Early Identification of IgA Anti-SARS-CoV-2 in Milk of Mother With COVID-19 Infection

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Abstract
Introduction: Human milk cannot currently be considered a major source of COVID-19 infection. On the other hand, it can contain specific antibodies that could modulate a possible newborn infection by SARS-CoV-2.
Main issue: A 32-year-old pregnant woman, gestational age 37 and 3/7 weeks, was admitted with a flu-like syndrome caused by COVID-19. The female newborn was appropriate for gestational age, with a birth weight of 2,890 g, length 48 cm, and head circumference 34 cm.
Management: The mother–infant dyad remained in the rooming-in unit during hospitalization, exclusively breastfeeding and following World Health Organization recommendations for contact and airway precautions. On the 3rd day after delivery, two mother’s milk samples (3 and 5 mL) were collected by hand expression. The samples were centrifuged for 10 min twice consecutively to separate fat, which was removed, and the remaining material was transferred to another tube to determine anti-SARS-CoV-2 Immunoglobulin A and Immunoglobulin G (ELISA, Kit EUROIMMUN AG, Luebeck, Germany). Anti-SARS-CoV-2 Immunoglobulin A was detected in the two samples evaluated, whose values were 2.5 and 1.9, respectively. No anti-SARSCoV-2 immunoglobulin G was detected. The exclusively-breastfed infant remained well through 45 days of age.
Conclusion: The presence of SARS-CoV-2 Immunoglobulin A in the milk of mothers infected with COVID-19 may be related to protection against the transmission and severity of the disease in their infants.

Keywords
breastfeeding, case study, COVID-19, human milk, infant, infant care, infant nutrition, pregnancy, SARS-CoV-2, vertical transmission

Resumo
Introdução: O leite humano não é considerado como fonte de transmissão de COVID-19 até o momento. Por outro lado, ele pode conter anticorpos que podem proteger o recém-nascido da infecção pelo SARS-CoV-2.
Descrição do caso clínico: Uma gestante de 32 anos, idade gestacional 37 3/7 semanas, foi admitida para realização do parto, com síndrome gripal causada por COVID-19. O seu recém-nascido, do sexo feminino, foi adequado para idade gestacional, pesou 2.890 gramas, comprimento 48 cm e circunferência craniana de 34 cm.
Tratamento: A mãe e seu recém-nascido permaneceram em alojamento conjunto durante a hospitalização, realizando aleitamento materno exclusivo, conforme as recomendações da Organização Mundial da Saúde em relação as precauções de contato e proteção de vias aéreas para nutrizes infectadas pelo COVID-19. No terceiro dia após o nascimento, coletou-se, por expressão manual, duas amostras de leite materno (2 e 5 mL) que foram centrifugadas por 10 min por duas vezes, para remoção da gordura e separação do material remanescente, que foi transferido para outro tubo para dosagem de anti-SARS-CoV-2 IgA e IgG (ELISA, Kit EUROIMMUN AG, Luebeck, Germany). Como resultado, foi detectado nas duas amostras de leite materno, a presença de IgA anti-SARS-CoV-2, cujos valores foram 2,5 e 1,9; respectivamente. Não se verificou a presença de IgG anti-SARSCoV-2. O recém-nascido permaneceu, clinicamente bem, em aleitamento materno exclusivo até a última avaliação que foi realizada aos 45 dias de vida.
Conclusion: A presença de IgA anti-SARS-CoV-2 no leite materno de mulher infectada pela COVID-19 pode se relacionar a proteção contra a transmissão e gravidade da doença nos recém-nascidos. Translation confirmed by Dr. Monica Pina.
Introduction

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is highly contagious, and the main transmission route of the virus is through aerosols and airway mucosal contact (Wang et al., 2020). So far, there is no evidence to confirm COVID-19 vertical transmission, even through breastfeeding. In a recent systematic review of 14 studies, 47 of 48 samples of human milk tested negative for the presence of SARS-CoV-2 (Lackey et al., 2020). Researchers have reported that none of the studies using nucleic acid detection for the COVID-19 virus had validation of their collection and analytical methods for use in human milk, or describe the presence of viable SARS-CoV-2 in samples (Cheema et al., 2020).

