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Preterm birth is not associated with asymptomatic/mild SARS-CoV-2 infection *per se*: Pre-pregnancy state is what matters

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Abstract

Evidence for the real impact of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection on preterm birth is unclear, as available series report composite pregnancy outcomes and/or do not stratify patients according to disease severity. The purpose of the research was to determine the real impact of asymptomatic/mild SARS-CoV-2 infection on preterm birth not due to maternal respiratory failure. This case-control study involved women admitted to Sant Anna Hospital, Turin, for delivery between 20 September 2020 and 9 January 2021. The cumulative incidence of Coronavirus disease-19 was compared between preterm birth (case group, n = 102) and full-term delivery (control group, n = 127). Only women with spontaneous or medically-indicated preterm birth because of placental vascular malperfusion (pregnancy-related hypertension and its complications) were included. Current or past SARS-CoV-2 infection was determined by nasopharyngeal swab testing and detection of IgM/IgG antibodies in blood samples. A significant difference in the cumulative incidence of Coronavirus disease-19 between the case (21/102, 20.5%) and the control group (32/127, 25.1%) ($P = 0.50$) was not observed, although the case group was burdened by a higher prevalence of three known risk factors (body mass index > 24.9, asthma, chronic hypertension) for severe Coronavirus disease-19. Logistic regression analysis showed that asymptomatic/mild SARS-CoV-2 infection was not an independent predictor of spontaneous and medically-indicated preterm birth due to pregnancy-related hypertension and its complications (0.77; 95% confidence interval, 0.41-1.43). Pregnant patients without comorbidities need to be reassured that asymptomatic/mild SARS-CoV-2 infection does not increase the risk of preterm delivery. Preterm birth and severe Coronavirus disease-19 share common risk factors (i.e., body mass index > 24.9, asthma, chronic hypertension), which may explain the high rate of indicated preterm birth due to maternal conditions reported in the literature.

Introduction

In December 2019, a novel coronavirus, termed Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified in Wuhan, China. The clinical presentation of infection with SARS-CoV-2 was referred to as Coronavirus disease 19 (COVID-19) and officially declared a pandemic by the World Health Organization on 11 March 2020 [1]. The WHO identified pregnant women as a vulnerable group based on preliminary reports of increased risk of stillbirth, preterm birth, and fetal growth restriction (FGR) and from accumulated knowledge of previous respiratory virus outbreaks, including the Severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome (MERS) [2].

SARS-CoV-2 infection during pregnancy is usually asymptomatic or mild [3–6] but can lead to serious illness in a small proportion of pregnant women. A multinational cohort study reported that the risk could be higher in the third trimester [7]. Compared to non-pregnant women of the same age, pregnant women are at higher risk for intensive care unit (ICU) admission and mechanical ventilation, but the risk of death does not seem to differ [8]. However, COVID-19 in pregnant women has been associated with poor perinatal outcomes [9], including preterm birth and preeclampsia and low birth weight [10, 11].

Preterm labor (both spontaneous and medically indicated) was initially reported as the most adverse pregnancy outcome in affected patients [12] and related to acute/chronic inflammation and vascular malperfusion, both tell-tale placental features of SARS-CoV-2 infection [13]. Rates of preterm birth (before 37 weeks gestation) of up to 30% were reported in early COVID-19 patient cohorts [10, 12]; more recent systematic reviews estimate the incidence at 15% [8]. Preterm births account for 75% of perinatal mortality and more than half of long-term morbidity; the impact of COVID-19 on preterm labor is a critical concern for obstetricians. Evidence for the impact of COVID-19 on preterm birth comes from series that have reported a high number of preterm deliveries which, however, were indicated (induction of labor or caesarean section) subsequent to worsening of maternal conditions due to severe COVID-19. This has made it difficult to determine the real impact of SARS-CoV-2 infection on obstetric outcomes.

With the present study we wanted to determine the impact of asymptomatic/mild COVID-19 (categorized according to previously published criteria) [14] on preterm birth (excluding medically-indicated preterm births due to COVID-19-related maternal respiratory failure). To do this, we compared the incidence of SARS-CoV-2 infection in a cohort of women matched for delivery period who gave preterm birth and those who gave full term birth during the present pandemic.

