



A Possible Case of Vertical Transmission of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in a Newborn With Positive Placental In Situ Hybridization of SARS-CoV-2 RNA

Ihab Alamar,^{1,a} Mohammad H. Abu-Arja,^{1,a} Taryn Heyman,² Drucilla J. Roberts,³ Niyati Desai,⁴ Pramod Narula,¹ and Beata Dygulska¹

¹Department of Pediatrics, New York Presbyterian Brooklyn Methodist Hospital, Brooklyn, New York, USA; ²Department of Obstetrics and Gynecology, New York Presbyterian Brooklyn Methodist Hospital, Brooklyn, New York, USA; ³Department of Pathology, Massachusetts General Hospital, Boston, Massachusetts, USA; ⁴Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts, USA

Little is known about the effects of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the coronavirus disease 2019 (COVID-19) on pregnant mothers and their infants. Moreover, there is no definitive evidence that SARS-CoV-2 can be vertically transmitted from an infected mother to the unborn fetus.

Key words. COVID-19; pregnancy; SARS-CoV-2; vertical transmission.

Currently, there is no clear evidence that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can be vertically transmitted. Here, we report on an infant born to a mother with coronavirus disease 2019 (COVID-19). The infant tested positive for SARS-CoV-2 by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) at 24 and 48 hours of life (HOL) and on day of life (DOL) 7. Moreover, placental in situ hybridization (ISH) revealed the presence of SARS-CoV-2 RNA.

METHODS

We retrospectively reviewed the maternal and infant's charts to obtain clinical and laboratory data. Institutional review board approval and written informed consent from the mother were obtained. Beginning on 22 March 2020, all pregnant women who present to the Labor and Delivery Unit at New York Presbyterian Brooklyn Methodist Hospital are tested for SARS-CoV-2 using qRT-PCR of a nasopharyngeal swab. The infants of positive mothers are screened at 24 hours of life (HOL) using a nasopharyngeal swab.

Placental pathology was performed using standard protocols with hematoxylin and eosin-stained samples. A CD68

immunohistochemical stain was performed on a block that was then forwarded to the Massachusetts General Hospital Pathology Department.

Medical SARS-CoV-2 RNA ISH was done at the Massachusetts General Hospital Pathology Department. An RNAscope 2.5 LS Probe-V-nCoV2019-S (cat no. 848568) and an RNAscope 2.5 LS Reagent Kit-RED (cat no. 322150; Advanced Cell Diagnostic) on an automated BondRx platform (Leica Biosystems) were used. We used 5- μ m-thick sections of formalin fix paraffin embedded biopsy tissue. All steps were done on the BondRx machine, including baking for 1 hour at 60°C and counterstaining with hematoxylin. RNA unmarking was done using Bond Epitope Retrieval Solution 2 for 15 minutes at 95°C, followed by protease treatment for 15 minutes and probe hybridization for 2 hours. The signal was amplified by a series of signal amplification steps followed by color development in red using Bond Polymer Refine Red Detection (Leica) in the format of red dots.

We used 3 sets of controls that were obtained from Massachusetts General Hospital. First, we used 122 historical "normal" controls from uncomplicated term deliveries pulled from the 2000–2004 database for the indication of maternal group B streptococcal positivity. Second, we used 130 "pathologic" controls that included term and late preterm newborns from a database of placentas from mothers with neonates with hypoxic-ischemic encephalopathy. Finally, for ISH probe specificity, we used 10 placentas of SARS-CoV-2-negative mothers. The 10 placentas included patients with RNA viral infections (Zika exposure, human immunodeficiency virus infection, and hepatitis C infection); intrauterine fetal demise; and histopathologies associated with congenital infections and coagulopathies, such as high-stage and grade acute chorioamnionitis, high-grade maternal vascular malperfusion

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^aI. A. and M. H. A.-A. contributed equally to this work.

Correspondence: Beata Dygulska, Department of Pediatrics, New York Presbyterian Methodist Hospital, 506 6th St, Brooklyn, NY 11215 (bed9017@nyp.org).

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(MVM), high-grade fetal vascular malperfusion, high-grade villitis of unknown etiology, chronic histiocytic intervillitis, and multiple intervillous thrombi.

RESULTS

An otherwise healthy, 32-year-old gravida 2 para 0 female presented at 35 + 6 gestational age with vaginal bleeding and contractions. She reported subjective fever, mild chills, fatigue, dysgeusia, and anosmia beginning 1 day before presentation. Her prenatal care started in the first trimester; her perinatal serologies were not significant. A 2630-gram (56th percentile) female was delivered via an urgent cesarean section due to bleeding secondary to placenta previa on 7 April 2020. The APGAR scores were 9 at both 1 and 5 minutes. The newborn displayed no signs of respiratory distress. The newborn's length was 49.5 cm (89th percentile), and the occipitofrontal circumference was 35 cm (97th percentile). The father had potential exposure to SARS-CoV-2 as he works as a respiratory therapist in an intensive care unit that treats patients with COVID-19. However, the father was never symptomatic and was never tested before or during the mother's pregnancy. The maternal screen for SARS-CoV-2 nucleic acid from nasopharyngeal swab was positive on postpartum day 1.

