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.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
sFlt-1/PlGF ratio in hypertensive disorders of pregnancy in patients affected by COVID-19

Chiara Maria Soldavini\textsuperscript{a}, Daniela Di Martino\textsuperscript{a}, Elisa Sabattini\textsuperscript{a}, Sara Ornaghi\textsuperscript{b}, Vittoria Sterpi\textsuperscript{a}, Roberta Erra\textsuperscript{a}, Francesca Invernizzi\textsuperscript{b}, Gabriele Tine\textsuperscript{c}, Valentina Giardini\textsuperscript{b}, Patrizia Vergani\textsuperscript{b,d}, Manuela Wally Ossola\textsuperscript{a}, Enrico Ferrazzi\textsuperscript{a,e,*}

\textsuperscript{a} Obstetrics Unit, Department of Woman Child and Newborn, Fondazione IRCCS Ca’ Granda, Ospedale Maggiore Policlinico, Milan, Italy
\textsuperscript{b} Obstetrics Unit, Department of Maternal Fetal Medicine, Fondazione MBBM, San Gerardo Hospital, University of Milano Bicocca, Monza, Italy
\textsuperscript{c} Department of Economics and Quantitative Methods, University of Milano Bicocca, Monza, Italy
\textsuperscript{d} University of Milano Bicocca, Monza, Italy
\textsuperscript{e} Department of Clinical and Community Sciences, University of Milan, Italy

\textbf{ARTICLE INFO}

\textbf{Keywords:}
COVID-19
Hypertensive disorders of pregnancy
sFlt-1/PlGF ratio
Pregnancy comorbidities
Preeclampsia

\textbf{ABSTRACT}

\textbf{Objectives:} To analyze soluble Fms-like tyrosine Kinase 1 (sFlt-1) and Placental Growth Factor (PlGF) ratio concentrations in COVID-19 pregnant patients with and without Hypertensive Disorders of Pregnancy (HDP), compared with non COVID-19 pregnant patients with HDP and a control group.

\textbf{Study design:} We recruited and obtained a complete follow-up of 19 COVID-19 pregnant patients with HDP and of 24 COVID-19 normotensive pregnant patients. Demographic, clinical and sFlt-1/PlGF ratio findings were compared with a group of 185 non COVID-19 pregnant patients with HDP and 41 non COVID normotensive patients. Findings were based on univariate analysis and on a multivariate adjusted model, and a case by case analysis of COVID-19 pregnant patients with an abnormal sFlt-1/PlGF ratio > 38 at recruitment.

\textbf{Main outcome measures:} sFlt-1/PlGF ratio.

\textbf{Results:} We confirmed a significant higher prevalence of HDP in women affected by COVID-19 compared to control population. sFlt-1/PlGF ratio was found high in HDP patients, with and without of Sars-Cov2 infection.

\textbf{Conclusions:} COVID-19 pregnant patients showed a higher prevalence of HDP compared to non COVID-19 controls, as well as higher comorbidity rates. In spite of the possible common endothelial target and damage, between Sars-Cov-2 infection and HDP, the sFlt-1/PlGF ratio did not correlate with the severity of this syndrome.

\section{1. Introduction}

Hypertensive Disorders of Pregnancy (HDP), among these preeclampsia and gestational hypertension, encompass a variety of diseases with a common downstream pathologic condition: high blood pressure caused by endothelial damage [1–6].

The introduction of molecular markers of placental vascular growth factors and their soluble blocking factors introduced a new fresh way to look into placental vascular growth, oxidative stress, endothelial dysfunction and the possible relationship between syncytiotrophoblast oxidative stress and hypertensive disorders of pregnancy [7]. Levine and co-authors reported a significant increase of the ratio between the soluble blocking factor, the soluble Fms-like tyrosine Kinase 1 (sFlt-1), and placental vascular growth factor (PlGF) in pregnant women affected by COVID-19.
Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health 27 (2022) 103–109
104

...preeclampsia. This ratio was significantly higher in cases of preeclampsia associated with fetal growth restriction than in cases with normally grown fetuses. These abnormal signaling cascades of oxidative stress represent a common pathway in worsening hypertensive disorders of pregnancy [8]. sFlt-1 also impairs nitric oxide (NO) production and sensitizes endothelial cells to angiotensin-II, a cascade that causes endothelial damage.

Recently, high values of sFlt-1 had been reported by Giardini and co-workers in patients affected by COVID-19 pneumonia vs. COVID-19 without pneumonia [9]. In addition, a large multinational cohort study reported a strong significant association of COVID-19 with preeclampsia and with gestational hypertension [10].

sFlt-1/PlGF ratio is a marker of oxidative stress of the endothelium, which is present in hypertensive disorders of pregnancy and COVID-19 syndrome. We hypothesized that in pregnant women with COVID-19 an unbalance between sFlt-1 and PlGF might reflect the increased risk of developing hypertensive disorders of pregnancy in Sars-Cov-2 infected patients [10] or a worsening of the hypertensive disorder itself through a synergistic action of endothelial damage. The aim of this study was to analyze sFlt-1 and PlGF concentrations and their ratio in pregnant patients Sars-Cov-2 positive with and without hypertensive disorders compared to Sars-Cov-2 negative pregnant patients with hypertensive disorders and uneventful pregnancies.

