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ORIGINAL ARTICLE

Placental pathology, neonatal birth weight, and Apgar score in acute and distant SARS-CoV-2 infection

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ABSTRACT

Background: Most research on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection during pregnancy has been on acute infections with limited data on the effect of distant infection.

Aim: We examined placental pathology and neonatal outcomes in distant SARS-CoV-2 infection earlier in pregnancy compared to acute infections late in pregnancy/at birth and to non-SARS-CoV-2 infected patients with other placental pathologies/clinical presentations.

Methods: Placentas birthed to unvaccinated patients with SARS-CoV-2 reverse transcription–polymerase chain reaction (RT-PCR) testing and serology testing results from time of delivery were included in this study. A total of 514 singleton placentas between April 18, 2020, and July 26, 2021, were included: 77 acute SARS-CoV-2 infection (RT-PCR positive and serology negative); 222 distant SARS-CoV-2 infection (RT-PCR negative but serology IgG-positive); and 215 non-SARS-Cov-2 infected (RT-PCR negative, serology negative, and history negative) with other placental pathologies: preeclampsia/hypertension, intrauterine growth restriction (IUGR), diabetes, chorioamnionitis, and meconium. Placental pathology findings, Apgar scores, and neonatal birth weights were compared.

Results: Placentas from the acute group had significantly more villous agglutination (10.4%, $P = 0.015$) and eosinophilic T-cell vasculitis (5.2%, $P = 0.004$) compared to placentas from the distant group (2.7% and 0%) and non-SARS-CoV-2 placentas (1.9% and 0.9%). One acute case showed SARS-CoV-2 placentitis and resulted in preterm delivery at 25 weeks. Both the preeclampsia/hypertension and the IUGR groups showed significantly more maternal vascular malperfusion findings compared to the acute (6.5%, 6.5% and 1.3%) and distant (7.7%, 7.7%, and 3.2%) groups. Fetal vascular malperfusion findings such as thrombosis of fetal vessels (17.4% $P = 0.042$) and intramural fibrin deposition (21.7% $P = 0.026$) were significantly higher in the IUGR group compared to acute (7.8%; 2.6%) and distant (3.6%; 8.1%) infection. Many neonates born to patients infected with SARS-CoV-2 had birth weights outside of 95% confidence range of observed birth weights. There was no association of Apgar scores with infection status or placental pathology.

Conclusion: Acute and distant SARS-CoV-2 infections present differing placental pathology.

Relevance for Patients: SARS-CoV-2 infection during pregnancy has demonstrable effects on the placenta with potential significant impacts for maternal and fetal health. Prevention of maternal SARS-CoV-2 infection, primarily through vaccination, remains the best mitigation strategy to prevent sequelae of maternal SARS-CoV-2 infection.

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1. Introduction

The impact of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection during pregnancy remains an area of ongoing study. As the pandemic spread, some obstetrics units began the practice of universal SARS-CoV-2 reverse transcription–polymerase chain reaction (RT-PCR) testing for all patients at the time of delivery [1,2]. The subsequent histopathologic analysis of placentas from RT-PCR positive patients allowed for early insight into the impact of acute SARS-CoV-2 infection on pregnancy. Placentas in these studies ranged from asymptomatic to symptomatic patients with acute SARS-CoV-2 infection and demonstrated increases in non-specific findings associated with fetal vascular malperfusion (FVM), maternal vascular malperfusion (MVM), or villitis of unknown etiology [3-7]. Much of these studies were limited to active infections in the third trimester of pregnancy, thus analysis of placentas from patients with SARS-CoV-2 infection earlier in pregnancy is limited [8]. Some studies with placental findings of massive chronic villitis, massive fibrin deposition, and trophoblast necrosis have suggested transplacental vertical transmission can rarely occur and even more rarely lead to fetal demise [9-12]. Other prenatal infections such as cytomegalovirus, rubella, syphilis, and toxoplasma have varying levels of morbidity and risk of transmission depending on which trimester maternal infection occurs [13-15]. In addition, maternal infections such as influenza infection during pregnancy are associated with increased risk of low birth weight, premature birth, and infant death [16]. Therefore, additional research into the impact of SARS-CoV-2 infections earlier in pregnancy (first and second trimester) is warranted. Due to limitations in testing availability early in the pandemic, and asymptomatic infections in pregnancy [2,17], pregnant patients infected early in pregnancy may have gone unidentified. However, antibodies mounted against SARS-CoV-2 infection remain and can be used to detect a history of infection [18].

This study aimed to examine placental pathology and neonatal outcomes in distant SARS-CoV-2 infection remote from delivery as compared to acute infections late in pregnancy, as well as to non-SARS-CoV-2 infected patients with other placental pathologies or clinical presentations.

