Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health 31 (2023) 32–37

Contents lists available at ScienceDirect

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

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

Role of biomarkers (sFlt-1/PlGF) in cases of COVID-19 for distinguishing preeclampsia and guiding clinical management

Guilherme M. Nobrega [a], Jose P. Guida [a], Juliana M. Novaes [a], Larissa M. Solda [a], Luciana Pietro [a,b], Adriana G. Luz [a], Giuliane J. Lajos [a], Carolina C. Ribeiro-do-Valle [a], Renato T Souza [a], Jose G. Cecatti [a], Indira U. Mysorekar [c,d], Tabata Z. Dias [a], Maria Laura Costa [a,*]

[a] Department of Obstetrics and Gynecology, School of Medical Sciences, University of Campinas, Campinas, SP, Brazil
[b] Institute of Health Sciences, Paulista University, Brazil
[c] Department of Medicine, Section of Infectious Diseases, Baylor College of Medicine, Houston, TX 77030, USA
[d] Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX 77030, USA

ARTICLE INFO

Keywords:
Maternal health
COVID-19
Hypertensive disorders of pregnancy
Renin-angiotensin system

ABSTRACT

Objectives: To analyze soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factors (PlGF) concentrations and their ratio in pregnant and postpartum women with suspected COVID-19, and further investigate conditions associated with an increased ratio (sFlt-1/PlGF > 38), including preeclampsia (PE) and severe acute respiratory syndrome (SARS).

Study Design: The present study is a secondary analysis of a prospective cohort. Blood samples were collected at time of COVID-19 investigation and the serum measurements of sFlt-1 and PlGF were performed. Clinical background, SARS-CoV-2 infection characteristics, maternal and perinatal outcomes were further analyzed.

Main outcome measures: Serum measurements of sFlt-1 and PlGF; obstetrics and clinical outcomes.

Results: A total of 97 SARS-CoV-2 unvaccinated women with suspected infection were considered, 76 were COVID-19 positive cases and 21 COVID-19 negative. Among COVID-19 positive cases, 09 presented with SARS and 11 were diagnosed with PE, of which 6 had SARS-CoV-2 infection in first and second trimester (04 with sFlt-1/PlGF ≥ 38) and 05 with PE and COVID-19 diagnosed at the same time, during third trimester (03 with sFlt-1/PlGF ≥ 38). Five presented with PE with severe features. sFlt-1/PlGF ratio was significantly higher in the COVID-19 positive/PE positive group compared to COVID-19 positive/PE negative group (p-value = 0.005), with no increase in cases complicated by SARS.

Conclusions: sFlt-1/PlGF ratio could be a useful tool for differential diagnosis and adequate counseling among cases of COVID-19 and PE, especially if severe disease. COVID-19 early in pregnancy could potentially be a risk factor for PE later during gestation.

1. Introduction

Hypertensive disorders of pregnancy, especially preeclampsia (PE), represent main contributors to maternal mortality and morbidity worldwide and most significantly in low and middle-income populations [1,2]. During the COVID-19 pandemic, these populations have also been disproportionately affected by severe infections due to delays in healthcare, difficulties in testing and managing the disease, restricted availability of intensive care units, and inadequate management of complications, all aggravated by low access to vaccination. Outcomes are particularly striking for COVID-19 infections during pregnancy and postpartum, with increased numbers reported for maternal morbidity and mortality not only due to the challenges with healthcare, but also because of pregnancy itself as a risk factor for severe disease [3–5].

The association between COVID-19 and PE has been recently highlighted by different studies, showing increased frequencies of PE among cases of COVID-19 [6]. The rationale to explain such findings is based on the similar pathophysiology and clinical presentation of both conditions [7].

PE is a syndrome with multisystemic organ involvement associated

* Corresponding author.
E-mail address: mlaura@unicamp.br (M. Laura Costa).

https://doi.org/10.1016/j.preghy.2022.11.008
Received 23 May 2022; Received in revised form 11 October 2022; Accepted 28 November 2022
Available online 5 December 2022

with inflammation and endothelial damage. Plasma concentrations of pro-angiogenic/anti-angiogenic factors released by the placental syncytiotrophoblast have been identified as markers of disease progression. In PE, soluble fms-like tyrosine kinase 1 (sFlt-1, an anti-angiogenic protein) increases, and placental growth factor (PlGF, a pro-angiogenic protein) decreases. Therefore, sFlt-1 and PlGF directly and inversely correlate, respectively, with disease onset [8–10]. COVID-19 has a few similarities as it also presents with multi-organ involvement and extensive inflammation and endothelial damage, therefore the differential diagnosis can be challenging especially in severe acute respiratory syndrome (SARS) cases, leading to what has been referred to as "PE-like syndrome" [11,12].

Adequate diagnosis is key in ascertaining appropriate clinical management, especially in cases of preterm gestation since the decision on the timing of delivery has a significant impact on perinatal outcomes. Because clinical parameters of hypertension, proteinuria, and target organ damage can be similar, biomarkers could be a useful tool in distinguishing and guiding such decision-making for cases that involve COVID-19 and preeclampsia [13]. The sFlt-1/PlGF ratio at a threshold of below 38 can provide reassurance for the absence of PE at that given time, as well as indicate a low likelihood of onset in the following week. However, there are still uncertainties about the levels of biomarkers in COVID-19 cases given previous reports of high values of sFlt-1 in patients with COVID-19 pneumonia vs COVID-19 without pneumonia [8].

