Underestimation of SARS-CoV-2 infection in placental samples

INTRODUCTION: COVID-19, caused by SARS-CoV-2, has emerged as one of the most serious global public health problems in recent history. Data from the Centers for Disease Control and Prevention suggest that approximately 86,000 pregnant women in the United States have been infected so far.[1] SARS-CoV-2 infects cells by binding to the angiotensin-converting enzyme 2 (ACE2) receptor. However, viral cell entry is dependent on the spike protein cleavage by the transmembrane serine protease 2 (TMPRSS2).[2,3] Some reports demonstrated placental tissue expression of both ACE2 and TMPRSS2, suggesting a potential mechanism for viral cell entry and an ability to infect the placenta.[2,3] However, it is unclear why placental SARS-CoV-2 infection is rarely demonstrated using reverse transcription polymerase chain reaction (RT-PCR) testing.[4,5] We report on 2 cases of pregnant women infected with SARS-CoV-2 and demonstrate the variability in the detection of SARS-CoV-2 in placental tissues using RT-PCR.

CLINICAL PRESENTATION: Case 1 is a pregnant 40-year-old G3P2002 Hispanic woman presenting at 28 weeks' gestation with no remarkable medical history or pregnancy complications. The patient tested positive for SARS-CoV-2 by RT-PCR and was admitted with severe pneumonia. She progressed rapidly to hypoxic respiratory failure and required admission to the intensive care unit and intubation. However, the patient's condition continued to deteriorate, and a repeat cesarean delivery was performed to deliver the baby at 29 weeks' gestation. The placenta was collected for histopathologic examination. The baby tested negative for SARS-CoV-2 by RT-PCR analysis of nasopharyngeal swabs collected on days 1, 2, and 5 after birth. The neonate's birthweight was appropriate for gestational age and the neonate had prematurity-related complications but had an otherwise uncomplicated course, including normal head ultrasounds and no retinopathy of prematurity. The baby was discharged at 36 weeks' postgestational age. COVID-19 immunoglobulin G (IgG) antibody testing was done at 6 weeks of life and was negative. Developmental testing at 6 months of age was within normal limits.

Case 2 is a 38-year-old G3P1011 Hispanic woman who presented at 36 weeks' gestation. Her medical history was noted for obesity (body mass index, 50.34 kg/m^2). She was admitted with COVID-19–related symptomatology, decreased fetal movements, and a positive COVID-19 test result determined by RT-PCR. She was diagnosed with intrauterine fetal demise (IUFD). Autopsy examination showed a moderately macerated, nondysmorphic female fetus. The fetus's bodyweight and measurements were appropriate for gestational age. No congenital anomalies were identified, and the developmental maturation of the organs was appropriate for gestational age. Based on the presence of maternal COVID-19, postmortem viral swabs were performed on the fetus. RT-PCR testing detected 2 proteins, namely the E and N2 proteins, on the nasopharyngeal swab, but the deep bronchial and rectal swabs were negative.

LABORATORY METHODS:

Placental samples

The placentas were collected immediately after birth. For case 1, 1 amniotic membrane sample (labeled as section A) and 10 full-thickness placental biopsies (labeled B to K) were obtained from random locations for histologic examinations. Similarly, for case 2, placental biopsies were obtained from 6 random locations (labeled A to F). Tissues were formalin fixed and paraffin embedded (FFPE). Two lung sections from autopsies of COVID-19 patients were used as positive control. The study was approved by the New York University Institutional Review Board.

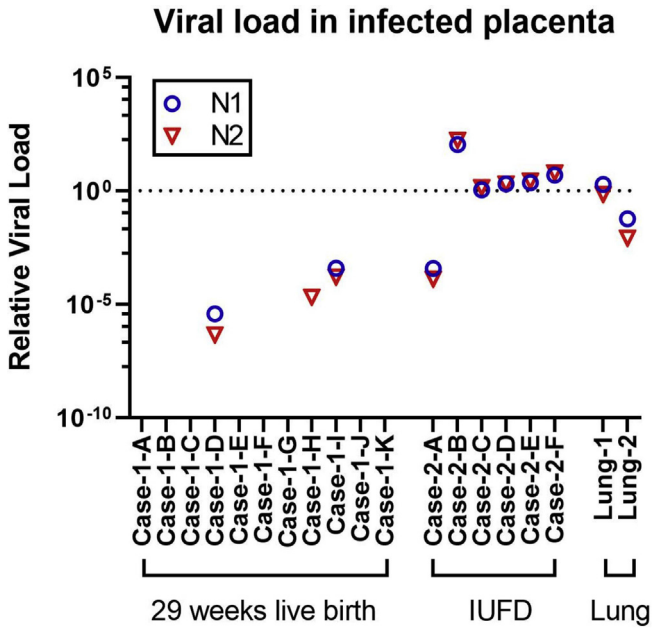
Detection of SARS-CoV-2 RNA in placental formalin-fixed and paraffin-embedded samples

