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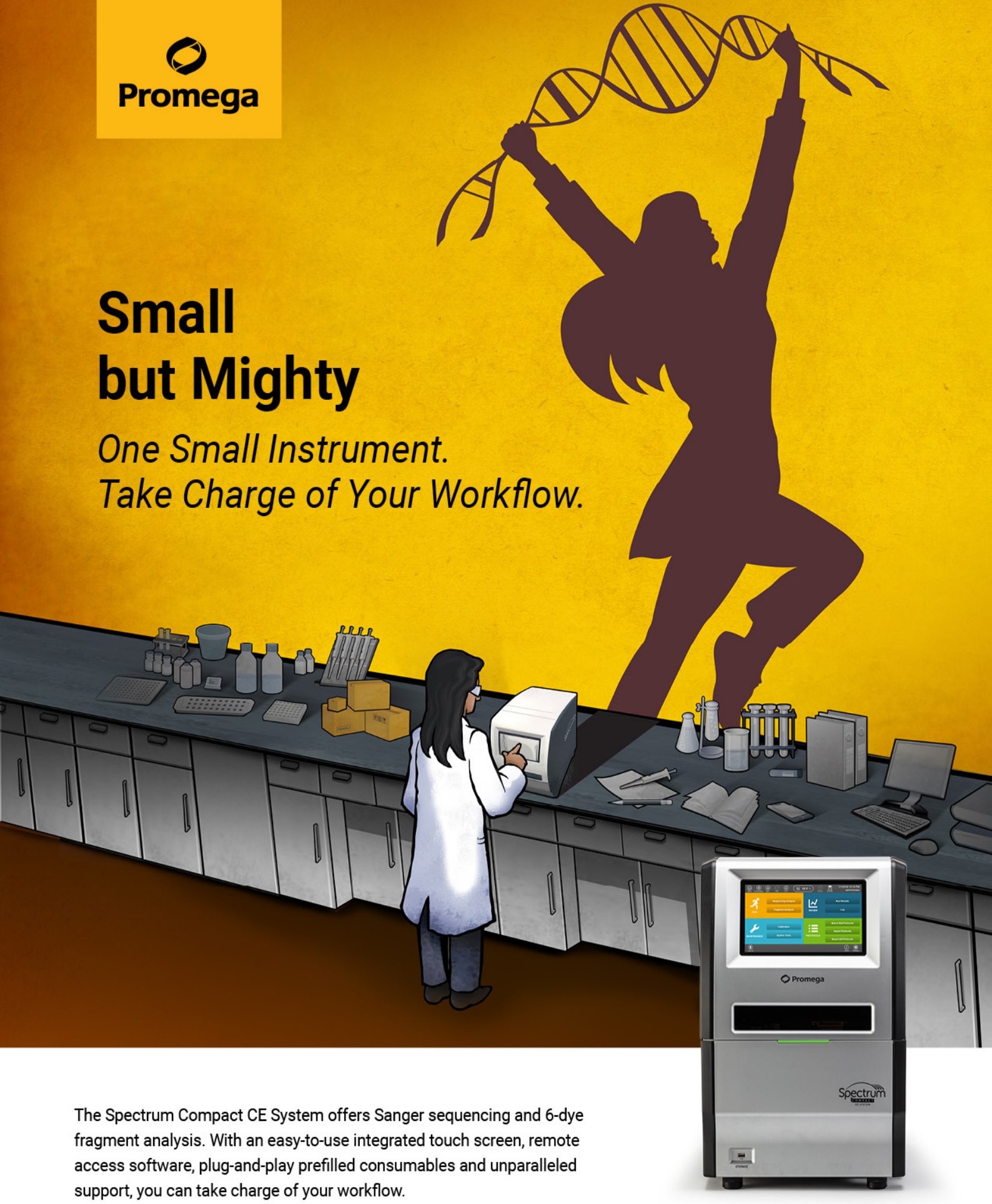

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Article type : Lesson of the Month

A rare but devastating cause of twin loss in a near-term pregnancy highlighting the features of severe SARS-CoV-2 placentitis

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From the start of the global COVID-19 pandemic, a lot of attention has been focused on how SARS-CoV-2 (severe acute respiratory syndrome coronavirus type 2) impacts pregnancy. The current evidence suggests that pregnant women may be at an increased risk for more severe COVID-19 disease and an increase in maternal death rate has been observed worldwide (1,2). However, mothers are not only concerned for their own wellbeing, but also for that of their unborn child. This concern might well be grounded, as a global increase in stillbirth (up to 28%) has been observed during this pandemic (2). Several studies also report an increase in adverse pregnancy outcomes in SARS-CoV-2-infected mothers, such as preterm delivery and low birth weight (1).

Many placental findings have been associated with both symptomatic and asymptomatic COVID-19. These mainly included non-specific signs of maternal or fetal vascular malperfusion, villitis and intervillositis. While all of these have been connected to fetal morbidity in the past, none seemed to be specific for a placental SARS-CoV-2 infection (3).

