Preeclampsia and superimposed preeclampsia: The same disease? The role of angiogenic biomarkers

Rafaela A. Costa, Mara S. Hoshida, Eliane A. Alves, Marcelo Zugaib, and Rossana P. V. Francisco

Department of Obstetrics and Gynecology of Faculdade de Medicina da Universidade de São Paulo, Sao Paulo, SP, Brazil; Department of Obstetrics and Gynecology of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Sao Paulo, SP, Brazil

ABSTRACT

Objective: We aimed to compare sFlt-1 and placental growth factor (PlGF) levels and the sFlt-1/PlGF ratio between women with preeclampsia and superimposed preeclampsia to, respectively, normotensive and chronic hypertensive ones.

Study design: We performed a prospective two-armed cohort in a tertiary teaching hospital in Sao Paulo, Brazil, including 37 normotensive and 60 chronic hypertensive pregnant women. We assessed the serum levels of sFlt-1 and PlGF at 20, 26, 32, and 36 gestational weeks by enzyme-linked immunosorbent assay.

Main outcome measures: Having preeclampsia and superimposed preeclampsia.

Results: Among normotensive and chronic hypertensive pregnancies, 4 (10.8%) and 14 (23.3%) women developed preeclampsia and superimposed preeclampsia, respectively. Compared with those who remained normotensive, the preeclampsia women presented higher sFlt-1 levels at 32 gestational weeks (4323.45 pg/mL vs. 2242.04 pg/mL, \( p = 0.019 \)), lower PlGF levels at 20 (183.54 pg/mL vs. 337.38 pg/mL, \( p = 0.034 \)), 32 (169.69 pg/mL vs. 792.53 pg/mL, \( p = 0.001 \)), and 36 gestational weeks (252.99 pg/mL vs. 561.81 pg/mL, \( p = 0.029 \)), and higher sFlt-1/PlGF ratios at 26 (9.02 vs. 1.84, \( p = 0.004 \)), 32 (23.61 vs. 2.55, \( p = 0.001 \)), and 36 gestational weeks (49.02 vs. 7.34, \( p = 0.029 \)). On the other hand, compared with those who remained chronic hypertensive, the superimposed preeclampsia women only presented a higher sFlt-1/PlGF ratio at 32 gestational weeks (9.98 vs. 2.51, \( p = 0.039 \)).

Conclusion: Although angiogenic imbalance is clearly related to preeclampsia, it seems to play a more modest role in superimposed preeclampsia, in which other mechanisms should also be investigated.

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Introduction

Despite being a major public health problem, the pathophysiology of preeclampsia is not completely understood. Preeclampsia progression comprises a pre-clinical stage and a clinical stage (1). The clinical stage defines the disease and is characterized by the onset of hypertension and proteinuria after 20 gestational weeks. In the absence of proteinuria, the onset of hypertension after 20 weeks is known as gestational hypertension (2). Hypertension...
before 20 gestational weeks determines a diagnosis of chronic hypertension. Chronic hypertensive subjects have a higher risk of developing superimposed preeclampsia, which can be a challenging diagnosis (3–5).

During the last decade, much work has been focused on identifying the pre-clinical stage of preeclampsia (6). Many researchers have clearly demonstrated an anti-angiogenic imbalance that is marked by higher levels of soluble fms-like tyrosine kinase-1 (sFlt-1) and lower levels of placental growth factor (PIGF) in the subjects who develop preeclampsia compared with those who do not (7–13). Although a growing number of studies in the high-risk population have shown the role of these biomarkers in diagnosing preeclampsia, superimposed preeclampsia remains partially understudied, and the literature regarding this subject continues to be relatively sparse as well as controversial (14, 15).

We aimed to evaluate the effect of maternal circulating sFlt-1 and PIGF levels on the diagnosis of superimposed preeclampsia in chronic hypertensive subjects in comparison to their effect on the diagnosis of preeclampsia in normotensive control subjects in a non-intervention cohort trial.

**Methods**

We conducted a prospective, single-center, two-armed observational cohort study involving chronic hypertensive and normotensive women who presented spontaneously or were referred from primary health care centers for antenatal care visits at a public tertiary teaching hospital in Sao Paulo, Brazil (Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo), from May 2011 to January 2014.

