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Low-level SARS-CoV-2 viremia coincident with COVID placentitis and stillbirth

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ARTICLE INFO

Keywords:
SARS-CoV-2
COVID
Pregnancy
Placentitis
Stillbirth
Viremia

ABSTRACT

SARS-CoV-2 infection in pregnancy and COVID placentitis are associated with an increased risk of stillbirth. We sought to investigate the presence of maternal viremia in people with SARS-CoV-2 infection during pregnancy who had histologic placentitis versus those without placentitis. SARS-CoV-2 qRT-PCR was performed on plasma from 6 patients with COVID placentitis and 12 matched controls without placentitis. SARS-CoV-2 infection occurred between 4/2020–1/2021; the latency between SARS-CoV-2 diagnosis and delivery was 0–76 days. Two placentitis cases demonstrated viremia (1 stillbirth and 1 well infant), while 12/12 controls were negative. Future research may consider viremia as a possible marker of COVID placentitis.

1. Introduction

Recent reports have identified an increased risk of stillbirth in pregnant people infected with SARS-CoV-2 \cite{1,2}. Perinatal pathologists have identified specific placental pathology, termed COVID placentitis, associated with a high risk of stillbirth and poor neonatal outcome \cite{3,4}. COVID placentitis, characterized by histiocytic intervillitis, increased perivillous fibrin deposition, and villous trophoblast necrosis, has been associated with direct viral infection of the syncytiotrophoblast layer of the placenta. Placentitis represents a clinical dilemma to obstetric providers as it can only be diagnosed on examination of the placenta after delivery. We hypothesized that, due to the interface between syncytiotrophoblast and maternal blood, patients with COVID placentitis would be more likely to demonstrate viremia than SARS-CoV-2-infected patients without placentitis.

2. Methods

As part of an active prospective observational cohort study, we identified patients who had SARS-CoV-2 infection during pregnancy and delivered at Northwestern Medicine Prentice Women’s Hospital in Chicago, IL, USA. We identified 6 patients diagnosed with COVID placentitis on pathologic exam and with maternal plasma samples collected and 12 matched controls who had SARS-CoV-2 infection without COVID placentitis. The control cases were matched for gestational age at birth and time between SARS-CoV-2 infection and delivery. COVID placentitis was diagnosed based on the presence of histiocytic intervillitis confirmed with immunohistochemical staining for CD68 and increased perivillous fibrin deposition in the context of maternal SARS-CoV-2 infection. The percentage of villous parenchyma involved was estimated using both gross and microscopic evidence of involvement.

Demographic and clinical data were collected through EMR review. Severity was defined according to National Institutes of Health criteria as asymptomatic, mild, moderate, severe, or critical illness \cite{5}. After

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, Coronavirus disease 2019; qRT-PCR, quantitative reverse transcription polymerase chain reactions; RNA, ribonucleic acid; CDC, Centers for Disease Control and Prevention.

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https://doi.org/10.1016/j.placenta.2022.03.003
Received 27 February 2022; Accepted 3 March 2022
Available online 9 March 2022
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birth, maternal blood was retrieved from as discards from the clinical laboratory. Blood was centrifuged, and plasma aliquots were stored at –80°C. This study was approved by the Institutional Review Board of Northwestern University (STU00212232) with a waiver of informed consent obtained.

Viral RNA was extracted from clinical specimens utilizing the QIAamp Viral RNA Minikit (Qiagen, cat. no. 52906). Testing for SARS-CoV-2 presence was performed by qRT-PCR with the CDC 2019-nCoV RT-PCR Diagnostic Panel utilizing the N1 probe in SARS-CoV-2 and RNase P probe for sample quality control as previously described (IDT, cat. no. 10006713) [6]. Specimens were run in technical duplicate with additional replicates performed to verify putative amplification. All specimens with an N1 probe cycle threshold (Ct) less than or equal to 35 were considered positive. Specimens with an N1 probe Ct value between 35 and 40 were considered positive only if all replicates amplified and on-target amplification was verified by TOPO cloning (CloneJET PCR Cloning Kit, ThermoFisher, cat. no. K1231) and Sanger sequencing of the N1 qPCR product.

### 3. Results

In this cohort, SARS-CoV-2 infections occurred between April 2020 and January 2021, prior to the emergence of variants of concern in Chicago (including Delta). Time between a positive SARS-CoV-2 test and delivery ranged from 0 to 76 days (Table 1). Of the 6 patients with COVID placentitis, 1 had asymptomatic SARS-CoV-2 infection, 4 were mild, and 1 had moderate COVID-19 severity. One placentitis case resulted in stillbirth at 29 4/7 weeks gestational age.

Of the 6 patients with placentitis, SARS-CoV-2 was amplified from maternal blood in 2 cases, including the case of stillbirth, while 0 controls were viremic at delivery. Cloning and Sanger sequencing of the qRT-PCR products confirmed specific on-target amplification of SARS-CoV-2 in these 2 samples. One additional case with placentitis showed amplification below the level of detection in 2 out of 6 replicates and was thus interpreted as negative. Of the 2 cases with confirmed low-level viremia, one woman was asymptomatic and delivered a stillborn infant 1 day following a positive test, while the other woman was mildly symptomatic and delivered a well infant.

