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Stillbirth due to SARS-CoV-2 placentitis without evidence of intrauterine transmission to fetus: association with maternal risk factors

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/uog.24906

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Keywords: COVID-19, pregnancy, intrauterine fetal death, placenta, SARS-COV-2-placentits, risk factors, thrombophilia, multiple sclerosis

Short title: Stillbirth and SARS-COV-2 placentitis

Contribution

What are the novel findings of this work?

This study provides a novel insight into the histology and pathophysiology of the potentially lethal for the fetus SARS-COV-2 placentitis, identifies thrombophilia and IUGR as independent predisposing factors for this lesion, and generates the hypothesis of a synergistic effect between the virus and underlying maternal risk factors.

What are the clinical implications of this work?

COVID-19 may uncommonly cause intrauterine fetal death due to a specific type of placental injury, SARS-COV-2 placentitis, potentially induced by specific predisposing factors. The lesion evolves rapidly and, in its initial stages, appears undetectable by ultrasound. Risk groups may include mothers with thrombophilia and prenatally detected IUGR.

Abstract

Objectives

To report the causative relationship of maternal COVID-19 with intrauterine fetal death, describe the specific placental pathology, findings of fetal autopsy and clinical characteristics, and identify potential risk factors.

Methods

This is a prospective case series. A cohort of 165 placentas of non-vaccinated pregnant women affected by COVID-19 in Greece were histologically examined and six cases of intrauterine fetal death associated with SARS-COV-2 placentitis were retrieved. Complete fetal autopsy was performed in three cases. Gross, histopathological, immunohistochemical, molecular, and electron microscopy examinations were carried out in the stillbirth placentas and fetal organs. The histological findings of SARS-COV-2 placentitis were compared with the 159 cases, in which maternal COVID-19 resulted in livebirth. Regression analysis was used to identify predisposing factors for SARS-COV-2 placentitis.

Results

All six stillbirth placentas showed severe and extensive histological changes of SARS-COV-2 placentitis, i.e. a combination of marked intervillositis with a mixed inflammatory infiltrate and massive perivillous fibrinoid deposition with trophoblast damage, associated with intensely positive immunostaining for SARS-COV-2 spike protein, the presence of virions on electron microscopy and a positive RT-PCR test in placental tissues. The histological lesions obliterated over 75% of the maternal intervillous space, accounting for intrauterine fetal death. Similar histological lesions affecting less than 25% of the placenta were recorded in 7 liveborn neonates, while the remaining 152 placentas of COVID-19-affected pregnancies with livebirths did not show similar findings. Complete fetal autopsy showed evidence of an asphyctic mode of death without evidence of viral transmission to the fetus. The mothers had mild clinical symptoms or were asymptomatic and the interval between maternal COVID-19 diagnosis and fetal death ranged from 3 to 15 days. Statistically significant predisposing

factors for SARS-COV-2-placentitis included thrombophilia and IUGR. Multiple sclerosis was seen in one case.

Conclusions

SARS-COV-2 placentits occurred uncommonly in COVID-19-affected pregnancies of non-vaccinated mothers and, when extensive, caused fetal demise, with no evidence of transplacental fetal infection. Thrombophilia and prenatally detected IUGR emerged as independent predisposing factors for the potentially lethal SARS-COV-2 placentitis.

Introduction

The COVID-19 global pandemic has raised, among many others, major concerns regarding the impact of infection on pregnancy course, resulting in regular updates of guidances and recommendations, based on emerging evidence and epidemiological studies.

Intrauterine transplacental transmission of SARS-COV-2 from mother to fetus is thought to occur, typically via a hematogenous route, but is uncommon[1, 2]. Most placentas studied had no evidence of infection, and of those tested, a minority tested positive for SARS-COV-2 genome (7.7 – 21%)[3, 4]. As the COVID-19 pandemic evolved during 2020, a small number of international reports began to emerge describing a particular pattern of inflammation in placentas of COVID-19 positive women, which was termed “SARS-COV-2 placentitis” in January 2021[5]. Placental involvement with SARS-COV-2 placentitis seemed to be uncommon, but had the potential to cause significant placental injury, potentially resulting in fetal compromise.

In this study we report six cases of intrauterine fetal death (IUFD) in Greece attributed to SARS-COV-2 placentitis, retrieved from a cohort of 165 COVID-affected pregnancies, describe the specific placental pathology and ancillary tests, the findings of fetal autopsy, compare with the histological findings in livebirths, and identify potential risk factors.

Methods

This is a prospective multicenter study, designed and supervised by the 3rd Clinic of Pediatrics, Attikon University Hospital, and the Unit of Perinatal Pathology, 1st Department of Pathology, Medical School, National and Kapodistrian University of Athens (NKUA), Greece. In the context of this study, from April 18, 2020 until August 31, 2021 a total of 165 placentas of COVID-19-affected pregnant women were sent for histological examination at the Units of Perinatal Pathology, University Laboratories of Pathology, Medical School NKUA, Athens, and the Laboratory of Pathology, Hippokration Hospital, Thessaloniki, both referral centers for perinatal and placental pathology in Greece. All women gave their informed consent for the examination of the placentas, and the study was approved by the Ethical Committee of Attikon University Hospital, Medical School, NKUA.

During the study period, six COVID-affected pregnancies were complicated with IUFD, and the parents opted for full fetal autopsy in three. The remaining 159 pregnancies resulted in livebirth. Clinical characteristics of the pregnancies, including maternal history, were available in 69 cases.