2. Materials and methods

A convenience sample of 514 patients who gave birth to a living singleton between April 18, 2020, and July 26, 2021, at a single New York City hospital who had a placenta sent for histologic evaluation, RT-PCR testing at time of delivery and serology testing on remnant clinical blood samples were included in this retrospective study. Placentas that were sent for pathology evaluation at the discretion of the obstetrics clinician for an indication of active or prior SARS-CoV-2 infection, preeclampsia/hypertension, intrauterine growth restriction (IUGR), diabetes, chorioamnionitis, or *in-utero* meconium [19] were included in this study (Table 1). All 514 patients had RT-PCR testing for SARS-CoV-2 infection performed at time of admission for delivery due to universal SARS-CoV-2 testing protocol implemented at the hospital [2,17]. In addition, semiquantitative serology testing (anti-Spike protein IgG) was performed on leftover clinical peripheral blood samples collected at time of delivery [17,20,21]. 513 patients were confirmed to not have received any vaccination against SARS-CoV-2 before delivery. One patient received one dose of an mRNA vaccine but tested RT-PCR positive within 3 days of receiving dose 1 and thus was considered unvaccinated at time of infection and was included in the study. 77 placentas were categorized as acute SARS-CoV-2 infection from patients who were RT-PCR positive and serology negative at time of delivery (n = 76) or within 10-days before delivery (n = 1); 222 placentas were categorized as distant SARS-CoV-2 infection from patients who were RT-PCR negative but serology IgG-positive at time of delivery; and 215 placentas from both RT-PCR negative and serology negative patients were used as samples demonstrating other placental pathology. Non-SARS-CoV-2 placentas were classified into subgroups based on a hierarchy of clinical indications (IUGR>preeclampsia/hypertension>diabetes>chorioamnionitis>meconium). Thus, if a placenta was received with an indication of IUGR it was classified in the IUGR group regardless of whether other indications such as diabetes, meconium, and chorioamnionitis were noted. Any placenta with an indication of preeclampsia and/or hypertension that was not IUGR was classified as preeclampsia/hypertension. This procedure followed for the other indications included in the study.

Placentas were grossly examined, and hematoxylin and eosin stained sections of umbilical cords, membranes, and discs, including fetal and maternal surfaces were performed using standard procedures [22]. Slides were reviewed as part of routine clinical pathologic interpretation by a perinatal pathologist. Histologic findings were classified based on the Amsterdam criteria [23], as previously described [8].

The post formalin-fixation placenta weights and neonatal birth weights were classified into four groups (<10^{th} percentile, in the range of 10^{th}–50^{th} percentile, in the range of 50^{th}–90^{th} percentile, and over 90^{th} percentile) with respect to gestational age at delivery [24,25]. We compared placenta weights and neonate birth weights (when available) from acute and distant SARS-CoV-2 infected patients using a Wilcoxon rank sum test. $P < 0.05$ was considered statistically significant.

To visualize population distributions across each group, placental weight Z scores, and neonatal birth weight Z scores were calculated per sample using the formula:

$$Z = \frac{(x - \mu)}{\sigma}$$

The distribution of placental weights and birth weights in each grouped population relative to gestational age at delivery assumes a t-distribution and is visualized by 95% confidence ellipse.

We studied incidence of pathological findings across grouped placentas using Chi-square test of independence for all categorical variables. Continuous variables were compared using Kruskal–Wallis Rank Sum Test or Wilcoxon rank sum test.

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Statistical analyses were performed using R 3.6.3, RStudio 1.1.463.

This study was approved by the institutional review board.

3. Results

3.1. Acute versus distant infection

The acute and distant infection groups had similar demographics including maternal age (mean 33.3 and 33.7 years), gestational age at birth (mean 38.5 and 38.6 weeks), and mode of delivery (67.5% and 67.1% vaginal) (Table 1).

Analysis comparing the acute and distant infections groups showed that the acute group had higher incidence of villous agglutination (10.4% vs. 2.7%, $P = 0.015$) and eosinophilic T-cell vasculitis (5.2% vs. 0%, $P = 0.004$) (Table 2). Further analysis of the acute and distant infection groups showed no statistically significant differences in other MVMs (villous infarcts, retroplacental hemorrhage, accelerated villous maturation, increased syncytial knots, decidual vasculopathy, and increased perivillous fibrin) or FVM findings (thrombosis of fetal vessels, intramural fibrin deposition, avascular villi, and villous stromal karyorrhexis) in the two SARS-CoV-2 infected cohorts (Table 2).

Although not statistically significant, there was one case of SARS-CoV-2 placentitis in a placenta from a patient with acute SARS-CoV-2 infection in our study. Similar to the previous reports [26-29], this case resulted in premature labor at 25 weeks and 3 days gestation and showed extensive chronic histiocytic-neutrophilic intervillositis with associated trophoblast necrosis and perivillous fibrin deposition. Clinically, the newborn required intubation and had Apgar scores of 1 at 1 min and 8 at 5 min. Multiple RT-PCR tests for SARS-CoV-2 were negative in the newborn and serology testing at delivery was negative for both IgM and IgG in the infant. In addition, during the study period there was a case of a maternal acute SARS-CoV-2 infection and intrauterine fetal demise at 30 weeks with evidence of massive chronic histiocytic intervillositis. However, since this study

