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First-Trimester SARS-CoV-2 Infection: Clinical Presentation, Inflammatory Markers, and Obstetric Outcomes

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Mini-Summary

What does this study add to current knowledge?

- First-trimester SARS-CoV-2 infection is usually mild or asymptomatic, with mild inflammatory response in symptomatic women. We did not find an increased risk of adverse outcomes, including placental insufficiency. However, pregnancy-associated plasma protein A expression was higher in symptomatic women and might be involved in COVID-19 inflammatory response.

What are the main clinical implications?

- Our results regarding obstetric outcomes of first-trimester SARS-CoV-2 infections are reassuring. The data provided can be useful in the clinical management and counselling of women with first-trimester SARS-CoV-2 infection, especially considering the high rates of infection currently observed among younger patients.

Keywords
Severe acute respiratory syndrome coronavirus 2 · COVID-19 · First trimester · Preeclampsia · Obstetric outcomes

Abstract

Objective: Second- and third-trimester SARS-CoV-2 infections may have an increased risk of obstetric complications. However, data on first-trimester infections are scarce. We sought to characterize the clinical and inflammatory presentations and pregnancy outcomes of first-trimester infections. ***Methods:*** A population-based multicenter study including 817 singleton pregnancies with SARS-CoV-2 serologic testing at 8–14 weeks between March and May 2020. Blood count, uterine artery Doppler, and pregnancy-associ-

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ated plasma protein A (PAPP-A) were performed in all women. Placental growth factor (PlGF), soluble fms-like tyrosine kinase 1 (sFlt-1), IL-6, and ferritin were determined in positive women. Obstetric outcomes were evaluated. ***Results:*** The prevalence of first-trimester infection was 15.2% (n = 124). 72.6% of positive women were asymptomatic. Symptomatic women had higher rates of lymphopenia (1.91×10^9/L vs. 2.16×10^9/L, $p = 0.017$) and increased levels of IL-6 (9.1% vs. 1.2%, $p = 0.051$), but lower rates of decreased ferritin (6.3% vs. 19.8%, $p = 0.015$). PAPP-A was higher in symptomatic women compared with asymptomatic and negative women (1.44 [IQR 0.90–1.82] vs. 1.08 [IQR 0.66–1.61] $p = 0.014$, vs. 1.08 [IQR 0.77–1.55] $p = 0.019$, respectively). Obstetric outcomes were not increased. ***Conclusions:*** First-trimester SARS-CoV-2 infections are mostly asymptomatic, with a mild increase of inflammatory markers in symptomatic women. Obstetric complications were not increased, but PAPP-A levels were higher in symptomatic women.

Introduction

The impact of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak on pregnancy is still a major concern for the obstetric community. The immune and respiratory physiologic adaptations to pregnancy pose a significant risk of severe respiratory infections [1]. Higher rates of preterm birth, preeclampsia (PE), and perinatal death have been reported in women developing COVID-19 disease during pregnancy, but these studies mostly included women in the second half of pregnancy [2, 3].

SARS-CoV-2 enters the host cells by interacting with the angiotensin-converting-enzyme 2 receptor, with increased levels in the placenta during pregnancy [4]. In the first trimester, angiotensin-converting-enzyme 2 expression is higher in the villous cytotrophoblast, syncytiotrophoblast, and decidua, making the placenta a potential target for SARS-CoV-2 [5]. Trophoblast invasion and spiral artery remodeling are essential for a successful placentation and pregnancy outcome [6]. Any interference in endothelial invasion and trophoblast proliferation could thus lead to impaired placentation and an increased risk of obstetric complications. Growing evidence suggests that placental dysfunction is associated with the dysregulation of angiogenic factors. An imbalance of these factors correlates with the development of PE and placental dysfunction syndromes [7]. Moreover, altered levels of pregnancy-associated plasma protein A (PAPP-A) also correlate with obstetric complications [8].

Although the impact of SARS-CoV-2 in the second and third trimester has been largely investigated [3, 9], data regarding early pregnancy is limited. A hyperactivation of the immune occurs in the first trimester, favoring implantation, and placental development [10]. We hypothesize that the endothelial dysfunction and prothrombotic state observed in COVID-19 patients [11] could aggravate this proinflammatory state, potentially affecting placental development and obstetric outcomes. The objective of our study was to characterize the clinical presentation and inflammatory response in first-trimester SARS-CoV-2 infected women and to evaluate obstetric outcomes of these pregnancies.