2. Methods

2.1. Study design

Since February 2021 we conducted a multicenter study (COvid in Obstetrics) to investigate COVID-19 infection in the obstetric population through hemodynamic, biochemical, and biophysical parameters. Pregnant patients were recruited from two COVID-19 Hub maternity hospitals: the Unit of Obstetrics at the Department of Woman, Child and Newborn, Fondazione IRCCS Ca’ Granda, Ospedale Maggiore Policlinico, Milan and the Unit of Obstetrics at the MBBM Foundation at San Gerardo Hospital, Monza, Italy.

Subjects were enrolled at their admission at COVID-19 wards at the Units of Obstetrics. Assessment of Sars-Cov-2 infection was made through PCR RNA analysis on nasopharyngeal swabs. Inclusion criteria were: maternal age ≥ 18 years, gestational age > 24 weeks, able to sign the informed consent. The present study is a sub-study of the Co-OST study approved by the Ethical Committee Milan Area 2 (Co-OST, n° 295_2021) and included only pregnant patients recruited consecutively from February 2021 to July 2021.

These observations were compared with findings regarding Sars-Cov-2 negative patients recorded during 24 months in the same Units from February 2020 to July 2021. These subjects were enrolled according to the same inclusion criteria at the time of the obstetric visit at the maternal fetal medicine outpatient clinics, or at admission to maternal fetal medicine wards, provided a negative Sars-Cov-2 nasopharyngeal molecular swab (Ethical Committee Milan Area 2, (MATER, n° 71_2020).

Exclusion criteria for all patients were: multiple pregnancy, fetal malformation, others maternal infections during pregnancy (toxoplasma, cytomegalovirus, rubella, varicella zoster virus, hepatitis B and C virus, human immunodeficiency virus).

All subjects underwent routine clinical assessment. HDP were diagnosed and classified according to ISSHP guidelines [11] and treated per standard clinical protocol. Patients affected by COVID-19 syndrome were treated by a multi-disciplinary team including maternal fetal medicine specialists, infectious diseases specialists, anesthesiologists, and neonatologists. Standard diagnostic tools included O2 saturation and Log10 of box-plot and whiskers of sFlt-1/PlGF of the groups were compared by Wilcoxon test. Statistical analysis was performed using SPSS Statistics software version 26.0 (IBM Corp, Armonk, NY).

3. Results

From February to July 2021 we recruited consecutive COVID-19 pregnant patients. Of these, 23 were affected by HDP (34%) while 45 were normotensive. Four HDP subjects were lost to follow-up. Among normotensive patients, 21 were discharged after they became negative for Sars-Cov-2. These 21 cases were delivered at their local maternity unit, or lost to follow-up. We compared findings with a cohort of uninfected pregnant patients. Therefore, we analysed data from four groups of subjects: 19 COVID-19, HDP patients; 24 COVID-19, non HDP patients; 185 non COVID-19, HDP patients; 41 normotensive controls (non COVID-19, non HDP).

Table 1 reports the demographic and prenatal data compared among the four groups. COVID-19 patients affected by HDP had a higher pregnancy BMI than COVID-19 normotensive patients and normotensive controls; also, among un-infected patients, BMI was higher in HDP subjects. COVID-19, HDP patients showed a greater prevalence of multiparity when compared to normotensive COVID-19 subjects and to un-infected ones.

Patients of non-Caucasian ethnicities were more represented in the COVID-19 cohort and in non COVID-19 hypertensive patients than in uneventful control pregnancies. Among COVID-19 subjects, one out of five in the HDP group showed Hispanic origin and nearly one out of three in the normotensive group was of Arabic origin.

Table 2 reports the perinatal outcome of the COVID-19 cohort, of non...
were of Hispanic, Arabic, or Asian origin (21%, 10%, 5%, respectively). All patients were of south European Caucasian ethnicity; all other subjects were of the uneventful control pregnancies. 