The mother wore a surgical mask and a disposable, nonsterile isolation gown throughout her hospitalization. She washed her hands and chest with soap and water before breastfeeding or skin-to-skin care. The infant was kept inside a closed incubator placed 6 feet away from the mother's bed in the same room. The mother fed her baby with formula and direct breastfeeding. On postpartum day 2, the mother started expressing her breast milk, and the nurse fed the baby.

The infant's nasopharyngeal qRT-PCR for SARS-CoV-2 was positive at 24 and 48 HOL. The infant's complete blood count, basic metabolic panel, and C-reactive protein were all within normal limits. The mother and infant remained afebrile, asymptomatic, and hemodynamically stable throughout

the hospitalization. The infant was discharged with the mother on DOL 4 with a daily telehealth visit plan up to DOL 14. The infant's nasopharyngeal qRT-PCR remained positive on DOL 7. She remained asymptomatic during the daily follow-up period. Serologic studies of COVID-19 immunoglobulin (Ig) M and IgG were not performed as testing facilities were not available. To the best of our knowledge, there was no transmission of SARS-CoV-2 to any other individual from this case.

The gross examination of the 469-gram trimmed placenta was notable for a hypercoiled umbilical cord. The placental histopathology showed no inflammation (chronic villitis, intervillitis, or chorioamnionitis). However, it showed 1 central infarct with peripheral increased perivillous fibrin, a finding that is common in bleeding placenta previa. On routine stains, the infarcted area showed fetal placental vascular rupture and villous necrosis. A CD68 immunohistochemical stain showed subjective increased numbers of CD68 immunoreactive Hofbauer cells in the villous stroma (Figure 1). ISH for SARS-CoV-2 RNA revealed a strong signal in the villous syncytiotrophoblast in a multifocal pattern (Figure 2). The placental signal was restricted to the syncytiotrophoblast, not present in villous stromal cells, Hofbauer cells, or villous endothelium.

DISCUSSION

In this case, a neonate born to a mother with COVID-19 had positive nasopharyngeal qRT-PCR at 24 and 48 HOL and DOL 7. The infant remained afebrile and asymptomatic with normal hematologic and inflammatory markers. There was no evidence of growth restriction or microcephaly. However, since the delivery time was close to the time of the maternal infection, we cannot rule out the risk of growth restriction and microcephaly in future cases. Although the mother wore personal protective equipment, the infant was not isolated immediately after delivery. The baby started breastfeeding at 1 HOL, and the first neonate nasopharyngeal qRT-PCR was done at 24 HOL.

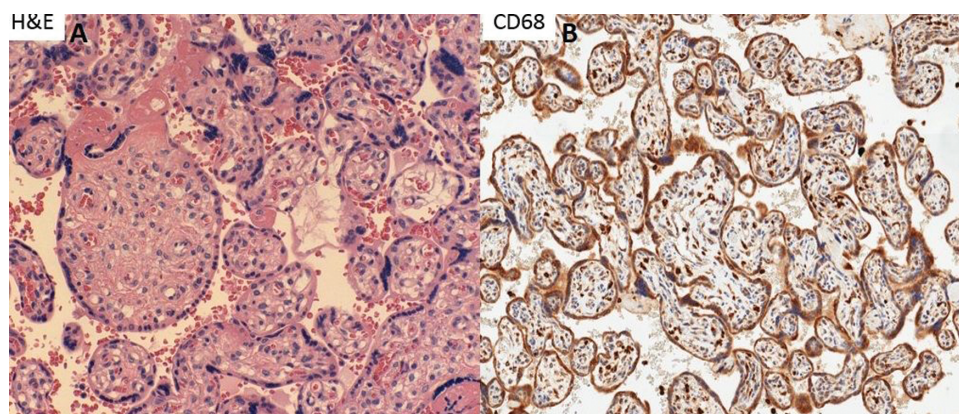


Figure 1. Placental villous tissue with (A) hematoxylin and eosin staining and (B) immunohistochemical staining of CD68 tissue macrophages (Hofbauer cells) within the distal villi. Scattered macrophages within the intervillous spaces are also present (200× magnification).