Case description of IUFD cases – Maternal medical history

All six IUFDs occurred in 6 different towns across Greece, between December 2020 and August 2021. Clinical information is shown in Table 1. The gestational age ranged from 21^{+1} to 39^{+6}w. IUFD occurred 3 - 15 days after maternal COVID-19 diagnosis, determined as the date of positive maternal PCR test of nasopharyngeal swabs or the onset of symptoms. Four mothers had mild clinical symptoms, according to NIH criteria of illness severity, including fever, cough, malaise and myalgias, without need of hospitalization. Two mothers were asymptomatic and were found SARS-COV-2-positive at routine PCR testing before delivery. Maternal history was remarkable for underlying conditions potentially associated with pregnancy complications in 4 cases. Mother #3 (Table 1) Gravida2/Para1, with history of multiple sclerosis, had stopped treatment during gestation. Mother #4 (Table 1), primigravida, was investigated after the event and was found to bear a homozygous pathogenic variant for the mild thrombophilia factor Plasminogen Activator Inhibitor-1 (PAI-1

4G/4G). Mother #5, Gravida 2/Para0 had a previous 2nd trimester miscarriage; in this pregnancy she was diagnosed with gestational diabetes, managed by diet. Mother #6, Gravida 2/Para0, had a previous 1st trimester miscarriage and was diagnosed with combined heterozygous MTHFR variants (C677T and A1298C) at thrombophilia testing; during this pregnancy she had received anticoagulant treatment. Mother #1 was thoroughly investigated after stillbirth, and there were no commorbidities or other contributing risk factors identified. Mother #2 was lost to follow-up. In this case, prenatal ultrasound monitoring before maternal COVID had shown oligohydramnios and abnormal Doppler, while in the remaining five cases, prenatal findings were normal (Table 1). Decreased fetal movements were recorded in 3 cases post-infection.

The following investigations were carried out in the placentas:

PCR-testing for SARS-COV-2 and other viruses: On admission at the pathology laboratory, four of six IUFD placentas were sampled fresh for RT-PCR-testing, using protective equipment, two were received in formalin and were not sampled. The fetal surface was cleaned with alcohol solution to minimize the possibility of viral RNA contamination from traces of maternal blood, amniotic fluid or other maternal fluids, and a section of 1cm was cut with a sterile blade from the placental parenchyma. Fresh tissue samples were also obtained from the lungs, liver, and spleen of two autopsied fetuses. Samples were delivered to the University Department of Microbiology, Medical School NKUA, Athens, and were tested for the presence of viral RNA using the genesig Real-Time PCR COVID-19 assay (2019-nCoV), following RNA extraction with Promega's Maxwell viral nucleic acid extraction with magnetic beads. The same samples were also tested for Cytomegalovirus (CMV), Herpes Simplex Viruses (HSV1 and 2), Parvovirus, Enterovirus, and Ebstein-Barr virus (EBV). Additional placenta samples obtained from 16 formalin-fixed/paraffin embedded placentas showing parenchymal inflammatory lesions were retrospectively tested for DNA viruses (CMV, HSV1/2, ParvoB19, EBV).

Pathological examination in all placentas was carried out with appropriate precautions by a standard protocol after fixation in 10% buffered formalin for 10 – 14 days before gross

examination. Slices of the fixed placentas were photographed for the evaluation of gross pathological changes. Ten tissue blocks of each placenta, including umbilical cord sections and membrane rolls were embedded in paraffin and stained with Harris haematoxylin - eosin (H-E) for histological evaluation.

Immunohistochemistry: Immunohistochemical stains were performed on selected sections using the following antibodies: SARS-COV-2 (COVID-19) Spike Antibody (GeneTex, 1A9, 1:100), and CD68 (KP1) DAKO M0814, 1:300); CD3 (DAKO A045201, 1:200); CD4 (DAKO M731001, 1:100); CD8 (DAKO M710301, 1:100); CD15 (DAKO M0733(C3D-1), 1:50); CD20 (DAKO M0755, 1:100), to identify leukocyte subpopulations, according to standard procedures. Negative controls for SARS-COV-2 immunohistochemistry were six age-matched placental specimens of SARS-COV-2-negative mothers, as well as sections from the six index placentas where the primary antibody was not applied.

Electron Microscopy: Four IUFD placentas were processed for electron microscopy after previous fixation in formalin. Small tissue fragments (1-2mm3) from each placenta were cut and washed with dH20 5min (x3) and re-fixed in 2.5% glutaraldehyde in PBS for 2h at RT and then overnight at 4oC. After washing with PBS, the specimens were post-fixed in 1% aqueous osmium tetroxide for 1h at 4oC, then dehydrated in a graded series of ethyl alcohol, followed by propylene oxide (PO), infiltrated gradually in a mixture of Epon/Araldite resins diluted in PO and finally embedded in fresh epoxy resin mixture. Ultrathin epoxy sections (70-80nm thickness) were cut on a Leica Ultracut R ultramicrotome, equipped with a Diatome diamond knife and observed with a FEI Morgagni 268 transmission electron microscope equipped with Olympus Morada digital camera.

Statistical analysis

Continuous variables were presented as medians and interquartile range values. Categorical variables were summarized as percentages. Mann Whitney U test was used for comparisons of continuous variables between the groups. Chi-square or Fisher's exact test were used for pairwise comparisons of proportions, as appropriate. Logistic regression (backward, by likelihood ratios) was performed for the incidence of SARS-COV-2 placentitis. Potential

predictors included thrombophilia, asthma, smoking, hypothyroidism, gestational diabetes mellitus, gestational hypertension, preeclampsia, IUGR (defined as: estimated fetal weight/EFW or abdominal circumference/AC <3rd centile; ESW or AC >3rd and <10th centile and Umbilical artery pulsatility index centile >95th; cerebroplacental ratio centile <5th), maternal age, gestational age at covid-19 infection, asymptomatic Covid-19 infection, mild Covid-19 infection, moderate Covid-19 infection and severe Covid-19 infection. Adjusted odds ratios, along with their 95% confidence intervals (CIs) were calculated. The analyses were performed on SPSS (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.) and open source software R 2.15.1 (The R Foundation for Statistical Computing).

Results

Placental pathology, histology and immunohistochemistry

One placenta (case #2) was small, the weight falling under the 10th centile, the remaining five were appropriately grown for age. On gross examination and cut sections, all six placentas were compact and stiff, with a mosaic pattern of whitish streaks running through the parenchyma and intervening hemorrhagic lesions (Fig. 1). This process indicated massive perivillous fibrinoid deposition and involved over 75% of the placental parenchyma. Histologically these lesions corresponded to a combination of massive perivillous fibrinoid deposition with severe diffuse intervillositis and intervillous thrombosis (Fig. 2). The inflammatory infiltrate was mixed and consisted of abundant macrophages and mononuclear cells, variable numbers of immature granulocytes and neutrophils, along with T-lymphocytes and sparse B-lymphocytes (Figure 3). The inflammatory cells appeared entrapped in a meshwork of interwoven fibers (Figure 2D), while intervillous thrombosis was also conspicuous (Figure 2A). Trophoblast damage was evident as necrosis, erosion and blebbing of the perivillous trophoblast (Figure 3A-inset). Viral immunostaining with anti-SARS-CoV2-spike antibody showed areas of intensely positive cytoplasmic staining in the perivillous and extravillous trophoblast, as well as in scattered cells of the villous stroma (Figure 3A). Interestingly, areas with massive deposition of perivillous fibrinoid and destruction of the trophoblast showed weak or absent perivillous positivity.

