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Detection of SARS-CoV-2 in placentas with pathology and vertical transmission

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Running title: SARS-CoV2 in placenta and vertical transmission

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Author contribution:

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Objective

COVID19 pandemic in New York City causes significant mortalities in adult population. Pregnant women at late gestational age positive for SARS-CoV-2 showed mild symptoms in comparison to non-pregnant adults. Rare cases of newborn babies testing positive for the SARS-CoV2 virus within first 24 hours have been reported [1, 2]. Miscarriages in second trimesters were also reported related to maternal COVID19 [3]. Placental pathology has been studied in small case series with controversial results and the vertical transmission was reported in some cases [4-6]. We sought to examine the placental pathology and to localize the SARS-CoV2 viral particles within the placental tissue.

Study design

The case series study was approved by the institutional review board. The placentas were submitted to routine pathology examination based on the institutional criteria of maternal and fetal conditions established previously. All placentas were fixed in formalin overnight and standard

four sections were taken for light microscopy. Routine pathology examination was performed based on the current Amsterdam placental examination guideline. Clinical and pathological features were collected and statistically analyzed using various programs of R-package. SARS-CoV2 in-situ hybridization was performed by using RNAscope reagent kit containing specific probe against viral M-spike RNA and Leica Bond III automated instrument following the manufacturer's instruction. Immunostaining for CD68 and CD42b was performed to highlight the tissue macrophages and platelet aggregates using the Leica Bond III automated instrument for routine clinical application as described previously.

Results

We examined 364 consecutive placentas from the mothers tested in our facilities since the universal testing policy was adopted in March 2020 including 74 positive and 290 negative for SARS-CoV2 by nasopharyngeal swab PCR method as previously described [1]. Pathological examination of the placentas after delivery was performed and the clinical characteristics of the mothers were similar to those described previously [1]. The placental pathological findings and clinical characteristics were listed in Table 1. There were no specific histopathologic features within the placentas to maternal SARS-CoV2

infection. The clinical diagnoses of preeclampsia and category 2 fetal heart tracing were shown to be negatively associated with COVID19 (Table 1). The odds ratio of specific placental and clinical features from COVID19 positive and negative mothers were calculated using the generalized linear model of R-package (Poisson model) (Figure 1) and the placental weight and gestational age distributions were plotted using conditional plot of R-package (Figure 2). SARS-CoV2 viral particles were tested within the placental tissue by automated in-situ hybridization (ISH) using specific COVID19 probe (RNAscope, Newark, CA). SARS-CoV2 viral particles can be demonstrated within the syncytiotrophoblasts associated with or without placental infarcts (Figure 3). There were increased maternal macrophages and platelet aggregates (thrombosis) surrounding the infarcts positive for CD68 and CD42b (Figure 3). SARS-CoV2 viral RNA signals were also detected within the atrophic endometrial glandular epithelium (Figure 3, bottom panel) and subchorionic plate (Langhan's fibrinoid) (Figure 4). No viral signals were identified within any other maternal, fetal or placental cell types. Totally 53 placentas from positive mothers were tested and there were two placentas positive for SARS-CoV-2 by ISH (2/53, 3.8%). No positive placentas were identified by ISH in 10 placentas from PCR-negative mothers. One positive placenta for SARS-CoV2 by ISH was delivered by C- section at 35 weeks 6 days due to placental previa

associated with placental infarcts, and the newborn baby was tested positive by swab PCR at 24 hours, 48 hours and 7 days. The neonate was asymptomatic and discharged home. The other positive placenta was from a mother with 40 week gestation associated with no significant clinical and pathological features, and the baby was tested negative for SARS-CoV2 by swab PCR method within the first 24 hours. ISH test for this placenta showed the presence of viral particles only within the endometrial glands but not in the syncytiotrophoblasts. All other neonates were tested negative by nasopharyngeal swab PCR methods.