As the pandemic progressed, rare reports were published on placental SARS-CoV-2 infection with diffuse viral localization in syncytiotrophoblast. Histologically, these cases showed variable syncytiotrophoblast necrosis and histiocytic intervillositis. Neonatal outcome in these cases was highly variable, ranging from asymptomatic babies to stillbirth in up to 45% (4).

Our first experience with severe SARS-CoV-2 placentitis was in early 2021, when a 22-year-old primipara carrying twins presented at 36 weeks with severe pre-eclampsia and rupture of membranes (case 1). A week earlier, she had tested positive for SARS-CoV-2, showing mild symptoms. Examination on admission revealed IUFD (intrauterine fetal death) of one twin and severe fetal distress in the other, for whom, therapy was stopped because of diffuse (hypoxic) cerebral damage. This dramatic turn of events raised one question: “could this be COVID?”.

On section, the dichorionic, diamniotic placenta was nearly diffusely affected by large, irregular, solid areas with whitish discoloration (*Figure 1A*). Microscopically, unusually prominent syncytiotrophoblast necrosis, involving 70% of the placenta, and a mild to moderate histiocytic intervillositis were seen (*Figure 1B-C*). The infiltrate was composed of histiocytes, intermixed with smaller numbers of CD8- or CD4-positive T-cells, and neutrophils. There was no villitis. An additional finding was strong, nearly diffuse, linear C4d deposition at the surface of syncytiotrophoblast (*Figure 2B*). SARS-CoV2 nucleocapsid protein immunohistochemistry showed diffuse, strong staining in villous trophoblast (*Figure 2A*). The presence of SARS-CoV-2 was confirmed by RT-qPCR on RNA extracted from formalin-fixed, paraffin-embedded (FFPE)

material. Sequencing of the virus failed, but mutation-specific PCRs did not show variants of concern (tested for the 20I/501Y.V1 (UK), 20H/501Y.V2 (South-African) and 20J/501Y.V3 (Brazilian) variants).

At autopsy the IUFD twin showed no signs of inflammation, thrombotic events or any other specific findings. Nasal swabs in both children tested negative for SARS-CoV-2.

One month later, a similar story ensued (case 2), when a SARS-CoV-2-positive 28-year-old woman, with a singleton pregnancy of 31 weeks, was admitted with contractions and signs of fetal distress, but luckily, this child did well. Placental examination showed similar findings, but with less extensive syncytiotrophoblast necrosis (20-30%) and more pronounced intervillositis. Remarkably, similar, strong C4d positivity was also observed. SARS-CoV-2 infection was confirmed immunohistochemically. Sequencing on FFPE-extracted RNA showed infection by the SARS-CoV-2 20I/501Y.V1 variant (UK; B.1.1.7). Nasal swabs were negative for the virus.

To test the robustness of our findings, we repeated SARS-CoV2 immunohistochemistry on 14 placentas of SARS-CoV-2-infected mothers, without signs of intervillositis or trophoblast necrosis. On 8 of these RT-qPCR was performed. No (false) positive results were observed. We also immunostained all 14 of the above-mentioned, as well as infarcted regions in 5 randomly selected placentas from SARS-CoV-2-negative mothers, for C4d, but none of these showed any positivity.

Case 1 illustrates the possible dramatic consequences of placental SARS-CoV-2 infection. In this case, it is highly likely that the massive trophoblast necrosis was responsible for the deleterious effect on the twins. It is also tempting to speculate that massive release of syncytiotrophoblast fragments in the maternal circulation could have played a role in the pre-eclampsia. However, how the COVID-19 virus provokes trophoblast necrosis and intervillositis is currently unknown. An interesting finding is that prominent C4d deposition was noted in both infected placentas, similar to, but more extensive than what has been observed in idiopathic chronic histiocytic intervillositis (5). This suggests that complement activation may play a role in SARS-CoV-2 placentitis, analogously as reported in SARS-CoV-2 lung infection (6). The way of infection is still unclear, as the main binding receptor of SARS-CoV-2, angiotensin-converting enzyme (ACE2), is expressed in a polarized pattern with highest expression on the stromal side of syncytiotrophoblast (3). A possible way for the virus to bypass the tight syncytiotrophoblast barrier could be by neonatal Fc receptors that transfer virus-IgG complexes through the fetomaternal barrier, similarly as implicated in placental cytomegalovirus infections (7). Unfortunately, we were unable to obtain fresh placental tissue or appropriate maternal blood samples for further investigation of complement activation.

In conclusion, we report 2 cases of severe SARS-CoV-2 placentitis, which is rare, but can have a dramatic effect on pregnancy outcome. The main histopathological features of SARS-CoV-2 placentitis are syncytiotrophoblast necrosis, histiocytic intervillositis and strong positive immunohistochemistry for SARS-CoV2 nucleocapsid protein. Deposition of C4d may be an additional hallmark, but deserves further study.

Ethics Approval: The use of medical information and tissue for this study was approved by the ethics committee of Ghent University Hospital (EC/045-2021/mf, 20/03/2021).