Material and Methods

We conducted a multicenter prospective population-based study involving two tertiary hospitals in Barcelona, Spain, (Hospital de la Santa Creu i Sant Pau and Hospital Clínic) between March 1st and May 31st, 2020. Pregnant women with a singleton pregnancy who had a first-trimester blood sampling for aneuploidy screening were invited to participate at the time of first-trimester ultrasound. This study was approved by the Ethics Committee of the Institutional Review Board at each participating hospital. All included women gave their written informed consent.

Serum samples from the first-trimester screening blood test were separated by centrifugation at 1,500 *g* for 10 min at 4°C, and stored at −80°C until analyzed. SARS-CoV-2 IgG and IgM/IgA antibodies were tested using COVID-19 VIRCLIA® Monotest, Vircell Microbiologist, Granada, Spain. All indeterminate results were retested with VITROS® Immunodiagnostic Products Anti-SARS-CoV2 Total Tests, Ortho Clinical Diagnostics, Rochester, NY, USA. All positive results for IgM/IgA but negative for IgG in women reporting no symptoms suggestive of COVID-19 were retested by a quantitative suspension array assay based on the xMAP Luminex technologies. A positive serological result was considered in the following situations: (1) positive IgG, (2) positive IgM/IgA in symptomatic COVID-19 women, and (3) positive IgM/IgA confirmed by two tests (Vircell and Luminex). See online supplementary material for additional details (for all online suppl. material, see www.karger.com/doi/10.1159/000523974).

In women with positive serology, serum concentrations of interleukine-6 (IL-6), placental growth factor (PlGF), soluble fms-like tyrosine kinase 1 (sFlt-1) (electrochemiluminescence immunoassays, fully automated on Cobas® e 601 analyzer; Roche Diagnostics), and ferritin (chemiluminescence immunoassay, fully automated on Architect® analyzer; Abbott Laboratories) were determined. Ferritin levels between 15 and 200 μg/L and IL-6 levels below 7 pg/mL were considered normal.

Results on leukocyte, lymphocyte, and platelet counts were recorded for all women. PAPP-A was available from aneuploidy screening (electrochemiluminescence immunoassays, fully automated on Cobas® e 601 analyzer; Roche Diagnostics, and AutoDELFIA® Automatic Immunoassay System; Perkin Elmer). Multiples of the median (MoM) values for PAPP-A and PlFG were considered.

