Intrauterine SARS-CoV-2 infection: a case confirming transplacental transmission followed by divergence of the viral genome

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SHORT RUNNING TITLE

Transplacental transmission of SARS-CoV-2

TWEETABLE ABSTRACT

COVID-19 can lead to intrauterine SARS-CoV-2 transmission with placental dysfunction and foetal distress.

CASE

A 27-year-old woman (gravida 2, para 1) was transported to the regional university hospital in gestational week (GW) 34 + 4 due to a three-day history of fever, abdominal pain and reduced fetal movements. She had developed a dry cough one day prior to the admission (Figure 1).

The woman, was slightly overweight (BMI 27 kg/m^2) but otherwise healthy. She had normal antenatal controls and an obstetric ultrasound at GW 32 + 2 showed a normal fetal weight deviation of +8%[1].

At admission, the patient was promptly isolated in a negative pressure room at the delivery unit and standard operating procedures and personal protective equipment (PPE) were used[2]. A combined nasopharynx (NPH) throat swab for SARS-CoV-2 using real time reverse transcriptase quantitative polymerase chain reaction (RT qPCR) was obtained and normal vital parameters (apart from fever 38.3 degrees Celsius) were registered. The admission cardiotocograph (CTG) test showed reduced baseline variability, absence of accelerations with recurrent prolonged, and late decelerations (Figure S1). In light of the pathological CTG pattern, the obstetric team made the prompt decision to deliver the patient by an immediate caesarean section (CS). An uncomplicated CS was performed in an operating theatre with negative pressure in line with the international recommendations for COVID-19[2]. The total blood loss was 200 mL. The amniotic fluid was of normal amount and there were no signs of meconium staining or premature rupture of the amniotic membranes.

The neonate showed no initial signs of spontaneous breathing and was ventilated by neonatal staff. A maximum of 80% supplemental oxygen was needed to maintain adequate saturation. At six minutes of age, the neonate established spontaneous breathing and continuous positive

airway pressure (5 cm H_2O) was maintained for an additional 24 minutes, whereafter further ventilatory support was not needed. At one minute of age, the neonate had an Apgar score of 1 (heart rate = 1, remaining items = 0), at five minutes of age Apgar 4 (heart rate = 2, muscular tonus = 1, reflex irritability = 1, remaining items = 0), and at ten minutes of age Apgar 8 (heart rate = 2, respiratory activity = 2, skin colour = 1, muscular tonus = 2, reflex irritability = 1). Validated umbilical cord blood gases[3] from both cord artery and vein showed a cord arterial pH of 7.20 and lactate 11 mmol/L. Figure 1 illustrates the timeline of events for mother and child.

After the CS, the mother was isolated in the postpartum ward and the NPH/throat swab taken upon admission returned positive for SARS-CoV-2. Analysis of maternal blood was also RT qPCR positive for SARS-CoV-2. Serology from the day of delivery revealed that the mother was weakly positive for immunoglobulin (Ig) M and negative for IgG. Along with lymphocytopenia (0.7 $x10^9$/L) and thrombocytopenia (98 $x10^9$/L); inflammatory markers including c-reactive protein (36 mg/L), ferritin (340 µmol/L) and lactate dehydrogenase (9.5 µkat/L) were found to be elevated. The clinical condition of the mother improved and she was discharged four days after delivery with thromboprophylaxis (Tinzaparin 4500 IE subcutaneously once daily) for a total six weeks postpartum. By day 11 postpartum, the mother was seropositive for anti-SARS-CoV-2 IgM and IgG. Breast milk analyzed day 14 postpartum was RT qPCR negative for SARS-CoV-2, and further, at day 35 postpartum, negative for anti-SARS-CoV-2 total immunoglobulin.

The neonate in the current case had no contact with any family member, including the mother, during the first 60 hours of life. Since neither skin-to-skin care nor any other contact with the mother occurred, the neonate was regarded as non-infected. In accordance with national

guidelines at the time[4], the neonate was tested for COVID-19 using a NPH swab 48 hours after delivery. This test returned positive for SARS-CoV-2 and the neonate was then regarded as contagious. Infection control routines were initiated to investigate a potential COVID-19 breakout at the neonatal ward and to rule out the possibility of postpartum transmission. All staff that had tended to the neonate (n=27) and all nearby patients (n=4) were tested for COVID-19. The NPH/throat swabs for SARS-CoV-2 RT qPCR returned negative in all cases (data not shown). Symptom surveillance in this group was continued for a further 14 days but no COVID-19 positive cases were discovered during this time.

