Increased levels of soluble fms-like tyrosine kinase-1 are associated with adverse outcome in pregnant women with COVID-19

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KEYWORDS: COVID-19; endothelial dysfunction; maternal death; placenta; placental growth factor; SARS-CoV-2

CONTRIBUTION

What are the novel findings of this work?
Soluble fms-like tyrosine kinase-1 (sFlt-1) multiples of the median is higher in symptomatic pregnant women with severe coronavirus disease 2019 (COVID-19) pneumonia compared to those with non-severe COVID-19 pneumonia and has the capability to predict serious adverse pregnancy events associated with COVID-19, such as severe pneumonia, intensive care unit admission, viral sepsis and maternal death.

What are the clinical implications of this work?
This is the first study to demonstrate the potential of sFlt-1 as a biochemical marker for the prediction of severe features and adverse outcome of COVID-19 in symptomatic pregnant women.

ABSTRACT

Objective In addition to the lungs, the placenta and the endothelium can be affected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) are markers of endothelial dysfunction and could potentially serve as predictors of severe coronavirus disease 2019 (COVID-19). We aimed to investigate the association of serum concentrations of sFlt-1 and PlGF with the severity of COVID-19 in pregnancy.

Methods This was a prospective cohort study carried out in a tertiary care hospital in Mexico City, Mexico. Symptomatic pregnant women with a positive reverse-transcription quantitative polymerase chain reaction test for SARS-CoV-2 infection who fulfilled the criteria for hospitalization were included. The primary outcome was severe pneumonia due to COVID-19. Secondary outcomes were intensive care unit (ICU) admission, viral sepsis and maternal death. sFlt-1 levels were expressed as multiples of the median (MoM). The association between sFlt-1 and each adverse outcome was explored by logistic regression analysis, adjusted for gestational age for outcomes occurring in more than five patients, and the predictive performance was assessed by receiver-operating-characteristics-curve analysis.

Results Among 113 pregnant women with COVID-19, higher sFlt-1 MoM was associated with an increased probability of severe pneumonia (adjusted odds ratio (aOR), 1.817 (95% CI, 1.365–2.418)), ICU admission (aOR, 2.195 (95% CI, 1.582–3.047)), viral sepsis (aOR, 2.318 (95% CI, 1.407–3.820)) and maternal death (unadjusted OR, 5.504 (95% CI, 1.079–28.076)). At a 10% false-positive rate, sFlt-1 MoM had detection rates of 45.2%, 66.7%, 83.3% and 100% for severe COVID-19 pneumonia, ICU admission, viral sepsis and maternal death, respectively. PlGF values were similar between women with severe and those with non-severe COVID-19 pneumonia.

Conclusion sFlt-1 MoM is higher in pregnant women with severe COVID-19 and has the capability to predict serious adverse pregnancy events, such as severe pneumonia, ICU admission, viral sepsis and maternal death. © 2021 International Society of Ultrasound in Obstetrics and Gynecology.

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**INTRODUCTION**

Pregnancy is considered a major risk factor for adverse outcome in women with severe acute respiratory coronavirus syndrome 2 (SARS-CoV-2) infection. Recent studies in women of reproductive age infected with SARS-CoV-2 have shown that pregnancy increases the odds of death (odds ratio (OR), 1.65 (95% CI, 1.30–2.09)) pneumonia (OR, 1.99 (95% CI, 1.81–2.19)) and admission to the intensive care unit (ICU) (OR, 2.25 (95% CI, 1.86–2.71))\textsuperscript{1,2}. In Mexico, the year 2020 ended with a maternal mortality ratio of 46.6, which represents a 33.2% increase when compared with 2019 (maternal mortality ratio, 31.1)\textsuperscript{3}, with coronavirus disease 2019 (COVID-19) being the leading cause of maternal death, overtaking obstetric hemorrhage and pre-eclampsia (PE)\textsuperscript{4}. In addition to the major effect of SARS-CoV-2 infection on the lungs, there is evidence suggesting that the most severe forms of COVID-19 may also affect the vascular endothelium\textsuperscript{5}, causing significant systemic effects, such as hypertension, renal impairment, thrombocytopenia and liver injury\textsuperscript{6–9}. Maternal SARS-CoV-2 infection generates inflammatory changes in the placenta due to direct viral invasion into the parenchyma and blood vessels, causing inflammation of the chorionic villi, lymphoplasmacytic villitis and chorangiosis, associated mainly with low-grade placental hypoxia\textsuperscript{10}. As a result of this inflammatory response, the placenta produces soluble fms-like tyrosine kinase-1 (sFlt-1) protein by stimulating toll-like receptors along with a critical inflammatory response of the tissue\textsuperscript{10,11}. The sFlt-1 protein is a splice variant of VEGFR-1, a receptor for vascular endothelial growth factor A (VEGF-A) and placental growth factor (PlGF). When binding to its circulating ligand, sFlt-1 inhibits the VEGF-A pathway, causing endothelial and cell homeostasis impairment\textsuperscript{12}.

