Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Excretion of SARS-CoV-2 in human breast milk

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To the Editor,

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing coronavirus disease 2019 (COVID-19) [1] emerged in China in December 2019 and has rapidly spread to reach 3 023 788 confirmed cases over time, according to the World Health Organization report on 29th April 2020 (https://experience.arcgis.com/experience/685d0ace521648f8a5beeeee1b9125cd). SARS-CoV-2 is detectable in different body fluids, although human-to-human transmission occurs mainly by the respiratory route. The virus is plausibly transmitted to neonates through the infected mothers' respiratory droplets during breastfeeding [2], but SARS-CoV-2 transmission may not occur through breast milk [3,4].

In March 2020, we studied two pregnant women (hereafter referred to as patient 1 and patient 2) admitted to our hospital (Fondazione Policlinico Universitario A. Gemelli IRCCS in Rome, Italy) who received a laboratory-documented diagnosis of COVID-19 (i.e. with nasopharyngeal swabs that were positive for SARS-CoV-2) [5].

Patient 1 presented with fever, shortness of breath, and diarrhoea, and patient 2 presented with a cough (Table 1). Neither patient had pneumonia. Laboratory investigations showed that lymphocytes were below the normal range (<1.0 × 10^9 cells/L) and C-reactive protein concentrations were elevated (>10 mg/L). The patients were in their third trimester and, 8 days after COVID-19 diagnosis, both underwent caesarean section following foetal distress (patient 1, on 28th March 2020) or a history of caesarean section (patient 2, on 26th March 2020). The patients were treated empirically with antimicrobial agents, and only patient 1 received oxygen support (nasal cannula) (Table 1). The neonate of patient 1 was born prematurely at 35 gestational age plus 5 days and had a birthweight <2500 g. Both neonates were without/did not develop any clinical symptoms and, as a precaution, did not receive breast milk.

We tested for the presence of SARS-CoV-2 in amniotic fluid, cord blood, placental tissue, neonatal throat swab, and breast-milk samples (obtained on subsequent days after the caesarean section) in both patients, using reverse-transcriptase polymerase chain reaction (RT-PCR) assays (Table 1). The real-time RT-PCR was performed using the Korea Ministry of Food and Drug Safety approved Seegene Allplex 2019-nCoV assay (Arrow Diagnostics): a single-tube assay detecting the three target genes (E gene, RdRP gene and N gene) as in the protocols recommended by the World Health Organization. All RT-PCRs ran on a Bio-Rad CFX96 Real-time Detection system. Each RT-PCR assay provided a cycle threshold: the number of cycles required for the fluorescent signal to cross the threshold for a positive test. The Seegene automated data analysis software (Seegene Viewer) identified the samples as positive if their cycle threshold value was <40. Use of the Logix Smart Novel Coronavirus (2019-nCoV) test kit (Co-Diagnostics)—a PCR assay detecting the RdRP gene—confirmed the test results. Procedures to prevent sample contamination and PCR carryover were in accordance with standard laboratory practices. Furthermore, we used a dedicated electric breast pump and aseptic conditions to collect breast-milk samples in disposable sterile bottles from the two women who wore facemask and gloves during the expression of their breast milk.

In patient 1 we detected viral RNA in both placental tissue and cord blood samples and, importantly, in multiple breast-milk

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Table 1
Summary of the features of two women with COVID-19