Therefore, the aim of this study was to analyze sFlt-1 and PlGF concentrations and their ratio in pregnant/postpartum women with suspected COVID-19, and further investigate conditions associated with the increased ratio (sFlt-1/PlGF $>$ 38), including preeclampsia and severe COVID-19 cases.

2. Methods

2.1. Participants

The present research is part of a large prospective cohort study included in REBRACO – Brazilian Network of COVID-19 in Obstetrics – a multicenter study of 15 Brazilian referral centers from different regions across the country aiming to understand the burden of disease on maternal and perinatal outcomes related to COVID-19 infection, with the University of Campinas (Unicamp) as coordinating center [14,15]. At Unicamp, SARS-CoV-2 quantitative reverse transcriptase polymerase-chain-reaction (RT-qPCR) assay testing for suspected cases started in April 2020 and routine universal screening was implemented in June 2020. COVID-19 suspected women had a diversity of biological samples collected at Unicamp, including peripheral blood – specifically serum. A total of 135 cases of unvaccinated pregnant or postpartum women who were admitted to the institution from June 2020 to July 2021 were included and considered for testing for SARS-CoV-2 infection by RT-qPCR assay in upper respiratory secretion samples. Of the 135 COVID-19 suspected women considered, 28 who had mild flu-like syndrome did not have their peripheral blood sampled. Of the remaining 107 cases, 5 delivered elsewhere and have no available data for analysis of outcomes – therefore these cases were further excluded. Thus, the total cases eligible for the current analysis were 102 women. The blood sampling occurred at different times in relation to SARS-CoV-2 infection – mostly during active infection or near recovery. Clinical background, maternal SARS-CoV-2 infection characteristics, socio-demographic information and maternal and perinatal outcomes of the included cases were analyzed. The cases were grouped by PE diagnosis, COVID-19 testing, and clinical severity (acute respiratory syndrome (SARS). Pharmacological or immunobiological methods were not administered to any study participants.

2.2. Blood serum samples

The blood serum samples were obtained by maternal peripheral blood collection in a dry vacuum tube with clot separator gel. Peripheral blood samples were processed within 1 h after sampling for sample integrity. The blood tubes were centrifuged for 10 min, at 1,200xG at room temperature (18 °C). After centrifugation, the blood serum samples were aliquoted in sterile cryotubes (around 600 μL per cryotube) inside a class II biological safety cabinet in a NB-2 laboratory. The samples in cryotubes were immediately stored in an Ultrafreezer at −80 °C temperature. The entire sample collection and processing team wore appropriate personal protective equipment (PPE) recommended for such pathogens, including disposable N95 masks, disposable apron, disposable gloves, disposable cap, eye protection (glasses or face shield) and closed shoes.

2.3. Electrochemiluminescence for determination of sFlt-1 and PlGF concentration

The measurements of angiogenesis-related factors sFlt-1 and PlGF in peripherical blood were performed in the Roche Cobas e411 device (ROCHE®), with automated Elecsys® sFlt-1/PlGF kits (ROCHE®), using 50 μL of maternal serum, through immunoassays for the quantitative determination of such biomarkers based on electrochemiluminescence technology. The protocol was implemented according to the manufacturer instructions. The concentration results obtained by the assay were expressed in pg of the analyte per mL of serum - pg/mL. The values obtained were also used for the calculation of sFlt-1/PlGF ratio. The sFlt-1/PlGF ratio had a threshold implemented of 38 for statistical analysis.

2.4. Clinical data

Medical charts were reviewed to retrieve information on sociodemographic characteristics including age; obstetric and clinical background – parity; nulliparous; multiparous; mean height (cm); mean weight (kg); mean BMI (kg/m2); obesity (IMC $>$ 30); hypertension and diabetes variables – and maternal/perinatal outcomes including, PE; PE with severe features; eclampsia; HELLP syndrome; confirmed COVID-19; heart rate (bpm); respiratory rate; systolic blood pressure; diastolic blood pressure; temperature; ICU admission; gestational age at delivery ($<$37 or $\geq$37 weeks); route of delivery (vaginal or cesarean section); mean birth weight at delivery (g); 5th minute APGAR score $<$ 7; stillbirth; neonatal death. For confirmed COVID-19 cases, data on gestational age at diagnosis, symptoms and severity of disease were considered (use of supplementary oxygen, intubation, ICU admission). For cases with the diagnosis of PE, detailed information on gestational age at diagnosis, severe features, and outcomes were also retrieved.

Suspected COVID-19 was based on the presence of fever and/or at least one respiratory symptom or sign of flu-like syndrome: sore throat, runny nose, cough, sputum production, shortness of breath, nasal or conjunctival congestion, pain swallowing, and O_2 saturation $<$ 95 %, signs of cyanosis, and/or flapping of the nose and dyspnea were considered signs of SARS. Other symptoms such as diarrhea, anosmia and dysgeusia were also considered [15].

