SARS-CoV-2 and Placental Pathology

Malperfusion Patterns Are Dependent on Timing of Infection During Pregnancy

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Abstract: The extent to which severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection at different points in the pregnancy timeline may affect maternal and fetal outcomes remains unknown. We sought to characterize the impact of SARS-CoV-2 infection proximate and remote from delivery on placental pathology. We performed a secondary analysis of placental pathology from a prospective cohort of universally tested SARS-CoV-2 positive women >20 weeks gestation at 1 institution. Subjects were categorized as having acute or nonacute SARS-CoV-2 based on infection <14 or ≥14 days from delivery admission, respectively, determined by nasopharyngeal swab, symptom history, and serologies, when available. A subset of SARS-CoV-2 negative women represented negative controls. Placental pathology was available for 90/97 (92.8%) of SARS-CoV-2 positive women, of which 26 were from women with acute SARS-CoV-2 infection and 64 were from women with nonacute SARS-CoV-2. Fetal vascular malperfusion lesions were significantly more frequent among the acute SARS-CoV-2 group compared with the nonacute SARS-CoV-2 group (53.8% vs. 18.8%; $P=0.002$), while frequency of maternal vascular malperfusion lesions did not differ by timing of infection (30.8% vs. 29.7%; $P>0.99$). When including 188 SARS-CoV-2 negative placentas, significant differences in frequency of fetal vascular malperfusion lesions remained between acute, nonacute and control cases (53.8% vs. 18.8% vs. 13.2%, respectively; $P<0.001$). No differences were noted in obstetric or neonatal outcomes between acutely and nonacutely infected women. Our findings indicate timing of infection in relation to delivery may alter placental pathology, with potential clinical implications for risk of thromboembolic events and impact on fetal health.

Key Words: placenta, pathology, SARS-CoV-2, pregnancy placental malperfusion

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The global pandemic of coronavirus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to concerns about the impact of this disease on pregnant women and their neonates.[1] Emerging data show pregnancy is an independent risk factor for severity of COVID-19.[2–4] However, the full extent to which infection may affect maternal and fetal outcomes, as well as the impact of SARS-CoV-2 infection at different points in the pregnancy timeline, remains unknown.

Placental pathology offers a unique window to evaluate pathophysiological changes that may result from SARS-CoV-2 infection. Early studies on placental pathology from SARS-CoV-2 positive women demonstrated evidence of vascular lesions and thromboses, although reports were conflicting whether such lesions occurred on the fetal or maternal side of the placenta.[5] In 1 early case series of 15 placentas from SARS-CoV-2 positive patients, there were significantly more maternal vascular malperfusion (MVM) lesions compared with historical controls.[6] MVM lesions are associated with adverse fetal outcomes including intrauterine growth restriction (IUGR), as well as maternal comorbidities such as preeclampsia, chronic hypertension, and maternal cardiovascular disease.[7,8] In contrast, we and others have noted fetal vascular malperfusion (FVM) as the predominant lesion in placentas of SARS-CoV-2 positive women during the peak of the first wave of the pandemic.[9–11] FVM lesions reflect obstruction of fetal blood flow and are associated with neonatal central nervous system abnormalities, IUGR, intrauterine fetal demise (IUFD), and, in the setting of stillbirth, viral infection.[12,13]

In the course of our clinical care of women with SARS-CoV-2 infection, we observed that differences in

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placental pathology appeared to be related to the timing of SARS-CoV-2 infection relative to delivery timing. We designed this study to test our hypothesis that placentas from patients who delivered during acute SARS-CoV-2 infection would be more likely to demonstrate FVM lesions, while placentas from patients with a history of SARS-CoV-2 infection remote from delivery would be more likely to demonstrate MVM lesions.

MATERIALS AND METHODS

This is a secondary analysis of a prospective cohort study of SARS-CoV-2 positive women >20 weeks gestation admitted for delivery at 1 institution in New York City (NYC) from March 22, 2020 to June 23, 2020 (Institutional Review Board approved, protocol 20-03021682).

Full details of the prospective cohort study have been previously published.[11] Briefly, all pregnant women admitted for delivery were tested for SARS-CoV-2 beginning on March 22, 2020 using a nasopharyngeal swab. SARS-CoV-2 reverse transcriptase polymerase chain reaction (RT-PCR) clinical testing platforms included: Altona (internally developed, Food and Drug Administration [FDA] emergency use authorization approved assay), Roche Cobas 6800 (FDA approved), and Cepheid Xpert Xpress (FDA approved). Serologic testing for SARS-CoV-2 IgG was performed using serum or plasma from peripheral blood in a subset of women with sample capture. Placental pathology was recommended for all women who were SARS-CoV-2 positive. A team of abstractors reviewed the electronic medical record for each subject and recorded demographic, clinical, obstetric, laboratory, and pathologic data.