Mother’s milk cannot be considered as a major source for COVID-19 infection. On the other hand, it can contain specific antibodies that could modulate a possible newborn infection by SARS-CoV-2 (Davanzo, 2020). Given the evident benefits of breastfeeding, the World Health Organization (WHO, 2020) strongly recommends that women with COVID-19 be encouraged and supported to breastfeed, wear masks, and adopt contact precautions.

The study participant in this report agreed with the publication of the case study, preserving identity confidentiality, and that this manuscript should only be used for scientific dissemination. The study participant signed a consent form and approved the final version. The aim of this case study was to follow this mother and her infant, and to test the mother’s milk to identify Anti-SARS-CoV-2 antibodies which can be a protective factor during breastfeeding.

History and Observational Assessment

A pregnant woman, aged 32 years, gestational age 37 and 3/7 weeks (as per the date of the last menstruation) who was single, a smoker, and had completed high school, was admitted to the Public Maternity, Gynecology, and Obstetrics Emergency Room with a flu-like syndrome. She had had severe cough for the previous past 3 days, associated with fever and dyspnea. Oxygen saturation at admission was 95%. She denied urinary complaints, myalgia, headache, and diarrhea. The obstetric evaluation indicated she was ready for delivery, which occurred 2 hr after hospitalization, by cesarean section due to persistent fetal tachycardia. The participant had had 10 prenatal visits, had immunity to toxoplasmosis, cytomegalovirus, Hepatitis B and C, a nonreactive VDRL and HIV, and her blood type was O+. She had a pregestational weight of 76 kg (154 cm, BMI = 32.0 kg/m²), and had gained 9 kg during pregnancy.

Management

After delivery, the team attempted to enable skin-to-skin contact, but the participant was not in a stable clinical condition. The delivery staff decided to separate the newborn from her mother. The postpartum participant received routine care, hydration, and oxygen therapy. Five hours after delivery, her clinical condition improved and she was sent to an isolation room in the puerperium unit, together with her newborn, exclusively breastfeeding.

The participant was instructed by the nursing team to wear surgical masks, especially when she was in contact with her newborn infant, and to wash her hands or use hand sanitizer before and after touching her baby or directly breastfeeding (Table 1). Her infant was kept at a 2 m distance from the participant’s bed. The surfaces were cleaned frequently and contact with relatives was restricted to the newborn’s father.

The participant remained under observation, afebrile, and with an oxygen catheter (1 L/min) for 24 hr (oxygen saturation 97%–98%). She was given ceftriaxone 2 grams/day (7 days), azithromycin 500 mg/day (5 days), low molecular weight heparin (1 mg/kg), and oseltamivir 75 mg (in a single dose). Tests for the investigation of the flu-like syndrome, inflammatory markers, and chest computed tomography (CT) were collected (Table 2).

The CT evidenced ground-glass opacity and sparse bilateral consolidation predominating in peripheral regions. The pulmonary extension involvement was 25% (mild extension) and was interpreted as acute respiratory distress syndrome (ARDS) meeting the 2020 Coronavirus-Disease Interim Case Definition as a probable case (presumptive laboratory evidence and clinical

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The lowest lymphocyte count was 1.118 \(10^3/\text{mm}^3\), with changes in aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), high-sensitivity C-reactive protein (PCR-us), and creatine phosphokinase (CPK; Table 2).

The newborn female was appropriate for gestational age (AGA), with Apgar 9 in the 1st minute, and 10 in the 5th minute, with a birth weight of 2,890 g, length 48 cm, and head circumference 34 cm. She remained in excellent general condition during hospitalization. The infant continued to exclusively breastfeed, while the participant followed the WHO (2020) recommendations for women with COVID-19.