Uregulation of sFlt-1 has been demonstrated in critically ill, non-pregnant COVID-19 patients\textsuperscript{13}, suggesting that sFlt-1 could play an important role in COVID-19-associated systemic endothelial dysfunction, which also involves the human placenta\textsuperscript{14,15}. As endothelial dysfunction becomes more evident in severe forms of COVID-19, angiogenic biomarkers may potentially serve as a valuable tool for predicting severe COVID-19 with severe endothelial dysfunction.

We hypothesized that these angiogenic markers are predictive of adverse outcomes related to SARS-CoV-2 infection in pregnant women, such as severe pneumonia, ICU admission, viral sepsis and maternal death. This study aimed to investigate the association of serum concentrations of sFlt-1 and PlGF with the severity of COVID-19 among symptomatic pregnant women.

**METHODS**

**Study design and participants**

This was a prospective cohort study conducted at the General Hospital of Mexico ‘Dr Eduardo Liceaga’, a tertiary-care hospital in Mexico City, Mexico, between May 2020 and February 2021. Inclusion criteria were all pregnant women who presented at the emergency department with clinical suspicion of SARS-CoV-2 infection, which was later confirmed by positive reverse-transcription quantitative polymerase chain reaction (RT-qPCR), and who fulfilled the criteria for hospitalization between July 2020 and January 2021. Criteria for hospitalization were positive RT-qPCR and an obstetric condition that required immediate care and/or a maternal condition according to the national protocol for SARS-CoV-2 in pregnancy, which includes any of the following characteristics for hospitalization: (1) a CURB-65 score for pneumonia > 1 point; (2) persistent fever > 39°C; (3) comorbidity such as chronic hypertension, chronic pulmonary obstructive syndrome, pregestational diabetes or treatment with immunosuppressant drugs; and (4) chest X-ray showing signs of pneumonia\textsuperscript{16}. Asymptomatic patients and patients with negative RT-qPCR for SARS-CoV-2 were excluded, as they did not require hospital admission or additional biochemical evaluation according to our protocol. The protocol was approved by the ethics and research internal review board (DI/20/112/04/24) of the General Hospital in Mexico City. All patients provided informed consent.

**Data collection**

The following data were collected from the medical records: age, gestational age at admission, pregestational body mass index (BMI), mean arterial pressure at admission, presence of chronic hypertension, pregestational diabetes, PE, pneumonia, sepsis, acute renal failure or organ dysfunction and mortality. Participants’ blood samples were obtained at hospital admission as part of routine testing for COVID-19 and the levels of the following laboratory variables were recorded: leukocytes, neutrophils, hemoglobin, hematocrit, platelets, glucose, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides, cholesterol, D-dimer, C-reactive protein (CRP) and procalcitonin. The blood sample for sFlt-1 and PlGF measurements was also drawn at admission.

**sFlt-1 and PlGF quantification**

At admission, an additional blood sample was processed by centrifugation (1000 g/10 min), and plasma was aliquoted and stored at −70°C until analysis. PlGF (Elecsys® PlGF, Roche Diagnostics, Rotkreutz, Switzerland) and sFlt-1 (Elecsys sFlt-1, Roche Diagnostics) levels were measured by electrochemiluminescence using an automated analyzer (Cobas® e411, Roche Diagnostics), according to the manufacturer’s instructions.