Preparation and examination of placentas, including gross examination and sectioning, was performed using standard procedures.[14] At our institution, 5 slides per case are reviewed by a pathologist. A single senior perinatal pathologist (R.N.B.) examined the hematoxylin and eosin-stained slides from the placentas of women with a positive RT-PCR result for SARS-CoV-2. This investigator was not blinded to clinical information. Control cases were reviewed by >1 perinatal pathologist. Lesions were diagnosed based on the Amsterdam criteria[8] and evaluated for the presence or absence of the following histologic lesion categories: FVM (representative images shown in Fig. 1), MVM (representative images shown in Fig. 2), acute chorioamnionitis, chronic villitis, meconium staining, umbilical cord abnormalities, chorangiosis, and intervillous thrombi. The histologic findings within each category are defined in Appendix Table 1 (Supplemental Digital Content 1, http://links.lww.com/PAS/B210).

For this analysis, all patients with SARS-CoV-2 or a clinical history of COVID-19 who had placental pathology performed during the study period were considered. Placentas from IUFD specimens were excluded due to disrupted specimens with surgical removal, as well as significant gestational age differences versus the vast majority of placentas available from SARS-CoV-2 positive women. To determine background prevalence of placental lesions in our patient population, control placentas from SARS-CoV-2 negative women during the study period were identified and included. At our institution, placental pathology for SARS-CoV-2 negative women is performed only if clinically indicated, at the discretion of the delivering provider.

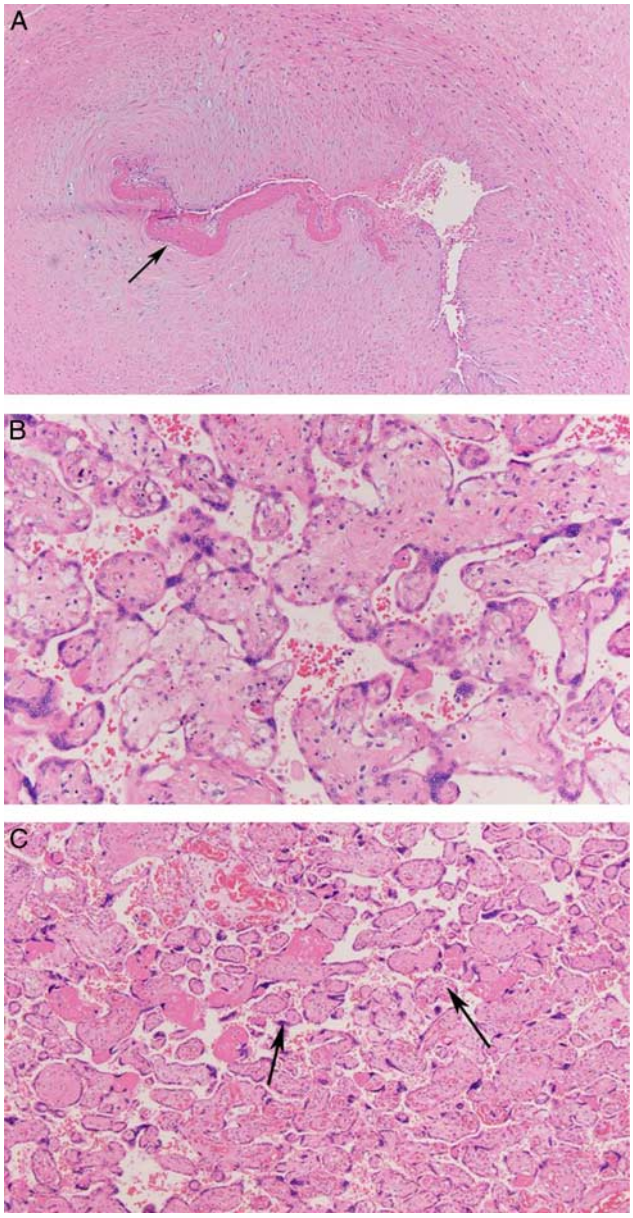

FIGURE 1. Representative FVM lesions. A, Intramural fibrin deposition: large chorionic plate vessel showing deposition of fibrin in the wall of the vessel. Hematoxylin and eosin (H&E). B, Villous stromal-vascular karyorrhexis: distal villi showing karyorrhectic debris in the villous stroma and beginning of loss of vasculature. H&E. C, Avascular villi (arrows): villi devoid of capillaries (avascular villi) with hyalinized stroma and a few remaining stromal cells present. H&E.

