Short communication

Asymptomatic SARS-CoV-2 infection is not associated with miscarriage in early pregnancy: a retrospective analysis

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SUMMARY

The prevalence of SARS-CoV-2 infection during pregnancy is relatively unknown. In this study we report the potential impact of undiagnosed SARS-CoV-2 infection on pregnancy loss in the first half of pregnancy by comparing the prevalence of the infection in a retrospective group of pregnant women with miscarriage (n=62) and a prospective control group with no pregnancy loss in the first trimester (n=218). Of 62 women who had miscarriage, 2 (3.2%) resulted IgM for SARS-CoV-2 negative and IgG seropositive, while of 218 pregnant women, 5 (2.3 %) resulted IgM for SARS-CoV-2 and IgG seropositive. The SARS-CoV-2 seroprevalence was not significantly different in the two groups of women, therefore excluding a significant role of SARS-CoV-2 infection in pregnancy loss. Therefore, our data show that SARS-CoV-2 infection within the first trimester does not seem to predispose to early pregnancy loss and that the impact of asymptomatic or mildly symptomatic SARS-CoV-2 infection on pregnancy appears limited.

Key words: SARS-Cov-2, infection, pregnancy, miscarriage

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The rapid spread of coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), raises particular concern for the health of a potentially vulnerable population, among which pregnant women [Cosma S *et al*., 2021]. COVID-19 is generally susceptible to all age groups, but the impact in pregnant women has drawn great attention because of the unique immunological state of pregnancy and the increased risk of respiratory infections. In particular, the characteristic immune responses during the different gestational periods are bound to be closely related to the outcome of infection [Jiao J, 2020]. Knowledge about the duration of immunity against SARS-CoV-2 is essential in maternal-fetal medicine, since maternal immunization can protect the newborn from COVID-19 infection during the first months of life [Carosso A *et al*., 2020]. Pregnant women who become SARS-CoV-2-positive are usually either asymptomatic or mild-to-moderately symptomatic, similar to non-pregnant women [Vivanti AJ *et al*., 2020; Gandhi RT *et al*., 2020], and many cases of infection remained undiagnosed, especially during the first pandemic wave [Crovetto F *et al*., 2020]. However, more severe symptoms have also been described during pregnancy, such as pneumonia or acute respiratory distress syndrome (ARDS). Concerning the risks for the fetus and the newborn, very limited information is currently available [Gandhi RT *et al*., 2020]. In symptomatic pregnant women, antibodies against SARS-CoV-2 have been detected from a few days to 3 weeks after onset of symptoms, with a median of 6 days [Tsatsaris V *et al*., 2020]. The presence of SARS-CoV-2 IgG antibodies is indicative of current or previous infection by SARS-CoV-2; however, the extent and duration of protection conferred by SARS-CoV-2 IgG antibodies remain unknown.

Comparing the number of miscarriages in 2020 to that in previous years, we reported an increased number of spontaneous abortions in 2020 (65 spontaneous miscarriages in 2019 vs 113 in 2020). This study aimed to evaluate the potential impact of undiagnosed SARS-CoV-2 infection on pregnancy loss in the first half of pregnancy by comparing the prevalence of the infection in a retrospective group of pregnant women with miscarriage and a prospective control group with no pregnancy loss in the first trimester.

Women who had been referred to our hospital for pregnancy loss care during the first trimester of pregnancy during the first SARS-CoV-2 pandemic wave (between March 3 and May 25, 2020) were contacted and enrolled retrospectively. Control pregnant women attending the Obstetrics and Gynecology Clinics of our Institution for routine ultrasound controls in the second and third trimester of pregnancy were enrolled between June 26 and August 30, 2020. Only pregnant women with last menstrual period between November 1 and February 22, 2020 (before the beginning of the pandemic period) were eligible for inclusion in the study, to exclude the possibility of seroconversion before

pregnancy. The last menstrual period of women who had miscarriage was between January 30 and April 20, 2020. Written, informed consent was obtained from all participants.

Blood samples of the control group were collected at enrollment, during routine ultrasound controls, while for women who had miscarriage blood samples were collected a median time of 152 (range 129-210) days after abortion.