The sample size was based on previous data from other authors (16, 17), with an estimated Cohen’s $d$ effect size coefficient of 1.547 for PIGF in the normotensive/preeclampsia group and of 0.98 for sFlt-1 in the hypertensive/superimposed preeclampsia group, assuming a statistical power of 80% and a significance level of 95%. Based on these calculations, considering the estimated incidence of preeclampsia and superimposed preeclampsia in our population, the sample size should be 36 in the normotensive cohort and 56 in the hypertensive cohort. We estimated a 10% loss to follow-up when recruiting subjects.

We included singleton pregnancies before 20 gestational weeks that did not present chronic diseases classically related to peripheral vasculopathy (except chronic hypertension), such as diabetes mellitus, systemic lupus erythematosus, cardiomyopathy, and thrombophilia. The gestational age was confirmed by one ultrasound during the first trimester or two ultrasounds during the second trimester. Each subject signed the informed consent form approved on 25 May 2011 by the ethics committee of the CAPPesq (Comissão de Ética para Análise de Projetos de Pesquisa), registration number 0273/10.

A participant was excluded if a fetal malformation was diagnosed before or after delivery or if either at least two blood samples were not collected during pregnancy or she abandoned antenatal care visits, resulting in a lack of outcome data.

The participants had antenatal care routines followed by practitioners who were unaware of the study protocol. We planned venous blood sample collection at the gestational ages of 20, 26, 32, and 36 weeks during antenatal visits or during hospitalization, as needed. After blood collection, the serum was promptly separated from the total blood samples in standard tubes, aliquotted, and stored at $-80^\circ$C until analysis, which was
performed after delivery. We assayed sFlt-1 and free PlGF by enzyme-linked immunosorbent assay (ELISA) using Quantikine® kits (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. The samples were run in duplicate by a single investigator blinded to the clinical outcomes. Intra and inter assay coefficients of variation were, respectively, 7.7% and 10.38% for PlGF, and 4.7% and 10.7% for sFlt-1. The detectable minimums are 3.5 pg/mL for sFlt-1 and 7.0 pg/mL for PlGF.

The primary outcome was the occurrence of preeclampsia, which was diagnosed by the practitioner in charge of antenatal care. Pregnancy outcome data were extracted from medical records. The first author of this paper (RAC) examined the medical charts to ensure that every case of preeclampsia was in agreement with the criteria of the National High Blood Pressure Education Program Working Group (18). Preeclampsia was diagnosed if the blood pressure was $\geq 140$ mmHg systolic or $\geq 90$ mmHg diastolic in women who were normotensive before 20 gestational weeks in association with proteinuria $\geq 300$ mg/24 h. The occurrence of hypertension without proteinuria after 20 gestational weeks was defined as gestational hypertension. Superimposed preeclampsia was defined as preexisting hypertension before 20 gestational weeks with a new onset of proteinuria or worsening of preexisting proteinuria and a sudden increase in the magnitude of hypertension. All cases were also in agreement with the current task force reported by the American College of Obstetricians and Gynecologists (4).

We used the IBM SPSS Statistics software for Macintosh 22.0 (IBM Corp., Armonk, NY, USA) and the G*Power 3.1 (Faul, Erdfelder, Lang and Buchner, 2009) (19) software for the statistical analysis. The normality of the data was assessed by the Shapiro–Wilk test. Continuous variables were summarized by the mean and standard deviation (SD) or by the median and interquartile range (IQR), as appropriate. For the comparisons between groups, we used Student’s $t$-test or Mann–Whitney U test, as appropriate for each parameter. The categorical variables were summarized by proportions and compared with the $\chi^2$ test or Fisher’s exact test, as appropriate.

We compared the circulating levels of sFlt-1 and PlGF and the sFlt-1/PlGF ratio at each gestational age between groups, according to diagnosed preeclampsia (normotensive vs. preeclampsia and chronic hypertensive vs. superimposed preeclampsia). Presented data were not transformed or treated. Only data from available blood samples were analyzed. A $p$-value of <0.05 was considered to be statistically significant (two-tailed tests).