SARS-CoV-2 positive cases were categorized based on timing of infection relative to timing of delivery to define the primary exposure (Table 1). Acute SARS-CoV-2 was

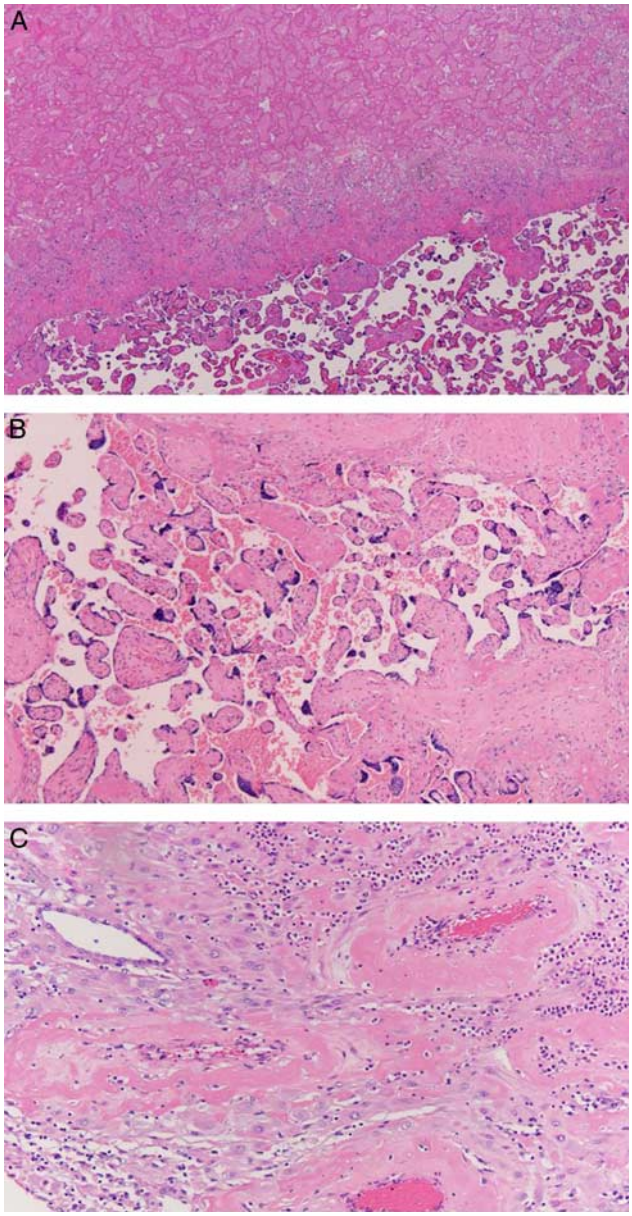

FIGURE 2. Representative MVM lesions. A, Villous infarct: complete infarction of villi (top of the image) with remaining viable villi below. Hematoxylin and eosin (H&E). B, Accelerated villous maturation: small chorionic villi and increased syncytial knots in a 37 weeks gestation. H&E. C, Decidual vasculopathy: abnormal decidual vessels demonstrating MVM. There is thickened vascular walls with deposition of fibrinoid and surround inflammation. H&E.

defined as women with a positive RT-PCR on admission for delivery who were (1) asymptomatic with no prior history of clinical symptoms of COVID-19, (2) had COVID-19 symptoms on admission, or (3) had a symptom history or laboratory-confirmed history of SARS-CoV-2 infection <14 days from admission. All patients with acute SARS-CoV-2 had negative IgG serologies, if available. Nonacute SARS-CoV-2 was defined as women with a positive or negative RT-PCR on admission for delivery who had (1) a positive RT-PCR ≥14 days before delivery admission date, (2) a history of COVID-19 symptoms ≥14 days before delivery admission date and positive IgG serologies (if available), or (3) no history of COVID-19 symptoms and IgG positive serologies (if available). The 14-day cutoff was chosen given the increased probability of antibody detection for SARS-CoV-2 infection in both the symptomatic and asymptomatic infected patients.[15,16]

The primary outcomes were incidence of FVM and MVM lesions. Secondary outcomes were incidence of chorioamnionitis, chronic villitis, meconium staining, umbilical cord abnormalities, chorangiosis, and intervillous thrombi.