Materials and methods

Consecutive women referred to our Hospital for preterm birth care (20 September 2020 to 9 January 2021) were contacted and enrolled (case group). Spontaneous preterm birth was defined as birth before 37 weeks of gestational age because of spontaneous labor or rupture of membranes. Indicated preterm birth was defined as birth following induction of labor or cesarean section for a maternal or fetal indication. Inclusion criteria were: spontaneous preterm birth, indicated preterm birth due to placental vascular malperfusion (pregnancy-related hypertension and its complications, such as hemolysis, elevated liver enzymes, and low platelet count [HELLP] syndrome or FGR). Exclusion criteria were: spontaneous preterm birth with a placental and fetal membrane sample positive for bacterial infection, indicated preterm birth for other obstetric reasons (diabetes, cholestasis, repeat cesarean delivery, multiple gestations, placental abnormalities) or for maternal severe COVID-19, SARS-CoV-2 infection before a positive pregnancy test, inability to give informed consent, and age less than 18 years.

Women at full term pregnancy admitted to our Hospital for delivery during the same time period (20 September 2020 to 9 January 2021) were the control group. These women, according to our institutional protocol, had undergone seromolecular testing for SARS-CoV-2 infection at 12-weeks of gestation, at the time of noninvasive prenatal diagnosis, and subsequently at 16 and 21 weeks of gestation. Only women with last menstruation no later than one month after the date of the first reported case of COVID-19 infection in Piedmont (22 February 2020) were eligible for inclusion in the study. This was done to exclude the possibility of COVID-19 seroconversion before pregnancy. This strict recruitment criterion allowed us to define seropositivity in the control group as seroconversion that had occurred during pregnancy.

The case and the control group underwent seromolecular testing for SARS-CoV-2 at Hospital admission for delivery and were invited to participate in the study. Nasopharyngeal (NP) swabs were taken for reverse transcriptase-polymerase chain reaction (RT-PCR) assay for detection of SARS-CoV-2; blood samples were collected for detection of antibodies against SARS-CoV-2. Viral RNA was extracted from the NP swabs using a MagNA Pure Compact nucleic acid isolation kit (Roche, Mannheim, Germany) and analyzed using a RT-PCR assay (CFX-96, Bio-Rad, Milan, Italy) with a Liferiver Novel Coronavirus 2019-nCov real-time RT-PCR kit that targets genes N, E, and ORF1ab (Liferiver Bio-Tech, San Diego, CA, USA). Semi-quantitative detection of IgM/IgG non-neutralizing antibodies (nNAbs) against the nucleocapsid viral proteins was performed by automated fluorescent lateral flow assay (AFIAS™ COVID-19, Boditech Med Inc, Gang-won-do, Korea); the AFIAS™ COVID-19 gives semi-quantitative results expressed as a cut-off index (COI), where a COI of >1.1 indicates a positive result. Semi-quantitative detection of anti-S1 and anti-S2 specific IgG neutralizing antibodies (NAbs) was performed using a chemiluminescent immunoassay (Liaison® SARS-CoV-2 S1/S2 IgG, DiaSorin, Saluggia, Italy); the antibody concentration is expressed as arbitrary units (AU)/mL and grades the results as positive when $\geq$15 AU/mL. Both serologic assays had emergency use authorization. Positive patients were defined as those who tested positive by NAbs or nNAbs or NP swab. Case group women with seroconversion during pregnancy were defined as those who tested positive by NP swab or IgM antibodies or IgG antibodies associated with COVID-19 related symptoms during pregnancy.

Demographics, COVID-19-related symptoms, and data on exposure to possible risk factors for severe SARS-CoV-2 infection and for preterm birth were collected by interview.