### Table 1

Data of patients with SARS-CoV-2 infection during pregnancy with and without COVID placentitis

| * | Gestational Age at delivery | SARS-CoV-2 infection date | Symptoms | Placentitis | Latency | Viremia | N1 Ct | RNAseP | Neonatal outcome |
|---|-----------------------------|---------------------------|----------|-------------|---------|---------|-------|--------|-----------------|
| P1 | 37 0/7                      | 4/2020                    | Mod      | 5%          | 62      | –       | ND    | 29.830 | SGA             |
| P2 | 29 4/7                      | 11/2020                   | Asympt   | Diffuse     | 1       | +       | 35.542| 28.869 | AGA             |
| P3 | 39 0/7                      | 11/2020                   | Mild     | 40%         | 29      | +       | 35.022| 31.185 | AGA             |
| P4 | 39 5/7                      | 12/2020                   | Mild     | Diffuse     | 0       | –       | ND    | 35.397 | AGA             |
| P5 | 40 4/7                      | 12/2020                   | Mild     | 10%         | 76      | –       | ND    | 35.198 | AGA             |
| P6 | 40 3/7                      | 1/2021                    | Mild     | 10%         | 63      | –       | ND    | 33.445 | AGA             |

*P = placentitis, C = control.
† Latency = time from SARS-CoV-2 PCR/symptoms to pregnancy outcome (stillbirth, delivery).
* P1 maternal sample at time of acute infection, others from delivery admission.

Growth categories: SGA = small for gestational age, AGA = appropriate for gestational age, LGA = large for gestational age.
4. Discussion

SARS-CoV-2 infection has affected over 173,500 pregnant persons reported to the Centers for Disease Control and Prevention as of February 2022 and remains a significant issue globally [7]. Concerning findings of placentalitis and higher incidence of stillbirth are among the many adverse outcomes of SARS-CoV-2 infection during pregnancy. Placentitis and stillbirth are not limited to patients with overt or severe infection, thus clinicians remain without tools to identify pregnancies with placentalitis or high risk of stillbirth until after birth.

Viremia in SARS-CoV-2 infection is rare. Approximately 1% of individuals with SARS-CoV-2 infection have detectable virus in their blood. While data on pregnant patients is scarce, one study of 127 pregnancies detected no cases of viremia [8]. Shook et al. recently reported SARS-CoV-2 viremia of ~10 genomes/ml in two cases of SARS-CoV-2 B.1.617.2 (delta) variant associated placentitis and stillbirth [9].

Our study expands this small and important body of literature by demonstrating viremia in pregnant patients in the pre-Delta era and in both stillbirth and those with mild, non-lethal placentalitis. Given the wide clinical availability of SARS-CoV-2 PCR testing, viremia could represent a target for screening pregnant patients with SARS-CoV-2 infection for potential COVID placentalitis and risk stratification of stillbirth.

However, there are several limitations to this study. First, the Ct values reported here are near or below the commonly used limit of detection (Ct value 35) for the CDC SARS-CoV-2 qRT-PCR assay used here. To mitigate the risk of false positive detection, we performed at least 4 technical replicates of each tentative positive sample, sequence verified on-target amplification, and included matched controls, none of which amplified. Nevertheless, the risk of false positives in widespread deployment remains possible. Additionally, while viremia was present in all 3 stillbirth cases reported (2 from Shook et al., 1 from this series), viremia was not detected in some placentitis cases we tested, including one case with diffuse placentitis involvement on pathologic examination. Thus, while the presence of viremia may portend an increased risk of placentitis-mediated stillbirth, the absence of detectable viremia may not exclude placentitis. Notably, 2 of the 6 cases of placentitis had >60 day latency between infection and maternal sample. Thus, transient viremia at the time of SARS-CoV-2 infection cannot be excluded. Further near-term research is needed to consider low-level viremia as a possible marker of COVID placentitis.

Funding

This work was supported by Friends of Prentice (to J.A.G.), the Stanley Manne Children’s Research Institute (to L.B.M.), and from institutional resources supported by the National Center for Advancing Translational Sciences [UL1TR001422]. J.A.G. is supported by the National Institute of Biomedical Imaging and Bioengineering [K88EB030120]. L.B.M. is supported by the National Institute of Allergy and Infectious Diseases [K23AI139337]. Additional funding was provided by the NIH-supported Third Coast CFAR P30 AI117943 (to J.F.H.); NIH grant R21 AI63912 (to J.F.H.); NIH grant U19 AI35964 (to E.A.O.); and through a generous contribution from the Walder Foundation’s Chicago Coronavirus Assessment Network (Chicago CAN) Initiative (to J.F.H. & E.A.O.).

Declaration of competing interest

The authors have no conflicts of interest to declare.

Acknowledgements

The authors wish to thank the staff of Northwestern Medicine Prentice Women’s Hospital Obstetric COVID Unit, the Northwestern Memorial Hospital Blood Bank, Chemistry Lab, and Pathology Gross Room for making this study possible. We also acknowledge the collaboration of the Northwestern Center for Pathogen Genomics and Microbial Evolution. This research was supported in part through the computational resources and staff contributions provided for the Quest high performance computing facility at Northwestern University, which is jointly supported by the Office of the Provost, the Office for Research, and Northwestern University Information Technology. We also acknowledge Allison Sackowicz, Antonia Willnow, Adwini Sunderraj, Raveena Aggarwal, Hooman Azad, Chiedza Mupanomunda, and Alexandra Isia for patient tracking and clinical data collection.

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