Of the remaining 159 placentas of COVID-19-affected pregnancies with liveborn neonates, 7 showed evidence of focal /segmental SARS-COV-2 placentitis involving less than 25% of the placenta (Fisher's exact test, p=0,0006). These cases showed positive immunostaining for SARS-COV-2, while PCR tests for other viruses (CMV, HSV, EBV and Parvo-B19) was negative (Table 2). Prenatal ultrasound had failed to detect SARS-COV-2 placentitis in either case of stillbirth or livebirth (Figure 4). The remaining 152 placentas of liveborn neonates did not show histological lesions of SARS-COV-2 placentitis. Comparative clinical and pathological data between the 6 cases of stillbirth and 24 selected control cases of livebirth matched for risk factors, are shown in Table 2.

RT-PCR

The Alpha B117 variant of SARS-COV-2 was detected in the four tested stillbirth placentas with low cycle threshold (Ct < 20), in favor of high levels of genomic RNA.

Samples from the fetal organs, lungs, liver and spleen, were negative for SARS-COV-2 genome. PCR tests on fresh placental and fetal samples were negative for all other viruses tested (CMV, HSV1 and 2, Parvovirus, Enterovirus, and EBV).

PCR tests on formalin-fixed samples obtained from 16 livebirth-placentas with parenchymal inflammatory lesions were negative for other viruses (CMV, HSV1/2, ParvoB19, EBV).

Electron Microscopy (E/M)

Electron microscopy of the placental tissues revealed viral particles morphologically consistent with coronavirus. They were mostly observed within the cytoplasm of syncytiotrophoblast cells, either grouped within enlarged vesicles (Fig. 5, Ai and Aii) or as single particles (Fig. 5B). On higher magnification the virions showed faint surface projections, with the helical nucleocapsid appearing as internal black dots (Fig. 5B). Occasional virions were also identified within the basement membrane (BM) of the syncytiotrophoblasts bordering the intravillous space (Fig. 5C). No virions were found on E/M in samples obtained from the lungs, liver and spleen of the autopsied fetuses.

Fetal Autopsy

Full postmortem examination of three fetuses was performed in case #3 (35 week-gestation) case #4 (27^{+2}w) and case #6 (21^{+1}w) (Table 1). The fetuses were appropriately grown for age, weighing 2480g (42^{nd} centile), 1090g (44^{th} centile) and 397g (54^{th} centile) respectively. There was evidence of an asphyctic mode of death, with pulmonary petechiae, meconium aspiration and visceral hemorrhages, without any histological evidence suggestive of infection. The brain of stillborn #3 showed focal subarachnoid hemorrhage with no evidence of hypoxic/ischaemic neuronal injury.

Logistic regression analysis

Out of 165 histologically examined placentas, clinical data entering the statistical analysis were available in 69 cases. Potential risk factors are presented in Table 3. Regression

analysis revealed thrombophilia (OR 29.014; 95% CI 1.672 – 503.482), IUGR (OR 24.250; 95% CI 1.072 – 544.081) and mild COVID-19 infection (rather than asymptomatic COVID-19 infection) (OR 17.366; 95% CI 1.955 – 154.228) to be significant independent predictors for SARS-COV-2 placentitis (Nagelkerke R2=0•593).

Discussion

With regard to fetal death, initial evidence obtained from meta-analyses covering the first phases of the pandemic was reassuring that, despite the globally increasing number of COVID-19 infections, there was no significant increase of adverse pregnancy outcomes. Data obtained at the subsequent phases of the pandemic, however, pointed towards an increase in the rates of fetal death, preterm birth, preeclampsia, and emergency cesarean delivery[6-8]

SARS-COV-2 placentitis and IUFD

The spectrum of placental histopathology in COVID-19-affected pregnancies is reportedly wide with variable, overall non-specific findings, generally related to uninfected neonates and a favorable pregnancy outcome[3, 9]. SARS-COV-2 placentitis", a particular pattern of placental pathology, appeared to emerge as the pandemic evolved during 2020, related to PCR-positive placentas and/or infected neonates[10-13], attributed to the Alpha variant in 2021[5], and currently becoming more widely adopted in association with stillbirth[14].

Our observations in the 6 herein reported cases of IUFD, occurring between December 2020 and August 2021, a period of prevalence of the Alpha B117 variant in Greece, confirm the detrimental potential of –at least– the Alpha variant on the placenta, causing in certain cases SARS-COV-2-placentitis, a constellation of severe histological lesions, potentially lethal when the placenta is diffusely affected. The lesions of SARS-COV-2-placentitis were identical in all six IUFDs, and were rapidly progressive, producing extensive occlusion of the intervillous space and compromising the maternal-fetal exchange. In our series, SARS-COV-2 placentitis consisted of a combination of massive perivillous fibrinoid deposition (MPFD), severe intervillositis with a mixed inflammatory infiltrate, trophoblast damage and intervillous thrombosis. This histology appears to differentiate SARS-COV-2 placentitis from the so far described COVID-associated "chronic histiocytic intervillositis"[5, 10, 11, 14], due to the mixed component – histiocytic, lymphocytic and granulocytic– of the inflammatory populations, which is more consistent with an infectious etiology. Nevertheless, the combination of a predominantly mononuclear inflammation with MPFD in the placenta is not unique for SARS-

COV-2, having been reported also in the context of other infectious etiology, e.g. cytomegalovirus and malaria [15, 16].

Comments on the E/M and fetal autopsy findings

The detection of virions within the cytoplasm and the basal membrane of perivillous syncytiotrophoblasts, suggests that the virus proceeded halfway within the placental barrier, but failed to enter the fetoplacental circulation. These observations underscore the crucial role of the perivillous syncytiotrophoblast as a key component of the placental barrier, but also demonstrate that at least the Alpha variant could cause fetal death by placental injury, without being transmitted to the fetus. These are further supported by the absence of any evidence of viral transmission in our 3 cases of autopsied fetuses, confirming that in these cases the virus failed to cross the placental barrier, but caused fetal death by asphyxia due to the diffuse occlusion of the intervillous space and the hypermetabolic inflammatory condition of the placenta, both compromising the fetomaternal exchange. This experience is not confined to Greece, and internationally a number of stillbirths have been attributed to SARS-COV-2 placentitis, continuing to occur during the period of prevalence of the Delta variant.[14, 17, 18].