Table 1. Patient demographics

Clinical variable	Total (*n*=514), *n* (%)	Acute (*n*=77), *n* (%)	Distant (*n*=222), *n* (%)	IUGR (*n*=23), *n* (%)	Preeclampsia/ HTN (*n*=48), *n* (%)	Diabetes (*n*=43), *n* (%)	Chorioamnionitis (*n*=65), *n* (%)	Meconium (*n*=36), *n* (%)
Maternal age (mean)	34.3 (5.5)	33.3 (5.6)	33.7 (5.9)	34.4 (4.7)	35.4 (3.9)	36.9 (5.3)	34.3 (4.9)	34.6 (5.3)
Gestational age (mean)	38.5 (2.2)	38.5 (3.0)	38.6 (1.8)	37.0 (3.0)	37.4 (2.5)	38.7 (1.1)	39.0 (2.5)	39.3 (1.5)
Mode of delivery								
Cesarean	195 (37.9)	25 (32.5)	73 (32.9)	13 (56.5)	25 (52.1)	19 (44.2)	26 (40)	14 (38.9)
Vaginal	319 (62.1)	52 (67.5)	149 (67.1)	10 (43.5)	23 (47.9)	24 (55.8)	39 (60)	22 (61.1)
Preterm birth (<37 weeks)	50 (9.7)	6 (7.8)	21 (9.5)	4 (17.4)	9 (18.8)	2 (4.7)	7 (10.8)	1 (2.8)

IUGR: Intrauterine growth restriction, HTN: Hypertension

Table 2. Placental findings in acute and distant severe acute respiratory syndrome coronavirus 2 infection

Placental pathology	Total (*n*=299), *n* (%)	Acute (*n*=77), *n* (%)	Distant (*n*=222), *n* (%)	*P*
Fetal vascular malperfusion				
Thrombosis of fetal vessels	14 (4.7)	6 (7.8)	8 (3.6)	0.236
Intramural fibrin deposition	20 (6.7)	2 (2.6)	18 (8.1)	0.161
Avascular villi	36 (12)	10 (13)	26 (11.7)	0.926
Villous stromal karyorrhexis	3 (1)	1 (1.3)	2 (0.9)	1
Maternal vascular malperfusion				
Villous infarct	46 (15.4)	6 (7.8)	40 (18)	0.05
Retroplacental hemorrhage	2 (0.7)	0	2 (0.9)	0.981
Accelerated villous maturation	22 (7.4)	5 (6.5)	17 (7.7)	0.933
Increased syncytial knots	22 (7.4)	5 (6.5)	17 (7.7)	0.933
Decidual vasculopathy	8 (2.7)	1 (1.3)	7 (3.2)	0.646
Villous agglutination	14 (4.7)	8 (10.4)	6 (2.7)	0.015*
Increased perivillous fibrin	8 (2.7)	0	8 (3.6)	0.201
Delayed villous maturation	6 (2)	2 (2.6)	4 (1.8)	1
Intervillous thrombus	71 (23.7)	16 (20.8)	55 (24.8)	0.579
Chronic histiocytic intervillositis	1 (0.3)	1 (1.3)	0	0.579
Eosinophilic T-cell vasculitis	4 (1.3)	4 (5.2)	0	0.004*
Chronic villitis	56 (18.7)	19 (24.7)	37 (16.7)	0.167
Chorangiosis	5 (1.7)	1 (1.3)	4 (1.8)	1
Low placental weight[a]	47 (15.7)	6 (7.8)	41 (18.5)	0.039*

[a]Low placental weight: <10th percentile for gestational age, *P <0.05

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excluded cases of intrauterine fetal demise it was not included in the analyses.

3.2. All populations

Additional population-based analysis of acute and distant SARS-CoV-2 infection groups compared with other placental pathologies including preeclampsia/hypertension, IUGR, diabetes, chorioamnionitis, and *in utero* meconium showed that patients with preeclampsia/hypertension and IUGR trended toward more Cesarean delivery (52.1% and 56.5%, $P = 0.069$) (Table 1).

Analysis of placental findings showed that the preeclampsia/hypertension and IUGR groups both had a higher incidence of MVMs such as accelerated villous maturation (27.1% and 26.1% $P < 0.001$), increased syncytial knots (27.1% and 39.1% $P < 0.001$), and decidual vasculopathy (12.5% and 13% $P = 0.004$). The IUGR group had more FVM findings such as thrombosis of fetal vessels (17.4% $P = 0.042$), and intramural fibrin deposition (21.7% $P = 0.026$) (Table 3). Analysis of the total number of different FVMs and MVMs seen per placenta showed that there is a greater proportion of multiple MVM findings observed among preeclampsia/hypertension and IUGR groups (29.2% and 39.1% $P < 0.001$) (Figure 1). Eight of 77 (10.4%) patients from the acute infection group and 41 of 222 (18.5%) patients from the distant infection group had a comorbidity of preeclampsia/hypertension and/or IUGR, and a sub-analysis excluding those cases did not alter the findings (data not shown).