### Table 1
Maternal demographic and prenatal data. Median and interquartile range and number of cases in brackets where appropriate.

| Variable                                    | COVID-19 HDP (19) | COVID-19 Normotensive (24) | Non COVID-19 HDP (185) | Normotensive Controls (41) | p-value | Post-hoc test * |
|---------------------------------------------|-------------------|---------------------------|------------------------|---------------------------|---------|-----------------|
| Maternal age (years)                        | 35 (31 – 38)      | 32 (27 – 33)              | 35 (31 – 38)           | 33 (31–36)                | 0.031   | †               |
| Multiparous women                           | 79% (15)          | 38% (9)                   | 46% (85)               | 44% (18)                  | 0.032   | † † †           |
| Caucasian ethnicity                         | 63% (12)          | 46% (11)                  | 76.2% (141)            | 98% (40)                  | <0.001  | • † † †         |
| Pre-pregnancy BMI (kg/m²)                   | 27 (25 – 34)      | 23 (20 – 26)              | 24 (21 – 29)           | 22 (20 – 26)              | 0.005   | † •             |
| Smokers                                     | 6% (1)            | 0% (0)                    | 5% (9)                 | 5% (2)                    | 0.821   | †               |
| Previous HDP/FGR                            | 0% (0)            | 0% (0)                    | 20% (38)               | 2.4% (1)                  | <0.001  | †                |
| Pre-pregnancy comorbidities *,†             | 53% (10)          | 13% (3)                   | 12% (22)               | 2% (1)                    | <0.001  | †                |
| Conceived with ART                          | 0% (0)            | 8% (2)                    | 16% (29)               | 2% (1)                    | 0.031   | †                |
| Gestational diabetes                        | 37% (7)           | 29% (7)                   | 19% (35)               | 0% (0)                    | 0.001   | †                |

HDP, Hypertensive Disorders of Pregnancy; BMI, Body Mass Index; FGR, Fetal Growth Restriction; ART, Assisted Reproductive Technology.

* Post-hoc test was used to calculate the intergroup significant correlation with p-value < 0.05 for:
† COVID-19 HDP vs. COVID-19 Normotensive;
• COVID-19 HDP vs. Non COVID-19 HDP;
‡ COVID-19 HDP vs. Normotensive Controls;
¶ COVID-19 Normotensive vs. Non COVID-19 HDP;
§ COVID-19 Normotensive vs. Normotensive Controls;
# Non COVID-19 HDP vs. Normotensive Controls.

### Table 2
Perinatal data. Median and interquartile range and number of cases in brackets where appropriate.

| Variable                                    | COVID-19 HDP (19) | COVID-19 Normotensive (24) | Non COVID-19 HDP (185) | Normotensive Controls (41) | p-value | Post-hoc test * |
|---------------------------------------------|-------------------|---------------------------|------------------------|---------------------------|---------|-----------------|
| Gestational age at recruitment (weeks)      | 32 (31 – 38)      | 37 (30 – 38)              | 35 (30 – 37)           | 24.7 (21.4 – 28.4)        | < 0.001 | ¦               |
| Gestational age at delivery (weeks)         | 38 (35 – 39)      | 39 (38 – 39)              | 37 (34 – 38)           | 40 (39 – 41)              | < 0.001 | • † † †         |
| Caesarean section rate                      | 3250 (1930 – 3500)| 3015 (2718 – 3375)        | 2450 (1607 – 3087)     | 3300 (3190 – 3625)        | < 0.001 | #               |
| NICU                                        | 28% (5)           | 17% (4)                   | 34.6% (64)             | 0% (0)                    | < 0.001 | #               |

HDP, Hypertensive Disorders of Pregnancy; NICU, Neonatal Intensive Care Unit.

* Post-hoc test was used to calculate the intergroup significant correlation with p-value < 0.05 for:
† COVID-19 HDP vs. COVID-19 Normotensive;
• COVID-19 HDP vs. Non COVID-19 HDP;
‡ COVID-19 HDP vs. Normotensive Controls;
¶ COVID-19 Normotensive vs. Non COVID-19 HDP;
§ COVID-19 Normotensive vs. Normotensive Controls;
# Non COVID-19 HDP vs. Normotensive Controls.

COVID-19 patients with HDP and of the uneventful control pregnancies. Newborn weight was significantly lower in non COVID-19, HDP patients. However, a large range of newborn weight was observed in the two groups affected by HDP (from 1930 to 3500 gr in infected patients, from 1607 to 3087 gr in un-infected ones).

Among the 19 COVID-19 HDP patients, we observed two cases of preeclampsia with fetal growth restriction (1745 g and 2230 g, delivered at 32 weeks and 37 weeks, respectively). In non COVID-19 HDP patients, 81 cases were affected by preeclampsia with fetal growth restriction. Gestational age at delivery and newborn weight were 34.7 ± 3.6 weeks and 1670 ± 540 g, respectively. The vast majority of normotensive COVID-19 patients were delivered vaginally at term.

Table 3 presents the clinical data of interest of subjects infected by Sars-Cov-2 with or without HDP. 63% (12/19) of HDP, COVID-19 patients were of south European Caucasian ethnicity; all other subjects were of Hispanic, Arabic, or Asian origin (21%, 10%, 5%, respectively). This high prevalence of non-Caucasian maternal ethnicity was found also in the normotensive COVID-19 cohort (12% Hispanic, 29% Arabic, 12% Asian).

Obesity was more represented among HDP patients (37% vs 8% in COVID-19 normotensive women).