**Outcomes**

The primary outcome was severe pneumonia due to COVID-19, defined according to the American Thoracic Society criteria.
Society criteria, which include either one major criterion (septic shock with vasopressor requirement or respiratory failure requiring mechanical ventilation) or three or more minor criteria (respiratory rate ≥ 30 breaths/min; ratio of partial pressure of arterial oxygen to fraction of inspired oxygen ≤ 250; multilobar infiltrates; confusion/disorientation; uremia (blood urea nitrogen level ≥ 20 mg/dL); leukopenia (white blood cell count < 4000/μL); thrombocytopenia (platelet count < 100 000/μL); hypothermia (core temperature < 36°C); or hypotension requiring aggressive fluid resuscitation)\textsuperscript{17,18}. Secondary outcomes were ICU admission, viral sepsis and maternal death due to SARS-CoV-2 infection. The need for ICU admission was determined according to the quick sequential organ failure assessment (QSOFA)\textsuperscript{17,18}. Viral sepsis was defined according to the Sepsis-3 International Consensus\textsuperscript{20}, in association with SARS-CoV-2 infection\textsuperscript{21}. PE was defined according to The American College of Obstetricians and Gynecologists\textsuperscript{22}; in women with new-onset hypertension, sFlt-1/PlGF ratio > 85 was used to differentiate PE from PE-like syndrome, as proposed by Mendoza et al.\textsuperscript{23}.

Statistical analysis

Descriptive and inferential statistics were used. The distribution of the data was assessed using the Kolmogorov–Smirnov test. Quantitative variables were reported as median and interquartile range, while qualitative data were reported as n (%). Differences in variables between cases with severe and those with non-severe COVID-19 pneumonia were evaluated using the Mann–Whitney U-test or chi-square test. PlGF and sFlt-1 measurements were log-transformed and converted to multiples of the median (MoM)\textsuperscript{24,25} according to the Fetal Medicine Foundation algorithms\textsuperscript{26}. sFlt-1 and PlGF MoMs were compared between the two groups using the Mann–Whitney U-test. Logistic regression analysis was performed to assess the association between sFlt-1 MoM and the primary and secondary outcomes. For outcomes occurring in more than five participants, gestational-age-adjusted logistic regression analysis was performed; for outcomes occurring in five or fewer patients, unadjusted logistic regression analysis was performed. For model construction, forward and backward stepwise logistic regression analyses were used, in which P ≤ 0.05 was used for model inclusion and P > 0.05 was the criterion for model elimination. The variance inflation factor (VIF) was used to evaluate the correlation between independent variables. VIF of 5–10 required further investigation, whereas VIF > 10 indicated serious multicollinearity and was a criterion for correction. Following logistic regression, the performance of the model was evaluated by receiver-operating-characteristics (ROC)-curve analysis. ROC curve analysis was also used to determine the best cut-off value of sFlt-1 MoM for the prediction of adverse outcome. Statistical analysis was performed using Stata 16 (StataCorp., College Station, TX, USA).

RESULTS

Study population characteristics

A total of 134 pregnant women with suspected SARS-CoV-2 infection were initially included in the cohort. Two patients were excluded following negative RT-qPCR result for SARS-CoV-2. A further 19 patients who tested positive for SARS-CoV-2 were excluded because they did not require hospital admission or additional biochemical evaluation as they were asymptomatic. Of the 113 included women, 31 (27.43%) had severe COVID-19 pneumonia, as defined by the composite definition, including five (4.42%) cases resulting in maternal death. Clinical and biochemical characteristics and the cause of death in the five cases of maternal death are provided in Table S1.

Table 1 shows the clinical and demographic characteristics of the study population according to the severity of COVID-19 pneumonia. Baseline characteristics were similar between women with non-severe (n = 82) and those with severe (n = 31) COVID-19 pneumonia. There was a statistically significant difference in the median gestational age at hospital admission between women with non-severe and those with severe COVID-19 pneumonia (35.2 vs 30.2 weeks; P = 0.002). In the severe and non-severe COVID-19 pneumonia groups, 22.58% and 17.07% of cases, respectively, developed PE features (new-onset hypertension and/or thrombocytopenia and/or elevated liver enzymes and/or proteinuria), all of which required antihypertensive drugs. There was no significant difference in the rate of PE-like syndrome between the severe and non-severe COVID-19 pneumonia groups.