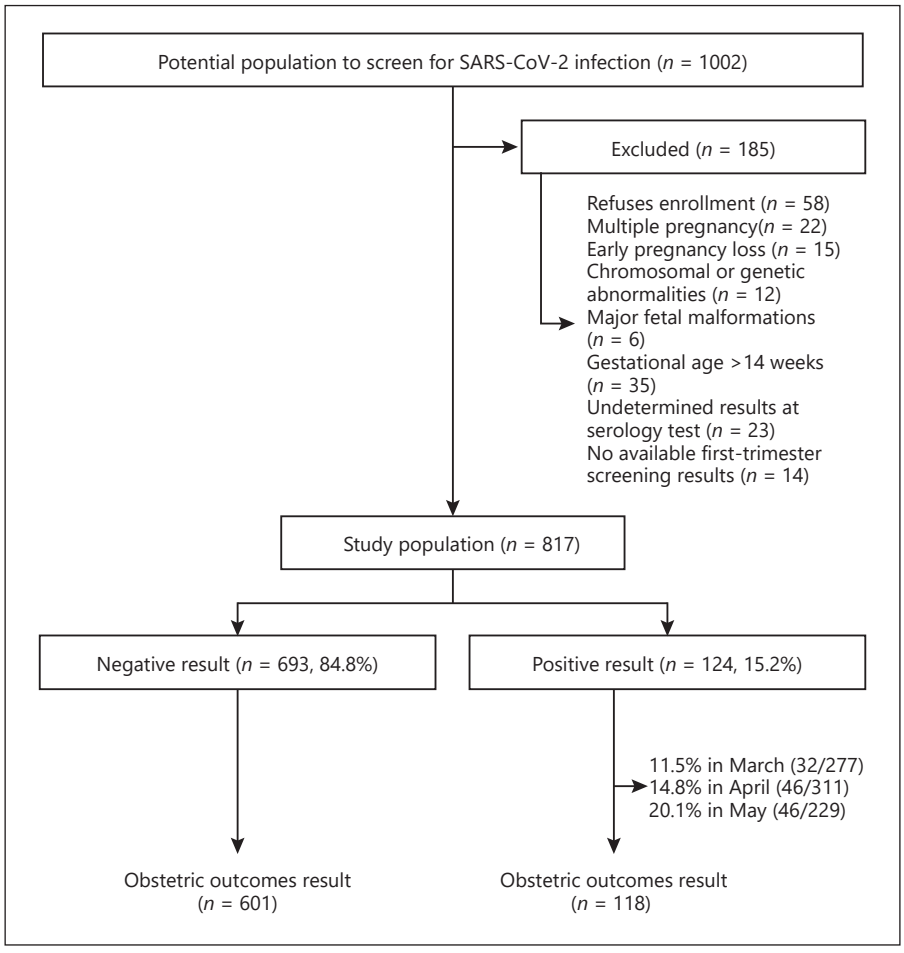

Fig. 1. Represents the flowchart of the study. The inclusion process, the causes for exclusion and the final number of patients included is detailed.

Nasopharyngeal swab samples for SARS-CoV-2 real-time polymerase chain reaction (RT-PCR) were obtained in all women attending delivery and in women presenting with COVID-19 symptoms later in pregnancy. Samples were collected in Micronics tubes with Zymo DNA/RNA Shield Lysis Buffer. RNA was extracted using the Quick-DNA/RNA Viral MagBead kit (Zymo) and the TECAN Dreamprep robot. Five microliters of RNA solution were added to 15 μL of rRT-PCR master mix (Luna Universal Probe One-Step RT-qPCR Kit; New England Biolabs) and used for amplification of SARS-CoV-2 N1 and N2 regions, as well as the human RNase P gene as control, as described in the CDC-006-00019 CDC/DDID/NCIRD/Division of Viral Diseases protocol released March 30, 2020. A SARS-CoV-2 positive result was considered if the Ct values for N1, N2, and RNase P were below 40. Samples discordant for N1 and N2 were repeated and samples with a Ct ≥ 40 for RNase P were considered invalid.

Maternal characteristics were recorded, including maternal age, ethnicity, parity, weight, height, method of conception, smoking habit, mean arterial pressure (MAP), obstetric history, and medical disorders. COVID-19 symptoms were collected using an online structured questionnaire focusing on symptoms noticed 12 weeks before inclusion.

Gestational age was determined according to crown-rump length in all cases, and mean uterine arteries pulsatility indices (UtA-PI) were evaluated. Multiples of the median values of ultrasound markers were calculated based on regression curves of locally derived normal medians using a validated screening model for early-onset PE [12]. All patients underwent early-onset PE screening, which was performed according to the institutional protocol of each participating hospital. Hospital Clínic uses a logistic regression-based model including maternal characteristics, UtA-PI, MAP, and PAPP-A [13]. Hospital de la Santa Creu i Sant Pau uses the above-mentioned multivariate Gaussian distribution model, including maternal characteristics, biochemical (PAPP-A, PlGF) and biophysical variables (UtA-PI, MAP). Both models have similar detection rates for early-onset PE. Patients at high-risk of PE were given 150 mg of aspirin at bedtime. Exclusion criteria were undetermined results at all SARS-CoV-2 serology tests, fetal major structural malformations, chromosomal or genetic abnormalities, and miscarriages before first-trimester ultrasound.

Data on pregnancy outcomes were collected from maternity records. SARS-CoV-2 reinfections were recorded. Likewise, new-onset infections occurring later in pregnancy or at birth were noted. PE was defined according to the guidelines of the International Society for the Study of Hypertension in Pregnancy and classified according to gestational age at delivery into early-onset (<34 weeks), late-onset (34–37 weeks), and term PE (>37 weeks). Small-

Table 1. Baseline characteristics of study participants according to SARS-CoV-2 serology test

Maternal and pregnancy characteristics	Negative serology (n = 693)	Positive serology (n = 124)	p value
General characteristics			
Maternal age,[a] years	33.9 (5.2)	33.1 (5.1)	0.093
Body mass index,[a] kg/m^2	23.6 (4.2)	24.2 (5.1)	0.210
Smoking during pregnancy[b]	57 (8.2)	5 (4)	0.080
Ethnicity[b]			
Caucasian	483 (69.7)	74 (59.7)	
Latin-American	132 (19)	29 (23.4)	
Asian	22 (3.2)	6 (4.8)	0.291
Afro-Caribbean	14 (2)	2 (1.6)	
Other	42 (6.1)	13 (10.4)	
Medical history[b]			
Chronic hypertension	19 (2.7)	4 (3.2)	
Thyroid conditions	59 (8.5)	14 (11.3)	
Diabetes mellitus	20 (2.9)	4 (3.2)	
Respiratory conditions	27 (3.9)	5 (4)	0.392
Hematologic conditions	19 (2.7)	1 (0.8)	
Autoimmune diseases	19 (2.7)	3 (2.4)	
Other	93 (13.4)	12 (9.7)	
Pregnancy history			
Nulliparous[b]	401 (57.9)	74 (59.7)	0.706
Previous PE[b]	14 (2.2)	1 (0.8)	0.268
ART conception[b]	73 (10.5)	7 (5.6)	0.072
MAP,[a] mm Hg	84.1 (7.9)	84.3 (8.3)	0.761
High-risk of PE[b]	66 (10.1)	7.8 (9)	0.422
Aspirin during pregnancy[b]	56 (8.1)	7 (5.6)	0.329