Serum samples were tested for detection of IgM and IgG antibodies to SARS-CoV-2 spike (S) protein. For detection of IgM, half-area 96-well microplates were coated for 1h with 5μg/ml recombinant receptor binding domain (RBD) of the Spike protein. After overnight blocking with 5% (wt/vol) skimmed milk, the plates were washed and incubated for 1h with human serum added to RF/adsorbent IgG (Euroimmun, Luebeck) in four-fold serial dilutions (starting from 1:50), then for 45 min with horseradish peroxidase-labeled goat IgM to human IgM and, finally, for 25 min with 5mg/ml orthophenylendiamine (OPD) before the addition of 4 N sulfuric acid. The optical density (OD) value of the serum incubated without RBD was subtracted from the OD value of the serum incubated with RBD. Cut-off of 0.100 net OD was calculated based on mean+2SD results of SARS-CoV-2 naive subjects at serum dilution 1:50. Serum dilution yielding 0.100 net OD value was considered as the RBD-binding IgM serum titer. IgG antibodies were detected by ELISA assay (Euroimmun, Luebeck, Germany), according to the manufacturer's instructions. The semi-quantitative (IgG) results are expressed as a ratio with respect to an internal calibrator: a ratio <0.8 was considered negative, ≥1.1 was considered positive and intermediate results were considered borderline. Pregnant women with positive IgG antibody for SARS-CoV-2 results were submitted to nasopharyngeal swab by real-time PCR assay. SARS-CoV-2 seroprevalence and maternal anamnestic data were compared in the two groups. Numerical variables were expressed as median value with range and compared with the Mann-Whitney U test, while categorical variables were expressed as percentage (and 95% confidence interval, CI) and compared with Fisher's exact test. Analysis was performed with GraphPad Prism version 6 and a p-value <0.05 was considered statistically significant.

A total of 62 women who had miscarriage during the first trimester (median gestational weeks: 8, range 5-12), except two who had miscarriage during the second trimester (21 and 24 gestational weeks), were enrolled and tested a median time of 152 (range 129-210) days after miscarriage: 2 women (3.2%, 95% CI: 0.4-11.1) resulted SARS-CoV-2-specific IgG positive/IgM negative. As controls, 218 pregnant women were enrolled and tested (median of gestational weeks: 21; range 17–37). Of these, 5 (2.3%, 95% CI: 0.7-5.3) resulted positive for SARS-CoV-2-specific IgG positive/IgM negative. All the seven SARS-CoV-2 IgG-positive/IgM-negative women from both miscarriage and control groups were negative for SARS-CoV-2 RNA in nasopharyngeal swab and never presented

symptoms related to SARS-CoV-2 infection. The SARS-CoV-2 seroprevalence was not significantly different in the two groups of women (Table 1), therefore excluding a significant role of SARS-CoV-2 infection in pregnancy loss. Moreover, while we are sure that seropositive women in the control group were infected during pregnancy, we cannot exclude that the 2 seropositive women in the miscarriage group were infected after pregnancy loss, since serostatus was tested 4-7 months after the end of pregnancy. The outcome of pregnancy in the 5 SARS-CoV-2 seropositive control women was listed as follows: median gestational weeks at delivery: 39 (range 34-40); 1 cesarean delivery (26%) and 4 normal deliveries; newborn median birth weight: 3110 (range 1645-3970) g and birth length 49 (50-51) cm; median head circumference 31.5 (range 29-34) cm. In the 213 SARS-CoV-2 seronegative control women, the following parameters at delivery were observed: median of gestational weeks at delivery: 40 (range 31-42); 59 cesarean delivery (28%), 159 normal delivery; newborn median birth weight: 3240 (range 1465-4220) g and birth length 51 (38-56) cm; median head circumference 34 (range 29-37) cm. No significant difference was observed between infected and non-infected women of the control group.

Anamnestic data of the two groups of women are reported in Tab. 1. Age, gravidity and delivery were not significantly different in the two groups, whereas a higher frequency of women with previous abortion ($p<0.01$) and from the Middle East ($p=0.02$) was observed in the miscarriage group.