Placental weight adjusted for gestational age was examined for each group. The incidence of low placental weight (<10th percentile) for gestational age is significantly different across groups ($P < 0.001$), with the highest incidence of low placental weight observed among IUGR (65.2%), preeclampsia/hypertension (25%), *in-utero* meconium (27.8%), and distant SARS-CoV-2

Table 3. Placental and neonatal findings in acute, distant and non-severe acute respiratory syndrome coronavirus 2 infected patients

Placental pathology	Total (*n*=514), *n* (%)	Acute (*n*=77), *n* (%)	Distant (*n*=222), *n* (%)	IUGR (*n*=23), *n* (%)	PREC/HTN (*n*=48), *n* (%)	Diabetes (*n*=43), *n* (%)	Chorio (*n*=65), *n* (%)	MEC (*n*=36), *n* (%)	*P*
Fetal vascular malperfusion									
Thrombosis of fetal vessels	29 (5.6)	6 (7.8)	8 (3.6)	4 (17.4)	5 (10.4)	2 (4.7)	1 (1.5)	3 (8.3)	0.042*
Intramural fibrin deposition	34 (6.6)	2 (2.6)	18 (8.1)	5 (21.7)	2 (4.2)	3 (7)	4 (6.2)	0	0.026*
Avascular villi	58 (11.3)	10 (13)	26 (11.7)	2 (8.7)	5 (10.4)	5 (11.6)	9 (13.8)	1 (2.8)	0.746
Villous stromal karyorrhexis	7 (1.4)	1 (1.3)	2 (0.9)	1 (4.3)	0	1 (2.3)	0	2 (5.6)	0.207
Maternal vascular malperfusion									
Villous infarct	84 (16.3)	6 (7.8)	40 (18)	7 (30.4)	8 (16.7)	7 (16.3)	9 (13.8)	7 (19.4)	0.206
Retroplacental hemorrhage	5 (1)	0	2 (0.9)	1 (4.3)	0	0	1 (1.5)	1 (2.8)	0.444
Accelerated villous maturation	50 (9.7)	5 (6.5)	17 (7.7)	6 (26.1)	13 (27.1)	1 (2.3)	6 (9.2)	2 (5.6)	<0.001*
Increased syncytial knots	53 (10.3)	5 (6.5)	17 (7.7)	9 (39.1)	13 (27.1)	3 (7)	4 (6.2)	2 (5.6)	<0.001*
Decidual vasculopathy	21 (4.1)	1 (1.3)	7 (3.2)	3 (13)	6 (12.5)	3 (7)	1 (1.5)	0	0.004*
Villous agglutination	18 (3.5)	8 (10.4)	6 (2.7)	1 (4.3)	2 (4.2)	1 (2.3)	0	0	0.019*
Increased perivillous fibrin	18 (3.5)	0	8 (3.6)	0	3 (6.2)	2 (4.7)	3 (4.6)	2 (5.6)	0.474
Delayed villous maturation	18 (3.5)	2 (2.6)	4 (1.8)	1 (4.3)	2 (4.2)	4 (9.3)	1 (1.5)	4 (11.1)	0.037*
Intervillous thrombus	101 (19.6)	16 (20.8)	55 (24.8)	1 (4.3)	6 (12.5)	8 (18.6)	7 (10.8)	8 (22.2)	0.059
Chronic histiocytic intervillositis	1 (0.2)	1 (1.3)	0	0	0	0	0	0	0.459
Eosinophilic T-cell vasculitis	6 (1.2)	4 (5.2)	0	0	0	0	2 (3.1)	0	0.008*
Chronic villitis	91 (17.7)	19 (24.7)	37 (16.7)	4 (17.4)	5 (10.4)	8 (18.6)	14 (21.5)	4 (11.1)	0.397
Chorangiosis	13 (2.5)	1 (1.3)	4 (1.8)	1 (4.3)	2 (4.2)	2 (4.7)	3 (4.6)	0	0.59
Low placental weight[a]	89 (17.3)	6 (7.8)	41 (18.5)	15 (65.2)	12 (25)	3 (7)	2 (3.1)	10 (27.8)	<0.001*
Small for gestational age birthweight[b]	61 (11.9)	5 (6.5)	28 (12.6)	12 (52.2)	4 (8.3)	1 (2.3)	6 (9.2)	5 (13.9)	<0.001*
Apgar score 1 min									
6	11 (2.1)	0	4 (1.8)	0	0	4 (9.3)	2 (3.1)	1 (2.8)	0.339
7	18 (3.5)	1 (1.3)	7 (3.2)	0	3 (6.2)	1 (2.3)	4 (6.2)	2 (5.6)	
8	78 (15.2)	8 (10.4)	39 (17.6)	5 (21.7)	8 (16.7)	6 (14)	8 (12.3)	4 (11.1)	
9	299 (58.2)	42 (54.5)	122 (55)	15 (65.2)	31 (64.6)	26 (60.5)	40 (61.5)	23 (63.9)	
Apgar score 5 min									
6	1 (0.2)	0	0	0	0	0	1 (1.5)	0	0.719
7	8 (1.6)	1 (1.3)	4 (1.8)	0	0	0	3 (4.6)	0	
8	24 (4.7)	2 (2.6)	7 (3.2)	0	2 (4.2)	5 (11.6)	5 (7.7)	3 (8.3)	
9	384 (74.7)	50 (64.9)	164 (73.9)	20 (87)	41 (85.4)	33 (76.7)	49 (75.4)	27 (75)	

[a]Low placental weight: <10th percentile for gestational age, [b]Small for gestational age birthweight: <10th percentile for gestational age using Fenton curves. IUGR: Intrauterine growth restriction, PREC: Preeclampsia, HTN: Hypertension, Chorio: Chorioamnionitis, MEC: Meconium, *P <0.05

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infection (18.5%) groups (Table 3). Distant infections led to significantly more placentas with low weight compared to acute infections (χ^2 4.25; P = 0.039). 10/77 (13.0%) placentas from acute SARS-CoV-2 infected patients and 31/220 (14.1%) placentas from distant SARS-CoV-2 infected patients fell outside of the 95% confidence ellipse (Figure 2).