Potential predisposing factors

As, the occurrence of SARS-COV-2 placentitis seemed to be uncommon among COVID-19-affected pregnancies, in accordance with other studies[19], with no obvious correlation between its occurrence and the severity of maternal disease, the question was raised on the potential contribution of predisposing factors. Four of the six mothers of the presented IUFD cases with SARS-COV-2 placentitis, and four of the five mothers with livebirth and less extensive SARS-COV-2 placentits –and available clinical information, had underlying conditions associated with pregnancy complications, namely multiple sclerosis, an autoimmune disease associated with an increased risk of infections[20], gestational diabetes, commonly associated with adverse pregnancy outcomes, thrombophilia factors, hypertension, and prenatally detected IUGR. Statistical analysis failed to confirm gestational/maternal diabetes as a significant predisposing factor for SARS-COV-2-

placentitis, but was significant for thrombophilia and IUGR. Two mothers had homozygous or compound heterozygous polymorphisms for the PAI-1 and MTHFR genes, associated with abnormal coagulation or impaired fibrinolysis, considered as mild thrombophilias of uncertain clinical significance with regard to adverse pregnancy outcomes. It is of note though, that immune-mediated diseases and thrombophilias have been associated with MPFD, a rare and poorly understood placental disorder[21, 22], which was one of the main components of SARS-COV-2 placentitis seen in all the IUFD cases, and to a lesser extent in 7 livebirths. In this context, given that coagulation and fibrinolysis abnormalities are common among COVID-19 patients[23], we suggest the hypothesis that certain maternal risk factors may act synergistically with SARS-COV-2 and result in the specific combination of placental lesions that characterize SARS-COV-2 placentitis. Maternal history in case #2 was undetermined, however the findings of fetal growth restriction and placental changes of maternal malperfusion suggest underlying uteroplacental insufficiency, potentially related to some predisposing maternal risk factor. The occurrence of these stillbirths in Greece and internationally during the periods when the Alpha and Delta variants prevailed, suggests a potential deleterious effect of these particular variants, when pregnant women with certain comorbidities or predisposing factors were infected. The number of cases is too small to draw safe conclusions, yet the synergistic action of SARS-COV-2 with particular predisposing factors may be plausibly assumed.

The strength of this study lies on the novelty of our observations, the broad spectrum of analyses, and the indication of potential predisposing factors for SARS-COV-2 placentitis, supporting the hypothesis of a synergistic effect between these factors and the coronavirus. The limitations include the small number of placentas with SARS-COV-2 RT-PCR results and the lack of placenta ultrasound images post-infection and before fetal demise, to correlate USS imaging with histopathology.

Future perspectives

At the time of this writing, the Alpha and Delta variants have been widely replaced globally, and as this disease and our experience continue to evolve, it is essential to highlight the

importance of the pathological investigation of fetal death and its provision of information critical for the management of pregnancy during the COVID-19 pandemic. Further pathological and epidemiological investigations appear even more important, as the impact of novel globally spreading SARS-COV-2 variants is still unknown, this challenge remaining to be confronted in the immediate future.

Aknowledgments

We thank Dr. Y. Koskosas, Dr. D. Koutsoulis, Dr. G. Stathoudakis and Dr. K. Soumakis for contributing their cases, and Mr. A. Pergaris for assisting to placenta sampling.

Prof. A. Konstantinidou is funded by REA Maternity Clinic, Athens, Greece, for conducting research in Perinatal Pathology of Stillbirth (NKUA SARG 11191).

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Figure legends

Figure 1 Gross pathology of placenta cut sections after formalin fixation in cases 3,4,5 and 6 of Table 1. The placentas are compact and stiff, with a mosaic pattern of whitish streaks running through the parenchyma and intervening hemorrhagic lesions. Variable coloration is due to variable degree of fixation.

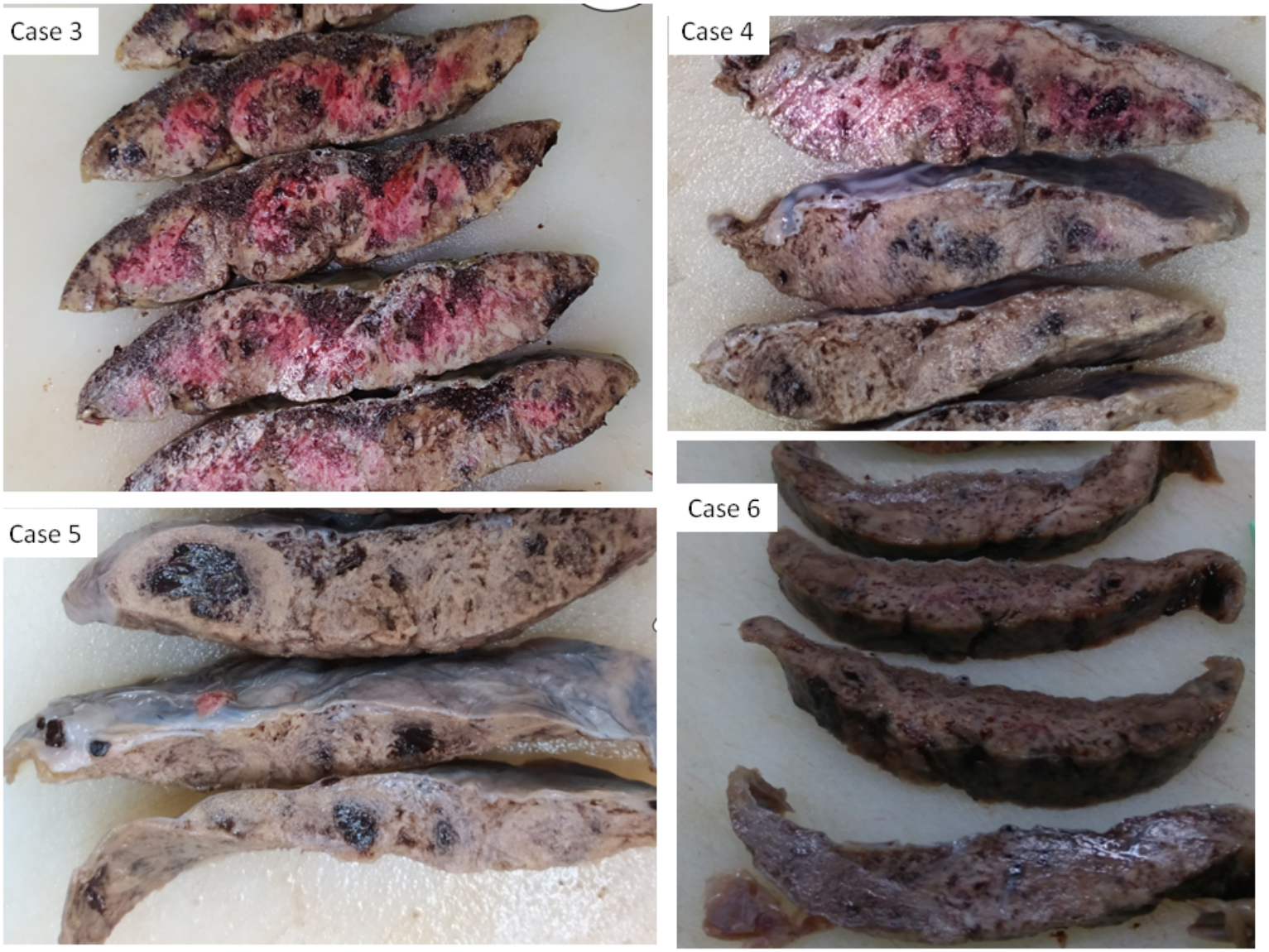
Figure 2 Histopathology of SARS-COV-2 placentitis. A: Fresh intervillous thrombosis (x45), B: Massive intervillous fibrinoid deposition (x100), C: Inflammation in the intervillous space (intervillitis) and damage of the perivillous trophoblast (x200), D: Inflammatory cells of mixed type within a meshwork of fibers in the intervillous space (x400).