**Results**

We recruited 102 women ($n = 40$ for the normotensive cohort and $n = 62$ for the chronic hypertensive cohort) for this trial. During the study period, three subjects from the normotensive and two subjects from the hypertensive arm were excluded either for failing to provide at least two blood samples or for abandoning hospital antenatal care.

Preeclampsia occurred in 4 (10.8%) of the 37 women in the first arm of the cohort at 35.75 (SD 2.39) gestational weeks and superimposed preeclampsia occurred in 14 (23.3%) of the 60 women in the second arm of the cohort at 31.28 (SD 5.29) gestational weeks. Since the literature is controversial about gestational hypertension, we excluded four (10.8%) subjects with this diagnosis. Therefore, 93 women remained in this analysis, from whom 342 blood samples were obtained at the gestational ages of 20.00 (SD 0.91), 26.22 (SD 1.06), 31.98 (SD 0.89), and 36.02 (SD 0.71) weeks. Clinical data of pregnancies
complicated by preeclampsia are listed in Table 1. Only one woman in the superimposed preeclampsia group had previously positive proteinuria and presented increase in blood pressure and worsening of previous proteinuria (158% proteinuria augmentation). There were no cases of hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome or eclampsia in our sample.

The maternal baseline characteristics and gestational outcomes are described in Table 2, based on the final diagnosis. We compared the serum levels of sFlt-1 and PlGF and the sFlt-1/PlGF ratio between normotensive vs. preeclampsia and between chronic hypertensive vs. superimposed preeclampsia.

Table 1. Clinical characteristics of pregnancies complicated by preeclampsia.

| Final diagnosis | Maternal age (years) | GA at PE diagnosis (weeks) | GA at delivery (weeks) | Anti-hypertensive drugs (n) | SGA | Positive PTU at antenatal care initiation (<300 mg/24 h) |
|-----------------|----------------------|---------------------------|------------------------|-----------------------------|-----|--------------------------------------------------|
| PE              | 26                   | 34.57                     | 37.71                  | 2                           | No  | –                                                |
| PE              | 26                   | 36.71                     | 36.86                  | 0                           | Yes | –                                                |
| PE              | 34                   | 33.14                     | 33.86                  | 0                           | Yes | –                                                |
| PE              | 39                   | 38.57                     | 38.57                  | 0                           | No  | –                                                |
| SIPE            | 29                   | 22.29                     | 37.43                  | 2                           | No  | –                                                |
| SIPE            | 42                   | 33.57                     | 36.14                  | 1                           | No  | –                                                |
| SIPE            | 35                   | 36.86                     | 37.14                  | 1                           | No  | –                                                |
| SIPE            | 36                   | 28.14                     | 37.14                  | 2                           | Yes | –                                                |
| SIPE            | 42                   | 27.00                     | 37.00                  | 2                           | No  | –                                                |
| SIPE            | 39                   | 36.86                     | 37.14                  | 1                           | No  | –                                                |
| SIPE            | 30                   | 35.43                     | 37.00                  | 0                           | No  | –                                                |
| SIPE            | 35                   | 26.14                     | 35.29                  | 1                           | Yes | –                                                |
| SIPE            | 24                   | 27.71                     | 33.86                  | 2                           | No  | –                                                |
| SIPE            | 38                   | 29.57                     | 33.00                  | 2                           | Yes | –                                                |
| SIPE            | 38                   | 35.14                     | 36.57                  | 2                           | Yes | –                                                |
| SIPE            | 35                   | 38.14                     | 38.14                  | 1                           | No  | –                                                |
| SIPE            | 31                   | 32.86                     | 37.00                  | 1                           | No  | –                                                |
| SIPE            | 48                   | 33.14                     | 36.57                  | 2                           | Yes | –                                                |

PE, preeclampsia; SIPE, superimposed preeclampsia; GA, gestational age; SGA, small for gestational age; PTU, proteinuria.  
1SGA was defined as birthweight < 10% for gestational age, according to Alexander’s Growth Chart (20).  
2Only chronic hypertensive women had their proteinuria assessed at first antenatal care visit; low-risk pregnancies did not have PTU assessed until there was clinical suspicion of preeclampsia.

