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sFlt-1/PlGF ratio in hypertensive disorders of pregnancy in patients affected by COVID-19

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ABSTRACT

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.
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.
Main outcome measures: sFlt-1/PlGF ratio.
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. COVID-19 patients with worse evolution of the disease showed greater rates of obesity and other comorbidities. sFlt/PlGF ratio proved not to be helpful in the differential diagnosis of the severity of this infection.
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 sFlt1/PlGF ratio did not correlate with the severity of this syndrome.

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 syncityotrophoblast 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

Abbreviations: sFlt-1, soluble Fms-like tyrosine Kinase 1; PlGF, Platelet Derived Growth Factor; COVID-19, COronavirus Disease 19; HDP, Hypertensive Disorders of Pregnancy; NO, Nitric Oxide; Co-OST, COvid in ObSTetrics; MBBM, Monza Brianza Baby and Mother Foundation; PCR, Protein Chain Reaction; ISSHP, International Society for the Study of Hypertension in Pregnancy; CAT, Computed Tomography; LMWH, Low Molecular Weight Heparin; BMI, Body Mass Index; OR, Odds Ratio; CI, Confidence Interval; ART, Assisted Reproductive Technology; NICU, Neonatal Intensive Care Unit.

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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 coworkers 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 $\geq$ 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. Standard diagnostic tools included O_2 continuous non-invasive monitoring, arterial emo-gas analysis and CAT scan when necessary; therapy included paracetamol, low molecular weight heparin (LMWH), corticosteroids (endovenous dexamethasone or methylprednisolone), antibiotics, O_2 respiratory support, when necessary according to local protocol. Dosage of low molecular weight heparin was selected according to maternal body mass index (BMI): we used Enoxaparin 4000 IU or 6000 IU subcutaneous injections if BMI was below or above 30 kg/m^2, respectively. We administered intramuscular Betamethasone to accelerate fetal lung maturation in case of risk of preterm delivery (below 34 weeks of gestation) within a week. If accelerated lung maturation was required without other maternal indication, we administered Betamethasone 12 mg every 24 h for two days. If accelerated lung maturation was required with also a maternal lung indication, we administered Betamethasone 6 mg every 12 h for 2 days, then Methylprednisolone 32 mg every 24 h for 8 days. If there was a maternal respiratory indication, but accelerated lung maturation was not required, we used Methylprednisolone 32 mg every 24 h for 8 days.

COVID-19 symptoms were considered severe, moderate or mild when symptomatic infection required mechanical ventilation, O_2 support respiratory therapy alone or none of these treatments, respectively.

Placental biomarkers sFlt-1 and PlGF were assayed on a maternal venous blood sample, taken at recruitment. Sampled blood was collected in a vial containing a separating gel. Tubes were bar-code labeled. Within three hours from collection, coded vials were centrifuged for 10 min. sFlt-1 and PlGF analysis was performed by Roche's Elecsys automated methods, which are immunoassays based on electrochemoluminescence technology. The limit of detection varies between 10 and 8500 pg/ml for sFlt-1 and between 3 and 10000 pg/ml for PlGF.

Demographic and clinical maternal and neonatal data were retrieved from electronical records.

2.2. Statistical analysis

Non-parametric tests were used to perform univariate analysis. We adopted the ANOVA of Kruskall-Wallis or Mann-Whitney *U* test for scalar variables and Marascuilo procedure for categorical variables, as appropriate. Data are expressed with median and interquartile range for continuous variables and with relative frequencies and absolute number for categorical variables, respectively. Frequencies for groups with$<$100 cases were rounded to the integer number. A multivariate model was adopted to adjust for maternal age, BMI and gestational age at recruitment and Log10 of box-plot and whiskers of sFlit-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 prepregnancy 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 uninfected 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

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)	*NormotensiveControls (41)*	p-value	Post-hoc test [a]
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/m^2)	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.
*pre-pregnancy comorbidities included chronic hypertension; diabetes, gastrointestinal disorders, immunodepression, autoimmune, cardiovascular, pulmonary, renal, urinary diseases
[a] 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 [a]
Gestational age at recruitment (weeks)	32 (31 – 38)	37 (30 – 38)	35 (30 – 37)	*24.7 (24.1 – 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	50% (9)	38% (9)	61.5% (114)	*22% (9)*	**<0.001**	#
Neonatal weight (gr)	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.
[a] 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-Causasian 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 or renal diseases. Conversely, only 12% (3/24) of normotensive COVID-19 patients had previous comorbidities.