Comparison of birthweight adjusted for gestational age at delivery revealed a high prevalence of small for gestational age

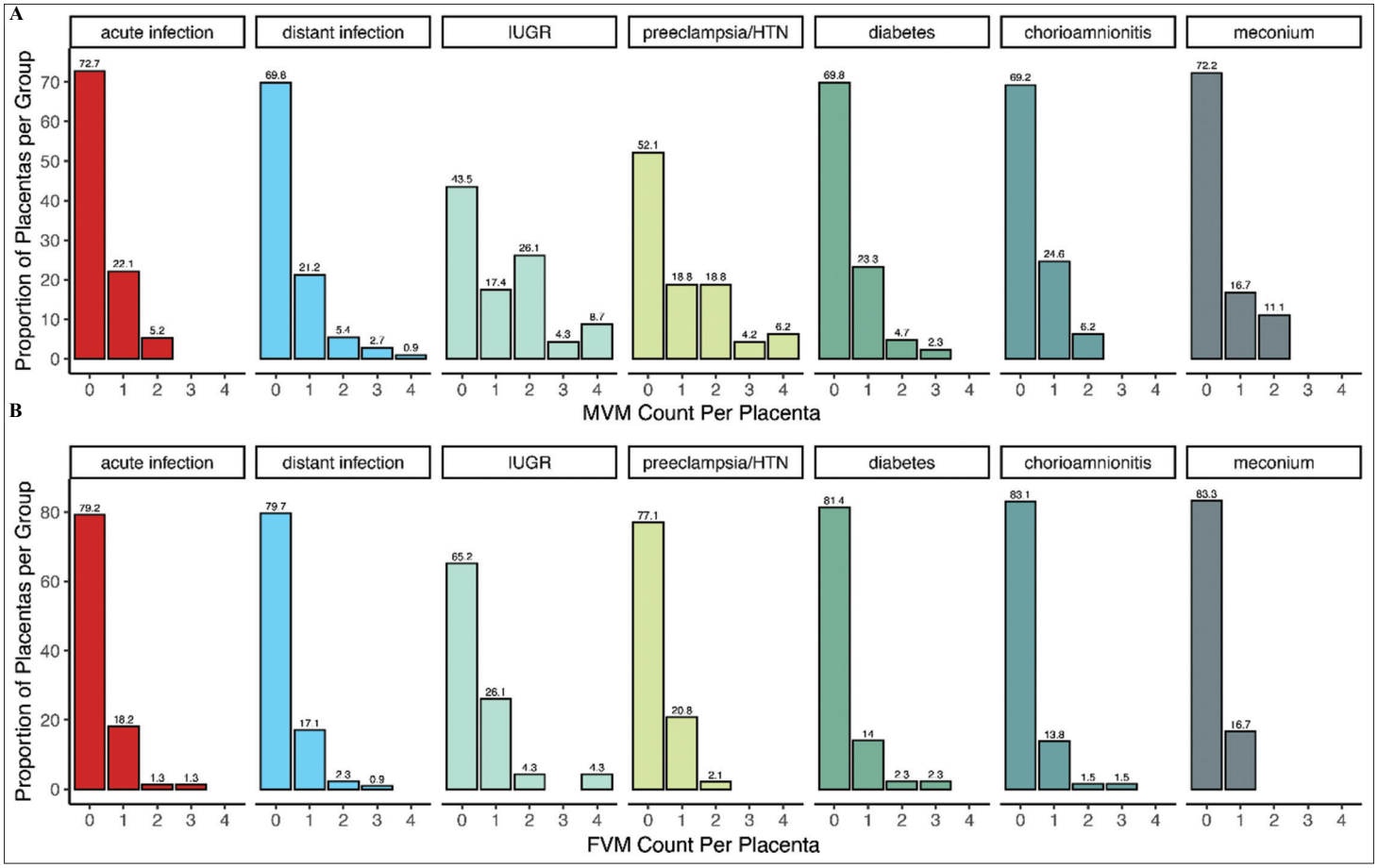

Figure 1. Tabulation of MVM and FVM findings in acute, distant and non-SARS-CoV-2 infected groups. Cumulative count of findings associated with MVM or FVM in acute, distant and non-SARS-CoV-2 control groups. MVM: Maternal vascular malperfusion, FVM: Fetal vascular malperfusion, IUGR: Intrauterine growth restriction, HTN: Hypertension.