| Characteristic/Outcome | NT (n = 29) | PE (n = 4) | p-value | CH (n = 46) | SIPE (n = 14) | p-value |
|-----------------------|------------|-----------|---------|------------|-------------|---------|
| Maternal age, years   | 29.68 ± 6.07 | 26.41 ± 6.17 | 0.322    | 33.97 ± 6.61 | 35.85 ± 6.09 | 0.518    |
| White race, n (%)     | 18 (62.07) | 4 (100.00) | 0.276    | 23 (50.00) | 6 (42.86) | 0.640    |
| BMI, kg/m^2           | 25.11 ± 3.70 | 29.32 ± 6.53 | 0.289    | 29.82 ± 5.68 | 29.91 ± 5.04 | 0.966    |
| Smoking, n (%)        | 4 (13.79) | 0 (0)     | 1.000    | 3 (6.52) | 0 (0)     | 1.000    |
| Primiparity, n (%)    | 16 (55.17) | 3 (75.00) | 0.620    | 11 (23.91) | 4 (28.57) | 0.734    |
| Previous PE, n (%)    | 1 (3.45) | 1 (25.00) | 0.231    | 12 (26.09) | 4 (28.57) | 1.000    |
| GA delivery, weeks    | 38.70 ± 3.03 | 36.75 ± 2.05 | 0.025    | 38.54 ± 1.36 | 36.39 ± 1.42 | <0.001   |
| Cesarean, n (%)       | 17 (58.62) | 3 (75.00) | 0.000    | 32 (69.57) | 12 (85.71) | 0.314    |
| Birth weight, grams   | 3177 ± 584 | 2343 ± 810 | 0.000    | 3283 ± 464 | 2584 ± 523 | <0.001   |
| SGA, n (%)            | 3 (10.34) | 2 (50.00) | 0.099    | 2 (4.35) | 5 (35.71) | 0.006    |
| Fetal distress, n (%) | 1 (3.45) | 2 (50.00) | 0.033    | 3 (6.52) | 1 (7.14) | 1.000    |

NT, normotensive; PE, preeclampsia; CH, chronic hypertension; SIPE, superimposed preeclampsia; BMI, body mass index; GA, gestational age; SGA, small for gestational age (birthweight < 10% for gestational age, according to Alexander’s Growth Chart (20)). Displayed data are either mean ± standard deviation or n (%).  
1Student’s t-test; 2Mann–Whitney U test; 3Fisher exact test; 4X^2 test.  
Italic values highlight results with p-value < 0.05.
preeclampsia. Subjects who developed preeclampsia presented statistically higher sFlt-1 levels at 32 gestational weeks (4323.45 (3410.66–13017.88) vs. 2242.04 (1511.19–3013.80), p = 0.019), lower PlGF levels at 20 (183.54 (115.54–228.02) vs. 337.38 (226.36–434.11), p = 0.034), 32 (169.69 (89.38–337.64) vs. 792.53 (635.69–1468.84), p = 0.001), and 36 gestational weeks (252.99 (116.87–288.25) vs. 561.81 (304.62–904.11), p = 0.029), and higher sFlt-1/PlGF ratios at 26 (9.02 (4.61–32.10) vs. 1.84 (1.16–3.41), p = 0.004), 32 (23.61 (10.7–226.04) vs. 2.55 (1.36–5.00), p = 0.001) and 36 gestational weeks (49.02 (19.27–53.29) vs. 7.34 (4.01–19.68), p = 0.029), as compared to normotensive controls. For subjects with superimposed preeclampsia, only the sFlt-1/PlGF ratio at 32 gestational weeks was statistically higher (9.98 (4.14–15.10) vs. 2.51 (1.06–7.16), p = 0.039) as compared to chronic hypertensive pregnant women (Table 3).