As far as gestational complications are concerned, gestational diabetes complicated one third of COVID-19 pregnancies (37% in HDP patients, 29% in normotensive patients). One out of ten fetuses was affected by growth restriction in both infected groups.

Preterm delivery before 34 weeks of gestation occurred in one out of five cases of COVID-19, HDP pregnancies (21%; at 31, 32, 33 and 34 weeks). In particular, one patient underwent an emergency caesarean section after eclamptic seizures and was later admitted to Intensive Care Unit (UTI). Two normotensive infected patients delivered before 34 weeks of gestation, at 27 and 33 weeks respectively. The former was a case of iatrogenic preterm birth due to critical maternal conditions of hypoxic pneumonia and acute respiratory insufficiency.

We classified COVID-19 syndrome in our patients according to the presence, type and severity of symptoms. Among normotensive patients, 29% (7/24) were symptomatic, which means they presented with one or

Table 3
. Demographic and Clinical data in COVID-19 patients. Number of cases in brackets.

Variable	COVID-19 HDP (n = 19)	COVID-19 Normotensive (n = 24)	p-value
Maternal ethnicity			
Caucasian	63% (12))	46% (11)	0.258
Asian	5% (1)	12% (3)	0.417
Arabic	10% (2)	29% (7)	0.136
Hispanic	21% (4)	12% (3)	0.451
Pre-pregnancy comorbidities			
Obesity (BMI > 30 kg/m^2)	37% (7)	8% (2)	0.594
Chronic Hypertension	26% (5)	0% (0)	–
Pregnancy complications			
Gestational Diabetes	37% (7)	29% (7)	0.594
Fetal Growth Restriction	10% (2)	8% (2)	0.806
Preterm delivery < 34 weeks	21% (4)	8% (2)	0.232
COVID-19			
Asymptomatic / pauci-symptomatic	79% (15)	71% (17)	0.545
Symptomatic	21% (4)	29% (7)	0.545
0_2 respiratory support	21% (4)	8% (2)	0.232
ICU admission	16% (3)	4% (1)	0.193

HDP, Hypertensive Disorders of Pregnancy; BMI, Body Mass Index; ICU, Intensive Care Unit.

a combination of the following symptoms: temperature, cough, pneumonia. Only one patient was admitted to ICU for severe pneumonia; overall, two patients required oxygen respiratory support. The other women of this cohort were asymptomatic or pauci-symptomatic with anosmia, ageusia, cold, or sore throat. Conversely, 21% (4/19) of HDP infected patients were symptomatic and one case developed an acute respiratory distress syndrome. Four subjects of this group required oxygen respiratory support, three of them were admitted to ICU and half of them were obese.

Newer medications had not been used yet in our COVID-19 Hub maternity wards during the period of recruitment. Among the 43 COVID patients recruited, 3 (7%) required therapy with LMWH, antibiotics and corticosteroids, 4 (9%) were treated with LMWH and corticosteroids, 7 (16%) with LMWH and antibiotics, and the others (68%) with LMWH only.

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 Stepan [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/PlGL 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 with HDP 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/

Table 4
sFlt-1/PlGF ratio stratified according to risk levels [17]. Median and interquartile range and number of cases in brackets where appropriate.

Variable	COVID-19 HDP (19)	COVID-19 Normotensive (24)	No COVID-19 HDP (185)	*Normotensive Controls (41)*	p-value	Post-hoc test [a]
sFlt-1/PlGF ratio at recruitment	34.6 (10.9 – 129.8)	9.7 (3.5 – 42.6)	88.3 (29.1 – 203.0)	*2.9 (1.6 – 5.0)*	**<0.001**	† ¶ #
Normal - Low risk (ratio ≤ 38)	53% (10)	71% (17)	30.8% (57)	*100% (41)*	**<0.001**	• ¶ § #
Medium risk (38 < ratio ≤ early 85/late 110)	21 % (4)	29% (7)	24.3% (45)	*0% (0)*	**0.005**	§ #
High risk (85/110 < ratio ≤ early 655/late 201)	21% (4)	0% (0)	33.0% (61)	*0% (0)*	**<0.001**	¶ #
Very high risk (ratio > early 655/late 201)	5% (1)	0% (0)	11.9% (22)	*0% (0)*	**0.037**	¶ #

[a] 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.