52% (10/19) of COVID-19, HDP infected women presented a pre-pregnancy comorbidity, and among them 5 subjects were affected by chronic hypertension; the other considered conditions were diabetes, gastrointestinal disorders, immunodepression, autoimmune, cardiovascular, pulmonary, renal, urinary diseases.
The ratio was then stratified into the risk levels for perinatal complications, as suggested by Stepans [13]. In agreement with these reported criteria, we adopted different cut-offs for the upper values of sFlt-1/PlGF ratio according to the time at onset of HDP, before or after 34 weeks of gestation. sFlt-1/PlGF ratio values resulted in normal range in 31% of HDP, non COVID-19 patients, in 53% of HDP, COVID-19 patients, in 71% of normotensive COVID-19 women and in 100% of normotensive un-infected controls. Conversely, we found a high and very high risk value of the ratio in 26% and 45% of HDP pregnancies with and without COVID-19, respectively. In normotensive patients, both infected and un-infected, we did not observe any case of high or extremely high risk sFlt-1/PlGF ratio value.

Fig. 1 reports the box-plot and whiskers of sFlt-1/PlGF ratio adjusted for maternal age, BMI and gestational age at recruitment. This adjusted model confirmed a significant difference between sFlt-1/PlGF ratio of COVID-19 versus HDP. COVID-19 vs. COVID-19 normotensive and between non-COVID-19 with HDP vs sFlt-1/PlGF ratio of all COVID-19 patients.

As far as the severity of HDP is concerned, among COVID-19, HDP patients, there were 3/19 (16%) cases of placental abruption and one (5%) case of severe pre-eclampsia. Among the 185 non COVID-19, HDP patients there were 4 (2%) cases of placental abruption (2%), 66 (36%) cases of severe pre-eclampsia or HELLP syndrome and 4 (2%) cases presenting both the complications mentioned.

Table 5 reports the clinical characteristics of interest of COVID-19 cases affected by HDP with abnormal sFlt-1/PlGF ratio. Significantly higher values in these groups compared with normotensive COVID-19 patients appears to be associated both with the severity of the maternal syndrome and/or the placental oxidative stress associated with fetal growth restriction.

Of interest, the highest ratio was observed in a patient with the most severe pulmonary insufficiency occurred in this COVID-19 cohort.

4. Discussion

4.1. Main findings

In our study, 34% of pregnancies complicated by COVID-19 were affected by hypertensive disorders of pregnancy (HDP). COVID-19 patients affected by HDP had a significantly higher prevalence of pre-pregnancy comorbidities and multiparity, than non COVID-19 patients with HDP.

COVID-19 did not worsen the antiangiogenic/angiogenic balance (sFlt-1/PlGF ratio) in pregnant patients with HDP compared with non COVID-19 patients with HDP. We observed a higher sFlt-1/PlGF ratio in pregnancies with HDP, regardless the concomitant presence or absence of COVID-19 syndrome. In normotensive COVID-19 patients the sFlt-1/PlGF ratio was significantly higher in non COVID-19 HDP patients.

Table 4 reports the overall sFlt-1/PlGF ratio at recruitment for the COVID-19 cohort, for the non COVID-19 HDP patients and for uneventful controls [12].

The overall median sFlt-1/PlGF ratio was significantly higher in non COVID-19 HDP patients.

The ratio was then stratified into the risk levels for perinatal complications, as suggested by Stepans [13]. In agreement with these reported criteria, we adopted different cut-offs for the upper values of sFlt-1/PlGF ratio according to the time at onset of HDP, before or after 34 weeks of gestation. sFlt-1/PlGF ratio values resulted in normal range in 31% of HDP, non COVID-19 patients, in 53% of HDP, COVID-19 patients, in 71% of normotensive COVID-19 women and in 100% of normotensive un-infected controls. Conversely, we found a high and very high risk value of the ratio in 26% and 45% of HDP pregnancies with and without COVID-19, respectively. In normotensive patients, both infected and un-infected, we did not observe any case of high or extremely high risk sFlt-1/PlGF ratio value.

Fig. 1 reports the box-plot and whiskers of sFlt-1/PlGF ratio adjusted for maternal age, BMI and gestational age at recruitment. This adjusted model confirmed a significant difference between sFlt-1/PlGF ratio of COVID-19 versus HDP. COVID-19 vs. COVID-19 normotensive and between non-COVID-19 with HDP vs sFlt-1/PlGF ratio of all COVID-19 patients.

As far as the severity of HDP is concerned, among COVID-19, HDP patients, there were 3/19 (16%) cases of placental abruption and one (5%) case of severe pre-eclampsia. Among the 185 non COVID-19, HDP patients there were 4 (2%) cases of placental abruption (2%), 66 (36%) cases of severe pre-eclampsia or HELLP syndrome and 4 (2%) cases presenting both the complications mentioned.