The results for quantitative variables are expressed as the mean ± standard deviation (SD) and qualitative categorical variables as frequency and percentages. Comparison of quantitative variables was performed using the t-test or the Wilcoxon-Mann–Whitney test based on normal or not distribution, respectively. Qualitative variables were compared using Pearson's chi-square test or Fisher's exact test, as appropriate. Logistic regression was performed to determine the impact of COVID-19 on preterm birth. The results are presented in terms of odds ratio (95% confidence interval [CI]). Assuming a prevalence of infection of 15% in the control group based on the semi-mechanistic Bayesian hierarchical model [15] and given a significance of 0.05 and power of -0.80, a sample size of 121 patients in each group was necessary to demonstrate an incidence of at least 30% in the case group.

Statistical analyses were performed using SAS software ver. 9.4 for Windows (SAS Institute, Carey, NC, USA).

The protocol for this case-control study was approved by the Institutional Review Board of the City of Health and Science of Turin (reference number: 00171/2020). Written, informed consent was obtained from all participants according to local ethic committee recommendations.

Results

A total of 229 women attending our Institute for delivery were included in the study. One hundred and two women in the case group and 127 women in the control group were enrolled. The patient adhesion rate was 82.2% (102/124) and 87.5% (127/145), respectively.

Fifty-three of the 229 women tested for anti-SARS-CoV-2 IgG and IgM antibodies were found to be seropositive or their NP swab tested positive, yielding an overall cumulative incidence of 23.1% at the end of pregnancy. There was no significant difference in the cumulative incidence of COVID-19 between the case patients (21/102, 20.6%) and the controls (32/127, 25.2%) ($P = 0.50$). The density incidence, calculated as cases/pregnant woman-month, did not differ between the two groups (21/603, 3.5%; 32/662, 4.8%) ($P = 0.23$).

Ten of the COVID-19 patients in the case group and 15 in the control group reported previous symptoms (10/21, 47.6% vs 15/32, 46.9%; $P > 0.99$) including fever (3/21, 14.3% vs 8/32, 25%; $P = 0.49$), cough (6/21, 28.6% vs 6/32, 18.7%; $P = 0.50$), sore throat (1/21, 50% vs 4/32, 12.5%; $P = 0.63$), dispnea (2/21, 9.5% vs 2/32, 6.2%; $P = 0.64$), diarrhea (1/21, 4.8% vs 2/32, 6.2%; $P = 0.99$), ageusia-anosmia (2/21, 9.5% vs 6/32, 18.7%; $P = 0.45$). No cases of pneumonia were recorded.

RT-PCR of the NP resulted positive in 10/21 (47.6%) case group and in 13/32 (40.6%) control group patients at term of pregnancy; 11/21 (52.4%) were only positive for SARS-CoV-2 antibodies in the case group and 19/32 (59.4%) in the control group. No difference in positivity for IgG NAbs was found between the case (5/11, 45.4%) and the control group (8/19, 42.1%) ($P = 0.68$).

Seroconversion during the course of pregnancy was determined for all positive women, except for two case group patients (asterisks in Fig 1). Isolated IgG antibody detection without referred symptoms did not allow us to determine the time of infection and to categorically exclude infection during the two months before pregnancy.

Table 1 presents the baseline characteristics and the risk factors for severe SARS-CoV-2 infection. Except for body mass index (BMI) > 24.9, asthma, and chronic hypertension, there were no statistically significant differences in risk factors for severe COVID-19 between the two groups. Risk factor variables for severe COVID-19 were entered into logistic regression analysis to determine the impact of SARS-CoV-2 infection in relation to confounders. There was no difference between the two groups in the odds of having a preterm birth, indicating that SARS-CoV-2 infection was not an independent predictor of preterm birth (0.77; 95% CI 0.41 - 1.43).

Subgroup analysis of risk factors for preterm birth between infected and healthy patients in the case group showed no statistically significant differences (Table 2).

Infected and healthy case group patients (preterm birth) shared also similar etiopathogenetic conditions for preterm birth (Table 3).

The mode of delivery and neonatal data differed between case and control groups, as expected (Table 4).