Discussion

The current study showed that SARS-COV2 viral particles are uncommon in placentas from PCR-positive mothers at late gestation. There appears largely no relationship between the maternal COVID19 status and placental pathology. Neonatal testing for SARS-CoV2 by swab PCR also showed rare positive cases from positive mothers. The negative association between the COVID19 status of the mothers and preeclampsia and category 2 fetal heart tracing clinically raised the possibility of disruption or de-coupling of oxygen sensing within the maternal tissue by the SARS-CoV2 viral infection, but the mechanism of viral pathogenesis remains unclear. The presence of SARS-CoV2 in

placenta suggests a high probability of vertical transmission in utero through fetoplacental circulation. Testing blood of the newborn instead of nasopharyngeal swab will likely yield more information of potential vertical transmission. Currently no clinical testing capability is available for testing SARS-CoV2 viral load (titer) within blood of either the mother or the fetus, and such information will likely be important in understanding the transient maternal and fetal viremic conditions. The presence of SARS-CoV2 viral particles within the atrophic endometrial glands raised the question of potential effects of SARS-CoV2 infection at early gestation and requires further study. Strictly, the endometrial glands are maternal tissues and the presence of the viral particles in endometrial glands should not be counted as transmission to fetal tissue, although the endometrial tissue is attached to the delivered placenta. The viral signals at the subchorionic plate (Langhan's stria, fibrinoid) and surrounding areas of infarcts with thrombosis raised the question of potential localiztion of SARS-CoV2 in platelets, but a limited number of ISH tests showed no SARS-CoV2 virus within the subcutaneous microthrombi and the bone marrow from COVID19 patients (not shown). Localization of SARS-CoV2 within the tissue using ISH test will likely provide critical information for better understanding of viral pathogenesis.

References:

1. Sutton, D., et al., *Universal Screening for SARS-CoV-2 in Women Admitted for Delivery.* N Engl J Med, 2020.
2. Chen, L., et al., *Clinical Characteristics of Pregnant Women with Covid-19 in Wuhan, China.* N Engl J Med, 2020.
3. Baud, D., et al., *Second-Trimester Miscarriage in a Pregnant Woman With SARS-CoV-2 Infection.* JAMA, 2020.
4. Patanè, L., et al., *Vertical transmission of COVID-19: SARS-CoV-2 RNA on the fetal side of the placenta in pregnancies with COVID-19 positive mothers and neonates at birth.* Am J Obstet Gynecol MFM, 2020: p. 100145.
5. Shanes, E.D., et al., *Placental Pathology in COVID-19.* Am J Clin Pathol, 2020.
6. Penfield, C.A., et al., *Detection of SARS-COV-2 in Placental and Fetal Membrane Samples.* Am J Obstet Gynecol MFM, 2020: p. 100133.

Figure legend:

Figure 1. Summary of Odds ratios of clinical and pathological findings of the 364 placentas from COVID19 positive and COVID19 negative mothers generated by using generalized linear model of R – package using the same data in Table 1.

Figure 2. Conditioning plot of gestational age and placental weight distributions from the 364 COVID19 negative and COVID19-positive mothers delivered pre-term (before 37 weeks) and term (37 weeks or later) using R-package.

Figure 3. H& E stains of placental villous tissue with placental infarcts and immunostaining for CD42b and CD68 for platelet aggregates and macrophages of 36 week placenta with ISH against specific SARS-CoV2. The bottom panel showed endometrial glands from the second positive patient by ISH (200x magnification).

Figure 4. H&E stain of placental subchorionic plate (Langhan's fibrinoid) with positive ISH signals (400x magnification).

Table 1. Baseline characteristics of clinical and pathologic findings of placentas from COVID19 positive and COVID19 negative mothers. 0 and -1 denote absence or presence of the specific feature. Cord issues include marginal or velamentous insertion, two vessel cord, true or false knots. Other issues include polyhydramnios, maternal history of 7 cholestasis, cancers, autoimmune diseases, thyroid diseases, IBDs, placental increta/percreta, previa, twins, MFI – maternal floor infarction (massive perivillous fibrinoid deposit). Lymphopenia denoted the lymphocyte percentage below the reference range (15.5%) at time of admission before delivery (reference 15.5-47.1%).