Table 5 reports the clinical characteristics of interest of COVID-19 cases affected by HDP with abnormal sFlt-1/PlGF ratio. Significantly higher values in these groups compared with normotensive COVID-19 patients appears to be associated both with the severity of the maternal syndrome and/or the placental oxidative stress associated with fetal growth restriction.

Of interest, the highest ratio was observed in a patient with the most severe pulmonary insufficiency occurred in this COVID-19 cohort.

4. Discussion

4.1. Main findings

In our study, 34% of pregnancies complicated by COVID-19 were affected by hypertensive disorders of pregnancy (HDP). COVID-19 patients affected by HDP had a significantly higher prevalence of pre-pregnancy comorbidities and multiparity, than non COVID-19 patients with HDP.

COVID-19 did not worsen the antiangiogenic/angiogenic balance (sFlt-1/PlGF ratio) in pregnant patients with HDP compared with non COVID-19 patients with HDP. We observed a higher sFlt-1/PlGF ratio in pregnancies with HDP, regardless the concomitant presence or absence of COVID-19 syndrome. In normotensive COVID-19 patients the sFlt-1/
PlGF ratio was normal in 71% of cases. In subjects infected by Sars-Cov-2, poorer clinical outcomes were seen in patients affected by obesity or other pre-pregnancy comorbidities, in pregnancies complicated by HDP or gestational diabetes or both. All these conditions underline a pattern of endothelial damage, thus presenting an altered sFlt-1/PlGF ratio. sFlt/PlGF ratio proved not to be helpful in the differential diagnosis of the severity of this infection; placental biomarkers did not correlate with the severity of symptoms.

Fig. 1. Box-plot and whiskers of Log10 of sFlt-1/PlGF adjusted for maternal age, BMI, and gestational age at recruitment of COVID-19 patients (red with HDP, yellow normotensive patients), and non COVID-19 patients with HDP (blue). In green, Log10 boxplot and whiskers of control normotensive pregnant women adjusted for the same variables. COVID-19 with HDP vs. COVID-19 normotensive P < 0.02; non COVID-19 with HDP vs all COVID-19 p < 0.001.

Table 5. Demographic data, COVID-19 symptoms and neonatal data of COVID-19 patients with values of sFlt-1/PlGF ratio above 38.

| Maternal age (years) | Hypertensive Disorders of Pregnancy | Gestational diabetes | COVID-19 a | Gestational age (weeks) | sFlt-1/PlGF ratio | Newborn weight (gr) | Newborn weight (percentile) b |
|---------------------|------------------------------------|----------------------|------------|------------------------|-------------------|---------------------|-----------------------------|
| 37                  | Yes                                | Yes                  | No         | 38                     | 67.7              | 1960                | 1                           |
| 32                  | Yes                                | No                   | No         | 38                     | 69.6              | 3420                | 60                          |
| 37                  | Yes                                | No                   | No         | 35                     | 95.8              | 2890                | 85                          |
| 38                  | Yes                                | No                   | No         | 39                     | 129.8             | 3330                | 58                          |
| 40                  | Yes                                | No                   | No symptoms| 37                     | 168.0             | 2230                | 4                           |
| 22                  | Yes                                | Yes                  | No symptoms| 31                     | 363.0             | 1780                | 67                          |
| 26                  | Yes                                | No                   | Mild       | 41                     | 70.3              | 3470                | 56                          |
| 29                  | Yes                                | No                   | Mild       | 32                     | 191.4             | 3350                | 70                          |
| 42                  | Yes                                | No                   | Severe     | 33                     | 762.1             | 1796                | 33                          |
| 37                  | No                                 | No                   | No         | 38                     | 43.1              | 2905                | 39                          |
| 27                  | No                                 | Yes                  | No         | 36                     | 47.6              | 3010                | 16                          |
| 35                  | No                                 | No                   | No         | 33                     | 62.3              | 1250                | 1                           |
| 25                  | No                                 | No                   | No         | 33                     | 109.5             | 1640                | 4                           |
| 35                  | No                                 | No                   | Mild       | 38                     | 53.9              | 4270                | 99                          |
| 30                  | No                                 | Yes                  | Mild       | 38                     | 62.3              | 2830                | 18                          |
| 27                  | No                                 | Yes                  | Moderate   | 36                     | 41                | 2705                | 45                          |

sFlt-1, soluble Fms-like tyrosine Kinase 1; PlGF, Placental Derived Growth Factor

a COVID-19 symptoms: Mild refers to symptomatic infection that does not requires O2 support respiratory therapy or mechanical ventilation; Moderate refers to infection that requires O2 support respiratory therapy; Severe refers to infection that requires mechanical ventilation.

b Newborn weight percentiles were calculated according to Italian newborn weight Charts for parity, sex, and gestational age [23].
4.2. Interpretation

The prevalence of hypertensive disorders in pregnant women affected by COVID-19 syndrome in this consecutive cohort was significantly higher than expected in the general population (34% vs 5–8%) [14]. This agrees with multinational surveys [10] in which HDP was observed in 40% of COVID-19 affected patients, and other reported systematic reviews on COVID-19 in pregnancy [15]. The small number of cases in our cohort study, as part of the ongoing Co-OST research, did not allow for a multiparametric model. However, maternal age, comorbidities, obesity, gestational diabetes were significantly reported in the COVID-19 patients affected by hypertensive disorders.