Compared with non-severe cases, women with severe COVID-19 pneumonia had higher levels of leukocytes, neutrophils, glucose, AST, ALT, LDH, CRP, procalcitonin and sFlt-1 and sFlt-1 MoM. PlGF values were similar between the two groups. Interestingly, women with severe COVID-19 pneumonia had lower levels of total cholesterol (Table 2).

Association between sFlt-1 MoM and adverse outcome

Leukocytes, neutrophils and LDH showed high multicollinearity (VIFs of 151.34, 142.14 and 10.81, respectively) and were removed sequentially from the multivariate regression analysis. The final multivariate model included maternal age, pregestational BMI, sFlt-1 MoM and fetal growth restriction. The only statistically significant independent predictor of severe COVID-19 pneumonia was sFlt-1 MoM (Table S2).

There was a significant association between sFlt-1 MoM as a continuous variable and severe COVID-19 pneumonia (adjusted OR (aOR), 1.817 (95% CI, 1.365–2.418); P < 0.0001) (Table 3). With regard to secondary outcomes, sFlt-1 MoM as a continuous variable was associated significantly with ICU admission (aOR, 2.195 (95% CI, 1.582–3.047)) and viral sepsis (aOR, 2.318 (95% CI, 1.407–3.820)). The association between sFlt-1 MoM and maternal death was determined...
to be significant on unadjusted logistic regression, with an OR of 5.504 (95% CI, 1.079–28.076).

**sFlt-1 MoM for prediction of severe COVID-19**

The area under the ROC curve (AUC) for sFlt-1 MoM in the prediction of severe COVID-19 pneumonia was 0.715 (95% CI, 0.582–0.828). The AUCs of sFlt-1 MoM for the prediction of ICU admission, viral sepsis and maternal death were 0.816, 0.939 and 0.976, respectively (Table 4, Figure 1). The detection rates for maternal death at 5% and 10% false-positive rates were 80% and 100%, respectively.

The best cut-off value of sFlt-1 MoM was 1.66, which had sensitivity and specificity of 61.54% and 79.31% for severe pneumonia, 72.22% and 79.57% for ICU admission, 100% and 73.83% for viral sepsis and 100% and 73.15% for maternal death, respectively (Table S3).

**DISCUSSION**

**Main findings**

The principal findings of this study are that, in symptomatic pregnant women with COVID-19, higher

| Characteristic | Non-severe pneumonia (n = 82) | Severe pneumonia (n = 31) | P |
|----------------|-------------------------------|--------------------------|---|
| Age (years)    | 30.4 (26.4–34.0)              | 31.2 (26.4–34.8)         | 0.576 |
| GA at diagnosis (weeks) | 35.2 (29.1–39.0)           | 30.2 (25.1–33.2)         | 0.002 |
| Pregestational BMI (kg/m²) | 30.0 (26.0–33.7)         | 26.8 (25.4–33.0)         | 0.294 |
| Smoker | 1 (1.22)                  | 1 (3.23)                 | 0.124 |
| Chronic hypertension | 1 (1.22)                  | 2 (6.45)                 | 0.796 |
| Gestational diabetes | 3 (3.66)                  | 0 (0)                   | 0.283 |
| Asthma | 2 (2.44)                  | 0 (0)                   | 0.382 |
| Chronic renal disease | 5 (6.10)                  | 0 (0)                   | 0.161 |
| MAP (mmHg) | 85.5 (80.3–93.3)             | 87.6 (80.0–95.3)         | 0.692 |
| MAP MoM | 1.0 (0.9–1.1)               | 1.0 (0.9–1.1)            | 0.41 |
| utA-PI | 0.7 (0.6–0.9)              | 0.9 (0.6–1.1)            | 0.05 |
| utA-PI MoM | 1.0 (0.9–1.2)              | 1.1 (0.9–1.3)            | 0.135 |
| SpO2 (%) | 94.4 (89.6–96.0)             | 90.0 (78.0–95.4)         | 0.036 |
| Fetal growth restriction | 5 (6.10)                  | 7 (22.58)                | 0.019 |
| Suspected PE | 14 (17.07)                | 7 (22.58)                | 0.522 |
| PE-like syndrome (normal sFlt-1/PlGF ratio) | 9 (10.98)                | 4 (12.90)                | 0.774 |
| True PE (abnormal sFlt-1/PlGF ratio) | 5 (6.10)                  | 3 (9.68)                 | 0.508 |
| ICU admission | 0 (0)                     | 18 (58.06)               | — |
| Duration of ICU admission (days) | —                     | 2 (1–10)                 | — |
| Viral sepsis | 0 (0)                     | 6 (19.35)                | — |
| Maternal death | 0 (0)                     | 5 (16.13)                | — |