PE was considered based on the presence of hypertension associated with proteinuria (proteinuria/creatinine $>$ 0.3 or 24 h proteinuria over 300 mg) after 20 weeks of gestation, in a previously normotensive pregnant woman. PE was also considered in the absence of proteinuria if there was target organ damage [16,17].

2.5. Data analysis

Initially, all cases of suspected and/or confirmed COVID-19 with available blood sample collection and clinical data (including childbirth outcomes) were selected. The biomarker level was considered using a threshold of 38 for the sFlt-1/PlGF ratio. Women were grouped according to such results and clinical data on sociodemographic characteristics, clinical background, and maternal and perinatal outcomes were compared among groups of sFlt-1/PlGF $\geq$ 38 and sFlt-1/PlGF $<$ 38.

Cases of confirmed COVID-19 during pregnancy that also presented with the diagnosis of PE were detailed to describe the timing of infection, the severity of disease and further association to PE.

A comparison between different groups was performed that considered sFlt-1 and PlGF serum concentrations and the sFlt-1/PlGF ratios, as well as diagnosis of PE and SARS associated with COVID-19-positive cases. The groups analyzed were classified as: COVID-19-positive cases with diagnosis of PE (COVID-19 + PE +) versus COVID-19-positive cases without diagnosis of PE (COVID-19 + PE -); and COVID-19-positive cases with diagnosis of SARS (COVID-19 + SARS +) versus COVID-19-positive cases without diagnosis of SARS (COVID-19 + SARS -).

Finally, biomarkers: PlGF ratios, sFlt-1 and sFlt-1/PlGF ratios were described among cases of suspected COVID, comparing those with positive and negative testing and no PE.

Comparisons between groups were performed using the Odds Ratio with 95 % confidence interval (CI) and Chi-square test for categorical variables, and Mann-Whitney test (*U* test) for continuous variables. Outliers were considered by the ROUT method. Statistical analysis tests were done using GraphPad Prism version 7 for Mac (GraphPad Software, San Diego, CA, United States) and *Epi*.Info 7.0 for Windows. A p-value $\leq$ 0.05 was considered statistically significant.

2.6. Ethical considerations

The research project followed all recommended rules for the use of human biological samples, with approval by the Research Ethics Committee of the University of Campinas, IRB #31591720.5.0000.5404. All women signed an informed consent form authorizing the collection, storage, and use of clinical samples and data.

3. Results

There were 135 COVID-19 suspected cases of unvaccinated pregnant and postpartum women included at Unicamp between June 2020 and July 2021. Of these, 97 cases were eligible for electrochemiluminescence assay for determination of sFlt-1 and PlGF concentration in blood serum. After laboratory analysis, 37 cases presented sFlt-1/PlGF ratio $\geq$ 38 and 60 sFlt-1/PlGF ratio $<$ 38. And among these, 76 were COVID-19 positive cases and 21 COVID-19 negative. In the COVID-19 positive cases, 11 were diagnosed with PE and 9 with SARS. And among the COVID-19 negative cases, 7 had only PE and 1 had PE and SARS (Fig. 1).

When grouping women according to a threshold ratio of biomarkers (sFlt-1/PlGF $\geq$ 38 or sFlt-1/PlGF $<$ 38) to investigate whether clinical diagnosis of PE and COVID-19 were associated with biomarker levels, there were no significant differences (Table 1). The presence of PE was more frequent in the sFlt-1/PlGF $\geq$ 38 (29.73 % vs 11.67; p = 0.051), but with no significance most likely due to the small number of cases and moment of sample collection (gestational age of COVID-19 investigation and not of PE diagnosis). For COVID-19 diagnosis, the frequency was similar in both groups (p = 0.72).

The detailed description of cases of COVID-19 and PE diagnosis are presented in Table 2. Among the 11 cases of PE and COVID-19, 5 presented PE with severe features and received magnesium sulfate due to severe hypertension and/or imminent eclampsia, such as headache, visual changes, nausea, and vomiting. Almost all considered cases were obese (10 cases), only two were previously diagnosed with chronic arterial hypertension, and over half of them developed gestational diabetes (6 cases). There was only one patient who needed supplementary oxygen use due to COVID-19 infection; she had asthma as a previous condition and did not need invasive ventilatory support. This patient developed secondary bacterial pneumonia and was treated with antibiotics.

Based on the timing of COVID-19 infection, only 3 cases presented a concomitant diagnosis of COVID-19 and PE, which was during the third