Figure 3 Immunohistochemistry of SARS-COV-2 placentitis. A: Intense positivity of the perivillous trophoblast for SARS-COV-2 spike protein (x 45) inset: Detail of the positively stained perivillous trophoblast showing cytoplasmic blebbing (x400) B: Prevailing macrophages / monocytes among the intervillous inflammatory population (CD68/Kp1, x45) C: large numbers of granulocytes among the polymorphous intervillous inflammatory populations (CD15, x100) D: Scattered T-lymphocytes among the mixed inflammatory cells (CD3, x200).

Figure 4 Ultrasound scan in SARS-COV-2 placentitis. Pregnancy 35w+3d. USS was taken post-infection, 4 days before delivery of a liveborn neonate. The placenta showed histological features of SARS-COV-2 placentitis involving <25% of the parenchyma. The lesions were undetectable on USS.

Figure 5 Electron Microscopy of SARS-COV-2 infected trophoblast. Ai) Low magnification of an infected syncytiotrophoblast with virion-containing vesicles (black box, white arrows) in the cytoplasm, Aii) higher magnification of the black box in Ai, depicting the vesicular structure (arrow) containing virions either with electron-lucent or dark center, B) high magnification of a syncytiotrophoblast showing an intracellular SARS-CoV2 particle (arrow) with visible surface projections and the helical nucleocapsid seen as internal black dots, C) high magnification of a syncytiotrophoblast showing a SARS-CoV2 particle (arrow) localized into the basement membrane (BM) of the syncytiotrophoblast. N: nucleus; BM: basement

membrane; black star: intervillous space; star: intravillous space. Original magnifications: Ai x8900; Aii x28000; B-C: x44000.



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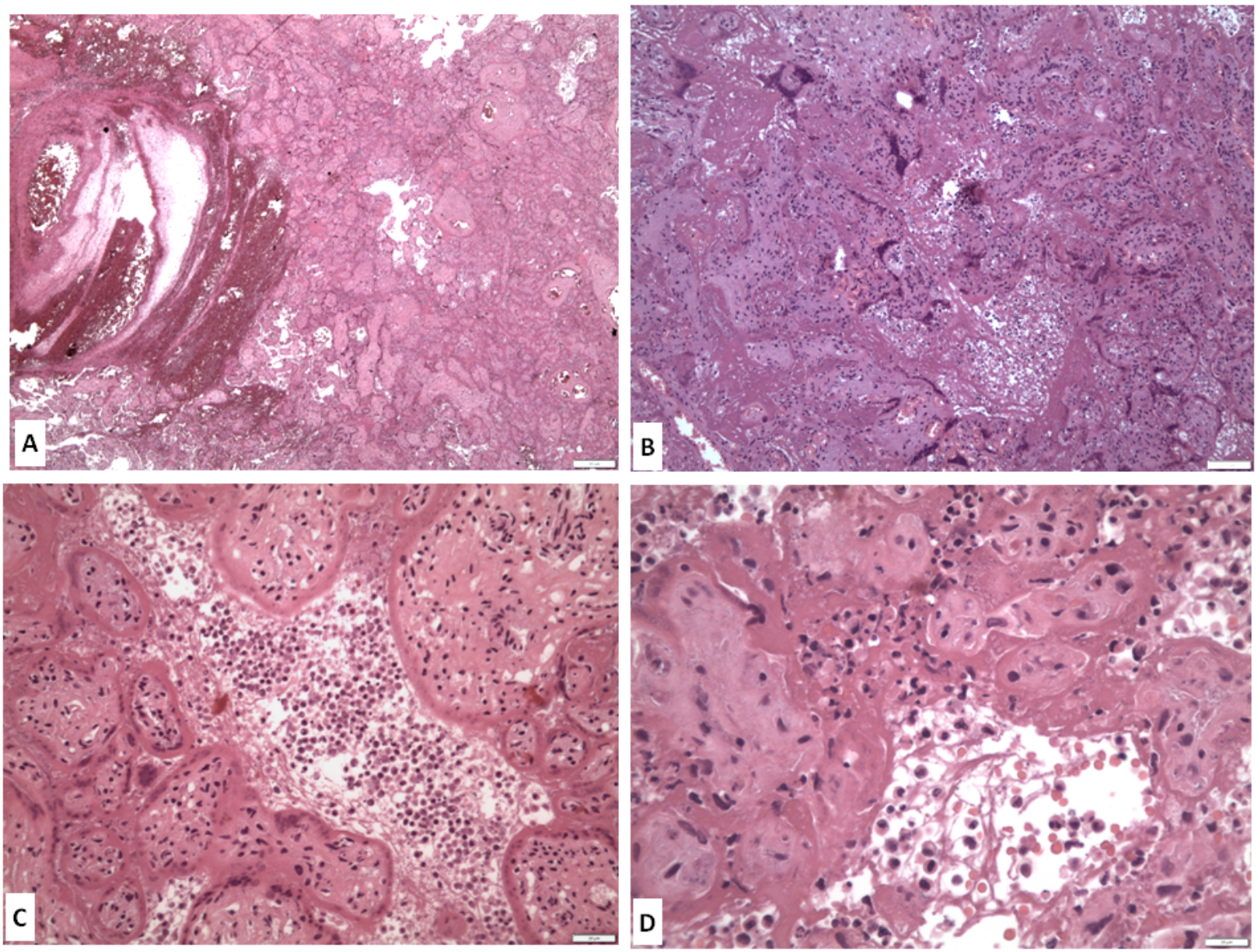