The SARS-CoV-2 N1 and N2 genes were assayed using a 2-step RT-PCR reaction. The total RNA was isolated using a miRNeasy FFPE Kit (catalog number 217504, Qiagen, Germantown, MD) and reverse transcription was carried out using the High-Capacity cDNA Reverse Transcription Kit (catalog number 4368814, ThermoFisher, Waltham, MA) with N1, N2, and RNase P gene primer premix solutions in the 2019-nCoV RUO Kit (catalog number 10006713, Integrated DNA Technologies, Coralville, IA). Real-time PCR was performed using Probes Master with TaqMan Gene Expression Assays using the 2019-nCoV RUO Kit on a QuantStudio 3 (Applied Biosystems, Foster City, CA). The cycle threshold (Ct) was calculated with StepOne Software (version 2.1, Applied Biosystems) and a Ct value in at least 1 of the replicates of below 40 was considered positive for PCR amplification. For assay validation, FFPE placental samples from uninfected individuals collected before May 2019, were spiked with a known amount of a synthetic SARS-CoV-2 RNA standard from the American Type Culture Collection. The relative viral load for FFPE samples was calculated by the delta-delta Ct method using a known reference point.

Immunoelectron microscopy

Paraffin-embedded placental tissue sections of 25 μm were used for electron microscopy (EM). Samples were exposed to a 1:200 dilution of a rabbit polyclonal anti-SARS-CoV-2 spike glycoprotein antibody—Coronavirus (catalog number ab272504, Abcam, Cambridge MA) and a 1:20 dilution of goat anti-rabbit IgG 10 nm gold antibody (Electron

FIGURE 1
RT-PCR detection of SARS-CoV-2



SARS-CoV-2 N1 and N2 genes were assayed by 2-step RT-PCR. For case 1 (preterm placenta at 29 weeks' gestation), only 3 out of 10 biopsies (labeled A—K) were positive for the SARS-CoV-2 N1 and N2 gene by RT-PCR. For case 2 (with intrauterine fetal demise, IUFD), the RT-PCR results were positive for all 6 placental biopsies (labeled A—F). Two lung tissue autopsy samples from COVID-19 patients were used as positive controls. The relative viral load in case #2 was 4 orders of magnitude higher than for case 1 and was similar to the control lung samples.

IUFD, intrauterine fetal demise; *RT-PCR*, reverse transcriptase—polymerase chain reaction.

Hanna. Underestimation of SARS-CoV-2 in placental samples. Am J Obstet Gynecol 2021.

Microscopy Sciences, Hatfield, PA). Grids were scoped on a Zeiss EM 900 transmission electron microscope retrofitted with an SIA L3C digital camera (SIA, Duluth, GA).

Immunohistochemistry

Immunohistochemistry (IHC) analysis was performed on FFPE placental samples using a rabbit specific HRP/DAB (ABC) Detection IHC Kit (catalog number ab64261, Abcam). Spike protein was detected with rabbit anti-SARS-CoV-2 spike glycoprotein antibody - Coronavirus (catalog number ab272504, Abcam). Normal rabbit IgG was used as a negative control.

Expression of angiotensin-converting enzyme 2 and transmembrane serine protease 2

The same procedures were followed as described above for the detection of SARS-CoV-2 RNA in FFPE placental samples. Sequence-specific oligonucleotide primers were used. The relative level of RNA was expressed as −dCt using CYC1 as an internal control.

RESULTS: Only 3 out of 10 biopsies from case 1 were positive for the SARS-CoV-2 N1 and N2 genes by RT-PCR (Figure 1). The amniotic membrane sample (case 1-A) was also negative. For case 2 (IUFD), the RT-PCR reactions were positive for all 6 placental biopsies. In addition, the relative viral load in case 2 was 4 orders of magnitude higher than that for case 1. The viral load in case 2 was similar to that of the 2 positive control samples (COVID-19 lung autopsy samples). As demonstrated in Supplemental Figure 1, SARS-CoV-2 was visualized using EM in a microvillus (case 1, section I).