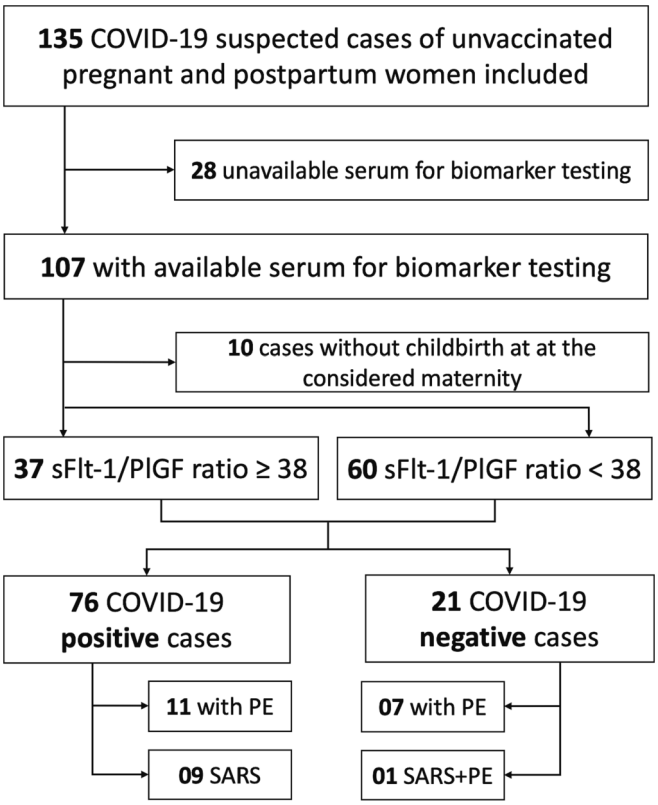

Fig. 1. Flowchart of the study included cases and cases grouping.

Table 1
Sociodemographic, clinical, obstetric characteristics and maternal and perinatal outcomes comparing cases of sFlt-1/PlGF ≥ 38 (increased ratio) and sFlt-1/PlGF < 38.

Variable	sFlt-1/PlGF ratio ≥ 38	sFlt-1/PlGF ratio < 38	p-value
N	37	60	
Age (Years)	30.49 ± 5.97	29.08 ± 6.29	0.279
Parity			0.401
Nulliparous	13 (35.14)	15 (25.00)	
Multiparous	24 (64.86)	45 (75.00)	
Mean height (cm)	1.62 ± 0.06	1.62 ± 0.05	0.785
Mean weight (kg)	85.25 ± 25.98	83.12 ± 21.26	0.698
Mean BMI (kg/m^2)	32.43 ± 9.60	31.87 ± 7.49	0.777
Obesity (IMC > 30)	16 (59.26)	28 (56.00)	0.972
Hypertension	12 (32.43)	10 (16.67)	0.121
Diabetes	14 (37.84)	20 (33.33)	0.816
Preeclampsia	11 (29.73)	7 (11.67)	0.051
Confirmed COVID-19	27 (72.97)	47 (78.33)	0.721
Preeclampsia with severe features	6 (16.22)	4 (6.67)	0.133
Eclampsia	0 (0.00)	0 (0.00)	–
HELLP Syndrome	2 (5.41)	0 (0.00)	0.068
Heart rate (bpm)	89.86 ± 13.02	92.94 ± 15.09	0.359
Respiratory rate	22.84 ± 11.49	19.32 ± 6.82	0.689
Systolic blood pressure	153.41 ± 183.08	134.15 ± 131.94	0.579
Diastolic blood pressure	75.27 ± 13.56	74.09 ± 11.46	0.672
Temperature	36.75 ± 0.82	36.53 ± 0.85	0.149
ICU admission	4 (10.81)	8 (13.33)	0.961
Gestational age at delivery			0.705
<37 weeks	12 (32.43)	16 (26.67)	
≥37 weeks	25 (67.57)	44 (73.33)	
Route of delivery			0.954
Vaginal delivery	20 (54.05)	31 (53.45)	
Cesarean section	17 (45.95)	27 (46.55)	
Mean weight at delivery (g)	2778 ± 828	2917 ± 813	0.420
5th minute APGAR < 7	2 (5.41)	2 (3.57)	0.669

Table 2
Detailed description of cases of confirmed COVID-19 and preeclampsia.

Cases	Maternal age	Parity	Type of pregnancy	Previous conditions	GA at COVID-19 Diagnosis (weeks)	COVID-19 severity*	GA at PE diagnosis (weeks)	GA At Birth	Weight At Birth (g)	5 Min Apgar	PE Severe Features**	Route Of Delivery	sFlt-1/ PlGF ratio	Prot/Crea
1	35	Multiparous	Single	Obesity, GDM	33	Mild	33	36	4595	10	No	C-Section	65.12	0.60
2	39	Multiparous	Single	Obesity, GDM	9	Mild	Puerperium	36	2175	9	Yes	C-Section	33.13	0.17
3	29	Multiparous	Single	Obesity	16	Mild	39	40	3645	9	No	C-Section	48.84	—
4	28	Primiparous	Twin	No	23	Mild	33	33	1120/ 1462	1–4	No	C-section	46.95	0.15
5	32	Multiparous	Single	Obesity, GDM	10	Mild	Puerperium	38	2750	9	No	C-Section	26.52	0.84
6	27	Primiparous	Single	Obesity, GDM	24	Asymptomatic	33	33	2085	10	Yes	C-Section	222.4	0.70
7	40	Multiparous	Single	Obesity, GDM, Hep B	35	Mild	37	37	3350	4	Yes	C-Section	12.82	0.15
8	34	Multiparous	Single	Obesity, GDM	19	Asymptomatic	36	36	3100	10	No	Vaginal	51.82	0.20
9	28	Primiparous	Single	Obesity, Asthma, CH	38	Severe	38	38	2880	9	Yes	C-Section	32.78	1.03
10	34	Multiparous	Twin	Obesity	34	Mild	33	36	2430/ 2535	9–8	No	C-Section	81.66	0.38
11	35	Multiparous	Single	Obesity	Postpartum	Asymptomatic	34	37	2465	10	Yes	Vaginal	212.26	2.65