Discussion

With this case-control study, we evaluated the impact of asymptomatic/mild COVID-19 on preterm birth in a cohort of pregnant women with SARS-CoV-2 infection as confirmed by RT-PCR assay of NP swabs or antibody testing. After excluding indicated preterm birth due to COVID-19-related maternal respiratory failure and adjusting for confounding factors, we found no association between preterm birth and SARS-CoV-2 infection. Since preterm birth because of placental vascular malperfusion was included, it is reasonable to assume that

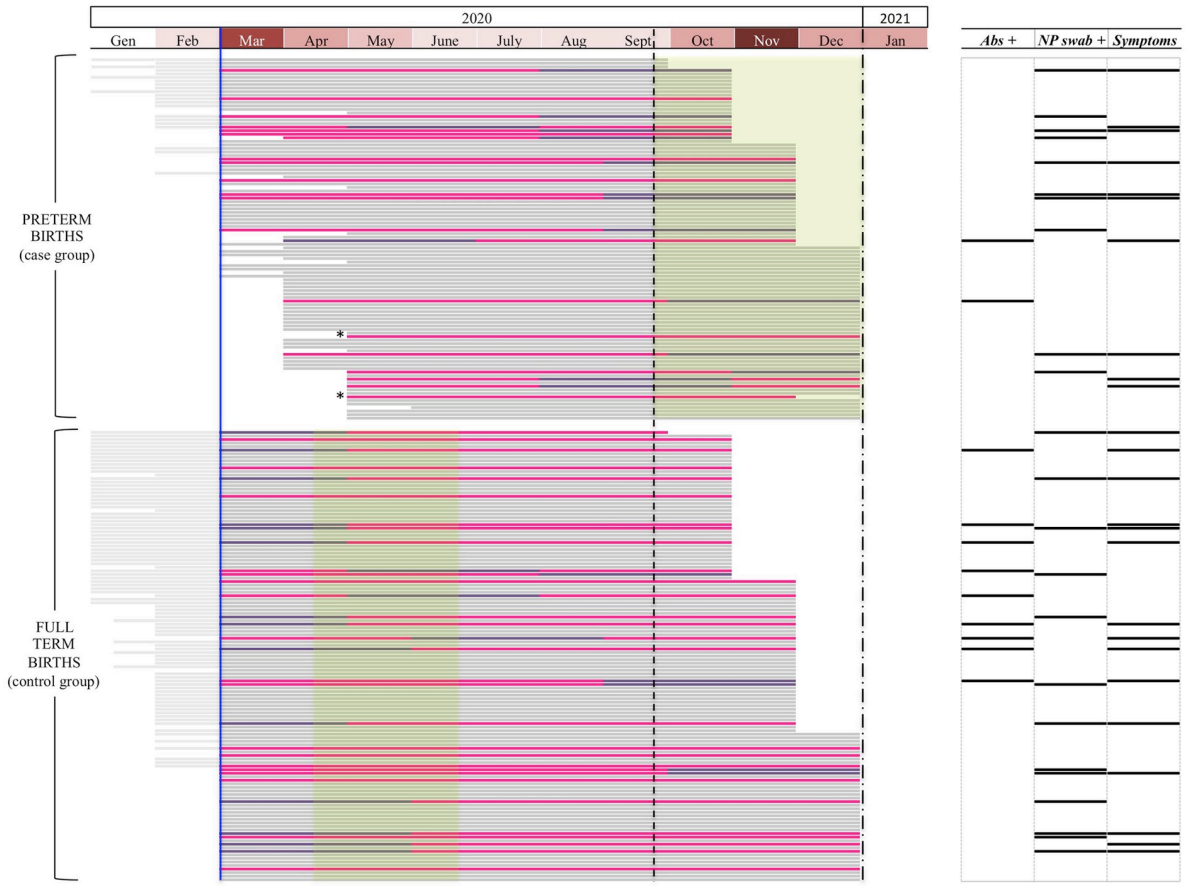

Fig 1. Study design and estimated pregnancy trimester of SARS-CoV-2 infection. Non-infected women (*horizontal grey line*); SARS-CoV-2 infected women (*horizontal fuchsia line*); estimated trimester of SARS-CoV-2 infection (*horizontal violet line*); diagnostic tests to estimate the time of infection (*horizontal black line*); first reported case of COVID-19 in Piedmont (*vertical blue line*); consecuitve enrollement of cases at delivery (20 September 2020 to 9 January 2021) and controls at early pregnancy (16 April to 22 June 2020) (*square green boxes*); time range between the first and the last delivery in the control group, corresponding to the case group consecutive recruitment (*time span between dotted and dash-dotted black vertical line*); exposure of the entire cohort to SARS-CoV-2 infection (*time span between vertical blue line and vertical dash-dotted black line*); patients with uncertain seroconversion during pregnancy (*asterisk*). Abbreviations: Abs, antibodies; NP, nasopharyngeal.