As already reported, we observed a significant prevalence of non-Caucasian ethnicities in COVID-19 pregnant patients [16]. As shown by Kahlil and co-workers [16], this is likely to be due to social deprivations as regards housing, manual works that cannot be avoided during lock-down, usage public transportation, and living with the most polluted pro-inflammatory air in poor neighborhood of large metropolitan areas.

The sFlt-1/PlGF ratio, a marker of syncytiotrophoblast [17] and endothelial oxidative stress [9] allowed us to cast a different light on possible biological associations. In non COVID-19 hypertensive disorders we observed significant higher sFlt-1/PlGF ratios compared to COVID-19 patients both with and without hypertension, with 69% of cases stratified as risk values. Overall, this finding underlines that maternal endothelial dysfunction associated with COVID-19, as observed in adult non-pregnant COVID-19 patients [9], did not add up to placental oxidative stress that is typical of hypertension in pregnancy. The small cohort of the Co-OST study allowed us to look at possible association of maternal and placental co-factors associated with abnormal sFlt-1/PlGF ratio in individual cases: gestational diabetes and symptomatic COVID-19 syndrome were observed in patients with an abnormal ratio. The highest value of sFlt-1/PlGF ratio (7.26) was observed in the only case of severe COVID-19 syndrome that required mechanical ventilation.

However, sFlt-1/PlGF ratio seems not to improve our knowledge in the evaluation, follow-up and treatment of COVID-19 patients with HDP comparing to HDP un-infected pregnancies. Therefore, COVID-19 infection does not appear to act as an additional trigger on endothelial cells whose function is already damaged by hypertensive disorders.

In addition to this, we also looked into a proxy of feto-placental growth, that is newborn weight percentile at birth. A low weight percentile is typical of early onset preeclampsia associated with the highest reported values of sFlt-1/PlGF ratio. This was already observed by Levine in 2006 [7]. These authors observed that cases of preterm preeclampsia with small-for-gestational-age infants had higher sFlt-1/PlGF ratio than cases of preterm preeclampsia with appropriately sized infants (47.9 vs. 17.2, p < 0.001). The small placenta with underdeveloped villi with their increased number of syncytial knots is the main source of soluble blocking factors, i.e. sFlt-1. This was also the case in 4 of our 16 cases of our study with abnormal sFlt-1/PlGF ratio. This signaling that originates from the dysfunctional placenta are the pathway of preeclampsia associated with fetal growth restriction [8]. It is of interest that, in our study, all but two cases of HDP in COVID-19 patients were associated with normal sized infants at birth, underlining how these clinical phenotypes of hypertensive disorders were more associated with “...predisposing cardiovascular or metabolic risks for endothelial dysfunction, as part of an exaggerated systemic inflammatory response” [18,19]. In these cases, placental oxidative stress is less severe, as it is also underlined by the different cut-off risk levels proposed by Stepan and coworkers [13].

4.3. Strengths and limitations of this study

Limitations of this study are obviously represented by the small number of cases recruited by this cohort study within the Co-OST ongoing research project. In addition to this, a significant number of COVID-19 cases not affected by HDP were lost to follow-up or had significant missing data since they were dismissed with negative Sars-Cov-2 swab and delivered at their local hospital. Non COVID-19 patients with HDP were collected in the two centers, but in a longer period of time bridging the COVID-19 pandemic. sFlt-1/PlGF ratio in controls was collected at a significantly lower age of gestation. However, observed ratios agreed with expected values in normal cases [20]. These data allowed us to provide comparable values for demographic and clinical data from within the same centers. In addition to this, the Co-OST consecutive cases were recruited in two COVID-19 Maternity Hubs in the metropolitan large area of Milan. This area is representative of a multiethnic one where approximately 23% of newborn babies are delivered by women of non-south European Caucasian ancestry. Patients lost to follow-up were likely to be the least symptomatic cases without additional obstetrical complications requiring monitoring and delivery in a referral center. The larger group of non COVID-19 hypertensive patients were collected in the same centers and their large number (one to four ratio) allowed for a more robust comparison of data and outcome. The introduction of sFlt-1/PlGF ratio and the differentiation of clinical phenotypes of hypertensive disorders according to their association with fetal growth restriction or appropriately sized infants [2,3,7,8], allowed us to observe the different possible links between COVID-19 syndrome, comorbidities and HDP.

