular morphogenesis and homeostasis through the angiopoietin-Tie system, Nat. Rev. Mol. Cell Biol. (2009).
[13] M. Scharpfenecker, et al., The Tie-2 ligand angiopoietin-2 destabilizes quiescent endothelium through an internal autocrine loop mechanism, J. Cell Sci. 771–780 (2005).
[14] A.L. Tranquilli, G. Dekker, L.E.A. Magee, The classification, diagnosis and management of the hypertensive disorders of pregnancy: a revised statement from the ISSHP, Pregn. Hypertens. (2014) 97–104.
[15] ACOG, Hypertension in pregnancy. report of the American College of obstetricians and gynecologists’ task force on hypertension in pregnancy, Obstetr. Gynecol. (2013) 1122–1131.
[16] K.M. Groom, et al., Patterns of change in uterine artery Doppler studies between 20 and 24 weeks of gestation and pregnancy outcomes, Obstet Gynecol. (2009) 332–338.
[17] M. Sanderson, et al., Association between level of delivery hospital and neonatal outcomes among South Carolina Medicaid recipients, Am. J. Obstet. Gynecol. 183 (6) (Dec 2000) 1504–1511.

[18] M.M. Bode, et al., Perinatal regionalization and neonatal mortality in North Carolina, 1968–1994, Am. J. Obstet. Gynecol. 184 (6) (May 2001) 1302–1307.
[19] J. Cifuentes, et al., Mortality in low birth weight infants according to level of neonatal care at hospital of birth, Pediatrics 109 (5) (2002) 745–751 ISSN 1098-4275.
[20] S. Rana, et al., Sequential changes in antiangiogenic factors in early pregnancy and risk of developing preeclampsia, Hypertension 50 (1) (2007) 137–142 ISSN 1524-4563.
[21] J.H. Lim, et al., Effective prediction of preeclampsia by a combined ratio of angiogenesis-related factors, Obstet. Gynecol. 111 (6) (2008) 1403–1409 ISSN 0029-7844.
[22] P.M. Villa, et al., Vasoactive agents for the prediction of early- and late-onset preeclampsia in a high-risk cohort, BMC Pregnan. Childbirth 13 (2013) 110 ISSN 1471-2393.
[23] A.C. Zhang, et al., Expression and clinical significance of platelet-derived growth factor A in placenta of pre-eclampsia, Zhonghua Fu Chan Ke Za Zhi 809–8011 (2009).
[24] M.R. Gurski, E. Gonzales, E.G. Brown, Immunochemical localization of platelet-derived growth factor in placnta and its possible role in preeclampsia, J. Invest. Med. 128–133 (1999).
[25] H. Morita, et al., Abundant expression of platelet-derived growth factor in spiral arteries in decidua associated with pregnancy-induced hypertension and its relevance to atherosis, Eur. J. Endocrinol. 271–276 (2001).
[26] M. Uzumcu, K.A. Dirks, M.K. Skinner, Inhibition of platelet-derived growth factor actions in the embryonic testis influences normal cord development and morphology, Biol. Reprod. 745–753 (2002).
[27] J. Andrae, R. Gallini, C. Betsholtz, Role of platelet-derived growth factors in physiology and medicine, Genes Dev. (2008) 1276–1312.
[28] J. Dingemann, et al., Abnormal platelet-derived growth factor signaling accounting for lung hypoplasia in experimental congenital diaphragmantic hernia, J. Pediatr. Surg. 1989–1994 (2010).
[29] M. Shao, et al., PDGF induced microRNA alterations in cancer cells, Nucl. Acids Res. 4035–4047 (2001).
[30] F.J. Schneuer, C.L. Roberts, A.W. Ashton, Angiopoietin 1 and 2 serum concentrations in first trimester of pregnancy as biomarkers of adverse pregnancy outcomes, Am. J. Obstet. Gynecol. 1–9 (2014).
[31] K. Hirokoshi, et al., Increase of serum angiopoietin-2 during pregnancy is suppressed in women with preeclampsia, Am. J. Hypertens. 1181–1188 (2005).
[32] M. Bolin, et al., Angiopoietin-1/angiopoietin-2 ratio for prediction of preeclampsia, Am. J. Hypertens. (2009) 891–895.
[33] S.Y. Han, et al., Angiopoietin-2: a promising indicator for the occurrence of severe preeclampsia, Hypertens. Pregnan. 189–199 (2012).
[34] E. Leinonen, et al., Maternal serum angiopoietin-1 and -2 and Tie-2 in early pregnancy ending in preeclampsia or intrauterine growth retardation, J. Clin. Endocrino (2010) 126–133.
[35] S.E. Maynard, S.A. Karumanchi, Angiogenic factors and preeclampsia, Semin. Nephrol. 31 (1) (2011) 33–46.
[36] J. Espinoza, et al., Identification of patients at risk for early onset and/or severe preeclampsia with the use of uterine artery Doppler velocimetry and placental growth factor, Am. J. Obstet. Gynecol. 196 (4) (2007) 326 ISSN 1097-6868.
[37] H. Stepan, et al., Predictive value of maternal angiogenic factors in second trimester pregnancies with abnormal uterine perfusion, Hypertension 49 (4) (2007) 818–824 ISSN 1524-4563.
[38] F. Crisp, et al., Predictive value of angiogenic factors and uterine artery Doppler for early- versus late-onset pre-eclampsia and intrauterine growth restriction, Ultrasound Obstet. Gynecol. 31 (3) (2008) 303–309 ISSN 1469-0705.
[39] A.E. Diab, et al., Angiogenic factors for the prediction of pre-eclampsia in women with abnormal midtrimester uterine artery Doppler velocimetry, Int. J. Gynaecol. Obstet. 102 (2) (2008) 146–151 ISSN 0020-7292.