PE, preeclampsia; MAP, mean arterial pressure; ART, assisted reproductive technologies; SD, standard deviation. [a] Data are presented as mean (SD) and analyzed by Student t test. [b] Data presented as n (%) and analyzed by χ^2 test.

for-gestational age infants were defined as neonates with a birthweight below the 10th centile according to local standards. Second- or third-trimester fetal demise was also recorded (online suppl. material).

Pregnancy characteristics and outcomes were compared between groups according to SARS-CoV-2 serology and the presence of COVID-19 symptoms. Women diagnosed with SARS-CoV-2 infection later in pregnancy or with reinfection were excluded from the outcome analysis in order to isolate the impact of first-trimester infections.

Statistical analysis was performed with IBM-SPSS software program, v.26.0 (IBM-SPSS Inc., Chicago, IL; USA). Data are shown as mean (standard deviation) or percentages for continuous and categorical variables, respectively, and median (interquartile range (IQR)) for nonparametric variables. Associations were evaluated using Student t test, χ^2 test or Mann-Whitney U test where appropriate. Unequal variances were assumed. p values <0.05 were considered statistically significant.

Multiple lineal regression analysis was performed to analyze the relation between first-trimester inflammatory markers and placental biochemical markers. Stepwise algorithm was performed to select variables with significant association at a p value cutoff of 0.05. Goodness-of-fit model was assessed by calculating R^2.

Results

A total of 1,002 women had blood sampling for first-trimester screening during the study period. Of those, 58 withdrew consent. From the remaining patients, 817 women fulfilled the inclusion criteria. Figure 1 depicts the flowchart of the study. Among the included patients, 124 (15.2%) women had a positive serology. Prevalence increased during the study period, from 11.5% in March 2020 to 20.1% in May 2020. Table 1 shows the baseline characteristics of the study population according to the SARS-CoV-2 serology test. No differences were observed between groups.

Both positive and negative women reported symptoms associated with COVID-19 disease during the study period. However, 72.6% of positive women remained asymptomatic. The most frequently reported symptoms were fever, dyspnea, anosmia, and/or ageusia, but none required hospitalization (online suppl. Table S1).

Table 2. Inflammatory and placental function markers according to SARS-CoV-2 serology test

Inflammatory markers and placental function evaluation	Negative (n = 693)	Positive (n = 124)	p value
Gestational age at first trimester blood sampling,[a] weeks	10.8 (1.19)	10.9 (1.42)	0.310
Inflammatory markers			
Leukocytes,[a] $\times 10^9$/L	8.17 (1.94)	7.93 (1.97)	0.222
Leukopenia[b]	3 (0.5)	2 (1.6)	0.434
Leukocytosis[b]	51 (8.1)	9 (7.3)	
Lymphocytes,[a] $\times 10^9$/L	2.11 (0.59)	2.10 (0.61)	0.785
Lymphopenia[b]	21 (3.3)	5 (4.1)	0.906
Lymphocytosis[b]	12 (1.9)	2 (1.6)	
Platelets,[a] $\times 10^9$/L	254 (58.6)	258 (65.4)	0.557
Thrombocytopenia[b]	9 (1.4)	1 (0.8)	0.103
Thrombocytosis[b]	10 (1.6)	6 (4.8)	
Inflammatory markers in patients with positive SARS-CoV-2 serology test			
Ferritin, µg/L	–	46.9 (41.16)	–
Decreased ferritin	–	18 (14.5)	–
Increased ferritin	–	2 (1.6)	–
IL-6 levels, pg/mL	–	2.5 (8.7)	–
Increased IL-6	–	4 (3.2)	–
Placental function biomarkers			
PAPP-A (MoMs)[c]	1.08 (0.77–1.55)	1.20 (0.7–1.69)	0.877
PlGF (MoMs)[a]	1.13 (0.38)	1.04 (0.41)	0.206
sFlt-1,[a] pg/mL	1,329.3 (467.9)	1,418.72 (586.7)	0.320
sFlt-1/PlGF ratio[a]	44 (19)	49 (21)	0.207
First-trimester ultrasound markers			
CRL,[a] mm	64 (8.0)	65 (8.7)	0.196
NT (MoMs)[c]	1.04 (0.94–1.17)	1.04 (0.94–1.18)	0.900
Mean UtA-PI (MoMs)[a]	1.02 (0.30)	1.01 (0.26)	0.740