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Table 1: Characteristics of Clinical and Placental features of COVID19 mothers

COVID19 (Placental pathology)	Negative (N=290)	Positive (N=74)	p value
Vasculopathy			0.729
- Classic type	119 (41.0%)	25 (33.8%)	
- Mixed type	25 (8.6%)	7 (9.5%)	
- Mural hypertrophy	14 (4.8%)	4 (5.4%)	
- No vasculopathy	132 (45.5%)	38 (51.4%)	
Weight	427.5 [366.0;526.0]	437.0 [390.0;519.0]	0.492
Gestational.age	39.0 [38.0;40.0]	39.0 [38.0;40.0]	0.705
Infarcts			1.000
0	265 (91.4%)	67 (90.5%)	
-1	25 (8.6%)	7 (9.5%)	
Chorioamnionitis			0.981
0	99 (34.1%)	26 (35.1%)	
-1	191 (65.9%)	48 (64.9%)	
Meconium			0.459
0	180 (62.1%)	50 (67.6%)	
-1	110 (37.9%)	24 (32.4%)	
Thrombosis			1.000
0	217 (74.8%)	56 (75.7%)	
-1	73 (25.2%)	18 (24.3%)	
Villitis			1.000
0	225 (77.6%)	57 (77.0%)	
-1	65 (22.4%)	17 (23.0%)	
Abruption			0.965
0	281 (96.9%)	71 (95.9%)	
-1	9 (3.1%)	3 (4.1%)	
Cord.issues			0.143
0	271 (93.4%)	73 (98.6%)	
-1	19 (6.6%)	1 (1.4%)	
MFI			0.555
0	275 (94.8%)	72 (97.3%)	
-1	15 (5.2%)	2 (2.7%)	
Others			0.946
0	258 (89.0%)	65 (87.8%)	
-1	32 (11.0%)	9 (12.2%)	
Cord coiling	3.0 [2.0; 5.0]	3.0 [2.0; 4.0]	0.428
Avascular.villi			0.614
0	277 (95.5%)	69 (93.2%)	
-1	13 (4.5%)	5 (6.8%)	
COVID19 (Clinical features)	Negative	Positive	p value
Delivery			0.048
- C	117 (40.3%)	20 (27.0%)	
- V	173 (59.7%)	54 (73.0%)	
Preeclampsia			0.003
0	240 (82.8%)	72 (97.3%)	
-1	50 (17.2%)	2 (2.7%)	
GDM2			0.449
0	264 (91.0%)	70 (94.6%)	
-1	26 (9.0%)	4 (5.4%)	

Table 1: Characteristics of Clinical and Placental features of COVID19 mothers

Category.2			0.000
	0	226 (77.9%)	74 (100.0%)
	-1	64 (22.1%)	0 (0.0%)
IUGR			0.632
	0	276 (95.2%)	72 (97.3%)
	-1	14 (4.8%)	2 (2.7%)
Lymphopenia			0.495
	0	143 (70.1%)	52 (75.4%)
	-1	61 (29.9%)	17 (24.6%)