We used descriptive statistics to describe differences in demographic characteristics between women with and without acute SARS-CoV-2, using parametric and nonparametric tests as appropriate. For the primary outcome analysis, we compared the incidence of FVM and MVM lesions, stratified by acute versus nonacute SARS-CoV-2, using descriptive statistics. We then included the SARS-CoV-2 negative controls and evaluated differences in demographics and histologic lesions across the 3 groups (acute, nonacute, and SARS-CoV-2 negative) using parametric and nonparametric tests.

We performed 2 sensitivity analyses to assess the validity of our findings. First, given that there is currently no accepted definition of acute or nonacute SARS-CoV-2 infection, we performed a sensitivity analysis to assess whether the relationship between the exposure and the outcomes of interest varied based on how the exposure was defined. We redefined acute SARS-CoV-2 as women with SARS-CoV-2 infection within 21 days of delivery, while nonacute SARS-CoV-2 was redefined as women with SARS-CoV-2 infection ≥21 days from delivery (Table 1). Women who could not clearly be reclassified based on the available data were excluded. We then repeated the comparison of placental histologic lesions by the newly defined exposure groups. Second, to account for lack of universal placental pathology among women without SARS-CoV-2, which may result in the selection of patients with greater degrees of clinical illness and consequently higher rates of abnormal placental pathology, we performed another sensitivity analysis to account for the inherent differences in the populations studied between SARS-CoV-2 positive patients, many of whom may otherwise be healthy, and SARS-CoV-2 negative patients. Two inverse probability of treatment weight analyses were undertaken for the outcomes of FVM and MVM. A propensity score was first estimated to predict SARS-CoV-2 status using the following covariates: gestational age < 37 weeks, asthma, diabetes, and hypertension. Once the propensity score was estimated, the inverse probability of treatment weight models were run separately to predict each outcome (FVM and MVM) using a binary exposure of SARS-CoV-2 status, positive versus negative.

No a priori sample size calculation was performed for this study. All analyses were performed in R Version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

TABLE 1. Definitions Stratifying Women With Acute Versus Nonacute SARS-CoV-2 Infection at Admission for Delivery

	Primary Analysis	Sensitivity Analysis
Acute SARS-CoV-2	Women with positive SARS-CoV-2 RT-PCR test on delivery admission and 1 or more of the following: Asymptomatic on admission and no history of symptoms during pregnancy Symptoms present on admission* History of symptoms or laboratory confirmed SARS-CoV-2 <14 d from admission date* Negative IgG serologies, if available	Women with positive SARS-CoV-2 RT-PCR test on delivery admission and 1 or more of the following: Asymptomatic on admission and no history of symptoms during pregnancy Symptoms present on admission* History of symptoms or laboratory confirmed SARS-CoV-2 <21 d of admission date* Negative IgG serologies, if available
Nonacute SARS-CoV-2	Women with positive or negative SARS-CoV-2 RT-PCR test on delivery admission and 1 or more of the following: Positive RT-PCR ≥14 d before admission date History of symptoms ≥14 d before admission date and IgG positive serologies* No history of symptoms and IgG positive serologies*	Women with positive or negative RT-PCR SARS-CoV-2 test on delivery admission and 1 or more of the following: Positive RT-PCR ≥21 d before admission date History of symptoms ≥21 d before admission date and IgG positive serologies* No history of symptoms and IgG positive serologies*

*Patient-reported symptoms of COVID-19 included fever, cough, sore throat, rhinorrhea, shortness of breath, diarrhea, other gastrointestinal symptoms, or myalgias.

Of note, the pathology of 29 placentas from the SARS-CoV-2 positive cohort were previously reported and compared with 106 placentas from SARS-CoV-2 negative women from this institution.[11,17] Here, we are presenting the placental pathology from a total of 90 placentas from SARS-CoV-2 positive pregnant women compared with 188 controls, stratified by SARS-CoV-2 acuity.

RESULTS

A total of 97 women who were SARS-CoV-2 positive were admitted for delivery during the study period, of whom 90 (92.8%) had placental pathology sent. Of these 90 placentas, 26 were from women with acute SARS-CoV-2 infection at delivery and 64 were from women with nonacute SARS-CoV-2.