The neonate was transferred and united with the mother at the postpartum ward isolation room at DOL 3 (60 hours after birth). Breastfeeding was thereafter initiated and the neonate did not receive any breastmilk before this time point. Repeated RT qPCR analyses showed the lowest neonatal CT-value at DOL 5 where after a gradual increase was seen. By DOL 20, SARS-CoV-2 was not detectable in NPH or throat swabs (Figure 1). Serology revealed that the neonate was anti-SARS-CoV-2 IgG negative at DOL 7 (IgM not analysed due to lack of material). At DOL 14, IgM was positive and IgG still negative and at DOL 20, the neonate was both IgM and IgG seropositive (Figure 1).

Viral genome sequencing

To determine the genetic clade and to fully investigate the viral genetic similarities, virus isolates from the mother (NPH/throat swab obtained on the day of delivery), and neonate (NPH swab obtained at 48 hours of age, labelled DOL 2, and further at DOL 5) as well as from placental tissue, were sent to the Public Health Agency of Sweden for whole-genome sequencing. Next-generation sequencing of samples produced several full length 29 903bp, SARS-CoV-2 genomes, all belonging to the genetic clade 20B/GR/B.1.1[5](Table S1). All four

sequences showed high identity. Further sequencing data analysis identified 12 variant positions in the sequences from isolates of the mother and placenta compared to the SARS-CoV-2 reference genome (NC_045512). These variants were also present in the sequences of the neonate isolates. Notably, an additional variant, A107G, was identified in the neonate samples but only present in 67 and 80%, respectively, of the sequences.

Placental pathology

The placenta was easily detached from the uterus during the CS. The remaining umbilical cord stump had a central insertion, was 9 cm long with a diameter of 1 × 1.5 cm and contained three vessels. The membranes had normal colour without signs of meconium staining. The trimmed weight of the placental disc was 342 grams, within the 10th to 90th percentile for GW 34+0 to 34+6[6]. At gross sectioning, fibrinoid depositions were evident as glistening white-grey-pink confluent lesions, encompassing approximately 50% of the total placental volume (Figure 2 A).

Microscopic examination confirmed the presence of confluent intervillous fibrinoid depositions accompanied by denudation of the villi from trophoblasts and syncytiotrophoblasts with dislocated syncytiotrophoblasts visible in the fibrinoid (Figure 2 B-C). There were multiple regions of dense intervillous infiltrates of neutrophilic granulocytes and macrophages (Figure 2 D). The areas devoid of intervillous fibrinoid depositions frequently showed chorangiosis (Figure 2 E). Immunohistochemistry confirmed that the inflammatory cell component of the intervillisotis was dominated by myeloperoxidase positive granulocytes and CD68 positive macrophages with sparse amounts of CD3 and CD20 positive lymphocytes (Figure 2 F-G).

Immunohistochemical detection of SARS-CoV-2 nucleoprotein was strongly positive in the cytoplasm and nucleus of villous cytotrophoblasts and syncytiotrophoblasts in areas with intervillositis and fibrinoid depositions, with some positive staining in the villous stromal cells (Figure 2 H-J). In contrast, SARS-CoV-2 nucleoprotein staining was focal or absent in most but not all areas devoid of intervillositis (Figure 2 K-L). Additionally, presence of ribonucleic acid (RNA) virus was confirmed in both cytotrophoblasts and syncytiotrophoblasts by *in-situ* staining for double stranded RNA (Figure 2 M). There were no signs of villitis or inflammation in the membranes or umbilical cord. Immunohistochemistry for SARS-CoV-2 nucleoprotein was absent or showed faint signal in the amniotic membranes and the foetal chorionic vessels.