IL-6, interleukine-6; PAPP-A, pregnancy-associated plasma protein-A; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase 1; MoM, multiples of the median; NT, nuchal translucency; Mean UtA-PI, mean uterine arteries pulsatility indices; CRL, crown-rump length; SD, standard deviation. [a]Data are presented as mean (SD) and analyzed by Student t test. [b]Data presented as n (%) and analyzed by χ^2 test. [c]Data are presented as median (IQR) and analyzed by Mann-Whitney U test.

Table 2 describes the laboratory findings and placental markers according to SARS-CoV-2 serology. We did not find any significant differences between negative and positive women in leukocyte, lymphocyte, and platelet counts. Ferritin and IL-6 levels in the positive group were within normal ranges for most women, with only 1.6% and 3.2%, respectively, having increased levels. We found no significant differences in biochemical or biophysical markers.

We further analyzed the group of positive women according to COVID-19 symptomatology (Table 3). Compared with asymptomatic women, symptomatic women had a lower lymphocyte count (1.91×10^9/L vs. 2.16×10^9/L, $p = 0.017$), lower rates of decreased ferritin (6.3% vs. 19.8%, $p = 0.015$) and increased levels of IL-6 (9.1% vs. 1.2%, $p = 0.051$), although these results did not reach statistical significance.

Regarding placental function evaluation within SARS-CoV-2 positive patients, no differences were found in angiogenic nor biophysical profiles. We did find increased PAPP-A levels in symptomatic women compared with asymptomatic women (1.44 [IQR 0.90–1.82] vs. 1.08 [IQR 0.66–1.61], $p = 0.014$). This difference remained significant when we compared this group with the group of negative women (1.44 [IQR 0.90–1.82] vs. 1.08 [IQR 0.77–1.55], $p = 0.019$). We analyzed the relation between PAPP-A levels, inflammatory and placental markers using multiple lineal regression analysis in positive women. Leukocytes, PlGF, and sFlt-1 were significantly associated with PAPP-A levels in the bivariate analysis, but only PlGF and sFlt-1 levels remained significantly associated with PAPP-A levels in the multivariate analysis (Table 4).

Table 3. Inflammatory and placental function markers according to the presence of COVID-19 symptoms within positive SARS-CoV-2 patients ($n = 124$)

Inflammatory markers and placental function evaluation	Asymptomatic ($n = 90$)	Symptomatic ($n = 34$)	p value
Gestational age at first trimester blood sampling,[a] weeks	10.9 (1.47)	10.7 (1.30)	0.378
Inflammatory markers			
Leukocytes,[a] $\times 10^9$/L	8.10 (2.09)	7.45 (1.54)	0.063
Leukopenia[b]	1 (1.1)	1 (3)	**0.043**
Leukocytosis[b]	9 (10)	0 (0)	
Lymphocytes,[a] $\times 10^9$/L	2.16 (0.64)	1.91 (0.45)	**0.017**
Lymphopenia[b]	2 (2.2)	9 (9.1)	0.156
Lymphocytosis[b]	2 (2.2)	0 (0)	
Platelets,[a] $\times 10^9$/L	260 (67.2)	252 (60.5)	0.509
Thrombocytopenia[b]	1 (1.1)	0 (0)	0.588
Thrombocytosis[b]	5 (5.6)	1 (2.9)	
Ferritin,[a] µg/L	47.2 (37.6)	46.33 (49.6)	0.930
Decreased ferritin[b]	16 (19.8)	2 (6.3)	**0.015**
IL-6,[a] pg/mL	1.6 (1.4)	4.7 (16.0)	0.271
Increased IL-6[b]	1 (1.2)	3 (9.1)	0.051
Placental function biomarkers			
PAPP-A (MoMs)[c]	1.08 (0.66–1.61)	1.44 (0.90–1.82)	**0.014**
PlGF (MoMs)[a]	1.04 (0.45)	1.06 (0.29)	0.773
sFlt-1,[a] pg/mL	1,386.2 (587.8)	1,502.2 (584.6)	0.346
sFlt-1/PlGF ratio[a]	49 (21)	49 (19)	0.950
First-trimester ultrasound markers			
CRL,[a] mm	65 (8.4)	64 (9.5)	0.515
NT (MoMs)[c]	1.03 (0.93–1.19)	1.04 (0.99–1.15)	0.551
Mean UtA-PI (MoMs)[a]	0.99 (0.25)	1.04 (0.30)	0.332