Demographic and clinical characteristics are outlined in Table 2. Women with acute SARS-CoV-2 were, on average, 4 years younger than women with nonacute SARS-CoV-2 (31.5 vs. 35.5 y, $P = 0.024$). All other characteristics were similar between the acute and nonacute groups, including gestational age at delivery, mode of delivery, maternal comorbidities, birthweight, and small for gestational age. Of note, all neonates tested negative for SARS-CoV-2. When including the 188 women with placentas sent from the SARS-CoV-2 negative control group, differences across groups were noted for mode of delivery ($P = 0.010$) and parity ($P < 0.001$) (Table 2).

The primary outcome analysis demonstrated that placentas from women with acute SARS-CoV-2 had a significantly higher frequency of FVM lesions compared with placentas from women with nonacute SARS-CoV-2 (53.8% vs. 18.8%; $P = 0.002$), while frequency of MVM lesions did not differ by exposure group (30.8% vs. 29.7%; $P > 0.99$) (Table 3). Placental weight was significantly lower in the nonacute group compared with the acute group (436 vs. 484 g; $P = 0.044$). There were no differences in frequency of other histologic lesions between placentas from acute and nonacute SARS-CoV-2 cases.

TABLE 2. Clinical and Demographic Characteristics of the Study Population, Stratified by Acute Versus Nonacute SARS-CoV-2 Infection

	Nonacute SARS-CoV-2 (N = 64)	Acute SARS-CoV-2 (N = 26)	*P*	SARS-CoV-2 Negative (N = 188)	*P**
Age, mean ± SD	35.5 ± 5.11	31.5 ± 1.06	**0.024**	35.4 ± 5.32	0.562
Parity, median (IQR)	1.00 (0.00-2.00)	1.00 (1.00-2.00)	0.198	0.00 (0.00-1.00)	**< 0.001**
Gestational age at delivery, median (IQR) (wk)	39.1 (38.0-39.9)	39.0 (37.9-39.6)	0.548	39.0 (37.2-39.6)	0.336
Mode of delivery, n (%)			> 0.99		**0.010**
Vaginal delivery	44 (68.8)	18 (69.2)		93 (49.5)	
Cesarean delivery	20 (31.2)	8 (30.8)		95 (50.5)	
Chronic HTN, n (%)	1 (1.56)	1 (3.85)	0.497	8 (4.26)	0.586
Preeclampsia/gestational HTN, n (%)	5 (7.81)	3 (11.5)	0.686	33 (17.6)	0.132
Small for gestational age, n (%)	11 (17.2)	2 (7.69)	0.333	37 (19.7)	0.328
Pregestational DM, n (%)	2 (3.12)	0 (0.00)	> 0.99	8 (4.26)	0.880
Gestational DM, n (%)	3 (4.69)	0 (0.00)	0.554	16 (8.51)	0.260
Chorioamnionitis, n (%)	2 (3.12)	1 (3.85)	> 0.99	13 (6.91)	0.650
Birthweight, mean ± SD (g)	3082 ± 627	3190 ± 631	0.457	3048 ± 680	0.595

Boldface indicates significant values.
**P*-value compares across all 3 groups.
DM indicates diabetes mellitus; HTN, hypertension; IQR, interquartile range.

TABLE 3. Placental Histologic Findings Among Women With and Without SARS-CoV-2 Infection, Stratified by Acute Versus Nonacute SARS-CoV-2

	Nonacute SARS-CoV-2 (N = 64)	Acute SARS-CoV-2 (N = 26)	*P*	SARS-CoV-2 Negative (N = 188)	*P**
FVM, n (%)	12 (18.8%)	14 (53.8%)	**0.002**	23 (13.2%)	**< 0.001**
MVM, n (%)	19 (29.7)	8 (30.8)	> 0.99	42 (24.1)	0.587
Histologic evidence of chorioamnionitis, n (%)					
None	58 (90.6)	22 (84.6)	0.466	148 (78.7)	0.094
Maternal response	6 (9.38)	4 (15.4)	0.466	25 (13.3)	0.625
Fetal response	4 (6.25)	2 (7.69)	> 0.99	13 (6.91)	> 0.99
Maternal and fetal response	4 (6.25)	2 (7.69)	> 0.99	12 (6.38)	0.927
Chronic villitis, n (%)			0.564		0.258
Absent	49 (76.6)	22 (84.6)		150 (86.2)	
Low-grade	11 (17.2)	2 (7.69)		13 (7.47)	
High-grade	4 (6.25)	2 (7.69)		11 (6.32)	
Meconium staining of placenta, n (%)	26 (40.6)	17 (65.4)	0.058	63 (36.2)	**0.018**
Umbilical cord abnormalities, n (%)	3 (4.69)	2 (7.69)	0.624	21 (12.1)	0.271
Chorangiosis, n (%)	1 (1.56)	1 (3.85)	0.497	8 (4.60)	0.597
Intervillous thrombi, n (%)	7 (13.5)	2 (8.00)	0.710	25 (14.4)	0.801
Placental weight, mean ± SD (g)	436 ± 93.1	484 ± 101	**0.044**	453 ± 113	0.162