COVID-19 outbreak cases in Piedmont: patient Hospital admission/month.

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asymptomatic/mild COVID-19 is not associated with an indirect increase in preterm birth due to an increase in pregnancy-related hypertension and its complications.

In addiction, the incidence of overweight, asthma, and chronic hypertension was higher among the preterm pregnant women. These are known risk factors associated with severe COVID-19.

The risk of preterm birth and preterm premature rupture of membranes (pPROM), varies in literature between 14.3% and 63.8% and between 6.4% and 16.1%, respectively [8, 16]. This wide range is likely linked to iatrogenic preterm birth due to maternal indications. An Italian Obstetric Surveillance System (ItOSS) study reported that preterm birth was more common in women with COVID-19: 13% of those with the disease gave birth before 37 weeks of gestation (almost double the national rate), in the majority of which (71%) preterm birth was medically indicated and the principal indication was pneumonia [17].

Table 1. Baseline characteristics, risk factors for severe SARS-CoV-2 infection, COVID-19 cumulative incidence in the case (preterm births) and the control (full-term births) group.

Clinical findings		Case (n = 102)	Control (n = 127)	*P*-value
Age, y		33.1 (± 4.8)	33.7 (± 4.4)	0.28
BMI prior to pregnancy, kg/m^2		24.6 (± 5.7)	22.9 (± 4.3)	0.01
Gravidity	0	60 (58.8)	73 (57.4)	0.20
	1	28 (27.4)	41 (32.2)	
	2	6 (5.8)	11 (8.6)	
	3	4 (3.9)	2 (1.5)	
	4	3 (2.9)	0	
	7	1 (0.9)	0	
COVID-19 positive		21 (20.6)	32 (25.2)	0.50
Chronic kidney disease		1 (0.9)	2 (1.5)	0.69
Chronic pulmonary disease		0 (0)	0 (0)	>0.99
Immunocompromised state		1 (0.9)	3 (2.3)	0.42
Cardiopathy		2 (1.9)	1 (0.7)	0.43
BMI > 24.9		37 (36.2)	29 (22.8)	0.02
Tobacco use		13 (12.7)	11(8.6)	0.31
Diabetes		17 (16.6)	12 (9.4)	0.10
Asthma		4 (3.9)	0 (0)	0.02
Chronic hypertension		6 (5.8)	1 (0.7)	0.04
Liver disease		1 (0.9)	0 (0)	0.26
Neurologic conditions		4 (3.9)	2 (1.5)	0.26
Thalassemia		1 (0.9)	2 (1.5)	0.69
Thrombophilia		3 (2.9)	5 (3.9)	0.68

Values are presented as number (%) or mean (± standard deviation).

Abbreviations: BMI, body mass index; COVID-19, Coronavirus disease 2019.

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The reported increased rate of preterm births in SARS-CoV-2 infected women does not seem to be related to the spontaneous preterm birth, which is reported to be relatively low (5-6%) and comparable to that of the general population [8, 18]. Evidence is conflicting, however. Data for the impact of COVID-19 on preterm birth have come mainly from case series, observational studies without a control group, and population surveillance systems [10, 19]. Prospective comparative findings are based on a small number of recent studies with composite outcomes [20–25]. Furthermore, because the rate of preterm birth varies by sampling frames, participant selection, and risk status of the participants, no definitive conclusions can be drawn. Unlike previous studies that compared SARS-CoV-2 infected (cases) and healthy (controls) women and evaluated the incidence of preterm birth in each group, we planned an innovative study design: comparison between women who had a preterm birth (cases) and those who had full term delivery (controls) and evaluation of the incidence of COVID-19 risk factor in each group. Consistent with this way of "seeing things from a different angle", we screened cases and controls for variables known to be risk factors for severe COVID-19. We found that the preterm birth group was burdened by a higher prevalence of three known risk factors (i.e. BMI > 24.9, asthma, chronic hypertension) for severe COVID-19 [26], which are also risk factors for preterm birth [27]. We can speculate that pregnant patients with such comorbidities are at greater risk for severe COVID-19 and consequently for indicated preterm birth because of COVID-19-related maternal respiratory failure. It follows then that the higher incidence of preterm birth reported in previous studies might be related to basal characteristics