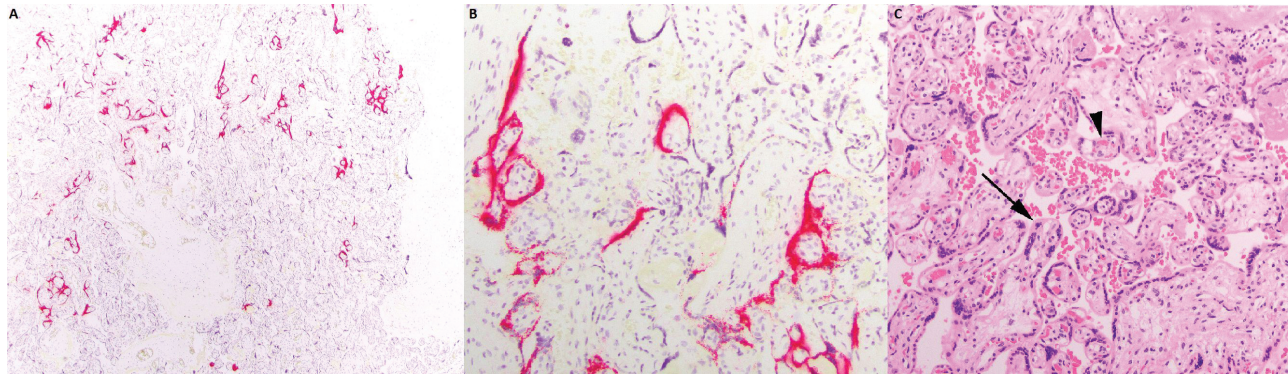


Figure 2. Noninfarcted chorionic villi from the placenta to illustrate severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in syncytiotrophoblast. **A**, In situ hybridization for SARS-CoV-2 RNA at 4× original showing clusters of positive red staining of villi. **B**, In situ hybridization for SARS-CoV-2 RNA at 20× original demonstrating a strong signal in syncytiotrophoblast in some but not all villi. **C**, Hematoxylin and eosin stained section of chorionic villi to identify syncytiotrophoblast (arrow) and vasculosyncytial membranes (arrowhead) 20× original.

Moreover, the mother's breast milk was not tested for SARS-CoV-2. Therefore, the neonate positive nasopharyngeal qRT-PCR may be secondary to contamination with maternal breast milk. Thus, the possibility of postnatal exposure cannot be eliminated. However, the placental villous syncytiotrophoblast was positive for SARS-CoV-2 by ISH, suggesting vertical transmission.

There are approximately 5000 births per year at our center. Between 22 March 2020 and 31 July 2020, 1463 mothers gave birth at our center, and all were universally screened for SARS-CoV-2 at admission. Of the 1463 mothers, 125 (8.5%) had a positive SARS-CoV-2 nasopharyngeal qRT-PCR. The only positive newborn was the one described here. Moreover, 53 placentas from positive mothers were tested by ISH for SARS-CoV-2 viral RNA. Only 2 placentas were positive. The first placenta was the one described here. The second placenta showed viral particles only within the endometrial glands but not in the syncytiotrophoblasts. The endometrial tissue is strictly maternal. Therefore, the viral presence does not reflect a vertical transmission. Accordingly, our data suggest a low probability of vertical transmission between positive mothers and their newborns.

Zhang et al and Hecht et al examined 74 and 19 SARS-CoV-2-exposed placentas, respectively [1, 2]. There were no specific histopathologic features within the placentas of positive mothers compared with controls in both cohorts [1, 2]. However, Shanes et al examined 16 placentas of mothers with SARS-CoV-2 [3]. They reported an increase in the rate of features of MVM and intervillous thrombi compared with historical controls [3]. Intervillous thrombi and MVM are associated with maternal hypertensive disorders such as hypertension and preeclampsia. However, both Zhang et al and Shanes et al [1, 3] found a negative association between preeclampsia and mothers who were positive for SARS-CoV-2. Therefore, more

studies are needed to evaluate the relationships between MVM, placenta coagulopathies, and SARS-CoV-2.

Viruses can infect stromal cells, especially the resident macrophages (Hofbauer cells), via either the receptors on the villous syncytiotrophoblast or the breaks in the villous trophoblast covering of the villous stroma. Therefore, in our case, the strong signal of SARS-CoV-2 in the syncytiotrophoblast may represent vertical transmission. Chronic villitis and/or intervillitis are considered a histopathological hallmark of vertically transmitted viral infections. However, RNA viruses can infect the placenta without causing characteristic histopathology, except for Hofbauer cell hyperplasia, chronic histiocytic intervillitis, and massive perivillous fibrin deposition [2]. In our case, the placental histopathology showed no chronic villitis, intervillitis, or chorioamnionitis. The lack of acute or chronic signs of placental inflammation in our case may be attributed to the lack of severe maternal systematic inflammatory response. However, the increased numbers of CD68 immunoreactive Hofbauer cells in the villous stroma may represent histiocytic intervillitis. Moreover, histiocytic intervillitis and Hofbauer cell hyperplasia were associated with other RNA viral placental infections, especially the Zika virus [4]. However, the lack of the SARS-CoV-2 signal in the Hofbauer cells necessitates further exploration of the possibility of maternal histiocytes carrying SARS-CoV-2.

Our report is limited by including only 1 case with no PCR testing of the amniotic fluid or maternal and newborn serum. Moreover, the mother started to have symptoms just 1 day before delivery. Therefore, the vertical transmission may suggest that the mother was viremic during her incubation period. However, clinical testing for SARS-CoV-2 viral load of

the mother's or fetus's blood was not available. More studies are needed to confirm the vertical transmissibility of SARS-CoV-2 and produce a consistent description of COVID-19 in newborns.

Notes

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