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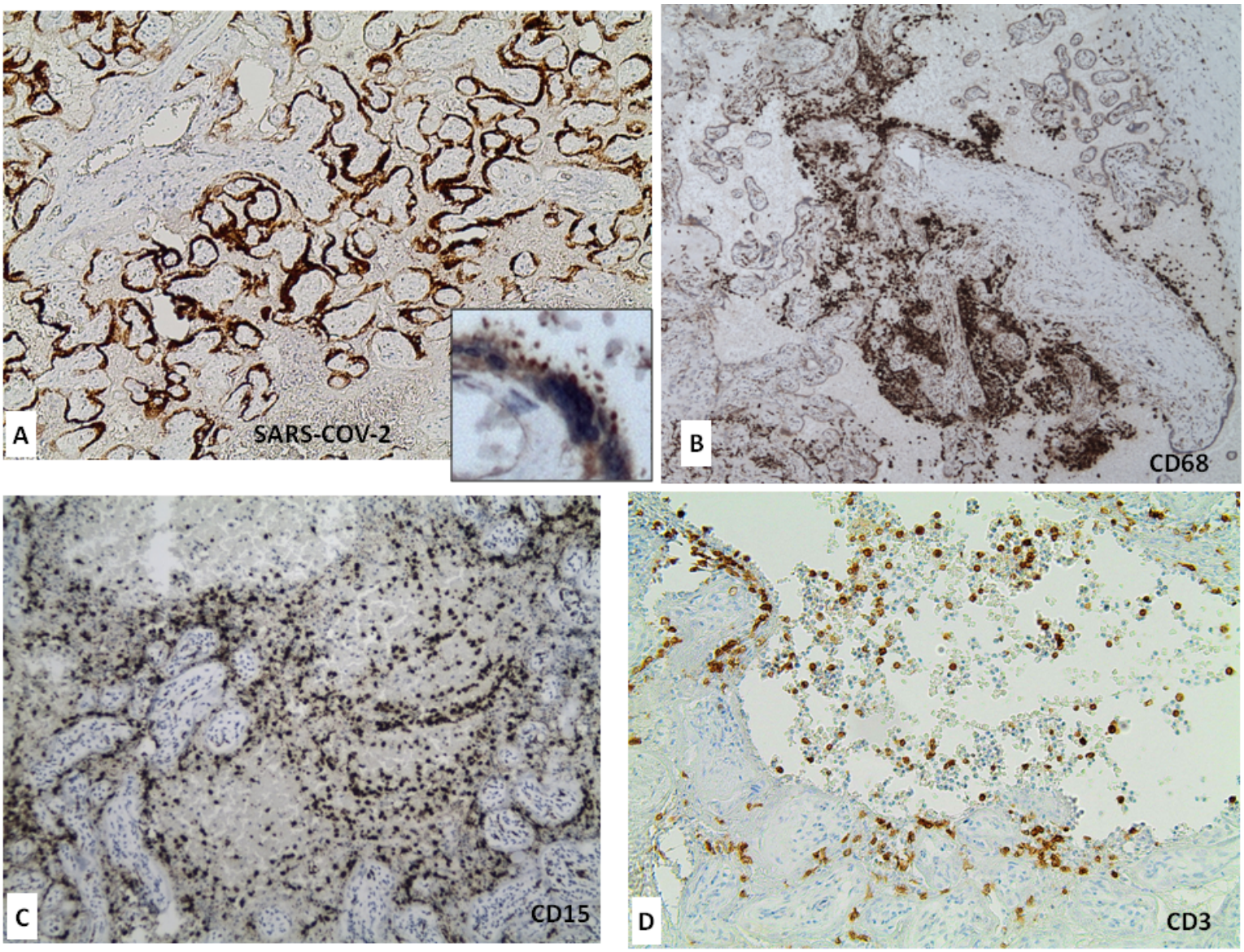