Table 2. Case group: Risk factors for preterm birth in SARS-CoV-2-positive and -negative patients.

Clinical findings	SARS-CoV-2-positive patients (n = 21)	SARS-CoV-2-negative patients (n = 81)	*P*-value
Ethnicity	3 (14.2)	6 (7.4)	0.32
Previous delivery-LM < 6 months	0	3 (3.7)	0.37
Previous abortions ≥ 1	6 (28.5)	13 (16.0)	0.18
ART therapy	0	4 (4.9)	0.29
BMI < 18.5	0	4 (4.9)	0.29
BMI > 24.9	10 (47.6)	27 (33.3)	0.22
Previous preterm delivery	3 (14.2)	4 (4.9)	0.13
Thyroid dysfunction	3 (14.2)	20 (24.6)	0.30
Asthma	1 (4.7)	3 (3.7)	0.82
Chronic hypertension	2 (9.5)	4 (4.9)	0.42
Psychiatric disease	0	4 (4.9)	0.29
Tobacco use	4 (19.0)	9 (11.1)	0.33
Previous conization	1 (4.7)	3 (3.7)	0.82
Uterine pathology	0	6 (7.4)	0.19
Multiple gestation	3 (14.2)	7 (7.5)	0.33

Values are presented as number (%).

Abbreviations: ART, assisted reproductive technique; BMI, body mass index; COVID-19, coronavirus disease 2019; LM, last menstruation.

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predisposing to the disease rather than actually associated with SARS-CoV-2 infection. This higher risk population group should receive intensive and dedicated care pathways once diagnosed with COVID-19, and ideally be vaccinated prior to infection.

Since some findings suggest that SARS-CoV-2 infection increases the risk of preeclampsia [28], the high rate of indicated preterm delivery reported in literature might have been due not only to COVID-19-related maternal respiratory failure but also to COVID-19-related preeclampsia. The placentas of SARS-CoV-2 infected patients often show decidual arteriopathy and other features of poor maternal vascular perfusion similar to placental changes in hypertensive disorders of pregnancy. This is not surprising, because COVID-19 can alter ACE2 expression and lead to a preeclamptic state; SARS-CoV-2 binding to this receptor may cause vasoconstriction resulting from renin-angiotensin system dysfunction. Therefore SARS-CoV-2 infection is thought to predispose pregnant women to severe preeclampsia, even if severe respiratory symptoms are absent [11]. It is unclear whether preeclamptic placental alterations lead to increased risk of preterm birth or aggravation of maternal conditions that would justify indicated preterm birth.

Table 3. Case group: Etiopathogenetic factors for preterm birth in SARS-CoV-2-positive and -negative patients.

Clinical findings	SARS-CoV-2-positive patients (n = 21)	SARS-CoV-2-negative patients (n = 81)	*P*-value
pPROM	11 (52.3)	45 (55.5)	0.79
Preterm labour	6 (28.5)	15 (18.5)	0.31
PE-HELLP syndrome-IUGR	4 (19.0)	17 (20.9)	0.84
Placental abruption	0 (0)	4 (4.9)	0.29

Values are presented as number (%).

Abbreviations: HELLP, hemolysis-elevated liver function tests-low platelets; IUGR, intrauterine growth restriction; PE, preeclampsia; pPROM, preterm premature rupture of membranes.

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Table 4. Delivery mode and neonatal data of the case (preterm births) and the control (full term births) group.