DISCUSSION

Vertical transmission is one of the major complications of viral diseases during pregnancy[7]. Pregnant women are more likely to need intensive care treatment related to COVID-19 as compared to non-pregnant women of reproductive age[8]. COVID-19 infection has also been associated with a higher rate of preterm birth, preeclampsia, CS, and perinatal death[9]. A number of reports have suggested postpartum transmission[10], but to the best of our knowledge, only Vivanti et al.[11] have convincingly reported a case of transplacental SARS-CoV-2 transmission. SARS-CoV-2 may be physically blocked by the placental barrier defense mechanisms, combated by immune-regulatory molecular pathways or, in the case of placental infection, mitigate a fierce immune response that may potentially reduce the risk for fetal SARS-CoV-2 transmission[12]. The placenta is therefore of key interest in understanding perinatal transmission.

Several studies have found SARS-CoV-2 in placental tissue, amniotic fluid and in cord blood [13-15]. Facchetti et al.[16] analyzed the placentas from 101 women and found SARS-CoV-2 proteins expressed in only of them. This placenta originated from a COVID-19 pregnant woman whose newborn tested positive for viral RNA soon after birth, however, it was unclear if postpartum neonatal infection had been ruled out. Similarly, severe placental pathology due to COVID-19 has been implicated in another case report that led to adverse neonatal outcome despite mild maternal disease[17]. Transplacental transmission of the virus can therefore have dire intrauterine consequences for the fetus in seemingly mildly symptomatic mothers secondary to masked placental dysfunction.

The mother presented with classic COVID-19 symptoms including fever and a dry cough[18] but abdominal pain and reduced fetal movements were also reported. Similar to previous

reports, we observed that the clinical condition of the mother improved rapidly after delivery[19, 20]. The mother also presented with elevated concentrations of several acute phase proteins including ferritin, procalcitonin and c-reactive protein, indicating systemic inflammation[21]. In addition, at the time of delivery, SARS-CoV-2 RNA was found in the maternal blood and RT qPCR indicated the highest viral load within the placenta. RT qPCR does not produce an exact quantification of viral load as different materials are analyzed. However, the CT-values were clearly the lowest in the placental specimen and histopathological placental analyses indicated high levels of SARS-CoV-2. Viral protein was found in the villous cytotrophoblasts, in the syncytiotrophoblasts and massive perivillous fibrin deposits covered over 50% of the placenta. Viremia in the blood is rare. According to Wang et al.[22] SARS-CoV-2 RNA was found in only 1% of blood samples taken from COVID-19 patients. The placental histopathological changes seen in this case are similar to several previous reports on SARS-CoV-2, as well as SARS-CoV-1 and MERS-CoV[11, 16, 17, 23].

The neonate in the current case suffered from transient asphyxia attributed to intrauterine hypoxia secondary to placental dysfunction. This was signaled by the pathological CTG registering and validated umbilical cord blood gases revealed a cord arterial and venous pH well below normal median reference values[3], along with high cord lactate values[24]. Following initial resuscitation, only standard supportive care of prematurity was needed. No evident signs of COVID-19 were observed and repeated RT qPCR testing revealed the lowest CT-values at DOL 5, suggestive of the highest in the upper respiratory tract at this time point. The CT-values later increased and by DOL 20, SARS-CoV-2 RNA was not detectable. Consistent with the observed viral clearance, neonatal IgM and IgG seroconversion was found. Previous knowledge of immunoglobulin transfer during pregnancy along with new data from the current COVID-19 pandemic confirm that anti-SARS-CoV-2 IgG can pass through the

placental barrier whilst IgM does not[25, 26]. In the current case, maternal serum was weakly positive for IgM and negative for IgG at the day of delivery. Thus, transplacental transfer of anti-SARS-CoV-2 immunoglobulin was not likely and we therefore conclude that the neonate seroconverted by its own means. The possibility of the neonate acquiring COVID-19 postpartum was ruled out by vigorous testing of all staff that had been in contact with the neonate during the first 48 hours of life, as well as surrounding patients and their attendees. Secondary symptom surveillance for two weeks revealed no new cases.