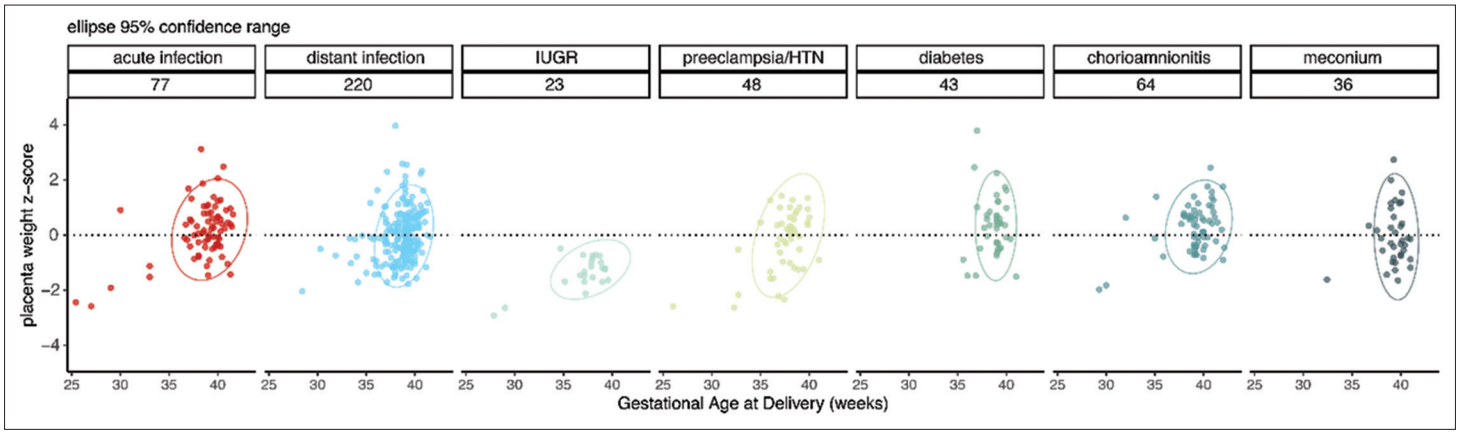

Figure 2. Post-fixation placenta weight in acute, distant and non-SARS-CoV-2 infected groups. Scatter plot of placental weight (Z-score) versus gestational age at time of delivery (weeks). Data points outside of ellipse fall outside of 95% confidence area. IUGR: Intrauterine growth restriction, HTN: Hypertension.

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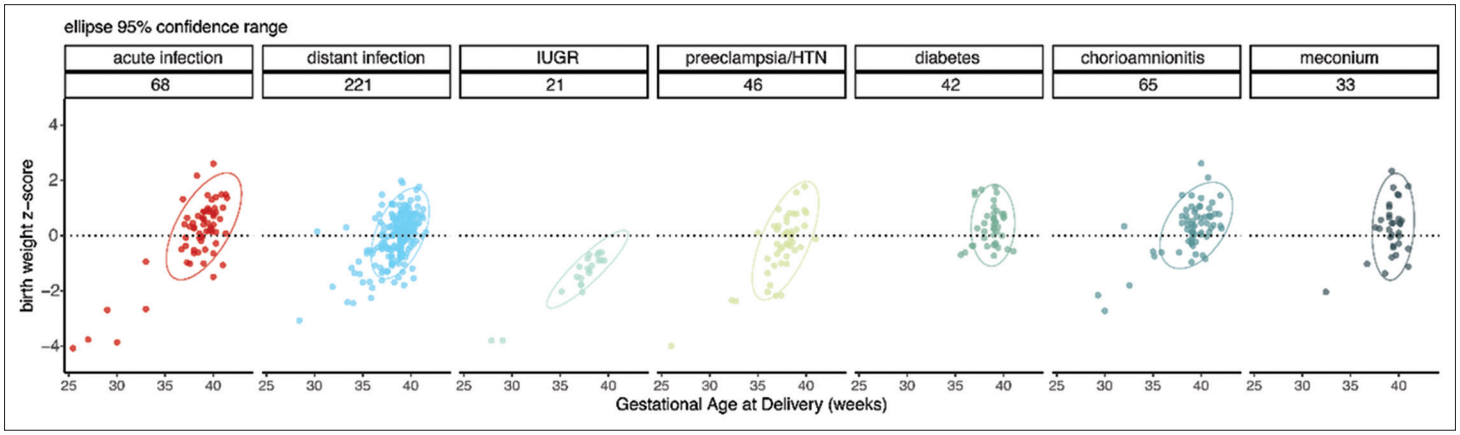

Figure 3. Neonatal birth weights from acute, distant and non-SARS-CoV-2 infected groups. Scatter plot of neonatal weight (Z-score) versus gestational age at time of delivery (weeks). Data points outside of ellipse fall outside of 95% confidence area. IUGR: Intrauterine growth restriction, HTN: Hypertension.

(<10th percentile) babies born to the IUGR (52.2%), *in utero* meconium (13.9%), distant SARS-CoV-2 infection (12.6%), preeclampsia/hypertension (8.3%), and acute SARS-CoV-2 infection (6.5%) groups ($P < 0.001$) (Table 3). 11/68 (16.2%) neonates born to acute SARS-CoV-2 infected patients had weights outside of the 95% confidence ellipse, and 32/221 (14.5%) neonates born to distant SARS-CoV-2 infected patients had weights outside of the 95% confidence ellipse of observed birth weights (Figure 3). There was no association of Apgar scores with infection status or placental pathology (Table 3).