Clinical findings	Case (n = 102)	Control (n = 127)	*P*-value
Gestational age at delivery, days	238.4 (±20.1)	275.5 (±8.3)	<0.0001
Vaginal delivery	47 (46.0)	86 (67.7)	0.001
Vacuum assisted delivery	1 (0.9)	8 (6.2)	0.06
Caesarean delivery	54 (52.9)	33 (25.9)	<0.0001
Birth weight, g	2203.1 (± 666.0)	3361.2 (± 374.0)	<0.0001
1' Apgar score	8.0 (± 1.6)	8.8 (± 0.9)	<0.0001
5' Apgar score	8.3 (± 1.6)	8.9 (± 0.8)	<0.0001
Umbilical artery pH	7.2 (± 1.0)	7.2 (± 0.1)	0.05
NICU admission	46 (45.0)	4 (3.1)	<0.0001

Values are presented as number (%) or mean (± standard deviation).

Abbreviations: NICU, neonatal intensive care unit.

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A previous study focused on the incidence of preterm birth in women with SARS-CoV-2 infection and healthy patients, used NP swab testing only at the time of delivery and did not include past infections during pregnancy. Hence, the incidence of medically-induced preterm birth because of COVID-19-related preeclampsia could have been underestimated [29].

The concomitant use of SARS-CoV-2 RT-PCR assay and serological testing allowed us to detect ongoing and previous SARS-CoV-2 infection and to include preterm delivery due to placental vascular malperfusion into the analysis. Unlike other comparative studies, this study reflects the conditions of patients with COVID-19 not only at the time of delivery but during the course of the disease in pregnancy. Most SARS-CoV-2-induced placental alterations need time to determine the related clinical outcome. Studies that focus only on active infection with RT-PCR-positive samples [19–21, 23–25] may rule out chronic conditions (chronic inflammation or placental vascular malperfusion) that can lead to preterm labor. Furthermore, the use of serological testing alone and not systematically associated to molecular sampling [25], may lead to the underestimation of those asymptomatic conditions negative for antibodies but positive for RT-PCR at testing.

Another strength of the present study is the use of the same seromolecular assays for all women recruited, allowing for a confirmed and not only presumed COVID-19 diagnosis, as previously reported [30]. Additionally, our data on pregnancy outcomes are highly reliable since all women were treated at the same Hospital.

The inclusion of a control group was key to correctly measure the impact of SARS-CoV-2 infection on pregnancy and reduce the risk of overestimation. The strict exclusion criterion that did not allow entry of patients into the study later than one month after the date of the first reported case of COVID-19 in Piedmont ensured the selection of a control group with an infection that most certainly occurred during pregnancy.

Vice versa, a major limitation of the present study is inaccuracy in backdating the time of infection in women with preterm birth whose last menstruation occurred after the first reported case of COVID-19 infection in Piedmont (8 patients). In the absence of an IgG avidity test, we defined as seroconverted during pregnancy only patients who tested positive for IgM antibodies or who self-reported COVID-19-related symptoms. On the basis of these criteria, seroconversion during pregnancy may be uncertain for two patients (asterisks in Fig 1).

Another limitation is that the patients delivering at term (control group) were selected from among those who had undergone seromolecular testing for SARS-CoV-2 infection throughout

their pregnancy, as required by our institutional protocol, whereas the majority of preterm pregnant women did not (case group).

Conclusions

Pregnant women without comorbidities can be reassured that asymptomatic/mild SARS-CoV-2 infection does not increase the risk of preterm delivery neither in itself nor indirectly because of an increase of pregnancy-related hypertension and its complications.

Data analysis of risk factors known to be related to severe COVID-19, in preterm and full term pregnant women, suggests that severe COVID-19 and preterm birth share some common risk factors. Pregnant women who are overweight or affected by asthma or chronic hypertension should get early prenatal care and should be invited to receive a SARS-CoV-2 vaccine to reduce the risk of indicated preterm birth due to severe COVID-19.

A future area of focus is to investigate outcome etiology and causal relationships, while avoiding confounders that may obscure the real effect of COVID-19 exposure. Restriction to spontaneous or indicated preterm births, matching for risk factors or stratification according to COVID-19 severity, are optimal ways by which confounders can be addressed.

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