We further compared angiogenic factor levels between preeclampsia and superimposed preeclampsia. We found statistically lower levels of sFlt-1 at 32 (2437.73 (1655.01–3894.96) vs. 4323.45 (3410.66–13017.88), p = 0.045) and 36 gestational weeks (2186.08 (1280.44–4080.74) vs. 6227.94 (5555.64–12400.76), p = 0.049) in women with superimposed preeclampsia as compared to the preeclampsia group.

The patterns of changes in sFlt-1 and PlGF levels and the sFlt-1/PlGF ratio along the pregnancy are represented in Figure 1. We observed similar patterns of the angiogenic factors changes between normotensive and chronic hypertensive women. On the same way, patterns of changes of the PlGF levels were similar between preeclampsia and superimposed preeclampsia groups, but the sFlt-1 levels, and so the sFlt-1/PlGF ratio, presented a more accentuated increase in the preeclampsia group from 32 gestational weeks on. Besides, both preeclampsia and superimposed preeclampsia groups presented an earlier decrease in the PlGF levels, as compared to normotensive and chronic hypertensive women.

**Discussion**

Although sFlt-1 (32 gestational weeks), PlGF (20, 32, and 36 gestational weeks), and the sFlt-1/PlGF ratio (26, 32, and 36 gestational weeks) levels were different between normotensive and preeclampsia pregnant women, only the sFlt-1/PlGF ratio at 32 gestational weeks was useful for distinguishing those presenting superimposed preeclampsia from those with chronic hypertension.

We found a very high rate of preeclampsia (10.8%) in our normotensive cohort. When evaluating baseline clinical characteristics of this group, it is important to address that 54.2% of those women were overweight/obese prior to pregnancy; besides, 24.3% of them also presented gestational diabetes, both risk factors for preeclampsia. Although these rates are very high, unfortunately they reflect the epidemiological transition of women in reproductive age in Brazil, especially in Sao Paulo city, with increasing prevalence of obesity and glucose tolerance impairment in adult population.

Our study has some strong points. Studies dedicated to chronic hypertensive and superimposed preeclampsia pregnancies are rare. Most published data regarding the diagnosis of preeclampsia in high-risk pregnancies include miscellaneous conditions, such as diabetes, nulliparity, obesity, chronic kidney disease, and a previous diagnosis of preeclampsia (17,21–25). Starting from a two-armed normotensive, chronic hypertensive cohort allowed us to longitudinally compare patterns of angiogenic marker profiles among
Table 3. Comparison of circulating levels of sFlt-1 and PlGF and the sFlt-1/PlGF ratio among groups according to final diagnose of preeclampsia.

| Biomarker/GA | NT | PE | PE vs. NT | CH | SIPE | SIPE vs. CH |
|--------------|----|----|-----------|----|------|-------------|
| sFlt-1 (pg/mL) |    |    |           |    |      |             |
| 20 weeks     | 28 | 1476.34 (1016.64–2430.80) | 4  | 1195.33 (687.02–3711.68) | 0.564 | 38 | 924.66 (665.28–1192.84) | 0.664 |
| 26 weeks     | 28 | 1566.86 (994.57–2073.31) | 4  | 2425.05 (1277.10–7918.05) | 0.230 | 42 | 1100.34 (733.12–1568.63) | 0.663 |
| 32 weeks     | 27 | 2242.04 (1511.19–3013.80) | 4  | 4323.45 (3410.66–13017.88) | 0.019 | 46 | 1458.92 (988.86–3148.50) | 0.060 |
| 36 weeks     | 26 | 4784.67 (2970.76–6287.07) | 3  | 6227.94 (5555.64–12400.76)* | 0.150 | 44 | 2914.91 (1631.14–5929.59) | 0.350 |
| PlGF (pg/mL) |    |    |           |    |      |             |
| 20 weeks     | 28 | 337.38 (226.36–434.11) | 4  | 183.54 (115.54–228.02) | 0.034 | 38 | 208.04 (146.99–373.05) | 0.219 |
| 26 weeks     | 28 | 819.78 (592.74–1367.88) | 4  | 284.82 (152.98–649.85) | 0.054 | 42 | 497.69 (288.63–860.82) | 0.677 |
| 32 weeks     | 27 | 792.53 (635.69–1468.84) | 4  | 169.69 (89.38–337.64) | 0.001 | 46 | 551.68 (293.39–1000.52) | 0.067 |
| 36 weeks     | 26 | 561.81 (304.62–904.15) | 3  | 252.99 (116.87–288.25)* | 0.029 | 44 | 385.73 (178.39–762.61) | 0.876 |
| sFlt-1/PlGF ratio |    |    |           |    |      |             |
| 20 weeks     | 28 | 5.28 (2.66–8.25) | 4  | 8.73 (4.98–19.46) | 0.279 | 38 | 3.56 (2.06–8.06) | 0.973 |
| 26 weeks     | 28 | 1.84 (1.16–3.41) | 4  | 9.02 (4.61–32.10) | 0.004 | 42 | 1.97 (1.29–4.49) | 0.235 |
| 32 weeks     | 27 | 2.55 (1.36–5.00) | 4  | 23.61 (10.7–226.04) | 0.001 | 46 | 2.51 (1.06–7.16) | 0.039 |
| 36 weeks     | 26 | 7.34 (4.01–19.68) | 3  | 49.02 (19.27–53.29)* | 0.029 | 44 | 8.95 (2.59–34.53) | 0.841 |