To account for the possibility that these findings may be from patients who are serology positive from an infection prior to pregnancy, a sub-analysis on deliveries between March and December 2020, where patients could only have been infected during pregnancy due to the onset of the pandemic in March 2020 was performed, and the results remain unchanged.

4. Discussion

To date there has been limited analysis on the impact of SARS-CoV-2 infection early in pregnancy on placental pathology and outcomes. Distant SARS-CoV-2 infection had significantly fewer findings of villous agglutination and eosinophilic T-cell vasculitis compared to acute SARS-CoV-2 infection, and these findings remained statistically significant when compared to non-SARS-CoV-2 placentas. While villous agglutination is a finding seen in approximately 2% of term pregnancies with normal outcomes [30], it is indicative of ischemic changes in the spectrum of MVM, occurring before development of a villous infarct. Interestingly, though not statistically significant ($P = 0.05$), infarctions were more commonly seen in the distant SARS-CoV-2 infection group (18%), compared to acute infection (7.8%), and perhaps indicating a temporal evolution of these lesions during gestation (Table 2).

Conversely, eosinophilic T-cell vasculitis is an uncommon chronic inflammatory condition with an estimated incidence of 0.2–0.7% [31]. A previous study [32] has reported eosinophilic T-cell vasculitis in a single placenta from a patient with SARS-CoV-2 infection. Histologically, these lesions demonstrate large fetal vessels, often chorionic plate vessels, with eosinophils, histiocytes and T-cells present within the vascular walls [31,33]. While these lesions can occur in combination with villitis of unknown etiology, they are often an isolated finding. Research has shown that the inflammatory cells in eosinophilic T-cell vasculitis are fetal [33]. Although specific clinical findings associated with this placental pathology remain uncertain, given the fetal origin of this inflammation, it is possible that in the context of SARS-CoV-2 infection, eosinophilic T-cell vasculitis indicates direct placental infection with a fetal inflammatory response. Recent studies have shown that stillbirths associated with SARS-CoV-2 demonstrate SARS-CoV-2 placentitis, a triad of chronic histiocytic intervillositis, perivillous fibrin deposition, and trophoblast necrosis [28,29]. During our study period we did note two cases with such pathology, one resulting in premature labor at 25 weeks and one with intrauterine fetal demise at 30 weeks.

Preeclampsia and maternal hypertension are known risk factors for MVM. Placental pathologies such as accelerated villous maturation, increased syncytial knots, and decidual vasculopathy were present significantly more in non-SARS-CoV-2 placentas from patients with preeclampsia/hypertension and IUGR compared to both acute and distant infection groups. Similarly, FVM findings (thrombosis of fetal vessels and intramural fibrin deposition) were more common in non-SARS-CoV-2 IUGR placentas than those in the acute or distant infection groups.

Like most placental pathology, MVM and FVM occur on a spectrum where the greater number of ischemic changes affecting a greater percentage of the placenta leads to more significant findings. By tabulating the number of findings for each placenta which are considered a type of MVM or FVM, we can visualize the increased burden of MVM in the preeclampsia/hypertension group compared to the acute and distant infection groups (Figure 1). In this study, 29.2% of placentas from the preeclampsia/hypertension and 39.1% of placentas from the IUGR group had multiple MVM findings while only 5.2% of acute infection and 9% of distant infection did. For comparison, in the literature, only 7.4% of term placentas with normal outcomes showed two or more features of

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MVM [30]. These findings suggest that the majority of mild acute and distant SARS-CoV-2 infection during pregnancy do not show significant MVM or FVM comparable to conditions associated with sequelae of MVM and FVM.

Compared to prior studies, this study did not show increased FVM among the acute or distant SARS-CoV-2 infection groups. These findings can be potentially attributed to differences between the study designs. The previous studies have relied on either solely RT-PCR testing or lack of symptomatic history without serology confirmation. In contrast, in this study, we utilize serology data in addition to RT-PCR testing, along with patient history to better capture past SARS-CoV-2 infection. In addition, non-infected controls were RT-PCR and serology negative. Given the high proportion of asymptomatic SARS-CoV-2 infection and limited testing early in the pandemic, it is possible that in other studies the control groups may have contained individuals with a history of distant infection. Our work is also an expansion on the previous publication from our institution, with the inclusion of a larger and minimally overlapped cohort between the Glynn *et al*. [8] study and our study. Glynn *et al*. predominantly included samples from March 2020; while the earliest sample in our study is from late April 2020 and extends through July 2021. Only 28 of 299 placentas from SARS-CoV-2 infected patients, and 32 of 215 placentas from non-SARS-CoV-2 infected patients were shared between the two study groups. In addition, our cohort did not include any of the placentas that the previous study categorized as acute infections. A recent study examining placental pathology in 67 non-acute, recovered COVID-19 patients [34] in which 49% of cases had pregnancy complications including gestational hypertension and diabetes, reported increased MVM and FVM findings compared to a cohort of GBS-colonized patients; however, there was no evidence of SARS-CoV-2 placentitis or current trophoblast viral infection. As previously described our study design of utilizing serology data in addition to RT-PCR testing, along with patient history to capture past SARS-CoV-2 infection including patients who may have been asymptomatic or not RT-PCR tested. In addition, there was a relatively low incidence of comorbidities such as preeclampsia/hypertension and/or IUGR among our acute and distant SARS-CoV-2 infection groups (10.4% and 18.5%, respectively). It is possible that in pregnant patients with comorbidities that predispose to MVM and FVM findings, SARS-CoV-2 infection leads to more pronounced MVM and FVM placental pathologies.