Figure 1

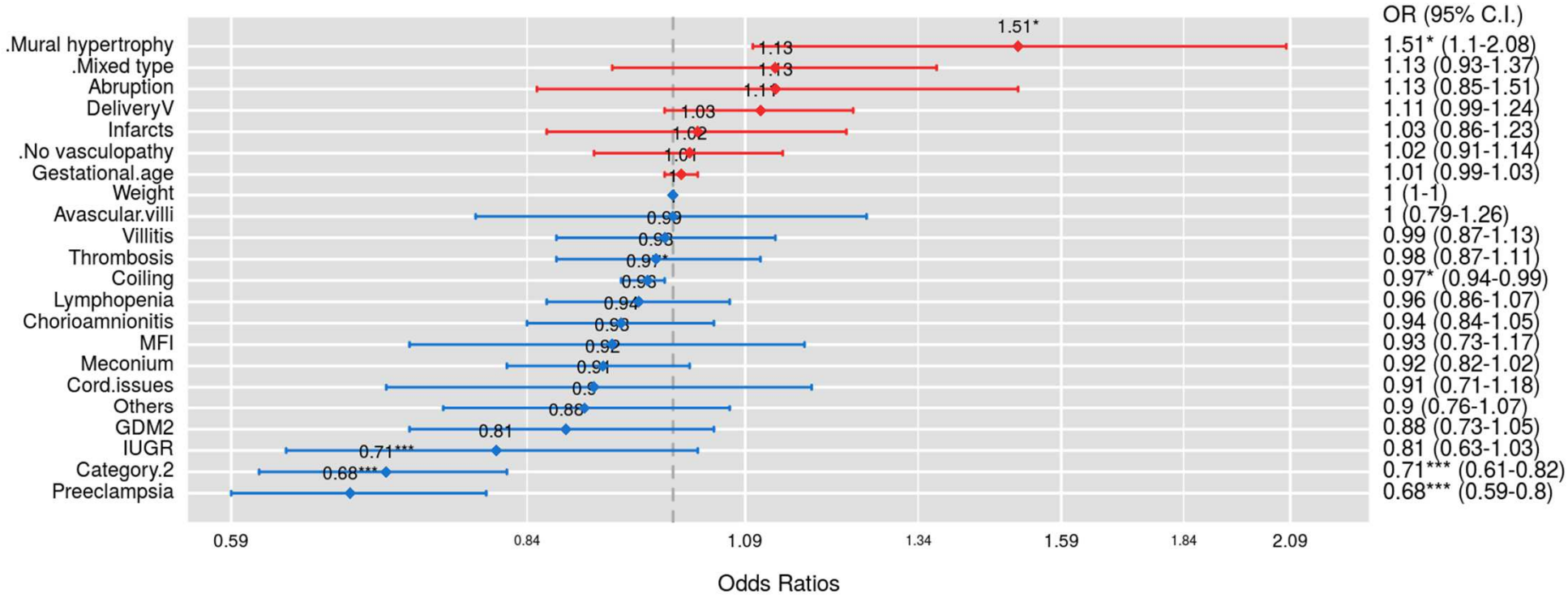

OR (95% C.I.)
.Mural hypertrophy
.Mixed type
Abruption
DeliveryV
Infarcts
.No vasculopathy
Gestational.age
Weight
Avascular.villi
Villitis
Thrombosis
Coiling
Lymphopenia
Chorioamnionitis
MFI
Meconium
Cord.issues
Others
GDM2
IUGR
Category.2
Preeclampsia
1.51* (1.1-2.08)
1.13 (0.93-1.37)
1.13 (0.85-1.51)
1.11 (0.99-1.24)
1.03 (0.86-1.23)
1.02 (0.91-1.14)
1.01 (0.99-1.03)
1 (1-1)
1 (0.79-1.26)
0.99 (0.87-1.13)
0.98 (0.87-1.11)
0.97* (0.94-0.99)
0.96 (0.86-1.07)
0.94 (0.84-1.05)
0.93 (0.73-1.17)
0.92 (0.82-1.02)
0.91 (0.71-1.18)
0.9 (0.76-1.07)
0.88 (0.73-1.05)
0.81 (0.63-1.03)
0.71*** (0.61-0.82)
0.68*** (0.59-0.8)
0.59
0.84
1.09
1.34
1.59
1.84
2.09
Odds Ratios