NT, normotensive; PE, preeclampsia; CH, chronic hypertension; SIPE, superimposed preeclampsia; GA, gestational age; IQR, interquartile range. Data are the median (interquartile range). *For the small sample, the total range is displayed.

1Mann-Whitney U test.

Italic values highlight results with \(p\)-value < 0.05.
normotensive, chronic hypertensive, preeclampsia, and superimposed preeclampsia pregnancies. In addition to reducing the risks of bias, performing a prospective observational study prevented us from analyzing long-term stored blood samples. Besides, concerned about the dynamic changes in angiogenic biomarkers throughout pregnancy, we sampled blood at fixed gestational weeks, avoiding comparisons among samples from different gestational ages. We also cautioned that every woman was followed-up until delivery, in order to assure that a late diagnosis of preeclampsia would not lead to subject misallocation.

In agreement with other studies, we found higher levels of sFlt-1 and the sFlt-1/PlGF ratio and lower levels of PlGF in women who presented preeclampsia in comparison to those who remained normotensive (11–13). As presented by other authors (13), PlGF levels were earlier reduced in pregnancies to develop preeclampsia, while sFlt-1 and the sFlt-1/PlGF ratio were augmented a little later in these pregnancies. Though our sample size, our results for the preeclampsia group matched those demonstrated in the literature, validating our method and supporting angiogenic biomarkers as promising elements in the pathophysiology of preeclampsia.

Oppositely, for the chronic hypertensive cohort, the results were different and more modest. Only the sFlt-1/PlGF ratio at 32 gestational weeks was higher in women who presented superimposed preeclampsia as compared to those who did not.

Our findings are supported by those of Powers et al. (14), who did not find differences in PlGF levels between chronic hypertensive and superimposed preeclampsia groups at any gestational age. The authors reported higher sFlt-1 levels in superimposed preeclampsia subjects at 26–30 gestational weeks and their results suggested

![Figure 1. Patterns of sFlt-1, PlGF, and the sFlt-1/PlGF ratio median levels along pregnancy in normotensive, preeclampsia, chronic hypertensive, and superimposed preeclampsia groups.](image-url)
that angiogenic biomarkers are less predictive for superimposed preeclampsia than for preeclampsia.

In contrast, Perni et al. (15) reported higher levels of sFlt-1 and the sFlt-1/PIGF ratio and lower levels of PlGF in an early superimposed preeclampsia group at 20 and 28 gestational weeks, compared with chronic hypertensive subjects.

We expected to find similar anti-angiogenic imbalance in preeclampsia and superimposed preeclampsia groups. Nonetheless, assessments of angiogenic markers presented differently between these groups when compared, respectively, to normotensive and chronic hypertensive pregnant controls. These novel simultaneous comparisons suggested that angiogenic imbalance could play different roles in preeclampsia and superimposed preeclampsia. We could not find significant differences in angiogenic marker levels between the chronic hypertensive and the superimposed preeclampsia subjects until 32 gestational weeks, unlike we observed between the normotensive and the preeclampsia groups.