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Figures legends:

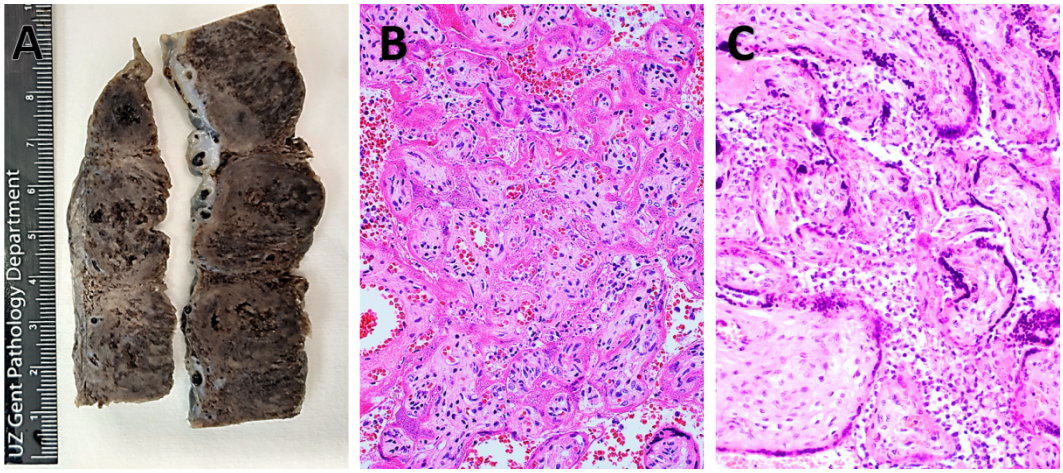
Figure 1

A: Macroscopy of the placenta of case 1 showing large, irregular, solid areas with whitish discoloration.
B: H&E section of the placenta of case 1, demonstrating necrotic syncytiotrophoblast, collapse of intervillous space and some histiocytes in the remaining intervillous spaces. Villous stroma is well preserved. (magnification: 200x)
C: H&E section of the placenta of case 2, illustrating prominent histiocytic intervillositis. Syncytiotrophoblast shows pyknotic nuclei and focal loss of nuclear staining, indicating necrosis. (magnification: 200x)

Figure 2

A: Immunohistochemistry for SARS-CoV-2 on the placenta of case 1 demonstrating diffuse positive staining of trophoblast (red). (magnification: 200x)

B: C4d immunohistochemistry on the placenta of case 1 showing strong and diffuse linear staining at the surface of syncytiotrophoblast. (magnification: 200x)



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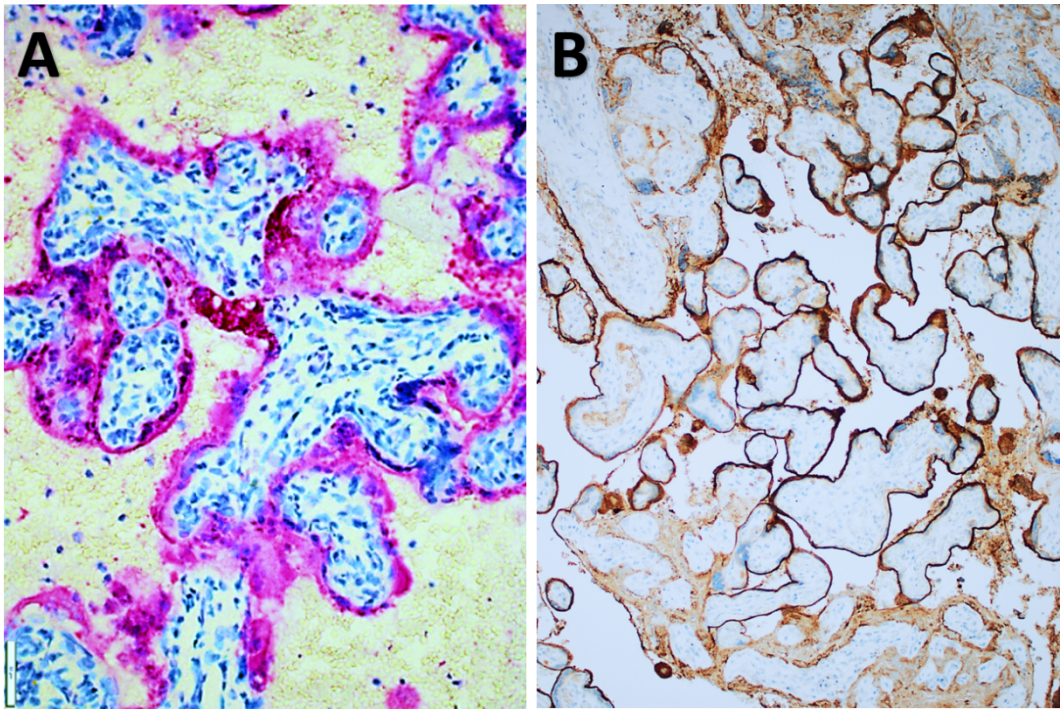

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