Figure 2

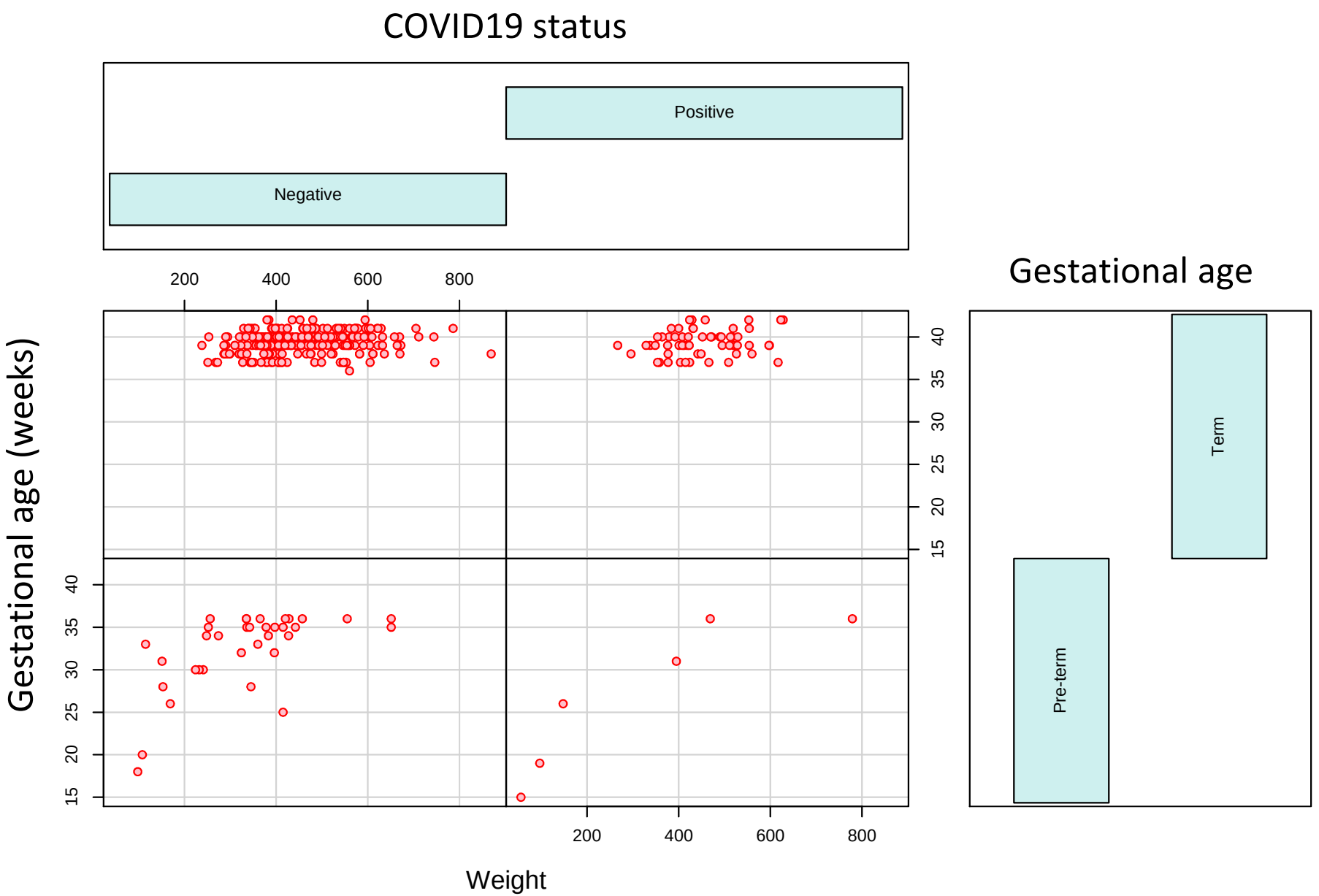

COVID19 status
Positive
Negative
200
400
600
800
Gestational age
Term
Pre-term
Gestational age (weeks)
15
20
25
30
35
40
Weight