IL-6, interleukine-6; PAPP-A, pregnancy-associated plasma protein-A; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase 1; MoM, multiples of the median; NT, nuchal translucency; Mean UtA-PI, mean uterine arteries pulsatility indices; CRL, crown-rump length; SD, standard deviation. Statistically significant p values (<0.05) are written in bold. [a] Data are presented as mean (SD) and analyzed by Student t test. [b] Data presented as n (%) and analyzed by χ^2 test. [c] Data are presented as median (IQR) and analyzed by Mann-Whitney U test.

Table 4. Multiple regression analysis evaluating the relation between PAPP-A levels,[a] inflammatory markers and angiogenic factors

	Bivariate analysis, p value	Multivariate analysis, $R^2 = 0.446$	
		coefficient	p value
Leukocytes ($\times 10^9$/L)	0.036	–	–
Lymphocytes ($\times 10^9$/L)	0.348	–	–
Platelet count ($\times 10^9$/L)	0.111	–	–
Ferritin (µg/L)	0.468	–	–
IL-6 (pg/mL)	0.896	–	–
PlGF (MoM)	**<0.001**	0.325	**0.036**
sFlt-1 (pg/mL)	**<0.001**	0.001	**<0.001**
sFlt-1/PlGF ratio	0.347	–	–

IL-6, interleukine-6; PAPP-A, pregnancy-associated plasma protein-A; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase 1; MoM, multiples of the median. Statistically significant p values (<0.05) are written in bold. [a] PAPP-A, dependent variable.

Table 5. Obstetric outcomes according to SARS-CoV-2 serology test

Pregnancy outcome	Negative (n = 575)	Positive (n = 113)	p value
Gestational age at birth,[a] weeks	39.6 (1.78)	39.5 (1.98)	0.651
Second trimester fetal loss[b]	7 (1)	2 (1.6)	0.633
Mode of delivery			
Vaginal delivery[b]	420 (75.9)	81 (73)	0.510
Cesarean section[b]	133 (24.1)	30 (27)	
Labour induction[b]	215 (38.9)	40 (36)	0.564
Birthweight,[a] g	3,271 (515)	3,255 (543)	0.788
Female gender[b]	252 (45.6)	46 (41.8)	0.469
Umbilical venous pH[a]	7.28 (0.10)	7.28 (0.07)	0.375
Umbilical arterial pH[a]	7.21 (0.09)	7.19 (0.10)	0.971
Overall PE[b]	28 (5.1)	8 (7.3)	0.368
Early-onset PE[b]	3 (0.5)	0 (0)	0.296
Late-onset PE[b]	5 (0.9)	3 (2.7)	0.153
Term PE[b]	20 (3.6)	5 (4.5)	0.649
Preterm birth[b]	22 (4)	7 (6.4)	0.286
SGA[b]	69 (12.5)	15 (13.6)	0.741
Neonatal admission to NICU[b]	20 (3.6)	4 (3.6)	0.992
Stillbirth[b]	1 (0.2)	0 (0)	0.546
Maternal hospitalization,[a] days	2.0 (0.95)	2.1 (0.84)	0.133

PE, preeclampsia; SGA, small-for-gestational age; NICU, neonatal intensive care unit; SD, standard deviation. [a] Data are presented as mean (SD) and analyzed by Student t test. [b] Data presented as n (%) and analyzed by χ^2 test.

