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Case report

Is SARS-CoV-2 vertical transmission still a current problem? A case report on a diagnosed SARS-CoV-2 infection with a positive sample of urines

Pier Luigi Bacchini[1], Antonino Sammartano[2], Piera Manfredi[1], Maria Luisa Bidetti[1], Monica Malpeli[2], Magda Magliani[2], Fabio Maradini[2], Luigi Ippolito[2]

[1]Pediatrics, Fidenza Hospital, Fidenza (PR), Italy; [2]Clinical Pathology Unit, Medical and Diagnostic Department, Fidenza (PR), Italy

Abstract. *Background and aim:* Current data suggest little to no possibility of original COVID-19 transmission in pregnant women to the fetus during pregnancy or childbirth. Warning with Omicron new variants has decreased. *Case report:* A clinical case of a SARS-CoV-2 virus transplacental infection of a newborn, born at the end of 2022, from a mother who tested positive for Sars-covid-2 and positive IgM SARS-CoV-2 anti-virus. The newborn tested positive for SARS-CoV-2 12 hours after birth, and was clinically symptomatic after three days, an increase in IgM antibodies was not found, although the virus was identified in the urine samples through molecular tests. The insufficient time to determine the presence of antibodies and the immune system's state of immaturity can explain the lack of IgM in the newborn's blood at 14 days after birth. *Conclusions:* The Omicron SARS-CoV-2 keeps provoking infections among newborns, especially if the mother contracts it during the third trimester. The host response is most likely influenced by the newborn's peculiar state of immune immaturity. Just before birth, a positive nasal swab and the presence of a positive urine examination confirmed the diagnosis of intraplacental exposure. Research on the virus through molecular tests of urines can represent an additional technique when an aetiological framework of the infection is necessary and a distinction between congenital and post-natal forms. (www.actabiomedica.it)

Key words: newborn, vertical transmission, SARS-CoV-2, nasal swab, Polymerase Chain Reaction SARS-CoV-2 test on urines

Introduction

There are three ways of vertical transmission of SARS-CoV-2: in the uterus through the maternal placental interface, intrapartum from the mother's blood or secretion, and lastly post-natal (1-2). To date, there aren't certain reports on the transmission of SARS-CoV-2 through breast milk (3). Italian National Health Institute (ISS) data from the 8th of November 2022 reported that, in Italy, the Omicron variant had a prevalence of 99,8% with the largely prevalent BA.5 sub-variant (4). The Omicron variant appears to determine infection in charge of the high respiratory tracts and no longer multisystem inflammation, similar to the original strain and SARS-CoV-2 Delta variant (5).

SARS-CoV-2 infection transmitted from the mother to the fetus is rare or improbable (2-3). Many pregnant women were in their third trimester (2,6). The majority of newborns infected are asymptomatic or show slight symptoms at birth (6). It is not always easy to distinguish infections through the placenta from forms by contact with secretions, during or after birth (1). We aimed to provide a rare neonatal

case. The newborn in our case was born from a positive mother and tested positive in the molecular swab 12h after delivery. He developed clinical symptomatology and alteration of a specific index of phlogosis; during the observation period, the newborn never was positive for IgM antibodies. To distinguish a certain vertical infection, a test of molecular nucleic acid amplification "Real-Time Polymerase Chain Reaction" (RT-PCR) was performed on urine samples, proving a positive result.

Case presentation

It involves a newborn at 41 weeks and 4 days of gestational age, female, born in November 2022, at 6.34 pm, without malformations, Apgar at the first minute 7 and fifth minute 9, the weight of g 3370 (87°p). The delivery happened two hours after the maternal membrane broke off. The mother was negative for HIV, hepatitis B and C virus, toxoplasmosis, and CMV. She received two shots of the SARS-CoV-2 vaccine, the last shot in September 2022. Five days before delivery, she had mild temporary rhinitis. Two days before delivery, she tested positive for SARS-CoV-2. At the time of delivery, the mother was asymptomatic. Measures for prevention of viral transmission were always applied (room of isolation, constant wearing of an FPP2 face mask, for both the mother and her partner, hands frequently washed, and distancing about 2 meters of the newborn's crib from her mother's bed). The newborn was breastfed, always with disinfected hands and an FPP2 face mask.

Due to reasons linked to the organization of the Clinical Pathology Laboratory, the newborn's molecular swab for SARS-CoV-2 was done (Table 1) sand processed the morning after, 12 h after birth, and tested positive. The newborn had never been feverish or presented with clinical symptoms of the respiratory tract. However, from the third day of life and for the next 48 h, the newborn showed a tendency to extend sleep, lower energy, and poor liveliness.

