Journal of the Pediatric Infectious Diseases Society

BRIEF REPORT

In Utero Severe Acute Respiratory Syndrome Coronavirus 2 Infection

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Evidence for in utero transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is growing but not definitive. We present a case of neonatal infection that supports in utero transmission of SARS-CoV-2 and provides insight into the hematogenous spread from mother to fetus.

Key words. cord blood; COVID-19; neonatal; vertical transmission.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is transmitted primarily via respiratory secretions. Evidence for in utero transmission is growing but not definitive. Reports to date describe the detection of SARS-CoV-2-specific immunoglobulin M (IgM) in neonatal serum [1], nucleic acids in neonatal serum and stool [2–4], nucleic acids in neonatal nasopharyngeal (NP) secretions [2, 4, 5], and nucleic acids on placental surfaces and tissues [4, 6]. To our knowledge, no one has reported SARS-CoV-2 RNA in cord blood associated with neonatal infection. We present a case of neonatal infection with viral RNA in cord blood that supports in utero transmission of SARS-CoV-2 and provides insight into the hematogenous spread from mother to fetus. This study was exempted by the Institutional Review Board at Holy Cross Hospital.

Received 6 August 2020; editorial decision 18 October 2020; accepted 20 October 2020; Published online October 22, 2020.

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Journal of the Pediatric Infectious Diseases Society 2020;9(6):769–71

DOI: 10.1093/jpids/piaa127

CASE DESCRIPTION

A 34-week gestation, 2414-g boy was born to a mother diagnosed 14 hours prior to delivery with SARS-CoV-2 infection via positive NP swab, obtained because of cough for 1 week. The mother sought medical attention for vaginal bleeding and cramping and was found to have thrombocytopenia, transaminitis, and hyperuricemia. She had a history of gestational diabetes controlled by diet and was not hypertensive. The infant was delivered via cesarean section because of concern for hemolysis, elevated liver enzymes, low platelet syndrome, suspicion for systemic COVID-19, and history of prior cesarean section. During delivery, the mother wore a non-rebreather face mask and medical personnel wore airborne-level personal protective equipment (PPE) including N-95 masks and face shields. Amniotic fluid was clear. The infant's cord was clamped after 30 seconds, and he received routine care 20 feet from his mother's head. Cord blood was collected immediately using standard procedure. Apgar scores were 7 at 1 minute and 9 at 5 minutes. The physical examination was normal. He was shown to his mother for a few seconds from a distance of 4 feet while she wore a surgical mask. The infant was placed in an incubator maintained outside the delivery room, transported to the adjacent neonatal intensive care unit (NICU), placed in a negative pressure room, and bathed shortly after birth. All NICU personnel wore airborne-level PPE while in contact with the infant. NICU personnel had no contact with the mother until after her hospital discharge, and the mother elected not to breastfeed or provide breast milk. The infant had no contact with family members until the day of life (DOL) 7 when his mother had recovered.

The infant continued to be asymptomatic with normal laboratory studies (see Supplementary Table) but NP swabs tested positive for SARS-CoV-2 RNA. The infant was discharged on DOL 8 and remained healthy through the first month of life.

Viral RNA Detected in Infant NP Secretions

NP swab for SARS-CoV-2 utilizing the Cepheid Xpert Xpress qualitative test for the presence of the SARS-CoV-2 target *Envelope protein* (*E*) and *nucleocapsid 2* (*N2*) genes was negative at 24 hours of life and then positive for only *N2* at 49 hours of life. Because of concern for false-positive results, multiple other specimens were tested with Xpert Xpress and a second qualitative test, the Roche Cobas SARS-CoV-2 assay, which utilizes *ORF1* and *E* gene targets (Table 1). Qualitative tests indicated an increasing viral burden in the nasopharynx over time based on decreasing cycle threshold (Ct) values.