Serologic surveillance among pregnant women in different countries between April and June 2020 was conducted to evaluate the consequences (for the mothers, the fetuses and the newborns) of SARS-CoV-2 infection during pregnancy [Crovetto F *et al*., 2020; Tsatsaris V *et al*., 2020; Flannery DD *et al*., 2020]. After the first pandemic wave, seroprevalence of SARS-CoV-2 infection in both women experiencing early pregnancy loss or control pregnant women enrolled in our study (2-3%) was lower than that reported in Spain (14%) [Crovetto F *et al*., 2020], but similar to that reported in France (4.5%) [Tsatsaris V *et al*., 2020] and in the USA (5-6%) [Flannery DD *et al*., 2020]. Despite the low prevalence among pregnant women [Flannery DD *et al*., 2020], a systematic review of recently arisen coronaviruses (SARS-CoV-2, SARS, MERS) reported higher rates of preterm birth, preeclampsia, cesarean delivery, and perinatal death [Di Mascio D *et al*., 2020] following SARS-CoV-2 infection. Congenital and intrapartum SARS-CoV-2 infection in the fetus/newborn is possible, but rare [Robaina-Castellanos GR, Riesgo-Rodríguez SC, 2021]. However, early gestational age at infection, maternal ventilatory supports and low birthweight remain the main determinants of adverse perinatal outcomes in fetuses with maternal SARS-CoV-2 infection [Di Mascio D *et al*., 2020]. Seasonal influenza has been associated with higher rates of miscarriage, and population level monitoring and upscale of community testing will be needed to ascertain whether this is also the case with COVID-19 [Dorélien A, 2019]. Sacinti *et al*., demonstrated that miscarriage incidence appears to have

increased by 25% in their population during the pandemic [Sacinti KG, 2021]. However, our data, in agreement with a recent report [Cosma S *et al*., 2021], show that SARS-CoV-2 infection within the first trimester does not seem to predispose to early pregnancy loss.

Regarding the possible causes of spontaneous miscarriage, the literature data report the following several reasons: infection, maternal age (especially in women >45 years), uterine abnormalities, hormonal irregularities, incompetent cervix, disease of immune system (such as lupus or other autoimmune disorders), placental problems, chromosome abnormalities, exposure to high levels of radiation or toxic agents, smoking, alcohol or use of illegal drugs, obesity, and diabetes [Garrido-Gimenez C, Alijotas-Reig J, 2015]. We could not analyze these data in our cohort, since they were not collected at the time of miscarriage. However, we found a significantly higher frequency of previous abortion in the 62 women with pregnancy loss. Therefore, we could hypothesize that underlying conditions are at the base of miscarriage, rather than SARS-CoV-2 infection, which was not more frequent in these women. Whether the increase in the rate of pregnancy loss could potentially be due to stress induced by the SARS-CoV-2 pandemic remains to be demonstrated. Nevertheless, the pandemic situation did not impact the quality of care for pregnant women; in our center, the number of pregnancies and deliveries (spontaneous or caesarean), did not change with respect to previous years, confirming the high quality of engagements guaranteed by healthcare personnel in order to offer adequate care to pregnant women.

The number of subjects examined in our study was relatively low (the sample size examined has 80% power to detect a significant difference if the prevalence of SARS-CoV-2 infection was six times higher in the miscarriage group). However, from these preliminary data the impact of asymptomatic or mildly symptomatic SARS-CoV-2 infection on pregnancy appears limited.

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Table 1. Sars CoV-2 seroprevalence and maternal characteristics in miscarriage and control groups

Parameter	Group		P-value
	Miscarriage (n=62)	**Control** (n=218)	
SARS-CoV-2 IgG positive, n (%; 95% CI)	2 (3.2; 0.4-11.1)	5 (2.3; 0.7-5.3)	0.65
Age, years (median, range)	34 (15-45)	33 (19-46)	0.62
Gravidity (median, range)	2 (1-5)	2 (1-7)	0.59
Delivery (median, range)	1 (1-3)	0 (0-4)	0.27
Previous abortion (median, range)	1 (0-3)	0 (0-5)	<0.0001
Country of origin, n (%)			
Europe	52 (83.8)	201 (92.2)	0.13
Africa	2 (3.2)	2 (0.9)	0.21
Asia	1 (1.6)	0 (0.0)	0.22
Middle East	7 (11.3)	8 (3.7)	0.02
South America	0 (0.0)	7 (3.1)	0.35