Data are given as median (interquartile range) or n (%). Comparisons were made using Mann–Whitney U-test for continuous variables and chi-square or Fisher’s exact test for categorical variables. BMI, body mass index; GA, gestational age; ICU, intensive care unit; MAP, mean arterial pressure; MoM, multiples of the median; PE, pre-eclampsia; sFlt-1, soluble fms-like tyrosine kinase-1; SpO2, oxygen saturation; utA-PI, uterine artery pulsatility index.

Table 2 Biochemical characteristics of 113 symptomatic pregnant women with COVID-19, according to severity of COVID-19 pneumonia

| Characteristic | Non-severe pneumonia (n = 82) | Severe pneumonia (n = 31) | P |
|----------------|-------------------------------|--------------------------|---|
| Leukocytes (×10/L) | 8.5 (7.1–10.1)               | 9.8 (7.5–14.0)           | 0.023 |
| Neutrophils (×10/L) | 6.3 (5.4–7.5)                | 8.8 (6.9–13.0)           | 0.0002 |
| Hemoglobin (g/dL) | 12.3 (11.3–13.5)             | 12.3 (11.0–13.9)         | 0.901 |
| Hematocrit (%) | 37.6 (34.3–40.7)             | 37.2 (34.0–41.9)         | 0.98 |
| Platelets (×10^3/L) | 216 (187–269)               | 200 (171–260)            | 0.246 |
| Glucose (mg/dL) | 177 (73–83)                  | 87 (75–119)              | 0.003 |
| Creatinine (mg/dL) | 5.5 (0.4–0.7)                | 0.5 (0.5–0.7)            | 0.932 |
| Aspartate aminotransferase (U/L) | 19 (15–30)                | 31 (24–49)               | 0.0001 |
| Alanine aminotransferase (U/L) | 16 (11–26)                | 26 (18–40)               | 0.001 |
| Lactate dehydrogenase (U/L) | 171.0 (140.0–202.5)          | 249.0 (192.0–375.0)      | 0.0001 |
| Triglycerides (mg/dL) | 214.5 (208.0–320.5)          | 281.0 (218.0–338.0)      | 0.305 |
| Cholesterol (mg/dL) | 231 (175–240)               | 149 (125–186)            | 0.0001 |
| D-dimer (ng/mL) | 206.5 (1262.0–3080.0)        | 1425.0 (1184.0–3322.0)    | 0.383 |
| C-reactive protein (mg/L) | 16.5 (6.6–60.6)             | 110.0 (63.8–200.0)       | 0.0001 |
| Procalcitonin (ng/mL) | 0.5 (0.03–0.15)             | 0.33 (0.18–0.60)         | 0.0001 |
| General urine test protein (mg/dL) | 20 (20–30)                 | 20 (10–70)               | 0.945 |
| PGF (pg/mL) | 146.0 (63.4–204.3)           | 127.1 (53.3–214.4)       | 0.839 |
| PGF MoM | 0.48 (0.19–0.71)            | 0.32 (0.21–0.64)         | 0.357 |
| sFlt-1 (pg/mL) | 1789 (1171–3286)            | 4050 (2099–11 490)       | 0.002 |
| sFlt-1 MoM | 0.76 (0.54–1.38)            | 1.91 (0.75–5.30)         | 0.001 |

Data are given as median (interquartile range). Comparisons were made using Mann–Whitney U-test. MoM, multiples of the median; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; SpO2, oxygen saturation; utA-PI, uterine artery pulsatility index.