Viral RNA Detected in Cord Blood and Infant Urine Without SARS-CoV-2-specific IgG and IgM Antibodies

We performed studies to determine the presence of viral RNA in cord blood, infant blood, and infant urine and of antibodies

Table 1. Infant SARS-CoV-2 Detection

	Test Kit					
Test (site)	Xpert Xpress (NP Secretions)		Roche cobas (NP Secretions)		ddPCR (Blood and Urine)	
Target	*E*, Result (Ct)	*N2*, Result (Ct)	*ORF1*, Result (Ct)	*E*, Result (Ct)	*N1*, Result (copies/mL)	*N2*, Result (copies/mL)
DOL 0					Cord blood: positive (424)	Cord blood: positive (589)
DOL 1	Negative	Negative				
DOL 2	Negative	Positive (44.1)	Negative	Negative (×2)	Infant urine: negative	Infant urine: positive (155)
DOL 3	Negative	Positive (42.3)	Negative	Positive (×2) (36.6, 35.9)	Infant urine: negative	Infant urine: negative
DOL 4	Positive (28.6)	Positive (32.3)	Positive (Ct not available)	Positive (Ct not available)		
DOL 5						
DOL 6					Infant blood: negative	Infant blood: negative
DOL 7	Positive (19.7)	Positive (22.0)	Positive (17.9)	Positive (17.7)		

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; NP, nasopharyngeal; *N, nucleocapsid*; DOL, day of life, with birth day being day 0; Ct, cycle threshold.

in cord blood. Samples were limited to those collected for clinical care that were still available after NP swab-confirmed infant infection. Blood and urine samples were refrigerated after collection and were subsequently frozen before being processed via the QIAamp Viral RNA Mini kit (Qiagen) according to the manufacturer's instructions.

To detect the presence of SARS-CoV-2, we utilized the Bio-Rad SARS-CoV-2 droplet digital polymerase chain reaction (ddPCR) Kit, a triplex assay for the nucleocapsid genes *N1* and *N2* with a human *RNase P* (*RP*) gene, according to the manufacturer's instructions. We found SARS-CoV-2 RNA corresponding to genes *N1* and *N2* in umbilical cord serum and the SARS-CoV-2 gene *N2* in DOL 2 urine (Table 1).

An immunoprecipitation assay was used to detect immunoglobulin G (IgG) to SARS-CoV-2 as previously reported [7]. The assay was modified to include antihuman immunoglobulin M (IgM) agarose beads [8] to detect IgM to SARS-CoV-2. Known SARS-CoV-2 seronegative and seropositive samples for IgG and IgM antibodies against nucleocapsid and spike proteins were used for assigning seropositive cutoff values and for standardization. Testing of both serum and plasma from the cord blood was seronegative for IgM and IgG antibodies against both proteins.

Placental Tissue Suggested Maternal Vascular Malperfusion Without Direct Evidence of Virus

The placenta was fixed in formalin on the day of birth, and several paraffin blocks were prepared for review. Clinical pathology showed no gross abruption. Two weeks after delivery, a larger sample of the placenta was transferred to ethanol. We performed both in situ hybridization and ddPCR studies on the placenta.

RNAscope 2.5-HD Assay-BROWN (Advanced Cell Diagnostics) was used according to the manufacturer's instructions for in situ hybridization of V-nCoV2019-S (complete genome) (Cat No. 848561 ACDBio) on paraffin-embedded samples. POLR2A (Cat No. 310451 ACDBio) mRNA labeling was used as a positive control for the RNA quality in samples. Sections of placenta showed hypermature villi with increased numbers of syncytial knots (44%), suggestive of maternal vascular malperfusion. (Figure 1) The RNAscope assay was negative in the placenta, membranes, and cord.

Preserved placental tissue was processed via the RNeasy FFPE kit to extract RNA and analyzed via ddPCR. Neither *N1* nor *N2* gene targets were detected in placental or umbilical cord tissue.

DISCUSSION

This infant most likely acquired SARS-CoV-2 hematogenously from the mother at or prior to delivery. All caregivers were aware of the mother's COVID-19 diagnosis prior to delivery, and it is extremely unlikely that the infant could have acquired infection from the mother via the respiratory route with such brief and distant exposure in the delivery room. While we cannot rule out microscopic maternal blood contamination of cord blood in this or any other delivery, cord blood collection procedures are designed to avoid gross contamination with maternal blood.