### Outcomes

Regarding the specific COVID-19 investigation, the participant tested negative in two nasopharyngeal reverse transcriptase-polymerase chain reaction (RT-PCR) swabs (3 and 6 days after the onset of symptoms). The participant newborn tested negative in one nasopharyngeal swab RT-PCR performed within 48 hr of life (Table 1). The participant had reactive Immunoglobulin M (IgM) and Immunoglobulin G (IgG) anti-SARS-CoV-2 serological tests (immunochromatography) 8 days after the onset of symptoms. On the 9th day after the onset of symptoms, she was submitted to quantitative Immunoglobulin A (IgA) and IgG anti-SARS-CoV-2 serological tests (ELISA, Kit EUROIMMUN AG, Luebeck, Germany). The results were positive, with values of 3.8 and 0.7, respectively.

On the morning of the 3rd day after delivery and the 6th day since the onset of symptoms, two mother’s milk samples (3 and 5 mL) were collected by hand expression. They were centrifuged for 10 min twice consecutively to separate fat, which was removed from the tube. The remaining material (serum) was transferred to plates to determine IgA and IgG anti-SARS-CoV-2 (ELISA, Kit EUROIMMUN AG, Luebeck, Germany). The IgA anti-SARS-CoV-2 was detected in both of the two samples evaluated, with values of 2.5 and 1.9, respectively. The IgG anti-SARS-CoV-2 was negative in both samples (Table 1).

The serological tests were performed according to standard practice in clinical analysis and manufacturer’s

### Table 1. Case Study Timeline.

| Time | Follow-up | Diagnostic Testing (Sample Source) | Interventions |
|------|-----------|------------------------------------|---------------|
| Birth | Mother: Afebrile, cesarean Infant: Term healthy newborn | Mother: Infant: | EBF; O² = 1L room-in |
| DOL 2 | Mother and infant healthy | Mother: Infant: | EBF; Room-in |
| DOL 3 | Mother: healthy Infant: phototherapy (physiological jaundice) | Mother: Infant: | EBF; room-in |
| | | | | + RT-PCR (NP swab) |
| | | | | + IgA SARS-CoV-2 (HM = 1.9 and 2.4) |
| | | | | + IgG anti-SARS-CoV-2 (HM) |
| DOL 5 | Mother healthy; Infant: phototherapy (physiological jaundice) | Mother: Infant: | EBF; room-in |
| | | | | + IgM and IgG anti-SARS-CoV-2 |
| DOL 6 | Mother and infant healthy; discharged home | Mother: Infant: | EBF; room-in |
| | | | | + IgA anti-SARS-CoV-2 ELISA test (3.8) |
| DOL 10 | Mother and infant healthy; isolated at home | | EBF |
| DOL 25 | Infant without symptoms; weight 3,500 g | | EBF |
| DOL 45 | Infant without symptoms | | EBF |

Note. DOL = day of life; EBF = exclusive breastfeeding; HM = human milk; NP = nasopharyngeal; RT-PCR = reverse transcription polymerase chain reaction.

### Table 2. Participant’s Postpartum Laboratory Values.

| Tests                          | Day 1 | Day 3 | Day 6 | Reference values |
|-------------------------------|-------|-------|-------|------------------|
| Lymphocytes (10^3/mm³)        | 1.118 | 2.830 | 2.290 | 2.5–5            |
| Alanine aminotransferase (U/L)| 7     | 16    | 98    | Up to 34         |
| Aspartate aminotransferase (U/L)| 20   | 31    | 50    | Up to 33         |
| Lactate dehydrogenase (U/L)   | 285   | 254   | 274   | 135–214          |
| C-reactive protein (mg/L)     | 54.13 | 56.65 | 13.43 | Up to 5          |
| Creatine phosphokinase (U/L)  | 399   | 130   | < 170 |                  |
recommendations in the Clinical Analysis Laboratory of Reference for the Diagnosis of COVID-19. The serological reactions were performed and compared with human serum negative and positive internal controls. The ELISA sensitivity of IgA and IgG anti-SARS-CoV-2 tests combined was 66.7%, the specificity of anti-SARS-CoV-2 IgA and IgG was 92.5% and 98.5%, respectively (ELISA, Kit EUROIMMUN AG, Luebeck, Germany). The human milk ELISA analysis for IgA and IgG anti-SARS-CoV-2 were tested when the human serum tests were done. The “activity” of the IgA was not tested against SARS CoV-2.