*Severity of COVID-19: Severe – hospitalization/oxygen supplementary use; Mild - no hospitalization, mild symptoms (cough, coryza, sore throat, anosmia, mild dyspnea, mild fever). ** PE Severe Features: Use of Magnesium Sulfate (imminence of Eclampsia), Eclampsia, HELLP Syndrome. GDM: gestational diabetes melittus; CH: chronic hypertension; HEP B: chronic hepatitis B; GA: gestational age; PE: pre-eclampsia; PROT/CREA: Protein/Creatinine ratio.

trimester. The majority presented COVID-19 prior to the diagnosis of PE, during the first/second trimester. The only reported case of fetal malformation was a case with COVID-19 at 9 weeks gestation, with Down syndrome (presented with fetal growth restriction and cardiac involvement). One case was diagnosed with COVID-19 postpartum, during the routine screening to visit the neonatal ICU (after a preterm delivery). Our data only included 2 cases with sFlt-1/PlGF ratio over 85, both ratios actually over 200 (cases 6 and 11); and both cases presented PE with severe features and short interval between PE diagnosis and delivery, with asymptomatic COVID-19 infection.

The results for sFlt-1 and PlGF serum concentrations and sFlt-1/PlGF ratios were further compared between groups categorized for PE and SARS diagnosis related to COVID-19-positive cases. The groups analyzed were classified as: COVID-19-positive cases with diagnosis of PE [COVID-19 + PE + (n = 11)] versus COVID-19-positive cases without diagnosis of PE [COVID-19 + PE-(n = 65)]; and COVID-19-positive cases with diagnosis of SARS [COVID-19 + SARS + (n = 09)] versus COVID-19-positive cases without diagnosis of SARS [COVID-19 + SARS-(n = 67)]. The findings revealed that the sFlt-1/PlGF ratio was significantly higher in the COVID-19 + PE + group compared to COVID-19 + PE-group (p-value = 0.0047). No significant difference was found in comparison of these groups related to sFlt-1 and PlGF concentrations when isolated. Considering COVID-19 cases grouped by SARS incidence, the analysis of COVID-19 + SARS + versus COVID-19 + SARS - revealed no significant difference in sFlt-1 and PlGF serum concentration, or in the sFlt-1/PlGF ratio (Fig. 2).

Regarding COVID-19 positive with COVID-19 negative cases, excluding cases with PE, no differences were observed in sFlt-1 levels only (p-value = 0.0861), PlGF levels only (p-value = 0.6473) and in sFlt-1/PlGF ratio (p-value = 0.5337) (**Supplemental Fig. 1**).

4. Discussion

This study presented the sFlt-1/PlGF ratio evaluated in women during their suspected and/or confirmed COVID-19 infection during pregnancy. Considering the threshold of 38 as abnormal, the findings support that this value, in association with clinical findings, can be used to identify cases of PE. COVID-19 alone did not impact these biomarker levels.

The rationale for the presented analysis was the reported increased frequency of PE among cases of COVID-19 and possible difficulty in the differential diagnosis of severe cases since both conditions can evolve to multiorgan damage, with inflammation and endothelial impairment[6]. Classical hypertension and proteinuria as diagnostic criteria might not be enough for such cases, nor the severity of laboratory findings such as altered liver enzymes, kidney, or coagulation. Therefore, biomarker evaluation could be a feasible approach [11]. There is still a lack of consistent data on the possible impact of COVID-19 alone on levels of sFlt-1 and/or PlGF [13].

COVID-19 and PE could possibly present common pathophysiological mechanisms [18–20]. One of the most considered is that SARS-CoV-2 binds ACE2-receptors in the placenta, leading to alteration of the placental renin-angiotensin system (RAS). As RAS regulates blood pressure, the virus may therefore increase adverse hemodynamic outcomes, such as PE [3,21].

Another possible pathway in common is NLRP3 activation. SARS-CoV-2 leads to direct activation of NLRP3 by a viral protein, and this activation has been strongly correlated to the inflammatory response in COVID-19 patients. This pathway can also be activated in PE, mainly in cases with severe features [22].

The timing of diagnosis and follow-up presents additional challenges. Previous studies have shown an increased risk of future PE among women that presented with COVID-19 during first/second trimesters [23]. This information can be valuable for counseling during pregnancy and adequate follow-up. Among the few cases of PE and confirmed COVID-19 that we reported here, nearly half presented with