All biochemical measurements and Covid-19 PCR tests and rapid antigen tests were performed at the Clinical Pathology laboratory, (Medical and Diagnostics Department P.O. Fidenza, AUSL Parma, Italy) by skilled technicians (7). The laboratory is licensed according to the ISO 15189 accreditation standard for clinical laboratories.

A complete blood count (CBC) showed leukopenia and lymphopenia, while the C-Reactive Protein (CPR) tested slightly abnormal for age. No pharmacologic therapeutic intervention was performed. Temperature, heart rate, oxygen saturation, and diuresis were always normal. At six days of life, the newborn was asymptomatic and in a healthy condition; the blood count and CPR were normal for her age. The molecular nasal swab was still positive, the anti-SARS-CoV-2 antibodies of the mother and newborn showed low titers of IgG and negative titers of IgM antibodies. We decided to perform a Real-Time Polymerase Chain Reaction (RT-PCR) analysis of the newborn's urine.

Urine was collected in aseptic mode and sent on ice to the laboratory. It was immediately processed, and the sample was positive. After three days, the RT-PCR nasal swab and urine samples tested positive, while IgM antibodies in the blood were still negative, and IgG was low titer positive. At the end of quarantine, the mother returned negative. The molecular nasal swab of the newborn was still positive 14 days after birth, while the test performed on urines was negative. The newborn's antibody dosage still showed low titer IgG and lacked IgM; meanwhile, the analysis of the mother's blood showed positive IgM and a very high IgG titer of over 40000 UA/ml. At 14 days after birth, the Infection Prevention and Control Office communicated to the parents the end of the newborn's quarantine. Therefore, they stopped any other tests and neurological follow-ups. They only agreed to have the ABR hearing test of the newborn done after 2 months. This turned out to be normal.

Discussion

To date, vertical transmission of SARS-CoV-2 during pregnancy remains controversial (8-10). Warning with new variants has decreased. An increase in the inflectional capacity of the virus was verified with the new variants and, at the same time, a decreasing capacity of its systemic diffusion. Newborns have a lighter illness, with a clinical presentation that differs from that of older

children and adults, with a prevalence of gastrointestinal symptoms and poor alimentation (11). The symptoms were lighter and shorter, even in the case described, but were linked to transient leukopenia and lymphopenia. In the field of the relative rarity of warnings and with the hypothesis that a transplacental infection could determine the result at a distance (12), the challenge is to distinguish an infection determined by the placental passage of the virus before birth that obtained through contact with maternal secretions containing the virus during delivery or after that (13). The nasal test alone is not sufficient (1,13) and as it was attended to according to the literature's warnings, the antibodies more frequently traceable in the newborn are the IgG, derived from the mother, and do not provide definitive answers (14).

In our case, the virus was isolated from the nasal secretions, and it was verified that there was an increase in weak clinical symptomatology. It was also possible to isolate the virus in the urine. This is compatible with hematic diffusion and can be used to classify the timing of mother-to-child transmission using appropriate samples, according to the World Health Organization (WHO) guidance (1). Moreover, repeated tests with appropriate samples are needed at various time phases to reduce the proportion of babies in whom vertical transmission cannot be confirmed despite their initial positive status. Consequently, we used a sample of urine, collected in the aseptic mode. Isolation of genetic material from the urines is possible (11,12), adding to the list of other useful tests to indicate the presence of an active infection and to deny the possibility of nasal contamination from viral material (1). The limitations are linked to the use of a technique on urines that is not completely validated, differing from that on nasal secretions. In newborn blood, anti-Spike IgG antibody titers were low. The mother was vaccinated, and the antibodies were transmitted to the fetus. An increase in IgM levels was not observed. This might have happened because 14 days

Table 1. Shows the exams we performed with their results.