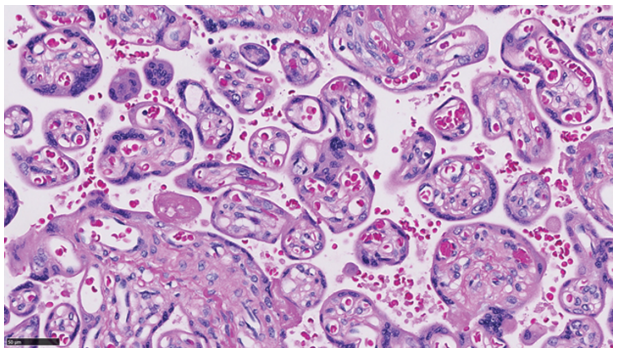

Figure 1. Villi near maternal plate showed more syncytial knots than expected for placental age (Hematoxylin and eosin stain, ×400).

Microscopic contamination would not explain the RNA levels observed in our patient's cord blood.

The scientific community has not agreed upon a case definition for in utero transmission of SARS-CoV-2, although 2 appealing approaches have been described [9, 10]. This case provides evidence for in utero hematogenous transmission of SARS-CoV-2: we found SARS-CoV-2 RNA in cord blood and in newborn urine and nasopharynx, with evidence for viral replication. We detected SARS-CoV-2 RNA in infant NP secretions on DOL 2, suggesting that the virus transmitted via cord blood seeded the nasopharynx and required 2 days for incubation and replication sufficient for detection. Furthermore, the Ct values of the qualitative tests for RNA in NP samples steadily decreased, indicating higher viral loads over time and suggestive of ongoing virus replication during the first week of life. Viral RNA in urine provides further evidence for the hematogenous spread in the newborn who remained well despite evidence of the virus in multiple organs.

The absence of anti-SARS-CoV-2 IgG and IgM in cord blood neither supports nor refutes the hypothesis that the virus was transmitted in utero. Seroconversion for IgG against the SARS-CoV-2 spike protein generally occurs more than a week after symptom onset in adults [7]. Since the mother experienced symptoms for 7 days before birth, there was likely insufficient time for IgG seroconversion and placental transfer. IgM, which cannot cross the placenta, can be detected in the blood of neonates with infections during the first weeks of life [11], but the seroconversion interval for neonates exposed to SARS-CoV-2 is unclear.

There are several possible reasons that we did not find SARS-CoV-2 RNA in placental tissue. First, the mother presented with bleeding indicating possible damage to placental structures, which may have caused direct viral seeding of the cord blood. Second, we did not examine the entire placenta and may have missed an area of local infection. Finally, the placenta was not preserved for the evaluation of RNA until approximately 2 weeks after delivery, which may have caused degradation of viral RNA to an undetectable level.

Hematogenous spread of SARS-CoV-2 from mother to fetus or newborn has at least 2 implications. First, since only a few cases of early-onset neonatal SARS-CoV-2 infection have been reported, hematogenous vertical transmission is likely uncommon. Second, the current recommendation of the American Academy of Pediatrics is to test the NP secretions of well newborns at 24 and 48 hours but not again in the absence of symptoms. This case suggests that some cases of SARS-CoV-2 in newborns may be detectable only after 48 hours of life.

CONCLUSIONS

This case adds to the emerging story of in utero transmission of SARS-CoV-2. Study protocols to identify the risk factors for in utero transmission of SARS-CoV-2 should be based on a clear case definition and include serial samples with optimal collection and processing [12]. Clinical recommendations should take into account that hematogenous spread is uncommon but possible, that infants may be infected with SARS-CoV-2 with few or no symptoms, and that newborn NP secretions may not contain detectable virus until after 48 hours.

Supplementary Data

Supplementary materials are available at the *Journal of The Pediatric Infectious Diseases Society* online (http://jpids.oxfordjournals.org).

Notes

Acknowledgments. We thank William A. Meyer III, PhD, D(ABMM), MLS(ASCP)^{CM} Quest Diagnostics, Baltimore, MD, for assistance in determining cycle times for patient samples run in his laboratory. We thank Dr Stefania Pittaluga at the National Cancer Institute for her laboratory's contribution to RNAscope assays and for her insightful comments about the work.

Financial support. This study was supported by the intramural research programs of the National Institutes of Health Clinical Center, the National Cancer Institute, the National Institute of Dental and Craniofacial Research, and the National Institute of Allergy and Infectious Diseases. M.J.R. is supported by NIGMS Postdoctoral Research Associate Training Program (1FI2GM137804-01).

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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