Boldface indicates significant values.
**P*-value compares across all 3 groups.

When the placentas from the SARS-CoV-2 negative control group were included, FVM lesions appeared more frequent among acute SARS-CoV-2 than either nonacute SARS-CoV-2 or control cases (53.8% vs. 18.8% vs. 13.2% for acute, nonacute and controls, respectively; $P < 0.001$) (Table 3). The frequency of MVM lesions between acute, nonacute and control placentas were not significantly different (30.8% vs. 29.7% vs. 24.1%; $P = 0.587$). In addition, distribution of the frequency of meconium staining of the placenta was different across the 3 groups (65.4% vs. 40.6% vs. 36.2% for acute, nonacute and controls, respectively; $P = 0.018$). All other outcomes were not significantly different between groups.

In the first sensitivity analysis, when infection acuity was redefined so that acute SARS-CoV-2 encompassed women with SARS-CoV-2 < 21 days from admission for delivery, there were 30 placentas from women with acute SARS-CoV-2 and 48 placentas from women with nonacute SARS-CoV-2. Twelve women could not be recategorized and were excluded. Demographic characteristics of the population were similar to the original categorizations (Appendix Table 2, Supplemental Digital Content 2, http://links.lww.com/PAS/B211). Consistent with the primary analysis, placentas from women with acute SARS-CoV-2 had a significantly higher frequency of FVM lesions compared with placentas from women with nonacute SARS-CoV-2 (53.3% vs. 18.8%; $P = 0.003$) (Appendix Table 3, Supplemental Digital Content 3, http://links.lww.com/PAS/B212). Apart from a shift to significance for frequency of meconium staining between the SARS-CoV-2 groups ($P = 0.003$), all other placental findings remained consistent with the primary analysis, including no differences in MVM by exposure group.

In the second sensitivity analysis, in which we tested whether the difference in histologic lesions across SARS-CoV-2 positive versus negative patients was driven by comorbidities among SARS-CoV-2 negative patients, the odds of FVM remained significantly higher in the placentas from women who were SARS-CoV-2 positive (odds ratio = 2.87, 95% confidence interval: 1.49-5.53, $P = 0.002$) as against SARS-CoV-2 negative women, while the odds of MVM in the placenta were not different between exposure groups (odds ratio = 1.53, 95% confidence interval: 0.84-2.79, $P = 0.165$) (Appendix Table 4, Supplemental Digital Content 4, http://links.lww.com/PAS/B213).

DISCUSSION

We found a significantly higher frequency of FVM lesions in the placentas from women infected with SARS-CoV-2 within 14 days of delivery admission compared with women infected with SARS-CoV-2 > 14 days before delivery admission; however, the frequency of MVM lesions did not differ based on timing of SARS-CoV-2 infection in relation to delivery. These findings were consistent in our sensitivity analyses, suggesting that they are not contingent on the exposure definitions. Collectively, our findings suggest that histologic lesions in the placenta may differ based on timing of SARS-CoV-2 infection during pregnancy.

The reported prevalence of FVM in the context of SARS-CoV-2 infection differs across cohorts universally tested for SARS-CoV-2. While 1 study demonstrated that only 8% of placentas from 50 SARS-CoV-2 positive women were found to have FVM lesions, another demonstrated that up to a third of placentas from 77 SARS-CoV-2 positive women had FVM lesions.[9,18] Our study demonstrates that the timing of infection, a factor not included in prior analyses, could account for the variability in the literature, as shown by the divergent rate of FVM lesions between our acute and nonacute cohorts.

Because our data suggest that placental lesions may evolve over the course of SARS-CoV-2 infection, the clinical interpretation of that lesion should take into account patient infection status at the time of delivery.