Table 6. Obstetric outcomes comparing SARS-CoV-2 positive symptomatic women and negative women

Pregnancy outcome	Negative (n = 575)	Positive symptomatic (n = 33)	p value
Gestational age at birth,[a] weeks	39.6 (1.78)	39.6 (1.44)	0.977
Mode of delivery			
Vaginal delivery[b]	420 (75.9)	24 (72.7)	0.679
Cesarean section[b]	133 (24.1)	9 (27.3)	
Labour induction[b]	215 (38.9)	10 (30.3)	0.314
Birthweight,[a] g	3,271 (515)	3,275 (579)	0.962
Female gender[b]	252 (45.6)	12 (36.4)	0.298
Umbilical venous pH[a]	7.28 (0.10)	7.27 (0.06)	0.602
Umbilical arterial pH[a]	7.21 (0.09)	7.23 (0.10)	0.449
Overall PE[b]	28 (5.1)	2 (6.1)	0.806
Early-onset PE[b]	3 (0.5)	0 (0)	0.555
Late-onset PE[b]	5 (0.9)	1 (3)	0.332
Term PE[b]	20 (3.6)	1 (3)	0.857
Preterm birth[b]	22 (4)	3 (9.1)	0.212
SGA[b]	69 (12.5)	4 (12.1)	0.952
Neonatal admission to NICU[b]	20 (3.6)	3 (9.1)	0.173
Stillbirth[b]	1 (0.2)	0 (0)	0.734
Maternal hospitalization,[a] days	2.0 (0.95)	2.1 (0.96)	0.632

PE, preeclampsia; SGA, small-for-gestational age; NICU, neonatal intensive care unit; SD, standard deviation. [a] Data are presented as mean (SD) and analyzed by Student t test. [b] Data presented as n (%) and analyzed by χ^2 test.

Obstetric outcomes of 719 women were recorded. Within the negative group, 34 (5.6%) women were diagnosed with SARS-CoV-2 later in pregnancy or at birth. Within the positive group, 7 (5.9%) women had a positive RT-PCR later in pregnancy and were, thus, considered as reinfections. Obstetric outcomes were evaluated after excluding these women. Table 5 illustrates these results. Groups were comparable in terms of gestational age at birth, birthweight, and mode of delivery. We did not find any significant differences in obstetric outcomes. In order to elucidate whether or not symptomatic infections had an increased risk for adverse outcomes, we further compared positive symptomatic women with negative women. We did not find differences between groups for any of the analyzed variables (Table 6).

Discussion

We found similar inflammatory markers in first-trimester infected women to those described in general population, including lymphopenia, increased ferritin, and IL-6 levels. However, these variations were mild and mainly present in symptomatic women. We did not find an increased risk of obstetric complications, regardless of the presence of symptoms.

A consistent pattern of lymphopenia, thrombocytopenia, coagulation abnormalities, increased ferritin and D-dimer, and higher levels of several ILs (including IL-6) and tumor necrosis factor-alpha (TNF-α) has been reported COVID-19 patients [14]. However, most of these findings have been described in nonpregnant populations, and data regarding pregnant women are lacking. Pregnancy is characterized by a unique immunologic state that can contribute to very different outcomes in the event of a viral disease [15]. Moreover, given the proinflammatory state described in the first trimester [10], this period itself has been suggested as a risk factor for a worse course of COVID-19 disease [16]. In our study, we confirmed some of the laboratory findings reported in COVID-19 patients. As expected, these results were observed in symptomatic women. However, the overall levels of inflammatory markers remained within normal ranges, which corroborates a mild inflammatory answer. However, inflammatory markers (IL-6 and ferritin) were only assessed in positive patients and comparisons with the negative group could not be performed. In a previous investigation regarding the clinical impact of SARS-CoV-2 infection, we found that first-trimester cases are more likely to be asymptomatic or mild [17]. We have now confirmed these results with a larger sample of women. Thus, from a clinical perspective, early stages of pregnancy do not seem to worsen the course of the disease.

It has also been suggested that women with first-trimester SARS-CoV-2 infection could be at an increased risk of impaired placentation [16]. An altered immunomodulation and an imbalance in cytokine expression might contribute to the pathogenesis of PE [18]. In particular, increases in IL-6 and TNF-α levels have been linked to trophoblast apoptosis and endothelial activation [19]. We sought to assess this relation by evaluating first-trimester placental markers. We determined first-trimester PlGF and sFlt-1 levels in positive women, as an early imbalance of these factors is useful for the risk-assessment of the condition [12]. No differences were found between groups, regardless of the presence of symptoms. The risk of obstetric complications such as PE or small-for-gestational age was also not increased. However, in our study, positive women were mostly asymptomatic or had a mild clinical course of the disease. Although first-trimester infections are usually mild, we cannot exclude the possibility of impaired placentation in the event of a more severe course of the disease.