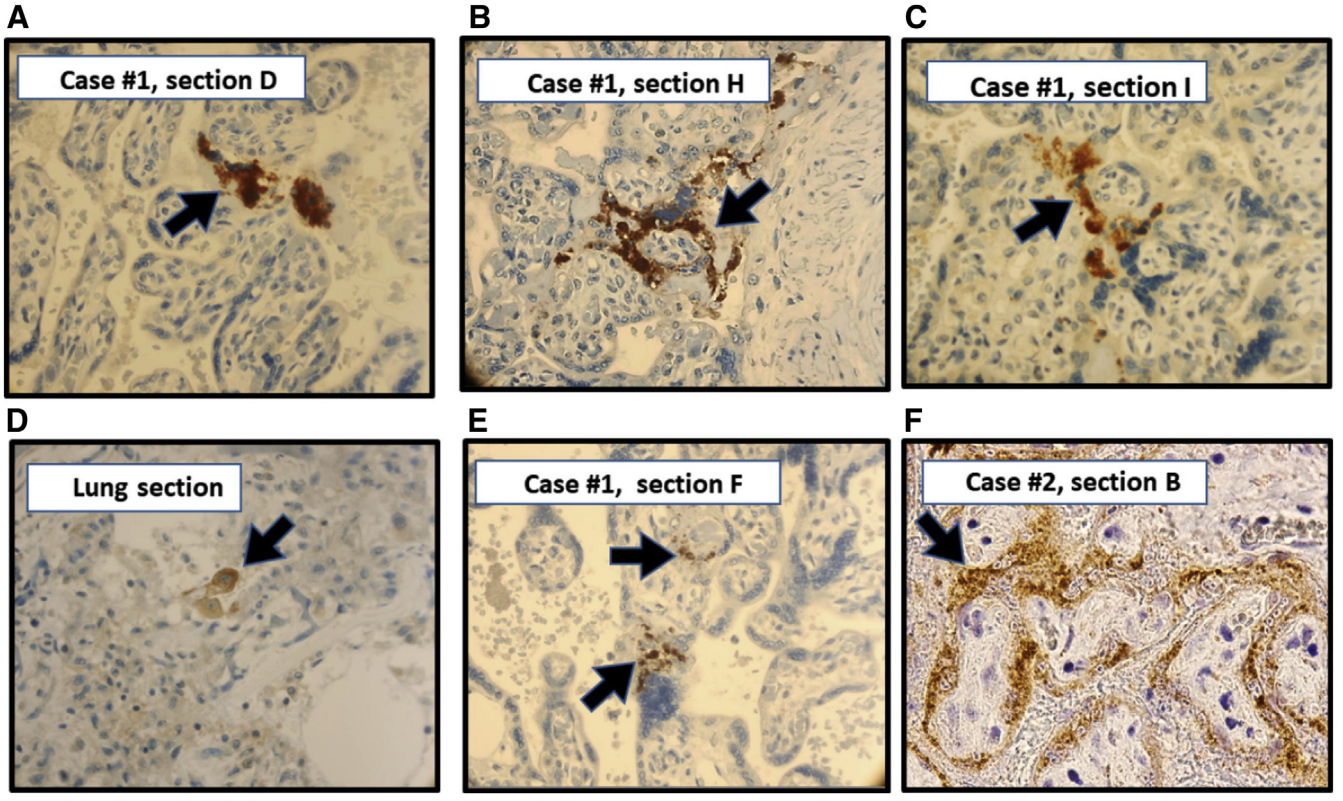
IHC demonstrated positive staining for the SARS-CoV-2 spike glycoprotein, mainly in the trophoblasts layer. In case 1, the placental sections that had positive RT-PCR results (section D, H, and I) all demonstrated positive staining for SARS-CoV-2 spike glycoprotein (Figure 2, A—C) as was also seen for the control lung sections (Figure 2, D). Interestingly, in all the placental sections from case 1 that had negative RT-PCR results, a faint staining was observed in sporadic areas of the slide (Figure 2, E). In case 2, staining for the SARS-CoV-2 spike glycoprotein was observed mainly in the trophoblast layer in all sections (Figure 2, F). Gene expression of the SARS-CoV-2 receptor, ACE2, and TMPRSS2 was evaluated in placental sections from case 1. The gene expression among all sections were comparable; there was no differential expression that could explain the sporadic SARS-CoV-2 infection observed in some sections but not in others (Supplemental Figure 2).

The placental histopathologic examination of case 1 demonstrated mature chorionic villi with focal villous edema and some areas of mild fibrin deposition, and decidual vasculopathy on the maternal surface. There were no histopathologic differences between sections that were positive and those that were negative for SARS-CoV-2 infection (as determined by PCR). In case 2, all placental sections had extensive perivillous deposition associated with extensive necrosis of the syncytiotrophoblast and chronic histiocytic intervillositis.