Figure 3

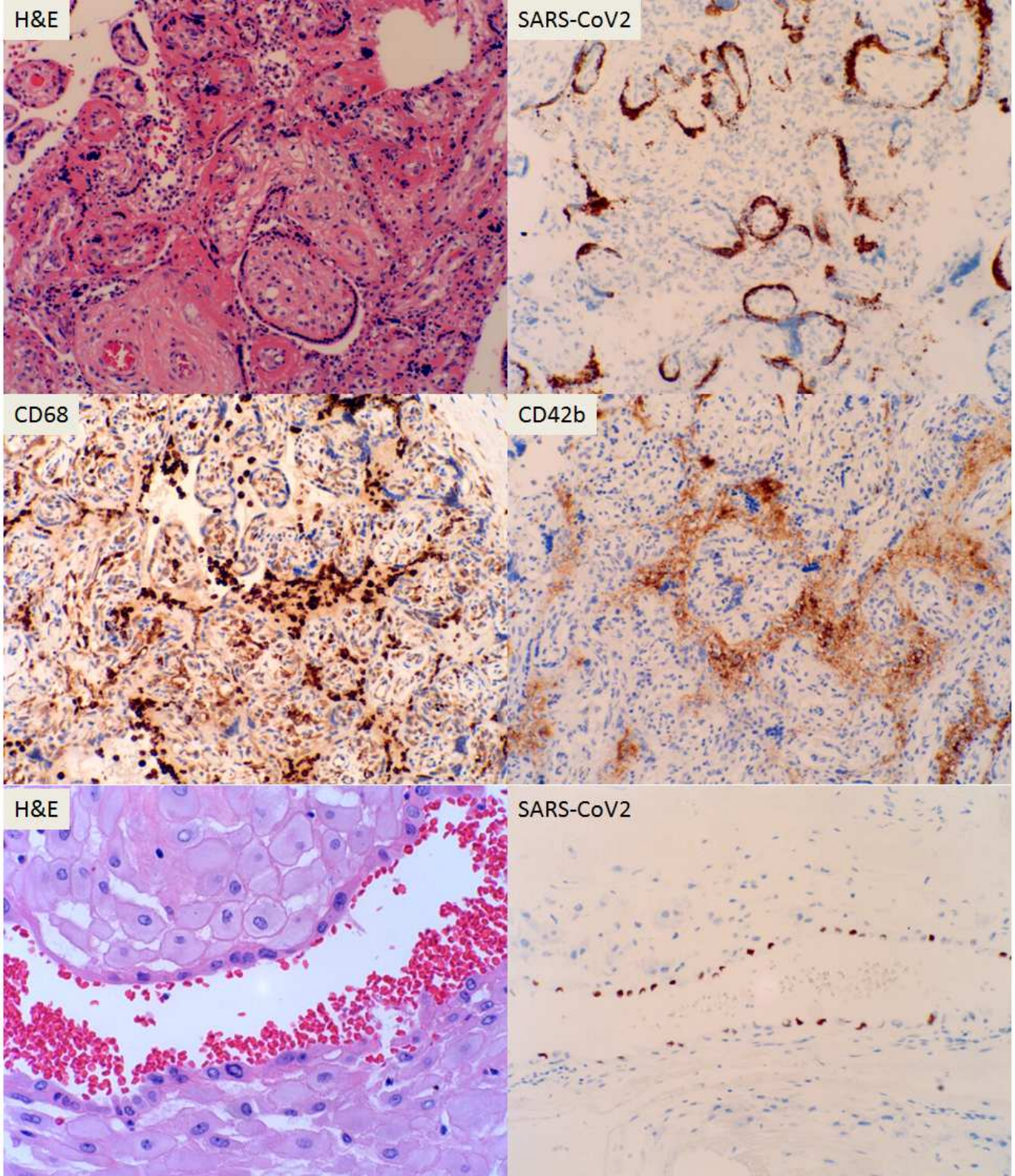
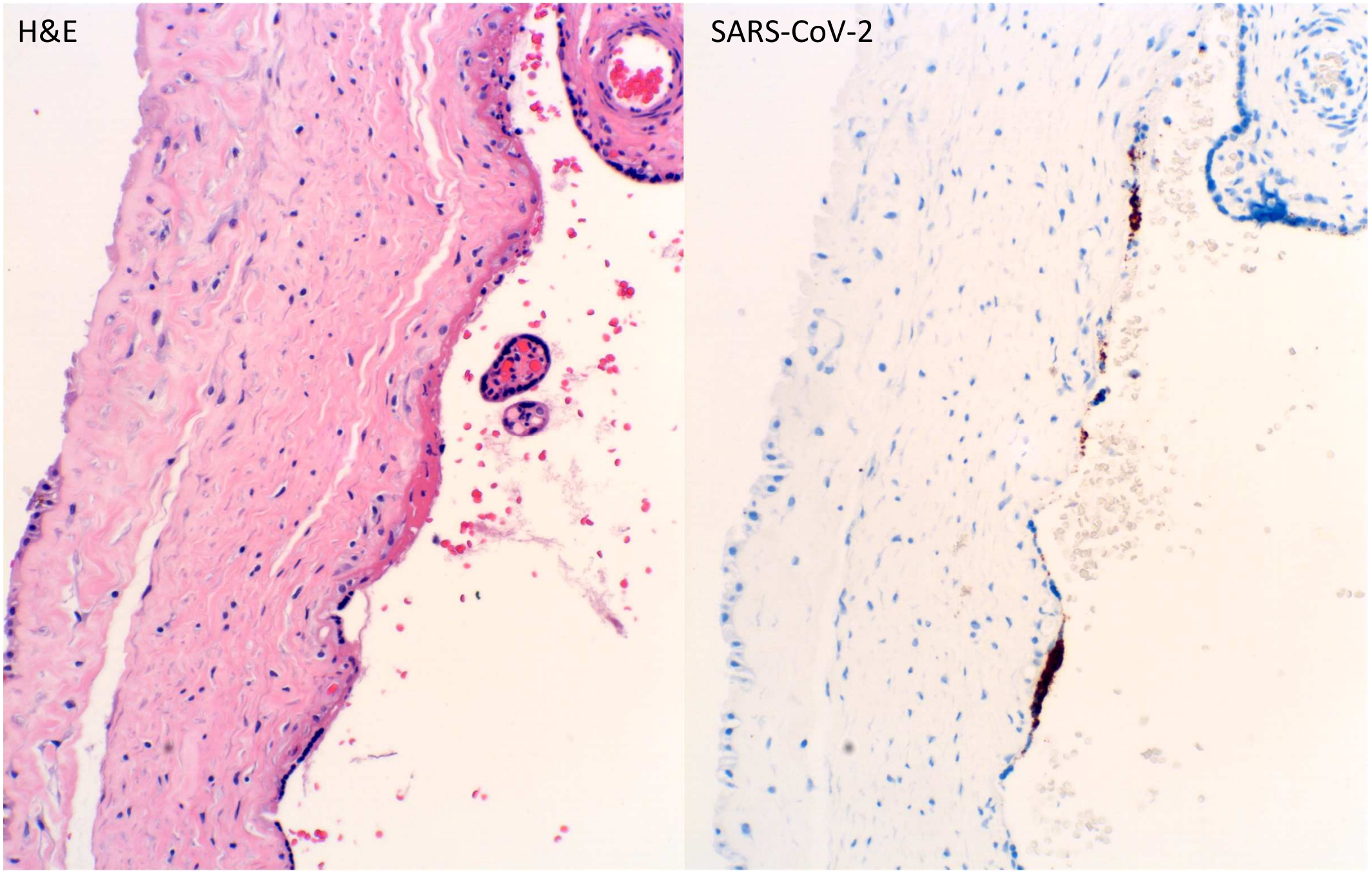


Figure 4



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Statement of authorship

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