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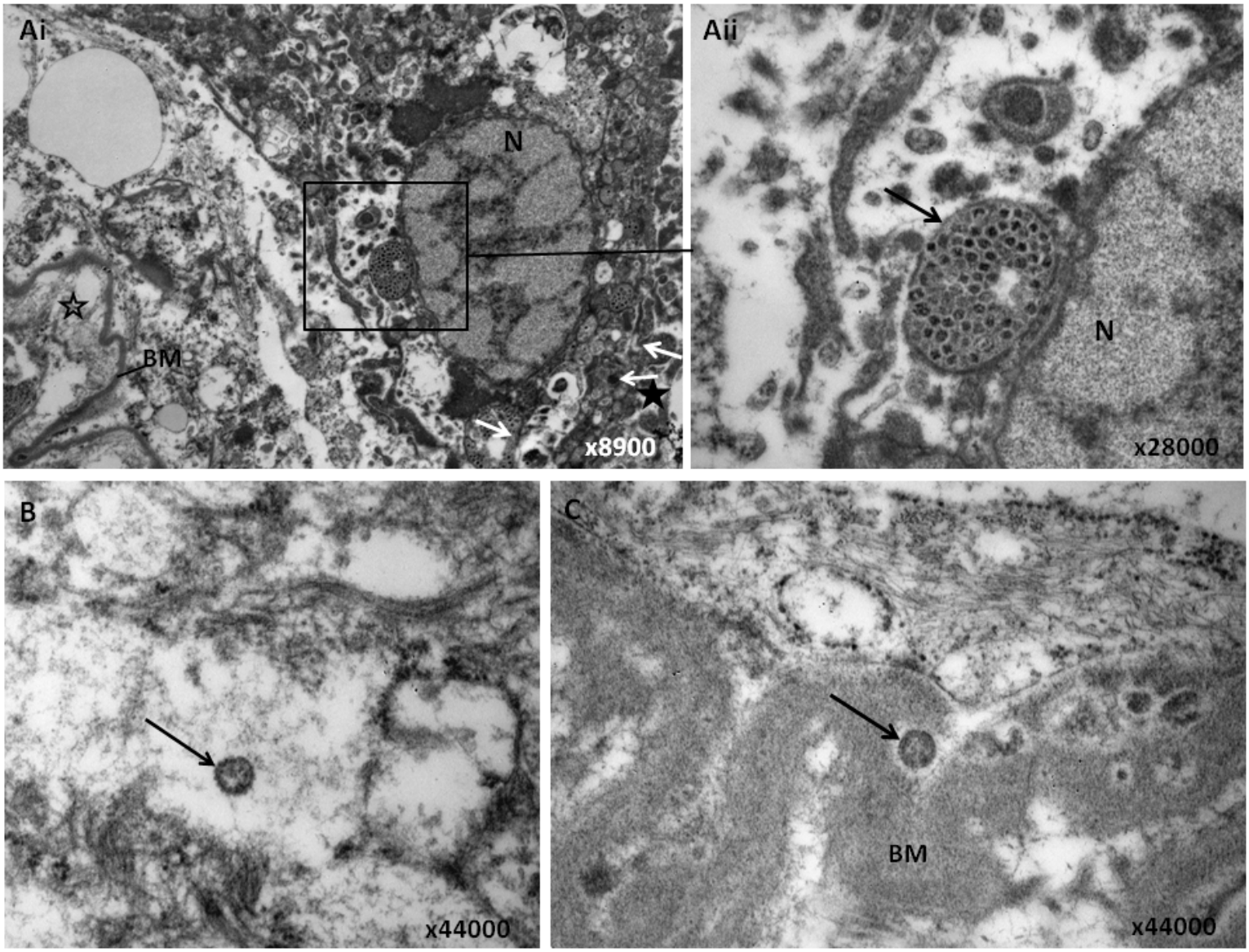

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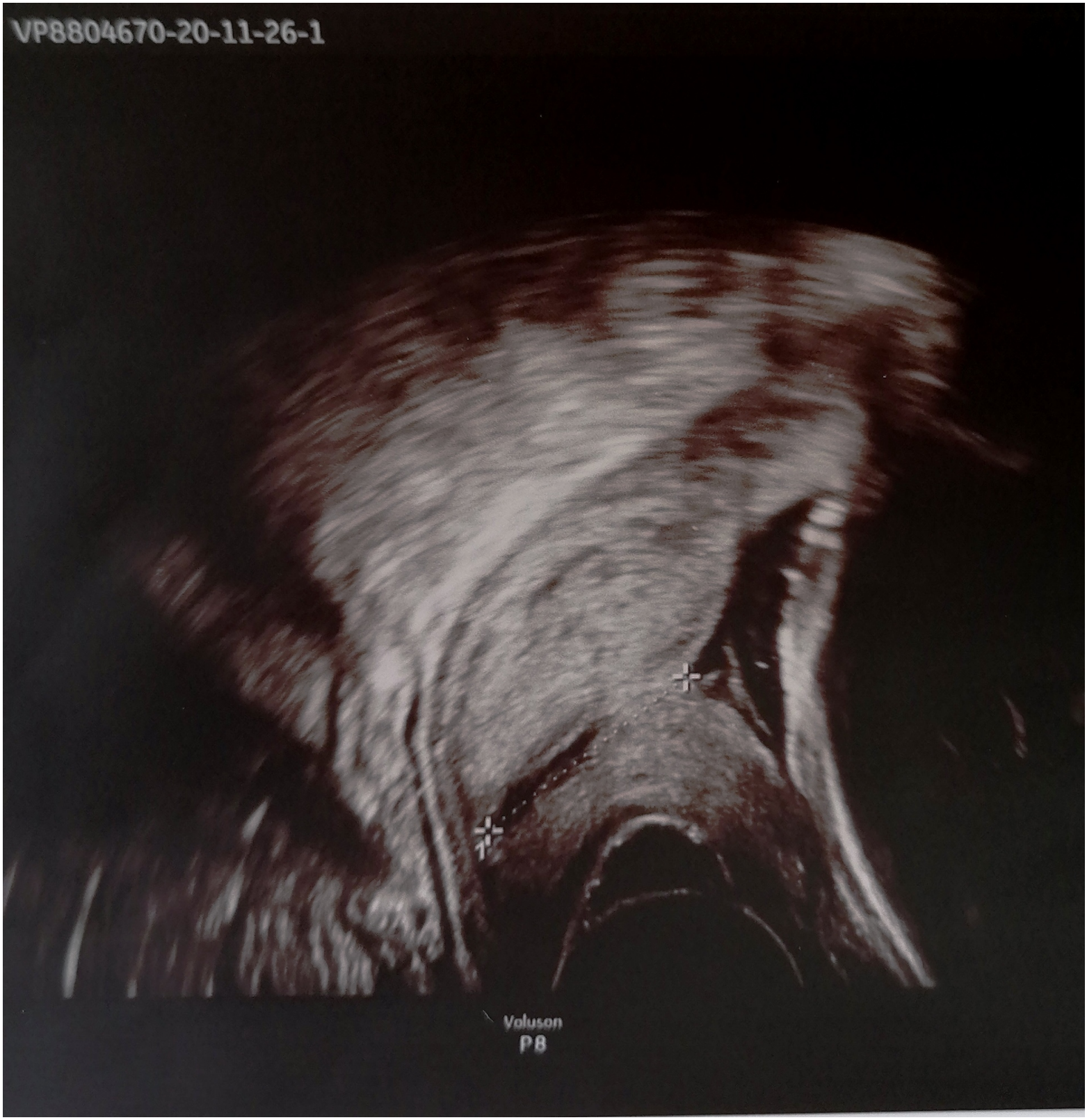