Date	Material	Exam	Results	Reference values
13.11.22	Nasal secretion	RT-PCR Virus CoViD 19	Positive	-
15.11.22	Blood	Leukocyte	3.590 /mm3	9,000 to 25,000/mm3
15.11.22	Blood	Leukocyte count	Neutrophils 2.440/ul; Lymphocytes 410 /ul	1,600 to 6,600/mm3 800 to 4,232/mm3
15.11.22	Blood	CPR	6.9 mg/l	< 5 mg/L
15.11.22	Blood	Anti-Spike IgM	Negative	-
15.11.23		ECG	Normal	-
16.11.22	Blood	Anti-Spike IgG	934 AU/ml	<40 AU/ml
18.11.22	Blood	Leukocyte	7.570 /mm3	9,000 to 25,000/mm3
18.11.22	Blood	Leukocyte count	Neutrophils 940/uL; Lymphocytes 5.330/ul	1,600 to 6,600/mm3 800 to 4,232/mm3
18.11.23	Blood	AST/ALT	116/41 U/L	<40 U/L
18.11.23	Urine	RT- PCR Virus CoViD 19	Positive	-
18.11.23	Blood	PCR	2.10 mg/L	< 5 mg/l
21.11.23	Nasal secretion	RT- PCR Virus CoViD 19	Positive	-
21.11.23	Urine	RT- PCR Virus CoViD 19	Positive	-
21.11.23	Blood	Anti-Spike IgM	Negative	-
21.11.23	Blood	Anti-Spike IgG	983 AU/ml	<40 AU/ml
28.11.23	Blood	Anti-Spike IgM	Negative	-
28.11.23	Blood	Anti-Spike IgG	736 AU/ml	<40 AU/ml
28.11.22	Urine	RT-PCR Virus CoViD 19	Negative	-

are not sufficient to reveal the presence, although, in scientific literature, two weeks seem sufficient (13,15).

Unfortunately, since the newborn recovered and finished her quarantine time, as the law indicated, it was not possible to continue the analyses. However, the lack of confirmation of the presence of IgM antibodies in our newborn, might not be just for the temporal limits of observation. Indeed, it could have a second interpretation: the combination of the state of immune immaturity of the newborn, the presence of maternal antibodies in her hematic circle, and the innate response of the viral infection might have somehow favored the absence of a strong reaction (14), typical of the obtained immunity, which, instead, was verified in the mother. We initially found that the maternal hematic values of IgG were not very high. Then anti-Spike IgM antibodies were positive with a high increase in anti-Spike IgG antibody titer. Moreover, the mother's vaccination seems not to have been protective from the transmission of the infection to the newborn, but the increase in just a light form of illness might have contributed. The viral strain caught is most likely different from the original of 2020, and this is proved by the appearance of IgM in the mother's blood, even though the mother had completed the vaccination for two months. Since the beginning, the newborn presented anti-Spike antibodies of maternal transplacental origin, which probably limited the expression of the illness.

To evaluate renal interest in the form systemic, was used the SARS-CoV-2 test on urines (12). It was relatively easy to perform, did not require additional technical difficulties, and was better tolerated by newborns by nasal or blood examination. Therefore, it could represent an additional technique, when evaluating the systemic interest in the infection is necessary.

Similar to other viruses, the hypothesis of a vertical passage raises the question of whether it might determine long-distance effects, especially on sensorial functions, cerebral growth, and child behavior development. Unfortunately, it was not possible to perform a complete follow-up.

Conclusions

Viral vertical congenital transmission may still occur even with new viral variants. Vaccination during pregnancy represents an element of protection against risks, as well as for the fetus.

Therefore, it is difficult to imagine how serology alone could contribute to a neonatal diagnosis, especially when the maternal infection is verified at the end of pregnancy, and there might have been insufficient time to develop antibodies. Some authors have proposed an algorithm for the diagnosis of vertical transmission of SARS-CoV-2 and raised the question of whether it might determine long-distance effects (15).

The WHO indicates, among the decisive exams, real-time PCR of blood (1). We propose to evaluate the possibility of examining the urines, easily collected and analyzed, as an element of proof of the viral and infective presence. Similar to what happens with other viruses, testing the SARS-CoV-2 presence with the urine's RT-PCR analysis, during the first or second days of life, could be useful to distinguish a congenital form from another obtained after birth. We also believe that PCR of newborn urines may be useful to distinguish a form limited just to the respiratory tract from systemic diffusion.

Other studies are still needed for the comprehension of the SARS-CoV-2 virus behavior, to enhance the management of pregnancy, and to offer new ways to prevent neonatal infection.

List of Abbreviations: ISS: Italian National Health Institute. WHO: World Health Organization. CPR: C-Reactive Protein. RT-PCR: Real-Time Polymerase Chain Reaction.

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Correspondence:
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Pier Luigi Bacchini, MD Pediatrics,
Fidenza Hospital
Via 5 Don Enrico Tincani 5,
Fidenza (PR), 43036 Italy
Phone: +390524515135-+390524515478
E-mail: pbacchini@ausl.pr.it
ORCID: 0009-0008-2764-0506