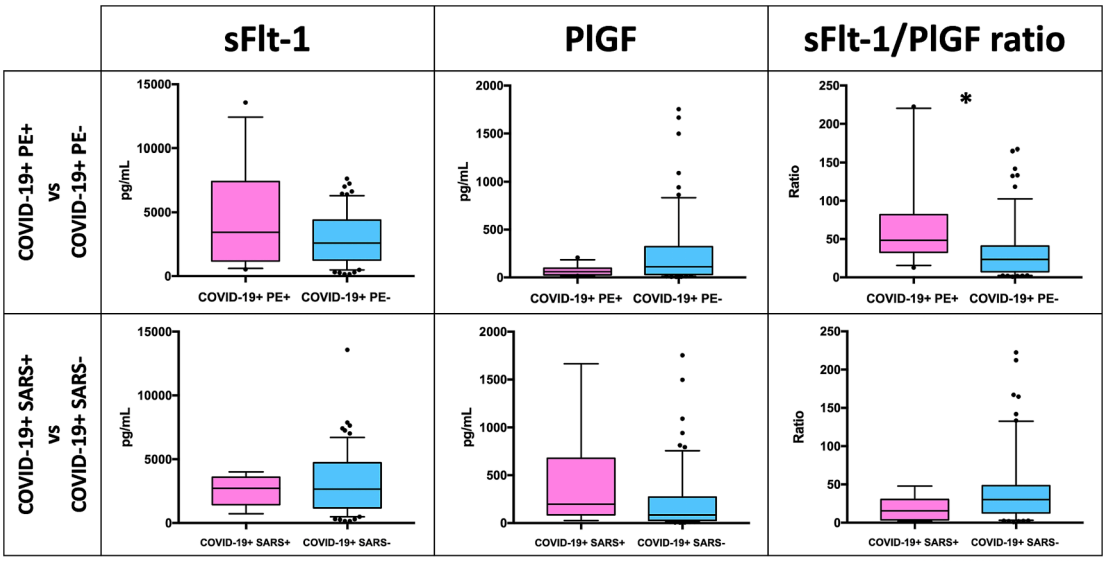

Fig. 2. Boxplots for values of sFlt-1, PlGF and their ratios (sFlt-1/PlGF) according to the presence of preeclampsia (PE) and severe acute respiratory syndrome (SARS). Boxes encompass a range from the 1st to the 3rd quartile, whiskers represent 10–90 percentile and points represent outliers. Mann-Whitney test (test U) was used to compare groups, with p-value ≤ 0.05 as significant.

SARS-CoV-2 infection during first/second trimesters and further developed PE, weeks or months later. And even with the serum collected at the infection moment, most showed an altered sFlt-1/PlGF ratio.

Increased sFlt-1/PlGF ratio or decreased PlGF values have been associated to adverse maternal and perinatal outcomes [3,13,24–26] Studies indicate that COVID-19 in pregnancy can induce higher incidence of adverse perinatal outcomes, as well as PE-like syndrome [24–26]. The same studies suggest that the biomarkers testing (such as PlGF and sFlt-1/PlGF ratio) could be used to differentiate PE from severe COVID-19 and improve clinical management [24,25].

Our data, when comparing outcomes among cases with altered sFlt-1/PlGF ratio (using 38 as the threshold) did not present significant differences. Nevertheless, we had limited number of cases and only very few with sFlt-1/PlGF ratio over 85 (the most used ratio for PE diagnosis) [27]. Regardless, biological samples were collected at the time of SARS-CoV-2 infection and not at the time of PE diagnosis, which may have experimental implications.

Other inflammatory markers can be used adjunctively for the management of COVID-19 cases, mainly the severe ones. COVID-19 cases commonly indicate elevated rates of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and D-dimer [28,29]. Other inflammatory markers that are altered are interleukins and cytokines, mostly decreasing the expression of IL-1β, which may indicate the state of clinical evolution depending on their high expression, especially in the cytokine storm state [30,31]. In our study, we did not evaluate these inflammatory markers.

This study included all case identification and sample collection prior to vaccination. Another similar cohort study is being conducted in which most women are already vaccinated, which may allow investigation into the question of whether vaccination plays any role in these associations. As we acknowledge the advances of such intervention among pregnant women, we also should consider the potential impact of the rise of new variants of concern (VOCs) [32,33]. Although there appears to be a recent reduction of the number of severe COVID-19 cases, the number of infected pregnant women are nevertheless still high and the effects on rates of hypertensive disease/PE remains a topic of relevant interest.

The relatively small number of cases included in this study is a limitation, especially considering PE and COVID-19 and also the moment of sample collection (with no biomarker levels at PE diagnosis). However, the present results provide detailed clinical outcomes tied to biomarker evaluations that add relevant evidence for the association of COVID-19 and PE. This should raise increased awareness about the risk of PE after COVID-19 diagnosis. More adequate pregnancy counseling considering patient information on the history of infection should be a key part of interventions moving forward.

Funding

This study was supported by *Fundo de Apoio ao Ensino, à Pesquisa e à Extensão* (FAEPEX-Unicamp) [Grant No 2300/20] and by the Washington University at Saint Louis, USA, McDonnell Academy seed grant for research on infectious diseases and the impact of COVID-19 (to IUM and MLC). MLC was also supported by *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP) [grant number 2021/09937-1] and by *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) [grant number 408407/2021-2]. GMN was supported by *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) [grant number 88882.329828/2019]. Roche Diagnostics (ROCHE®) supported the measurements of angiogenesis-related factors sFlt-1 and PlGF, providing automated Elecsys® sFlt-1/PlGF kits.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2022.11.008.

References

[1] E. Abalos, C. Cuesta, A.L. Grosso, D. Chou, L. Say, Global and regional estimates of preeclampsia and eclampsia: a systematic review, Eur. J. Obstet. Gynecol. Reprod. Biol. 170 (1) (2013) 1–7.