DISCUSSION: Our results suggest that SARS-CoV-2 can infect placental tissues; however, the infection is not uniform in the same placenta. It is unclear why some placental tissue sections showed infection whereas others did not. Because many research studies use RT-PCR to detect SARS-CoV-2 placental infections,[4,5] the prevalence of placental SARS-CoV-2 infection may be considerably underestimated if only 1 placental section is examined. In our first case, only 30% of the samples from the same placenta were positive for SARS-CoV-2 using RT-PCR. In the second case, the RT-PCR reactions were positive for all samples collected from the same placenta. This discrepancy can potentially be explained by the presence of a high viral load in case 2.

The IHC results confirm that the viral infection is not consistent throughout each placental slide and that virus localization is more concentrated in some areas relative to others. According to our data, the site of infection did not correlate with differential ACE2 or TMPRSS2 placental expression. Interestingly, the IHC analysis showed positive results in all samples from both cases, suggesting that IHC is a more sensitive analysis method when compared with RT-PCR.

FIGURE 2
IHC analysis of FFPE placental sections



IHC was performed on FFPE placental sections for the SARS-CoV-2 spike glycoprotein. For case #1, the placental sections that had positive RT-PCR test results demonstrated positive staining for SARS-CoV-2 spike glycoprotein in the trophoblast layer. **A,** Positive RT-PCR test result in case #1, representative section D. **B,** Positive RT-PCR test result in case #1, representative section H. **C,** Positive RT-PCR test result in case #1, representative section I. **D,** The positive control lung tissue samples demonstrated positive staining for SARS-CoV-2 spike glycoprotein. **E,** All other PCR negative placental sections had faint staining observed in sporadic areas of the slide (section F was selected as a representative section). **F,** For case #2, high-density staining for the SARS-CoV-2 spike glycoprotein was observed mainly in the trophoblast layer in all sections (section B was selected as a representative section).

FFPE, formalin fixed paraffin embedded; *IHC*, immunohistochemistry; *RT-PCR*, reverse transcriptase—polymerase chain reaction.

Hanna. Underestimation of SARS-CoV-2 in placental samples. Am J Obstet Gynecol 2021.

Our 2 cases demonstrated the variable effect of the virus on the placental histopathologic examination. In case 1, the histopathologic examination of the placental sections was mostly unremarkable. In contrast, the placental histopathologic examination for case 2 (high viral load) demonstrated extensive perivillous deposition with extensive necrosis of the syncytiotrophoblast and chronic histiocytic intervillositis.

In conclusion, this report suggests that the SARS-CoV-2 placental infection is patchy within the same placenta and that using RT-PCR testing from a single placental biopsy may lead to underestimation of the incidence of SARS-CoV-2 placental infection. The SARS-CoV-2 detection results using RT-PCR reactions might depend on the maternal viral load and if the site of the placental biopsy is coincidentally infected. Furthermore, our results may suggest that IHC is a more sensitive test for the detection of SARS-CoV-2 placental infections than RT-PCR. We suggest that RT-PCR testing should be done using multiple placental sections before declaring that the placental RT-PCR test result is negative for SARS-CoV-2 infection (Supplemental Video). ■

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The authors report no conflict of interest.

This study was funded by grant number 1R01HD098258-01A1 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD).

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 https://doi.org/10.1016/j.ajog.2021.07.010

Coagulation assessment with viscoelastic testing in asymptomatic postpartum patients with SARS-CoV-2 infection: a pilot study