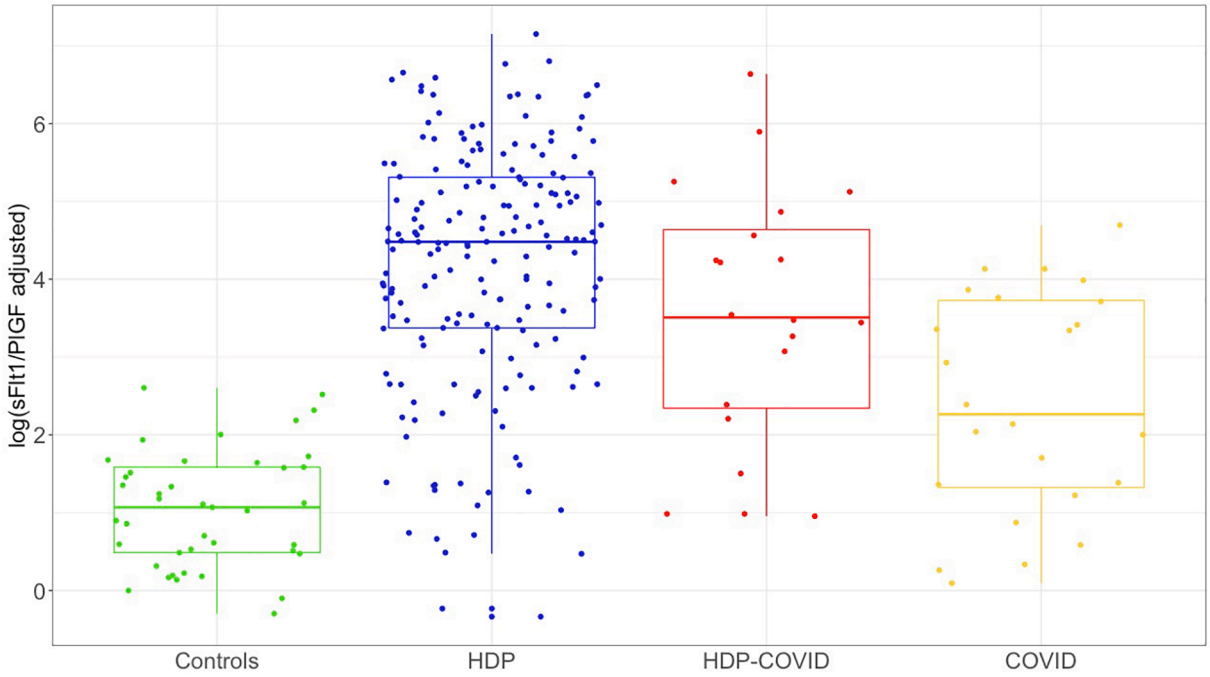

Fig. 1. Box-plot and whiskers of Log10 of sFlt-1/PlGF adjusted for maternal age, BMI, and gestational age at recruitment of COVID-19patients (red with HDP, yellow normotensive patients), and non COVID-19patients with HDP (blue). In green, Log10 boxplot and whiskers of control normotensive pregnant women adjusted for the same variables. COVID-19 with HDP vs. COVID-19 normotensive $P < 0.02$; non COVID-19 with HDP vs all COVID-19p < 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 symptoms	38	**67.7**	1960	1
32	**Yes**	No	No symptoms	38	**69.6**	3420	60
37	**Yes**	No	No symptoms	35	**95.8**	2890	85
38	**Yes**	No	No symptoms	39	**129.8**	3330	58
40	**Yes**	**Yes**	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 symptoms	38	**43.1**	2905	39
27	No	**Yes**	No symptoms	36	**47.6**	3010	16
35	No	No	No symptoms	33	**62.3**	1250	1
25	No	No	No symptoms	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 O_2 support respiratory therapy or mechanical ventilation; Moderate refers to infection that requires O_2 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].

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.

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 (726) 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, underling 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-1PlGF 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-1PlGF 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-1and 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].

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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.

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