4.4. Clinical and research implications of our findings

sFlt-1/PlGF ratio is an important marker of placental oxidative stress and maternal endothelial dysfunction. High sFlt-1 values seem to be associated with Sars-Cov-2 pathogenetic mechanisms [9]. We suggest that these molecular markers should be measured in COVID-19 pregnant patients as an additional monitoring tools both of ongoing placental function in the evolution of COVID-19 syndrome. Future research is required to compare the sFlt-1/PlGF ratio in COVID-19 pregnant patients without HDP and uneventful pregnancies to assess if and how much the possible inflammatory cascade of Sars-Cov-2 infection might affect the angiogenic balance in these pregnancies.

5. Conclusions

In our cohort of COVID-19 pregnant patients, part of an ongoing research project, we confirmed a significant prevalence of HDP. sFlt-1/PlGF ratio was found to be higher in HDP patients, regardless of the presence of Sars-Cov-2 infection. Indeed, COVID-HDP patients did not have higher values than non-COVID HDP patients, as we could have expected by the combined mechanisms of placental oxidative stress described in hypertensive disorders of pregnancy and the endothelial dysfunction observed in adults as a consequence of symptomatic Sars-Cov-2 infections. Indeed, this confirm reported findings by Nayeri and co-workers [21] that observed how sFlt-1 and PlGF are not influenced by corticosteroids modulation of inflammatory cytokines such as IL-6 in patients with severe preeclampsia, suggesting independent pathways of inflammation and angiogenic balance in these cases.

However, present findings and a case by case analysis of COVID-19 pregnant patients with an abnormal sFlt-1/PlGF ratio at recruitment, allowed us to observe possible multiple associations between abnormally high sFlt-1/PlGF ratio and pre-pregnancy comorbidities, hypertension and gestational diabetes, fetal growth restriction associated with hypertension and severity of COVID-19 syndrome. COVID patients with worse evolution of the disease showed higher rates of obesity and various comorbidities, including hypertensive disorders. However, the sFlt-1/PlGF ratio proved not to be helpful in the differential diagnosis of the severity of this infection; placental biomarkers did not correlate with the severity of symptoms, except for cases of severe respiratory failure, as described by Giardini and coworkers in non pregnant patients [9]. The highest value of sFlt-1/PlGF ratio was observed in the case of a...
severe COVID-19 pulmonary insufficiency requiring mechanical ventilation [22].

Funding

This project was supported through research funding from the Scientific BB Branch of the Fondazione IRCCS Ca Granda, Ospedale Maggiore Policlinico, Milan, Italy.

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.