UOG_24906_Figure 5.jpg

Table 1. Maternal characteristics, fetal/placental pathology, prenatal findings and ancillary investigations in six cases of intrauterine fetal death in Greece

	Case #1	**Case #2**	**Case #3**	**Case #4**	**Case #5**	**Case #6**
Time of IUFD month-year	December 2020	February 2021	March2021	May 2021	May 2021	August 2021
Gestational age (weeks)	$39w^{+6d}$	$24w^{+4d}$	35w	$27w^{+2d}$	37w	$21w^{+1d}$
Maternal characteristics						
Maternal age	39y	40y	36y	26y	38y	36y
Clinical severity of COVID-19	asymptomatic	asymptomatic	mild	mild	mild	mild
COVID-19- to-IUFD interval (days)	4	3	13	7	15	10
Maternal history	none	unknown	MS	thrombophilia PAI-1 4G/4G	gestational diabetes	thrombophilia MTHFR C677T/A1298C
Placental / fetal findings						
Fetal body weight / centile	3700g / 75c	280g /<1c	2480g /42c	1090g / 44c	3200g/75c	397g /54c
Placental weight /centile	460g / 25-50c	114g / <10c	391g / 25-50c	262g /50-75c	430g/25-50c	126g / 25c
Extent of SARS-COV-2 placentitis	90%	75%	90%	75%	90%	90%
Other findings	umbilical cord true knot	placenta abruption / MMP	--	--	--	--
Prenatal data						
Fetal anatomy	normal	normal	normal	normal	normal	normal
Fetal growth	normal	FGR	normal	normal	normal	normal
Risk for FGR / SGA PAPP-A, PLGF	low risk	n/a	n/a	low risk	low risk	low risk
Uterine Doppler	normal	abnormal	n/a	normal	normal	n/a
Amniotic fluid	normal	oligohydramnios	n/a	normal	polyhydramnios	normal
Placenta ultrasound	normal	n/a	n/a	normal	normal	n/a
Fetal Autopsy	no	no	yes asphyxia	yes asphyxia	no	yes asphyxia
Other investigations						
RT-PCR –placenta SARS-COV-2 variant	n/a	n/a	positive Alpha variant	positive Alpha variant	positive Alpha variant	positive Alpha variant
RT-PCR-fetal organs	n/a	n/a	negative	negative	n/a	negative
E/M-placenta	n/a	n/a	virions	virions	virions	virions
E/M fetal organs	n/a	n/a	–	–	n/a	–

n/a: non available data

MS: multiple sclerosis; MMP: maternal malperfusion; CA: chromosomal abnormalities

Table 2. Maternal risk factors and placental pathology in control cases of COVID-19-affected pregnancies with live births compared with 6 cases of stillbirth

		Placental histopathology				Outcome	
Maternal risk factors	**Nr of cases**	**SARS-COV-2 placentitis** (extent)	**Increase in fibrinoid deposition** *non-specific*	**Inflammation (villitis, intervillositis)** *non-specific*	**Other findings** *non-specific*	**Live births**	**Stillbirths**
Gestational Diabetes	13	1 (90%) 2 (<25%)*	4	5*	11 (RVM, IV thrombi subchorionic thrombosis)	12	1 (Case 5)
Thrombophilia							
PAI-1 (4G/4G)	1	1 (75%)					1 (Case 4)
MTHFR (C677T / A1298C)	1	1 (90%)					1 (Case 6)
FV-R2 heterozygosity	1	no	1 (mild)	1*	1 (MMP, DV, RVM)	1	
Antiphospholipid syndrome	1	1 (<25%)*				1	
Autoimmune disease							
Multiple Sclerosis	1	1 (90%)					1 (Case 3)
Hypothyroidism/Thyroiditis	4	no	2 (mild)	2*	2 (MMP,RVM)	4	
Hypertension	1	1 (<25%)*			1 (MMP, DV)	1	
Miscellaneous	5	no	2 (mild)	1*	4 (MMP, DV, RVM)	5	
Total nr of cases with known risk factors	**28**					**24**	4
Maternal history stated free	**41**	1 (90%) 1 (<25%)*	23	11	34 (MMP, DV, RVM, FMP, FIR, chorionitis, deciduitis, IV thrombi, subchorionic thrombosis)	**40**	1 (Case 1)
Maternal history non available	**96**	1 (75%) 2 (<25%)*	n/a	n/a	n/a	**95**	1 (Case2)
Total nr of cases histologically examined	**165**					**159**	**6**

n/a: data non available or not included in this study
RVM: retarded villous maturation; IV: intervillous; MMP: maternal malperfusion; DV: decidual vasculopathy; FMP: fetal malperfusion; FIR: fetal inflammatory response
* In 16 cases of livebirth with parenchymal inflammatory placental lesions, PCR test for Cytomegalovirus, Herpes Simplex Virus, Ebstein-Barr and Parvovirus was negative.

Table 3. Clinical characteristics of the pregnancies with and without SARS-COV-2 placentitis

	Placentitis (10)	No Placentitis (59)	P
Thrombophilia, n (%)	3 (30%)	1 (1.7%)	0.008
Asthma, n (%)	0	2 (3.4%)	0.729
Smoking, n (%)	0	2 (3.4%)	0.729
Hypothyroidism	0	6 (10.2%)	0.376
Gestational Diabetes Mellitus DM, n (%)	3 (30%)	10 (16.9%)	0.280
Gestational Hypertension, n (%)	1 (10%)	0	0.145
Preeclampsia, n (%)	0	2 (3.4%)	0.729
IUGR, n (%)	1 (10%)	2 (3.4)	0.380
Asymptomatic Covid-19 infection	5 (50%)	52 (88.1)	0.007
Mild Covid-19 infection	4 (40%)	3 (5.1%)	0.007
Moderate Covid-19 infection	0	2 (3.4%)	0.729
Severe Covid-19 infection	1 (10%)	2 (3.4%)	0.327
Maternal age (Median, IQR)	35 (5.3)	30 (9.5)	0.007
Gestational age at infection (Median, IQR)	35.5 (12)	38 (4.5)	0.041