Placental pathology is inherently linked to neonatal outcomes. An extensive analysis by Badr *et al*. [35] found that SARS-CoV-2 infection in late second and early third trimesters increased the risk for adverse obstetric (preterm delivery, cesarean delivery, postpartum hemorrhage, and deep vein thrombosis or pulmonary embolism) and neonatal outcomes (fetal distress during labor, NICU admission, Apgar scores of <7 at 5 min, umbilical artery pH abnormalities, and low birthweight). In this study, Apgar scores were similar between the acute and distant groups with an average 1 min score of 8 and a 5 min score of 9 for both groups, although we were unable to accurately time the infections for the acute and distant groups. The distant SARS-CoV-2 infection group had 18.6% placentas with low placental weight and 12.7% small for gestational age neonatal birth weights; although the distant infection group still had fewer low placental weights and small for gestational age birth weights compared to the IUGR group ($P < 0.001$).

While clinically, some COVID-19 symptoms may overlap with varied clinical presentations of other pathologies, the distinct findings in placental pathology in the different cohorts confirm that these processes have different pathophysiology and progression. Our findings of the changes in placental pathology in distant and acute infections as well as placental weight and neonatal weight highlight the importance of studying whether additional clinical surveillance of pregnancies might identify clinical and placental pathologies in the setting of COVID-19, especially given the known presence of asymptomatic infections in pregnant patients. It is important to note that in our cohort of patients infected with SARS-CoV-2, of those documented with symptomatic infections, most had mild disease; thus, it is likely that severe infections could lead to a more drastic impact on both placental pathology and neonatal outcomes.

The distinct histological findings between acute and distant SARS-CoV-2 infections highlight the need for further studies to determine if there are differing placental findings depending on which trimester SARS-CoV-2 infection occurs. In addition, the possibility of distinguishing the effects of SARS-CoV-2 infection with more severe clinical presentation or long-haul symptoms compared to those without on placental findings could be beneficial. The above findings argue for a need to expand longitudinal neonatal follow-up to include all babies with putative *in utero* SARS-CoV-2 exposure as compared to only those born to acutely infected mothers.

Our paper is a large study of placentas with more distinct subcategorization of both SARS-CoV-2 infection and other non-SARS-CoV-2 placental pathology populations. We utilize both RT-PCR and serology testing. There are several limitations to our study. First, due to the nature of routine clinical pathologic interpretation, the perinatal pathologists reviewing the cases were not blinded to the SARS-CoV-2 RT-PCR testing status of the mother. However, serology testing results were not known at the time of pathologic interpretation. In addition, the placental weights are all post formalin-fixation due to SARS-CoV-2 precautions. While this may slightly increase the placental weight compared to a fresh weight; all placentas were fixed under the same protocol. Since our study is based on convenience sampling of placentas which are sent for placental pathology at the discretion of the treating clinicians, there is also inherent sampling bias. This study does not have comparisons to completely "normal" controls since placentas are only sent for pathologic evaluation due to a clinician indication, that is, preeclampsia, IUGR, and *in utero* meconium. By comparison, most of the placentas in the acute or distant SARS-CoV-2 groups would not have otherwise been sent for pathologic evaluation if they did not have a clinical history of SARS-CoV-2 infection. This dichotomy limits the comparability between SARS-CoV-2 and non-SARS-CoV-2 groups. In addition, while the serology testing provided a more precise way of

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identifying distant SARS-CoV-2 infection, we could not further pinpoint the timing of infection. We could only classify patients as acute or distantly infected but could not exactly determine when someone was infected or in which trimester infection occurred as only 15 patients out of our distant cohort had documented dates of RT-PCR positive test results. There is a possibility that with the onset of the pandemic in our care area in early 2020, mothers who delivered after December 2020 maybe have been infected before pregnancy. Given this possibility, a sub-analysis limiting deliveries between March and December 2020 was performed and the conclusions remain unchanged. In addition, patients are likely to revert to seronegative status with more time elapsed since infection so we may have also missed some distant infections.

5. Conclusion

Based on the parameters collected in this study, villous agglutination and eosinophilic T-cell vasculitis were associated with acute SARS-CoV-2 infection during pregnancy and in rare cases were suggestive of placental involvement. Although rare, SARS-CoV-2 placentitis was identified, which has been previously linked to severe adverse outcomes such as premature delivery and intrauterine fetal demise. Some maternal and FVM findings were significantly more prevalent in the preeclampsia/hypertension and/or IUGR groups compared to both the acute and distant SARS-CoV-2 infection. Placentas from distant infections had significantly more low placental weights for gestational age compared to acute infections. Many neonates born to SARS-CoV-2 infected patients had birth weights outside of the 95% confidence range of birth weights; though Apgar scores were high and similar across groups. Primary prevention of maternal SARS-CoV-2 infection, primarily through vaccination, remains the best mitigation strategy to prevent sequelae of maternal SARS-CoV-2 infection.

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Conflict of Interest

The authors declare that there are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

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