Despite these reassuring findings regarding pregnancy outcomes, we did find an increase in PAPP-A levels among symptomatic women compared with asymptomatic and negative women. PAPP-A and its substrates promote trophoblast proliferation, and low levels of PAPP-A correlate with a higher risk of PE [8]. However, PAPP-A can be synthesized in other tissues besides the placenta [20], and its production is regulated by inflammatory cytokines [21]. Previous research showed that PAPP-A expression can be regulated by proinflammatory cytokines and growth factors, including TNF-α and IL-1ß [21]. In our study, PAPP-A levels did not correlate with other inflammatory markers such as IL-6 or ferritin, but TNF-α and IL-1ß were not assessed. We suggest that PAPP-A might be involved in the inflammatory response to SARS-CoV-2. Thus, the role of PAPP-A in COVID-19 disease should be further investigated.

Our study has several limitations. First, the diagnosis of SARS-CoV-2 infection was based on serological testing. Serologic tests are effective for SARS-CoV-2 diagnosis [22], but they provide limited information regarding the time of infection. In Spain, the coronavirus outbreak was declared at the end of February. The inclusion period of the study was limited from March to May in order to ensure the inclusion of first-trimester women. At that time, RT-PCR was not performed in the absence of severe symptoms. Even now, programs for SARS-CoV-2 screen-

ing do not target first-trimester pregnant women. Considering that most first-trimester cases are asymptomatic or mild, seroprevalence studies are essential to understand the impact of the disease, assess obstetric outcomes, and provide useful information for counselling. Second, although many biomarkers are involved in the inflammatory answer to COVID-19, we could only evaluate IL-6 and ferritin. However, IL-6 levels are used to assess the intensity of the systemic answer in patients with sepsis. In addition, lymphocyte and platelet count, ferritin, and IL-6 have been proposed as prognostic factors of COVID-19 [23]. Therefore, we believe these biomarkers fulfill the aims of our study. Third, this was a consecutive case cohort study, based on availability of patients during the study period, and lacked power calculation. Thus, our study could be underpowered for detecting increased risks of rare obstetric complications linked to first-trimester COVID-19 infections. Finally, our results are limited to the strains of COVID-19 circulating in Spain in March–May 2020 (SEC7 and SEC8). Other COVID-19 strains could lead to poorer obstetric outcomes.

Despite these limitations, our study also has some strengths. Data regarding first-trimester infections are very limited. Studies reporting first-trimester seroprevalence included small numbers of women and focused on seroprevalence and miscarriage risk [24, 25]. Ours is the largest study targeting this population and providing data on obstetric outcomes. Second, we evaluated obstetric outcomes after excluding women with reinfection or infection later in pregnancy in order to avoid potential bias. Thus, we believe that the data provided are reliable and useful in terms of clinical management and counselling of these women.

Conclusion

The inflammatory response in first-trimester SARS-CoV-2 infection is mild and correlates with symptomatology. We found no evidence of increased risk of obstetric complications. The data provided can be useful in the clinical management and counselling of women with first-trimester SARS-CoV-2 infection.

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Statement of Ethics

This research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Approval from the Ethics Committee of the Institutional Review Board of each participating hospital was obtained. For Hospital de la Santa Creu i Sant Pau: IRB case number IIBSP-COV-2020-38. For Hospital Clínic: HCB-2020-0434. All subjects have given their written informed consent and the study protocol was approved by the Institute's Committee on Human Research.

Conflict of Interest Statement

Dr. E. Gratacós reports grants from Stavros Niarchos Foundation, Santander Foundation, and "La Caixa" Foundation, during the conduct of the study. Prof. E. Gratacos is the editor-in-chief of the Fetal Diagnosis and Therapy journal. Dr. E. Llurba and Dr. F. Crispi are also editorial board members of the journal. The remaining authors report no conflicts of interest.

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Author Contributions

All the authors of the manuscript have significantly contributed to this research. Josefina Mora and Imma Mercadé were responsible for all the laboratory analysis of the serum samples performed in our study. Anna Mundo, Carmen Medina, Monica Cruz-Lemini, Elisa Llurba, and Cristina Trilla were responsible for the data gathering, ultrasound examination, and pregnancy fol-

low-up at Hospital de la Santa Creu i Sant Pau. Marta Camacho, David Boada, Marta Tortajada, Francesca Crovetto, and Fatima Crispi were responsible for the data gathering, ultrasound examination, and pregnancy follow-up at Hospital Clínic – Maternitat. Francesc Figueras, Eduard Gratacos, and Elisa Llurba were key in the study design, data analysis, and interpretation of the results. Finally, Cristina Trilla was responsible for the redaction of the manuscript, with supervision and contributions from the other authors. The final manuscript has been read and approved by all the authors.

Data Availability Statement

Data supporting the findings of this article are available from the corresponding author.

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