To fully determine viral genome similarities between the mother, neonate and the placenta, whole-genome sequencing was performed. All four isolates revealed 29 903bp SARS-CoV-2 genomes, belonging to the genetic clade 20B/GR/B.1.1. Further analysis of the sequencing data showed that the isolate from mother and placenta had 11 single-nucleotide polymorphisms (SNPs) and one multiple-nucleotide polymorphism (MNP) differences compared to the reference Wuhan genome of SARS-CoV-2 (Figure S2). Interestingly, the two neonate isolates, from DOL 2 and DOL 5, both had a mixed population of the virus. In addition to a population of the virus with the same genotype as the isolates from the mother and placenta, the neonate isolates contained another population of virus (80% in DOL 2 and 67% in DOL 5) with an additional SNP, e.g. A107G. Intrapatient genetic variation has previously been described in both MERS-CoV and SARS-CoV-2[27, 28]. To the best of our knowledge, this is the first case of ongoing genetic change in neonatal COVID-19 in the unique setting of intrauterine transmission. Possibly, the observed genetic drift is a response to a change in the external environment for the virus. Overall however, all virus isolates from mother, child and the placenta, displayed a clear similarity and shared a majority of the SNP's.

Given these genetic findings and the series of events presented above, along with the marked placental pathology and the high viral load, it can therefore be concluded that the neonate was infected *in utero*. The two main clinical lessons that can be learnt from the current case are; I) COVID-19 during pregnancy may cause severe placental dysfunction and fetal compromise and II) intrauterine SARS-CoV-2 transmission may not necessarily lead to severe neonatal outcome.

ACKNOWLEDGMENTS

The authors would like to thank the COVID-19 in Pregnancy and Early Childhood (COPE) study for allowing the use of a maternal blood sample, Dr Yang De Marinis for donating a COVID-19 serological kit from ZetaGene, Dr Ylva Lindroth and Dr David Nygren from Department of Clinical Microbiology, Skåne University Hospital Lund, Sweden. The authors would like to acknowledge Mia Brytting at the Public Health Agency of Sweden for assistance in the interpretation of sequencing results as well as Niklas Edner for preliminary clinical consulting and facilitation of whole genome sequencing. The authors also gratefully acknowledge the researchers from the originating laboratories responsible for obtaining the specimens and the submitting laboratories where genetic sequence data were generated and shared via the GISAID initiative, on which this research is based.

CONTRIBUTION TO AUTHORSHIP

M.Z, A.M.H and P.T conceived the project, performed the literature search, prepared the tables, figures, merged and interpreted all the data and wrote the manuscript draft. L.J, M.Z and A.M.H managed the mother. M.Z interpreted the maternal clinical picture and laboratory tests. P.T, J.S, and O.A managed the neonate, interpreted the neonatal clinical picture and laboratory tests. A.S.S interpreted the SARS-CoV-2 diagnostic data. M.L.K and O.K.L performed the whole genome sequencing and data analysis. S.R.H, D.G.N, M.L.K and O.K.L helped in data interpretation and revision of the manuscript. D.G.N performed the pathological examination, prepared the figures and co-authored the text. All authors critically reviewed the manuscript for important intellectual content and approved it in its final version.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS

The mother and father have provided written informed consent to publication, available upon request. The case study was performed in agreement with principles of the Declaration of Helsinki.

FUNDING

MZ was supported by research grants from Region Skåne and the Medical Faculty, Lund University, Sweden (ALF). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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LEGENDS

Figure 1.

Timeline of events for mother and neonate. SARS-CoV-2; severe acute respiratory syndrome coronavirus-2, PCR; Real time reverse transcriptase quantitative polymerase chain reaction, GW; gestational week, NPH; nasopharynx, Ig; Immunoglobulin.

Figure 2.

Placental pathology. (A) Transected placenta with confluent accumulation of fibrinoid demarcated (white broken line) (B-C) Massive intervillous fibrinoid deposition surrounding denuded villi with extravillous syncytiotrophoblasts (circles) located in the fibrinoid (D) Acute intervillositis with karrhyorectic neutrophils in the intervillous space and degeneration of the villous trophoblast layer (E) Representative region of chorangiosis (F-G) Immunohistochemical staining for myeloperoxidase (MPO) and CD68 with positivity in inflammatory cells in areas of intervillositis (H-J) Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) nucleoprotein (NP) detected in nucleus and (circle in I) and cytoplasm in villous trophoblasts and syncytiotrophoblasts as well as in the nucleus of villous stromal cells (J) in areas of intervillositis (K-L) Areas without intervillositis showed absent or focal staining for SARS-CoV-2 nucleoprotein of villi. M. Double stranded RNA (dsRNA) detected in villous trophoblasts and syncytiotrophoblasts (circles).