Clinical characteristics	Patient 1	Patient 2	SARS-CoV-2 RNA testing	Patient 1		Patient 2	
Age (years)	38	42	Sample type	Sample date	Result (cycle threshold per target)	Sample date	Result (cycle threshold per target)
Gestational age on admission	34 weeks, 4 days	37 weeks, 3 days	Breast milk 1	March 31, 2020	Positive (E: 34.3; RdRP: 37.1; N: 36.7)	March 29, 2020	Negative
Onset to delivery (days)	8	8	Breast milk 2	April 1, 2020	Positive (E: 33.8, RdRP: 34.3; N: 37.3)	March 30, 2020	Negative
Complications	Foetal distress	None	Breast milk 3	April 2, 2020	Negative	March 31, 2020	Negative
Signs and symptoms			Breast milk 4	April 3, 2020	Positive (E: 37.3; RdRP: 36.1; N: 38.3)	April 1, 2020	Negative
Fever	Yes	No	Breast milk 5	April 4, 2020	Negative	April 2, 2020	Negative
Myalgia/Arthralgia	No	No	Breast milk 6	April 6, 2020	Negative	April 5, 2020	Negative
Cough	No	Yes	Amniotic fluid	March 28, 2020	Negative	March 26, 2020	Negative
Dyspnoea	Yes	No	Cord blood	March 28, 2020	Positive (E: 28.3; RdRP: 29.9; N: 28.9)	March 26, 2020	Negative
Sore throat	No	No	Placental tissue	March 28, 2020	Positive (E: 22.6; RdRP: 23.5; N: 23.5)	March 26, 2020	Negative
Chest pain	No	No	Neonatal throat swab 1	March 28, 2020	Negative	March 26, 2020	Negative
Diarrhoea	Yes	No	Neonatal throat swab 2	March 31, 2020	Negative	March 28, 2020	Negative

Laboratory characteristics	Patient 1	Patient 2
White blood cell count ($\times 10^9$ cells/L)	5.37	12.92
Lymphocyte count ($\times 10^9$ cells/L)	0.64	0.80
Platelet count ($\times 10^9$ cells/L)	106	405
C-reactive protein concentration (mg/L)	91.3	125.7

Treatments after delivery	Patient 1	Patient 2
Supplemental oxygen	Yes	No
Lopinavir/ritonavir	Yes	Yes
Azithromycin	Yes	Yes
Hydroxychloroquine	Yes	Yes

E, envelope protein gene; RdRP, RNA-dependent RNA polymerase gene; N, nucleocapsid protein gene.

samples that were collected and tested after the first lactation (Table 1). Three of six breast-milk samples (50%) had a cycle threshold value <40 (the value interpreted as positive for SARS-CoV-2 RNA), indicating that patient 1 excreted virus in her breast milk, albeit intermittently (Supplementary Material Fig. S1). In patient 2 we did not detect viral RNA in any of the samples tested (Table 1).

We showed the potential for mother-to-child transmission of the virus by extra-respiratory routes, suggesting that testing for SARS-CoV-2 in breast-milk samples could be of value in preventing neonatal infections. However, further studies on more women are needed before recommending such testing as part of routine evaluation of pregnant women with SARS-CoV-2 infection. Meanwhile, breastfeeding may not be practised before a SARS-CoV-2-infected mother's isolation period is ended and viral clearance is assessed [4]. Aseptic precautions during breast-milk sample collection exclude the likelihood of contamination by the mother's respiratory droplets. Although we did not document SARS-CoV-2 infection in the neonate born from the mother with a positive RT-PCR result for the placenta, testing of intrauterine tissue samples may also be important [6]. In this regard, maternal–foetal transmission was suspected in eight (4.5%) of 179 cases of neonates tested for SARS-CoV-2 at birth (five with positive nasopharyngeal RT-PCR and three with specific IgM) who were born from pregnant women infected in the third trimester of pregnancy [7]. In any case, all clinical samples testing positive for SARS-CoV-2 RNA should be assessed for live virus, which is basic to understanding infectivity [8] but was not performed in the current study.

In conclusion, we believe that investigations of pregnant women with COVID-19 symptoms should necessarily include testing from various body sites or fluids. This will help to improve the sensitivity and reduce false-negative test results.

Author contributions

SC and BP contributed equally to this article, and both should be considered first author. SC, BP, GV and PC worked on concept/design of the study; DB, PV, ET and BC worked on data collection; SM, PC and MS performed laboratory work; SC, BP and AL worked on data analysis/interpretation; MS acquired funding; SC, BP and MS drafted and critically revised the manuscript. All authors read and approved the final draft.

Transparency declaration

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2020.05.027.

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