[2] L. Say, D. Chou, A. Gemmill, Ö. Tunçalp, A.-B. Moller, J. Daniels, et al., Global causes of maternal death: a WHO systematic analysis, Lancet Glob. Heal. 2 (6) (2014) e323–e333.

[3] D. Kumar, S. Verma, I.U. Mysorekar, COVID-19 and pregnancy: clinical outcomes; mechanisms, and vaccine efficacy, Transl. Res. (2022).
[4] M.L.S. Takemoto, M.O. Menezes, C.B. Andreucci, R. Knobel, L. Sousa, L. Katz, et al., Clinical characteristics and risk factors for mortality in obstetric patients with severe COVID-19 in Brazil: a surveillance database analysis, Available from: BJOG An Int. J. Obstet. Gynaecol. [Internet]. 127 (13) (2020) 1618–1626 https://obgyn.onlinelibrary.wiley.com/doi/abs/10.1111/1471-0528.16470.
[5] S. Verma, E.B. Carter, I.U. Mysorekar, SARS-CoV2 and pregnancy: an invisible enemy? Am. J. Reprod. Immunol. [Internet] (2020) e13308. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/aji.13308.
[6] A. Conde-Agudelo, R. Romero, SARS-CoV-2 infection during pregnancy and risk of preeclampsia: a systematic review and meta-analysis, Am. J. Obstet. Gynecol. 226 (1) (2022) 68–89.e3.
[7] K. Narang, E.A.L. Enninga, M.D.S.K. Gunaratne, E.R. Ibirogba, A.T.A. Trad, A. Elrefaei, et al., SARS-CoV-2 infection and COVID-19 during pregnancy: a multidisciplinary review, Mayo Clin. Proc. 95 (8) (2020) 1750–1765.
[8] C.E. Powe, R.J. Levine, S.A. Karumanchi, Preeclampsia, a disease of the maternal endothelium: the role of antiangiogenic factors and implications for later cardiovascular disease, Circulation. 123 (24) (2011) 2856–2869.
[9] H. Zeisler, E. Llurba, F. Chantraine, M. Vatish, A.C. Staff, M. Sennström, et al., Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia, N. Engl. J. Med. 374 (1) (2016) 13–22.
[10] E. Jung, R. Romero, L. Yeo, N. Gomez-Lopez, P. Chaemsaithong, A. Jaovisidha, et al., The etiology of preeclampsia, Am. J. Obstet. Gynecol. 226 (2S) (2022) S844–S866.
[11] M. Mendoza, I. Garcia-Ruiz, N. Maiz, C. Rodo, P. Garcia-Manau, B. Serrano, et al., Pre-eclampsia-like syndrome induced by severe COVID-19: a prospective observational study, BJOG 127 (11) (2020) 1374–1380.
[12] A. Garcia Rodriguez, S. Marcos Contreras, S.M. Fernandez Manovel, J.M. Marcos Vidal, F. Diez Buron, C. Fernandez Fernandez, et al., SARS-COV-2 infection during pregnancy, a risk factor for eclampsia or neurological manifestations of COVID-19? Case report, BMC Pregnancy Childbirth. 20 (1) (2020) 587.
[13] C.M. Soldavini, D. Di Martino, E. Sabattini, S. Ornaghi, V. Sterpi, R. Erra, et al., sFlt-1/PlGF ratio in hypertensive disorders of pregnancy in patients affected by COVID-19, Pregnancy Hypertens. 27 (2022) 103–109.
[14] M.L. Costa, R.T. Souza, R.C. Pacagnella, S.F. Bento, C.C. Ribeiro-do-Valle, A.G. Luz, et al., Facing the COVID-19 pandemic inside maternities in Brazil: a mixed-method study within the REBRACO initiative, PLoS One [Internet] 16 (7) (2021) 1–19, https://doi.org/10.1371/journal.pone.0254977. Available from:.
[15] M.L. Costa, R.T. Souza, R.C. Pacagnella, S.F. Bento, C.C. Ribeiro-do-Valle, A.G. Luz, et al. Brazilian network of COVID-19 during pregnancy (REBRACO: a multicentre study protocol). Metelus S, Castro L, Pabon S, Soeiro R, Silva AD, Junior PSR, et al., editors. BMJ Open [Internet]. 2021;11(12). Available from: https://bmjopen.bmj.com/content/11/12/e051284.
[16] A.L. Tranquilli, G. Dekker, L. Magee, J. Roberts, B.M. Sibai, W. Steyn, et al. The classification, diagnosis and management of the hypertensive disorders of pregnancy: a revised statement from the ISSHP. Vol. 4, Pregnancy hypertension. Netherlands; 2014. p. 97–104.
[17] J.C. Peraçoli, V.T.M. Borges, J.G.L. Ramos, R.de.C. Cavalli, S.H.de.A.M. Costa, L.G. de Oliveira, et al., Pre-eclampsia/Eclampsia TT - Pré-eclâmpsia/Eclâmpsia, Rev. Bras Ginecol. Obs. 41 (05) (2019) 318–332.