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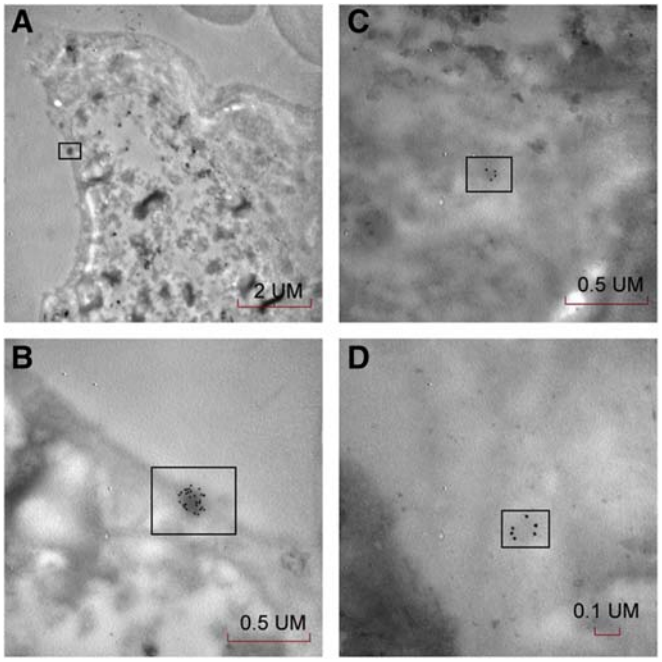
OBJECTIVE: Starting in the late 2019, the COVID-19 pandemic caused by SARS-CoV-2 infection has affected millions of people worldwide. Since then, multiple publications have described a hypercoagulable profile among patients with severe COVID-19.[1] This apparent underlying hypercoagulable state is expected to be more deleterious during the immediate postpartum period when the risk for thromboembolic events is already markedly elevated than in nonpregnant individuals.[2] In this study, we aimed to evaluate the coagulation profile of asymptomatic patients with COVID-19 during the immediate postpartum period by utilizing viscoelastic testing.

STUDY DESIGN: This was a single center cohort study. The institutional review board committee approved the protocol, and informed consent was obtained from all participants. At our institution, universal screening for SARS-CoV-2 on admission, using polymerase chain reaction (PCR) testing of nasopharyngeal swabs, is routine. Asymptomatic patients who presented for delivery and who had a positive PCR test result were approached by research staff for inclusion. Based on the availability of research staff, a convenience sample of individuals with a negative PCR test result was used as the control group. Patients with known coagulopathies were excluded. The coagulation profiles for both groups were determined using viscoelastic testing (Quantra QPlus System, Hemosonics, LLC, Charlottesville, VA) on a 2 mL blood sample, collected from each participant.[3] This device measures clotting time (which reflects the clotting factor function responsible for initial fibrin formation) and clot stiffness (which provides an independent value for the fibrinogen and platelet contribution to the overall stiffness of the clot).

RESULTS: A total of 34 patients were included (15 with COVID-19 and 19 controls without COVID-19). Most of the patients were Hispanic (n=32) and delivered vaginally

SUPPLEMENTAL FIGURE 1
SARS-CoV-2 was visualized using EM in a microvillus (case 1, section I)

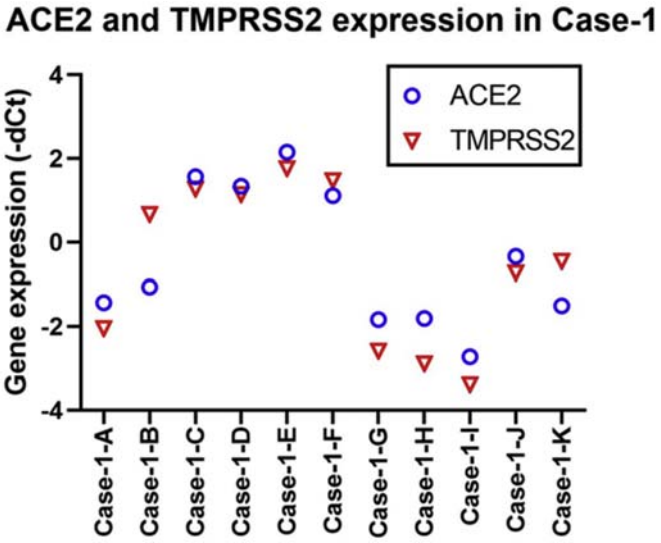


A, The boxed area shows the 10 nm gold particles attached to the spike glycoprotein of SARS-CoV-2 virions on the cell surface. **B,** Higher magnification image of the image in (**A**). **C,** Intracellular localization of the SARS-CoV-2 spike glycoprotein. **D,** Highest magnification image showing the approximate size of the virion at 100 nm.

EM, electron microscopy.

Hanna. Underestimation of SARS-CoV-2 in placental samples. Am J Obstet Gynecol 2021.

SUPPLEMENTAL FIGURE 2
Gene expression levels of ACE2 and TMPRSS2



The gene expression levels of SARS-CoV-2 receptors ACE2 and TMPRSS2 were evaluated in placental sections from case 1. The gene expression levels in all sections were comparable, with no correlation observed between the expression levels and the SARS-CoV-2 PCR-positive sections (sections D, H, and I).

ACE2, angiotensin-converting enzyme 2; *TMPRSS2*, transmembrane serine protease 2.

Hanna. Underestimation of SARS-CoV-2 in placental samples. Am J Obstet Gynecol 2021.