The best cut-off value of sFlt-1 MoM was 1.66, which had sensitivity and specificity of 61.54% and 79.31% for severe pneumonia, 72.22% and 79.57% for ICU admission, 100% and 73.83% for viral sepsis and 100% and 73.15% for maternal death, respectively (Table S3).
sFlt-1 MoM is associated with a 1.8-fold increase in the odds of severe pneumonia, a 2.2-fold increase in the odds of ICU admission, a 2.3-fold increase in the odds of viral sepsis and a 5.5-fold increase in the odds of maternal death. At a 10% false-positive rate, sFlt-1 MoM achieved detection rates of 45.2%, 66.7%, 83.3% and 100% for severe pneumonia, ICU admission, viral sepsis and maternal death, respectively.

Table 3 Association between soluble fms-like tyrosine kinase-1 multiples of the median and adverse outcome in symptomatic pregnant women with COVID-19

| Outcome              | OR (95% CI)  | P      | aOR (95% CI) | P     |
|----------------------|--------------|--------|--------------|-------|
| Severe pneumonia     | 1.726 (1.323–2.253) | < 0.0001 | 1.817 (1.365–2.418) | < 0.0001 |
| ICU admission        | 2.089 (1.534–2.844)  | < 0.0001 | 2.195 (1.582–3.047)  | < 0.0001 |
| Viral sepsis         | 2.302 (1.405–3.773)  | 0.0010  | 2.318 (1.407–3.820)  | 0.0010  |
| Maternal death       | 5.504 (1.079–28.076) | 0.0400  | —             | —      |

aOR, odds ratio adjusted for gestational age; ICU, intensive care unit; OR, odds ratio.

Table 4 Performance of soluble fms-like tyrosine kinase-1 multiples of the median in the prediction of adverse outcome in symptomatic pregnant women with COVID-19

| Outcome              | AUC (95% CI)             | DR (%) (95% CI) at FPR of: |
|----------------------|--------------------------|-----------------------------|
|                      |                          | 5%                          | 10%                          |
| Severe pneumonia     | 0.715 (0.582–0.828)      | 38.7 (9.7–61.3)             | 45.2 (29.0–64.5)             |
| ICU admission        | 0.816 (0.662–0.949)      | 55.6 (16.7–77.8)            | 66.7 (38.9–88.9)             |
| Viral sepsis         | 0.939 (0.886–0.998)      | 66.7 (16.7–100)             | 83.3 (50.0–100)              |
| Maternal death       | 0.976 (0.950–1.000)      | 80.0 (40.0–100)             | 100 (60.0–100)               |

AUC, area under the receiver-operating-characteristics curve; DR, detection rate; FPR, false-positive rate; ICU, intensive care unit.

Figure 1 Receiver-operating-characteristics (ROC) curves showing the performance of soluble fms-like tyrosine kinase-1 multiples of the median in the prediction of severe pneumonia (area under the ROC curve (AUC), 0.7153) (a), intensive care unit admission (AUC, 0.8157) (b), viral sepsis (AUC, 0.9393) (c) and maternal death (AUC, 0.9762) (d), in symptomatic pregnant women with COVID-19.

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Comparison with existing literature

Our findings of higher sFlt-1 and unchanged PI GF levels are in contrast to those observed in cases with PE, in which sFlt-1 is increased and PI GF is decreased27,28. Although COVID-19 has been shown to mimic PE, the angiogenic imbalance is different because the endothelial source of sFlt-1 associated with COVID-19 is primarily systemic and not placental29.