References

[1] L. Myatt, C.W. Redman, A.C. Staff, S. Hansson, M.L. Wilson, H. Laiviari, et al., Global Pregnancy CoLaboratory Strategy for standardization of preeclampsia research study design, Hypertension 63 (2014) 1293–1301. https://doi.org/10.1161/HYPERTENSIONAHA.113.02664.
[2] F. Ferrari, T. Stampalija, L. Monasta, D. Di Martino, S. Vonck, W. Gyselaers, Maternal hemodynamics: a method to classify hypertensive disorders of pregnancy, Am. J. Obstet. Gynecol. 218 (124) (2018) e1–e124.e11. https://doi.org/10.1016/j.ajog.2017.10.226.
[3] F.L. Foo, A.A. Mahendru, G. Masini, A. Fraser, S. Cacciatori, D.A. Maclntyre, et al., Association Between Prepregnancy Cardiovascular Function and Subsequent Preeclampsia or Fetal Growth Restriction, Hypertension 72 (2018) 442–450. https://doi.org/10.1161/HYPERTENSIONAHA.118.11092.
[4] J. Tay, L. Foo, G. Masini, P.R. Bennett, C.M. McEniery, I.B. Wilkinson, et al., Early and late preeclampsia are characterized by high cardiac output, but in the presence of fetal growth restriction, cardiac output is low: insights from a prospective study, Am. J. Obstet. Gynecol. 218 (517) (2018) e1–517.e12. https://doi.org/10.1016/j.ajog.2018.02.007.
[5] H.Z. Ling, G.P. Guy, A. Biquerda, L.C. Poon, N.A. Kametas, Maternal hemodynamics in screen-positive and screen-negative women of the ASPRE trial, Ultrasound Obstet. Gynecol. 54 (2019) 51–57. https://doi.org/10.1002/uog.20125.
[6] T.R. Easterling, D.H. Watts, B.C. Schmacker, T.J. Benedetti, Measurement of cardiac output during pregnancy: validation of Doppler technique and clinical observations in preeclampsia, Obstet. Gynecol. 69 (1987) 845–850.
[7] R.J. Levine, C. Lam, C. Qian, K.F. Yu, S.E. Maynard, B.P. Sachs, et al., Soluble endoglin and other circulating antiangiogenic factors in preeclampsia, N. Engl. J. Med. 355 (10) (2006) 992–1005. https://doi.org/10.1056/NEJMoa053252.
[8] C.W.G. Redman, A.C. Staff, J.M. Roberts, Syncytiotrophoblast stress in preeclampsia: the convergence point for multiple pathways, Am. J. Obstet. Gynecol. 30002-9378 (2020) 3115–3117. https://doi.org/10.1016/j.ajog.2020.09.047.
[9] 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 (8) (2020) E188-E191, https://doi.org/10.1002/ajh.25862.
[10] A.T. Papageorghiou, F. Bernelle, R.B. Gunier, S. Rauch, P.K. García-May, M. Mhatre, et al., Preeclampsia and COVID-19: results from the INTERCOVID prospective longitudinal study, Am. J. Obstet. Gynecol. S0002-9378 (21) (2021) 00561-565. https://doi.org/10.1016/j.ajog.2021.05.016.
[11] M.A. Brown, L.A. Magee, L.C. Kenny, S.A. Karumanchi, F.P. McCarthy, S. Saito, et al., International Society for the Study of Hypertension in Pregnancy (ISSHP), The hypertensive disorders of pregnancy: ISSHP classification, diagnosis & management recommendations for international practice, Pregnancy Hypertens. 13 (2018) 291–310. https://doi.org/10.1016/j.preghy.2018.05.004.
[12] 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. https://doi.org/10.1056/NEJMoa1414338.
[13] H. Stepan, I. Herraz, D. Schlembach, S. Verlohren, S. Brennecke, F. Chantraine, et al., Implementation of the sFlt-1/PlGF ratio for prediction and diagnosis of preeclampsia in singleton pregnancy: implications for clinical practice, Ultrasound Obstet. Gynecol. 45 (3) (2021) 241–246. https://doi.org/10.1002/uog.14799.
[14] M. Umesawa, G. Kobashi, Epidemiology of hypertensive disorders in pregnancy: prevalence, risk factors, predictors and prognosis, Hypertens. Res. 40 (3) (2017) 213–220, https://doi.org/10.1038/hr.2016.126.
[15] J. Alloey, E. Stalling, M. Bonet, M. Yap, S. Chatterjee, T. Kew, et al., For PregCOVID-19 Living Systematic Review Consortium, Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis, BMJ 370 (2020), m3320, https://doi.org/10.1136/bmj.m3320.
[16] A. Khalil, R. Hill, S. Ladhani, K. Pattisson, P. O’Brien, Severe acute respiratory syndrome coronavirus 2 in pregnancy: symptomatic pregnant women are only the tip of the iceberg, Am. J. Obstet. Gynecol. 223 (2020) 296–297, https://doi.org/10.1016/j.ajog.2020.05.065.
[17] C.W.G. Redman, A.C. Staff, J.M. Roberts, Syncytiotrophoblast stress in preeclampsia: the convergence point for multiple pathways. Am J Obstet Gynecol. 8, S0002-9378(20)31115-7. 10.1016/j.ajog.2020.09.047.
[18] K.B. Sole, A.C. Staff, K. Laine, Maternal diseases and risk of hypertensive disorders of pregnancy across gestational age groups, Pregnancy Hypertens. 25 (2021) 25–33, https://doi.org/10.1016/j.preghy.2021.05.004.
[19] E.A. Steegers, P. van Dadelszen, J.J. Duvekot, R. Fijnheer, Pre-eclampsia, Lancet 376 (9741) (2010) 631–644. https://doi.org/10.1016/S0140-6736(10)60629-6.
[20] S. Verlohren, I. Herraz, O. Lapaire, D. Schlembach, H. Zeisler, P. Calda, et al., New gestational phase-specific cutoff values for the use of the soluble fms-like tyrosine kinase 1/placental growth factor ratio as a diagnostic test for preeclampsia, Hypertension 63 (2) (2014) 346–352. https://doi.org/10.1161/HYPERTENSIONAHA.113.01787.
[21] U. Nayeri, I. Buhimschi, C. Laky, S. Cross, C. Durzy, W. Ramma, et al., Antenatal Corticosteroids Impact the Inflammatory Rather Than the Antiangiogenic Profile of Women With Preeclampsia, Hypertension 63 (6) (2014) 1246–1292, https://doi.org/10.1161/HYPERTENSIONAHA.113.04117.
[22] A. Jayaram, I.A. Buhimschi, H. Aldassoq, J. Hartwig, T. Owens, G.L. Elam, C. Buhimschi, Who said differentiating preeclampsia from COVID-19 infection was easy? Pregnancy Hypertension. 26 (2021) 8–10, https://doi.org/10.1016/j.preghy.2021.07.248.
[23] E. Bertino, E. Spada, L. Occhi, A. Coscia, F. Giuliani, L. Gagliardi, et al., Neonatal Anthropometric Charts: the Italian neonatal study compared with other European studies, J. Pediatr. Gastroenterol. Nutr. 51 (3) (2010) 353–361, https://doi.org/10.1097/MJP.0b013e3181d238e.