FVM encompasses multiple histologic findings of thrombosis that indicate obstruction of fetal blood flow, usually secondary to cord obstruction or a hypercoagulable state, and risk factors are similar to other coagulopathic processes.[8,12] Our findings may represent pregnancy-specific sequelae of COVID-19-associated coagulopathy, which may result from a combination of an inflammatory response to SARS-CoV-2 and microvascular injury through direct viral infection of endothelial cells.[19] The presence of FVM lesions may hint at increased risk of adverse fetal outcomes including IUGR and stillbirth.[20] Although we did not find differences in birthweight or small for gestational age between acute and nonacute groups, or when compared with the control group, we were limited by the small size of our cohort, short timeline between acute infection and delivery, and women with stillbirth were excluded. However, we did find a significantly lower placental weight among the nonacute cohort, perhaps suggesting long-term sequelae in response to SARS-CoV-2 infection, which could have clinical implications for the fetus. Although ultrasound screening for fetal growth, and consideration for antenatal testing, have been part of obstetric practice at many centers caring for women with SARS-CoV-2 in pregnancy, our findings may represent the placental basis for this practice. Given that FVM encompasses multiple histologic findings, further research is needed to understand the impact of SARS-CoV-2 infection during pregnancy on the development of the specific lesions that comprise FVM.

Our data did not support our hypothesis that MVM lesions are more prevalent among placentas from women with nonacute SARS-CoV-2 at delivery. Although a review of placental pathology associated with SARS-CoV-2 found 46% of placentas from 12 studies demonstrated evidence of MVM, only 1 study found the frequency of this lesion to be significantly different from controls.[5,6] Our rate of MVM was ~30% for both acutely and nonacutely infected women and was not different from controls. While MVM lesions are associated with hypertensive disorders of pregnancy and growth restriction, we similarly did not note an increased frequency of these clinical conditions in our cohort.

Overall, the clinical implications of histopathologic lesions in the context of the timing of SARS-CoV-2 infection in pregnancy are not fully clear. Our cohort of women with nonacute SARS-CoV-2 infection mostly reflects women with new infection during the third trimester. SARS-CoV-2 infection during earlier stages of pregnancy may influence both the fetal and maternal sides of the placenta more significantly, perhaps resulting in an increased frequency of adverse clinical outcomes. Although the placenta is capable of withstanding significant levels of insult, our findings warrant continued monitoring of women infected early in pregnancy. However, it is clear more data is needed to understand the spectrum of SARS-CoV-2 infection on FVM and MVM development and placental lesions more generally.

There are several strengths to our study. First, using a combination of clinical data, RT-PCR data, and serologic data allowed us to more closely time the true onset of infection in our study cohort. Second, our findings in our primary analysis remained valid in our sensitivity analysis where we varied the definition of acute SARS-CoV-2 infection, suggesting that the dichotomous exposure definition is not affecting our findings. Finally, we were able to capture 93% of SARS-CoV-2 affected placentas during our study period, resulting in a cohort of 90 placentas to examine, which is among the highest number of studied placentas reported in the literature to date.

Our study also has some limitations. First, lack of sample capture prevented serologic testing from being performed on all study participants. It is therefore possible that we misclassified some individuals. For instance, subjects defined as being SARS-CoV-2 negative based on a negative history and RT-PCR test may have had positive serologies and thus were actually nonacutely infected asymptomatic individuals. In addition, symptom history was obtained retrospectively at the time of admission to Labor and Delivery, introducing potential symptom recall bias. We also did not control for severity of COVID-19 or presence of symptoms in our analyses, which may also be associated with histopathologic lesions. Fourth, given that we excluded placentas from IUFD specimens, we are unable correlate the implications of our histologic findings of FVM to this clinical outcome. As with any study on placental pathology, histologic evaluation consists of representative sampling, rather than evaluation of the entire placenta, which may lead to biases in the frequency of lesion detection, although such a bias is likely at random. In addition, >1 pathologist reviewed the placentas from SARS-CoV-2 negative cases, which may introduce the possibility of interobserver differences. All placentas from SARS-CoV-2 positive women were reviewed by a single placental pathologist, to minimize interobserver variability; however, our pathologist was not blinded to the SARS-CoV-2 status, which introduces the possibility of bias in interpretation of the pathology for placentas from SARS-CoV-2 positive women. Nevertheless, the distinction between acute and nonacute cases was blinded to pathologic interpretation, limiting the potential bias of differences in frequency of lesions observed in this study.