The sFlt-1 protein is a splice variant of VEGFR-1, a receptor for VEGF-A and PI GF. When bound to its circulating ligand, sFlt-1 inhibits the VEGF-A pathway, causing endothelial and cell homeostasis impairment12. Overexpression of sFlt-1 has been demonstrated to play an important role in the pathogenesis of various diseases, especially those characterized by angiogenic imbalance, inflammation, endothelial dysfunction and hypertension. sFlt-1 contains a heparan sulfate binding site that binds to the extracellular matrix, causing intracellular sFlt-1 accumulation50. This mechanism includes activation of inflammatory pathways that convert arachidonic acid into prostaglandins and thromboxane, causing significant cytokine release31. In pathological conditions, such as viral infection or maternal sepsis, mononuclear cells, polymorphonuclear cells and peripheral blood platelets can also produce sFlt-112,31–33. The findings of this study suggest that inflammatory pathways are activated during COVID-19, leading to overproduction of sFlt-1. The placenta reacts to the viral infection in a similar way to the endothelium, showing increased production of sFlt-1. Other diseases, such as PE, have similar clinical and biochemical characteristics to those of COVID-19, which is known as PE-like syndrome. Even though we did not find a significant difference in the incidence of PE between severe and non-severe COVID-19 pneumonia cases in our sample, the findings of this study show that abnormal angiogenic status could be used to identify actual PE in pregnant women with COVID-19, regardless of the degree of severity23.

Strengths and limitations

The main strength of this study is that COVID-19 cases were recruited consecutively using a specialized database built for research purposes, thus minimizing potential bias. In addition, clinicians were blinded to the results of the biochemical analysis for sFlt-1 and PI GF to avoid selective outcome bias. Another strength is the inclusion of five cases of maternal death, which has been a very uncommon event in other consecutive cohort studies34–36. This was possible because our hospital is a tertiary referral center for COVID-19 in pregnancy, which encounters the most severe cases in Mexico. Baseline clinical characteristics were similar between pregnant women with severe and those with non-severe COVID-19 pneumonia, which diminishes the probability of selection bias. Furthermore, data were complete for each patient, allowing us to rule out other causes of sFlt-1 elevation, such as PE, fetal growth restriction, placental abruption and hemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome.

This study also has some limitations. First, the incidence of rare outcomes, such as maternal death (n = 5) and viral sepsis (n = 6), was low and the presented findings should be considered with caution, as the 95% CIs for the association between sFlt-1 and these severe adverse outcomes were wide. Another limitation is the inclusion only of women in the second half of pregnancy, which precluded evaluation of the utility of sFlt-1 in the first half of pregnancy and which highlights the need for further investigation into risk factors in this population. The small sample did not allow us to make statistical adjustment for several covariates for the outcome of maternal death; since death is a rare outcome, external validation of this model may be challenging. However, maternal death is rare globally, which makes the inclusion of five maternal deaths in this study a methodological strength despite the statistical limitations. Finally, although sFlt-1 MoM had good performance for short-term prediction of severe pneumonia, ICU admission, viral sepsis and maternal death in symptomatic pregnant women with COVID-19, these findings need clinical validation in independent cohorts before implementing it as a prognostic marker in real-life settings. Although fetal growth restriction was more prevalent in women with severe COVID-19 than in those with non-severe COVID-19, it was not associated with severe COVID-19 pneumonia in the final multivariate logistic regression model, and the only independent predictor was sFlt-1 MoM.

Clinical interpretation

Our data suggest that sFlt-1 could serve as a biomarker of endothelial dysfunction associated with adverse outcome in symptomatic pregnant women with COVID-19. Previous work has demonstrated that maternal risk factors, such as age ≥ 35 years, pre-existing diabetes, chronic hypertension, chronic renal disease and obesity1,37, are associated with COVID-19-related death, and this is the first study to demonstrate a potential biochemical marker for the prediction of severe COVID-19. The discovery of an inexpensive and widely available biomarker, such as sFlt-1, for the prediction of adverse outcome in pregnant women with COVID-19 may help develop strategies for optimized pregnancy care and management.

Conclusions

We have demonstrated that sFlt-1 MoM is higher in symptomatic pregnant women with severe COVID-19 pneumonia than in those with non-severe COVID-19 pneumonia and that sFlt-1 has the capability to predict serious adverse pregnancy events, such as severe pneumonia, ICU admission, viral sepsis and maternal death. Further research in larger prospective cohorts is needed to validate the potential of sFlt-1 as a biomarker for adverse outcome in pregnant women with COVID-19.