Our study has some limitations. For the preeclampsia group, although the sample size was smaller, we found statistically lower levels of PlGF from 20 gestational weeks and higher sFlt-1/PIGF ratios from 26 gestational weeks, when compared to normotensive pregnancies, evidencing a large effect size of the angiogenic markers on the diagnosis of preeclampsia. Nonetheless, for the superimposed preeclampsia group, despite the larger sample size, we did not achieve the expected statistical power from the tests. In a post-hoc analysis of our data (not provided), the effect sizes of the angiogenic markers (estimated by the Cohen’s d effect size coefficient) were mostly medium or small for the superimposed preeclampsia group. In order to reach a statistical significance of 0.95 and a statistical power of 0.80, with the effect sizes we observed from our sample, we should include, for example, 2366 women in our chronic hypertensive cohort for assessing the PlGF levels at 26 gestational weeks and 1606 women for the PlGF levels at 32 gestational weeks, suggesting clinical limitations when using angiogenic biomarkers for the diagnosis and/or prediction of superimposed preeclampsia when compared to what has been broadly presented for preeclampsia, including our results.

We speculated some explanations to this lack of statistical difference in angiogenic marker levels between chronic hypertensive and superimposed preeclampsia.

The first possibility would be a higher susceptibility in chronic hypertensive women, who would have lower thresholds for angiogenic imbalance to the development of preeclampsia. Recent studies have addressed the existence of a “normal angiogenic profile” preeclampsia, a condition characterized by more obese women, who are more likely to have preexisting diabetes and present at later gestational ages (26). Previous data showed that preeclampsia women with a normal angiogenic balance presented significantly higher blood pressure at midpregnancy than preeclampsia women with an abnormal angiogenic balance, suggesting that occult endothelial damage could increase susceptibility to preeclampsia (27).

Secondly, clinical presentation of chronic hypertension can be very heterogeneous among individuals regarding the duration and the severity of the disease, as well as the presence or absence of end-organ damage. So, even among chronic hypertensive pregnancies, endothelial susceptibility to preeclampsia might vary. In this sense, stratification of the chronic hypertension severity would be relevant in future studies on pathophysiology of superimposed preeclampsia.

Finally, superimposed preeclampsia could be a consequence of a distinct pathophysiologic pathway, in which other mechanisms could overlap the angiogenic imbalance. Preeclampsia
is a multifactorial disease and it is unlike that a single pathway could explain it. Since superimposed preeclampsia is a more specific situation, other mechanisms might also be involved and could be even more relevant. We observed that sFlt-1 levels were significantly higher in preeclampsia subjects as compared to superimposed preeclampsia, suggesting that sFlt-1 augmentation could be more decisive in pathophysiology of preeclampsia than superimposed preeclampsia. Underlying inflammatory status, oxidative stress and disturbances in vasoactive agent expressions are all mechanisms studied in the pathophysiology of preeclampsia and could be possibly more relevant for superimposed preeclampsia.

In summary, our results suggest caution when extrapolating the effect of angiogenic imbalance from preeclampsia to superimposed preeclampsia. Few studies have focused on this group, in which preeclampsia is more prevalent and difficult to diagnose. Further evaluation is necessary to understand the role of angiogenic factors in this subtype of hypertensive disorder during pregnancy.

**Conclusions**

Although an anti-angiogenic imbalance can be clearly demonstrated in preeclampsia, it is not so pronounced in superimposed preeclampsia. Since preeclampsia is a multifactorial disease, angiogenic imbalance might play a minor role in pathophysiology of superimposed preeclampsia as compared to its role in preeclampsia.

**Declaration of interest**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper. Financial and infrastructure support for this study was provided by the Department of Obstetrics and Gynecology of the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo.

**ORCID**

Rafaela Costa http://orcid.org/0000-0001-8828-6725
Mara Hoshida http://orcid.org/0000-0003-2232-4631
Marcelo Zugaib http://orcid.org/0000-0003-1155-2671
Rossana Francisco http://orcid.org/0000-0002-9981-8069

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