[18] V. Giardini, A. Carrer, M. Casati, E. Contro, P. Vergani, C. Gambacorti-Passerini, Increased sFLT-1/PlGF ratio in COVID-19: a novel link to angiotensin II-mediated endothelial dysfunction, Am. J. Hematol. 95 (2020) E188–E191.
[19] S. Espino-Y-Sosa, R.J. Martinez-Portilla, J. Torres-Torres, J.M. Solis-Paredes, G. Estrada-Gutierrez, J.A. Hernandez-Pacheco, et al. Novel Ratio Soluble Fms-like Tyrosine Kinase-1/Angiotensin-II (sFlt-1/ANG-II) in Pregnant Women Is Associated with Critical Illness in COVID-Viruses. 2021;13(10).
[20] R. Marín, F.H. Pujol, D. Rojas, L. Sobrevia, Sars-,, CoV-2 infection and oxidative stress in early-onset preeclampsia, Available from: Biochim. Biophys. Acta Mol. Basis Dis. [Internet]. 1868 (3) (2022), 166321 https://europepmc.org/articles/PMC8668602.
[21] S. Verma, C.S. Joshi, R.B. Silverstein, M. He, E.B. Carter, I.U. Mysorekar, SARS-CoV-2 colonization of maternal and fetal cells of the human placenta promotes alteration of local renin-angiotensin system, Available from, Med [Internet]. 2 (5) (2021) 575–590.e5, https://www.sciencedirect.com/science/article/pii/S2666634021001586.
[22] P.R. Nunes, M. Romao-Veiga, V.R. Ribeiro, J.C. Peracoli, M.T.S. Peracoli, L. De Oliveira, COVID-19: A new risk factor or just a new imitator of preeclampsia? NLRP3 activation: a possible common mechanism, J. Med. Virol. United States (2022).
[23] J.I. Rosenbloom, N. Raghuraman, E.B. Carter, J.C. Kelly, Coronavirus disease 2019 infection and hypertensive disorders of pregnancy, Am. J. Obstetr. Gynecol. 224 (2021) 623–624.
[24] B. Serrano, E. Bonacina, I. Garcia-Ruiz, M. Mendoza, P. Garcia-Manau, P. Garcia-Aguilar, et al., Confirmation of preeclampsia-like syndrome induced by severe COVID-19: an observational study, Am. J. Obstet. Gynecol. MFM (2022) 100760.
[25] A. Naeh, A. Berezowsky, M.H. Yudin, I.A. Dhalla, H. Berger, Preeclampsia-like syndrome in a pregnant patient with coronavirus disease 2019 (COVID-19), J. Obstetr. Gynaecol. Canada: JOGC 44 (2022) 193–195.
[26] B. Serrano, M. Mendoza, P. Garcia-Aguilar, E. Bonacina, I. Garcia-Ruiz, P. Garcia-Manau, et al., Shared risk factors for COVID-19 and preeclampsia in the first trimester: an observational study, Acta Obstet. Gynecol. Scand. 101 (7) (2022) 803–808.
[27] M.L. Costa, R.de.C. Cavalli, H.A. Korkes, E.V.da Cunha Filho, J.C. Peraçoli, Diagnosis and management of preeclampsia: suggested guidance on the use of biomarkers, Rev. Bras Ginecol e Obstet Rev da Fed Bras das Soc Ginecol e Obstet. (2022).
[28] R. Yamamoto, H. Asano, T. Umazume, M. Takaoka, K. Noshiro, Y. Saito, et al., C-reactive protein level predicts need for medical intervention in pregnant women with SARS-CoV2 infection: a retrospective study, J. Obstet. Gynaecol. Res. 48 (4) (2022) 938–945.
[29] R. Qaisieh, M. Al-Tamimi, N. El-Hammuri, M. Shalabi, M.M. Kilani, H. Taha, et al., Clinical, laboratory, and imaging features of COVID-19 in a cohort of patients: cross-sectional comparative study, JMIR Public Heal Surveill. 7 (9) (2021) e28005.
[30] M.L. Sherer, J. Lei, P.S. Creisher, M. Jang, R. Reddy, K. Voegtline, et al., Pregnancy alters interleukin-1 beta expression and antiviral antibody responses during severe acute respiratory syndrome coronavirus 2 infection, Am. J. Obstet. Gynecol. 225 (3) (2021) 301.e1–301.e14.
[31] D.C. Cornelius, X. Wang, A. Griffin, R. Morris, K. Wallace, Preeclampsia and COVID-19: the role of inflammasome activation, Curr. Hypertens. Rep. 24 (9) (2022) 341–348.
[32] S.K. Saxena, S. Kumar, S. Ansari, J.T. Paweska, V.K. Maurya, A.K. Tripathi, et al., Characterization of the novel SARS-CoV-2 Omicron (B.1.1.529) variant of concern and its global perspective, J. Med. Virol. 94 (4) (2022) 1738–1744.
[33] F.R.R. Moreira, M. D'arc, D. Mariani, A.L. Herlinger, F.B. Schiffler, Á.D. Rossi, et al., Epidemiological dynamics of SARS-CoV-2 VOC Gamma in Rio de Janeiro, Brazil, Virus Evol. 7 (2) (2021) veab087.