The dyad remained rooming-in and continued to exclusively breastfeed. The participant remained afebrile, with progressive improvement of the cough and other symptoms. The newborn remained stable, with no changes in clinical signs, requiring phototherapy for physiological jaundice. The participant and her newborn were discharged together, on the 7th day postpartum, with guidance about maintaining exclusive breastfeeding with contact precautions. The infant was evaluated after discharge on Days 10, 25, and 45 of life. The infant did not show any flu-like symptoms or other clinical problems, remained exclusively breastfeeding, and presented proper development, growth, and weight gain. The participant fully recovered from the COVID-19 infection.

**Discussion**

In this case study we demonstrated the presence of anti-SARS-CoV-2 IgA in mother’s milk in a puerperal woman with COVID-19, during the first 72 hr after delivery. A recent preprint (not peer-reviewed) also identified anti-SARS-CoV-2 IgA by the ELISA method in 14 human milk samples from women with COVID-19 (Fox et al., 2020). However, in this study, the authors collected human milk samples 14–30 days after delivery and did not clinically assess the infants who continued breastfeeding.

Although all immunoglobulins are found in colostrum and milk, IgA is considered the most important. IgA produced by the maternal mammary glands undergoes proteolytic cleavage to release secretory IgA, permitting transport into human milk (Brandtzaeg, 2010). While secretory IgA is responsible for 80%–90% of the total immunoglobulins in human milk, only about 10% is absorbed in the intestines and transferred to the bloodstream. Its action is fundamentally within the gastrointestinal tract (Brandtzaeg, 2010).

Human milk IgA provides a critical antimicrobial defense for the neonatal gastrointestinal tract by inhibiting pathogen attachment to mucosal surfaces, neutralizing microbial toxins, and providing passive immunity (Nolan et al., 2019). Breastfed infants show a 47%, 63%, and 57% reduction in the risk of death from infectious diseases, acute diarrheal disease, and hospitalizations due to respiratory diseases, respectively (Victoria et al., 2016). This can be explained by the complex immunology of human milk, including carbohydrates, long-chain polyunsaturated fatty acids, cells, and immunoglobulins. In this case study, the infant remained exclusively breastfeeding and had no symptoms related to COVID-19 infection, despite the participant’s symptomatic infection. The presence of anti-SARS-CoV-2 IgA in the participant’s milk may have been protective for the infant.

IgA in human milk protects the infant against enteric infections caused by rotavirus, *Escherichia coli*, poliovirus, and retrovirus (Van de Perre, 2003). Pregnant women vaccinated against meningococcus, influenza, and pneumococcus had higher concentrations of secretory IgA specific for these microorganisms in human milk and, in most of them, there was a reduced risk of developing these diseases in their infants (Maertens et al., 2014; Schlaudecker et al., 2013).

In this case study, the participant and her infant tested negative for COVID-19 by RT-PCR. Despite this, the participant had clinical characteristics for COVID-19 infection, and serological testing that was positive in blood and human milk samples. Some factors can influence the absence of RT-PCR positivity (e.g., time of disease, difficulty in clearly identifying the onset of symptoms, and collection method). The combination of different laboratory serological tests can increase the sensitivity of the COVID-19 diagnosis (Benoit et al., 2020).

Strengths of this study include the infant remaining exclusively breastfeeding and the followed up to 45 days of life without symptoms of clinical complications related to COVID-19 infection, despite being exposed. The ELISA method used to evaluate SARS-CoV-2 IgA and IgG is new and has not been standardized for use in human milk, which is a weakness of this single case study and, thus, validation studies using this method are necessary.

In conclusion, SARS-CoV-2 IgA in the milk of women infected with COVID-19 may be related to protection against the transmission and severity of the disease in their infant. Follow-up studies with an adequate number of mother–infant pairs included, that account for mother and infant proximity, amount of breastfeeding, and have long-term follow-up, would improve our knowledge about the protective effects of SARS-CoV-2 IgA in the mother’s milk.

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