In sum, we found that the frequency of FVM lesions is significantly higher among the placentas from women with acute SARS-CoV-2 infection compared with women with nonacute SARS-CoV-2 infection at delivery. These findings raise new questions regarding the etiology, clinical consequences, and potential downstream effects of FVM lesions in the setting of acute SARS-CoV-2 infection. Future studies with placentas from women infected across all 3 trimesters are needed to further elucidate the relationship of timing of SARS-CoV-2 infection during pregnancy and placental pathology.

REFERENCES

1. Qiao J. What are the risks of COVID-19 infection in pregnant women? *Lancet*. 2020;395:760–762.
2. Zambrano LD, Ellington S, Strid P, et al. Update: characteristics of symptomatic women of reproductive age with laboratory-confirmed

SARS-CoV-2 infection by pregnancy status-United States, January 22-October 3, 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69:1641–1647.
3. Oakes MC, Kernberg AS, Carter EB, et al. Pregnancy as a risk factor for severe coronavirus disease 2019 using standardized clinical criteria. *Am J Obstet Gynecol MFM*. 2021;3:100319.
4. Martinez-Portilla RJ, Sotiriadis A, Chatzakis C, et al. Pregnant women with SARS-CoV-2 infection are at higher risk of death and pneumonia: propensity score matched analysis of a nationwide prospective cohort (COV19Mx). *Ultrasound Obstet Gynecol*. 2021;57:224–231.
5. Sharps MC, Hayes DJL, Lee S, et al. A structured review of placental morphology and histopathological lesions associated with SARS-CoV-2 infection. *Placenta*. 2020;101:13–29.
6. Shanes ED, Mithal LB, Otero S, et al. Placental pathology in COVID-19. *Am J Clin Pathol*. 2020;154:23–32.
7. Ernst LM. Maternal vascular malperfusion of the placental bed. *APMIS*. 2018;126:551–560.
8. Khong TY, Mooney EE, Ariel I, et al. Sampling and definitions of placental lesions: amsterdam placental workshop group consensus statement. *Arch Pathol Lab Med*. 2016;140:698–713.
9. Patberg ET, Adams T, Rekawek P, et al. COVID-19 infection and placental histopathology in women delivering at term. *Am J Obstet Gynecol*. 2021;224:382.e1–382.e18.
10. Mulvey JJ, Magro CM, Ma LX, et al. Analysis of complement deposition and viral RNA in placentas of COVID-19 patients. *Ann Diagn Pathol*. 2020;46:151530.
11. Prabhu M, Cagino K, Matthews KC, et al. Pregnancy and postpartum outcomes in a universally tested population for SARS-CoV-2 in New York City: a prospective cohort study. *BJOG*. 2020;127:1548–1556.
12. Redline RW, Ravishankar S. Fetal vascular malperfusion, an update. *APMIS*. 2018;126:561–569.
13. Iwasenko JM, Howard J, Arbuckle S, et al. Human cytomegalovirus infection is detected frequently in stillbirths and is associated with fetal thrombotic vasculopathy. *J Infect Dis*. 2011;203:1526–1533.
14. Langston C, Kaplan C, Macpherson T, et al. Practice guideline for examination of the placenta: developed by the Placental Pathology Practice Guideline Development Task Force of the College of American Pathologists. *Arch Pathol Lab Med*. 1997;121:449–476.
15. Kubiak JM, Murphy EA, Yee J, et al. SARS-CoV-2 serology levels in pregnant women and their neonates. *Am J Obstet Gynecol*. 2021; 225:73.e1–73.e7.
16. Sethuraman N, Jeremiah SS, Ryo A. Interpreting diagnostic tests for SARS-CoV-2. *JAMA*. 2020;323:2249–2251.
17. Baergen RN, Heller DS. Placental pathology in Covid-19 positive mothers: preliminary findings. *Pediatr Dev Pathol*. 2020;23:177–180.
18. Gulersen M, Prasannan L, Tam Tam H, et al. Histopathologic evaluation of placentas after diagnosis of maternal severe acute respiratory syndrome coronavirus 2 infection. *Am J Obstet Gynecol MFM*. 2020;2:100211.
19. Connors JM, Levy JH. COVID-19 and its implications for thrombosis and anticoagulation. *Blood*. 2020;135:2033–2040.
20. Saleemuddin A, Tantbirojn P, Sirois K, et al. Obstetric and perinatal complications in placentas with fetal thrombotic vasculopathy. *Pediatr Dev Pathol*. 2010;13:459–464.