MANAGEMENT AND FOLLOW-UP OF NEONATES BORN TO SARS-COV-2 POSITIVE MOTHERS AFTER DISCHARGE: A PROSPECTIVE COHORT STUDY

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ABSTRACT Introduction: To understand SARS-CoV-2 infection in neonates, our department implemented an ambulatory follow-up program for neonates born to SARS-CoV-2 mothers. Our study aimed to characterize this population of neonates born to SARS-CoV-2 mothers, analyze SARS-CoV-2 antibodies evolution and evaluate these neonates after hospital discharge. **Material and Methods:** Single-center prospective cohort study enrolling all neonates born to mothers with active perinatal SARS-CoV-2 infection in a tertiary hospital in Portugal from April 2020 to October 2021. **Results:** There were 3138 deliveries, and 73 neonates (2.3%) were born from mothers with perinatal SARS-CoV-2 infection. Three neonates (4%) had perinatal SARS-CoV-2 infection. Post-discharge follow-up was achieved in 93% (n=68) of neonates. The first call by a physician was performed at a median of 6 days after discharge. During the first appointment, 46% (n=68) of exclusive breastfeeding was found in mothers.

We performed cerebral ultrasound (CUS) in 93% (n=68) of the newborns during the first month of life, and 35% (n=24) of the newborns had nonspecific abnormal cerebral findings. Regarding serology, 55.1% (n=38) of infants had positive anti-nucleocapsid antibodies at the first 24 hours of life. 52.6% (n=20) of these neonates maintained positive antibodies at one to three months of life. There was a significant decrease in anti-nucleocapsid antibodies titer since birth ($p<0.001$, Wilcoxon signed-ranks test), except in four neonates, three of them with perinatal SARS-CoV-2 infection.

Conclusion: We describe our experience following neonates born to mothers with SARS-CoV-2 infection after discharge, considering our hospital COVID-19 policies. The findings of this study suggest a limited impact of maternal, perinatal SARS-CoV-2 infection on early neonatal outcomes. Rooming-in mothers and newborns can be safe, as transmission rates of SARS-CoV-2 seem to be low. This article may contribute to a better understanding of SARS-CoV-2 infection in the neonatal period and its short-time consequences.

KEYWORDS SARS-CoV-2, neonate, perinatal infection, follow-up, antibodies

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Introduction

Neonatal SARS-CoV-2 infection is rare and can occur by one of three mechanisms: intrauterine infection (transplacental or ascending), intrapartum infection (transmission during birth/labour) and postpartum infection (through nasal secretions, environmental exposure).[1,2]

There is still a paucity of epidemiological data on neonatal COVID-19. The current evidence also indicates that the average pooled incidence rate of vertical transmission is low, ranging from 5.6 per 10,000 live births [3], an incidence of 0.03% [4], and an incidence of 16 per 1000 newborns born to mothers with SARS-CoV-2 infection) [5]. Another study demonstrated that 30% of cases of neonatal SARS-CoV-2 infections were likely due to vertical transmission, either intrapartum or congenitally and 9% of these cases were confirmed vertical infections (3.3% for intrapartum and 5.7% for congenitally transmitted infections, respectively). [6]

The data also suggests that the rate of premature delivery has increased, and the SARS-CoV-2 infection rate is proportionately higher in premature neonates, which appears to be related to premature delivery for maternal reasons rather than an increase in spontaneous preterm labour [3,7,8]. Just like the influenza A/PR/8/34 (H1N1) outbreak of 1918 (Spanish influenza), studies in past pandemics suggest that these children face the potential risk of long-term health outcomes and should be followed up [9].

Also, recent studies propose that neonates with SARS-CoV-2 infection require follow-up since it is not yet known whether this virus can cause long-term sequelae such as heart disease, hypertension, diabetes, kidney disease and gastric disease and perinatal complications such as prematurity and low birth weight [9-11]. In order to understand the impact of SARS-CoV-2 virus infection on the neonate, our department implemented an ambulatory follow-up program to support families and neonates born to SARS-CoV-2 mothers. Our study aimed to characterize this population of neonates born to SARS-CoV-2 mothers, analyze SARS-CoV-2 antibodies evolution and evaluate these neonates after hospital discharge.

Materials and Methods

We performed a single-centre observational prospective study enrolling all neonates born to mothers with active perinatal SARS-CoV-2 infection in the neonatal intensive care unit (NICU) and Nursery at a tertiary hospital in Lisbon, Portugal, between April 2020 and October 2021. Our study was exploratory to evaluate the neonatal period of newborns from mothers with perinatal SARS-CoV-2 infection after hospital discharge.

All data was obtained from patient records and a coded number was attributed to each participant to secure confidentiality. During our study period, as part of the Portuguese protocol, all pregnant women admitted to the delivery room were tested with real-time polymerase chain reaction (RT-PCR) for SARS-CoV-2 from both nasopharyngeal and oropharyngeal (NP/OP) swabs. Active perinatal SARS-CoV-2 infection was defined as mothers with a positive NP swab RT-PCR for SARS-CoV-2 virus between 14 days prior to and up to 48 hours after delivery. In addition, we used the classification system for perinatal SARS-CoV-2 transmission of the Scandinavian workgroup [2]: 1) congenital infection in a live neonate born to a mother with SARS-CoV-2 infection, defined by the detection of the virus with RT-PCR in umbilical cord blood or neonatal blood collected within the first 12 hours of birth and 2) intrapartum acquired neonatal infection defined by the detection of the virus with RT-PCR in NP swab at birth (collected after cleaning the baby) and at 24-48 hours of age in neonates born to mothers with SARS-CoV-2 infection.

The delivery room was divided into two circuits: one for positive SARS-CoV-2 women and another for non-infected women. In addition, our hospital isolation practices were updated according to national and international recommendations. From April 2020 to December 2020 (period A), all neonates from positive mothers were separated and isolated in the NICU in a negative pressure room until discharge. Since January 2021 (period B), rooming-in was allowed (in an individual room of the obstetric COVID-19 area, keeping the newborn in the crib two meters away from the mother's bed) if the mother was asymptomatic or mildly symptomatic and able to accomplish safety measures (hand and breast hygiene, and use of face mask). Instructions for contact, respiratory and breastfeeding precautions were explained to the mothers.

Maternal sociodemographic data, pregnancy complications, and the presence of symptoms were collected. The clinical spectrum of SARS-CoV-2 infection in mothers was classified, following Centers for Disease Control and Prevention (CDC) [12] guidelines, as a mild disease (fever, cough, sore throat, malaise, headache, myalgia, anosmia, dysgeusia or gastrointestinal symptoms, without shortness of breath, dyspnea or abnormal chest imaging); moderate disease (evidence of lower respiratory disease during clinical assessment or imaging and oxygen saturation (SatO2) $\geq$ 94% on room air at sea level); severe disease (SatO2 < 94% on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO2/FiO2) <300 mmHg, respiratory rate > 30 breaths/min or lung infiltrates > 50%) and critical disease (respiratory failure, septic shock and/or multiple organ dysfunction). Mothers with symptomatic disease were encouraged to pump and bottle-feed breast milk rather than directly breastfeed. Mothers with asymptomatic or mildly symptomatic infection were encouraged to breastfeed with a mask and practice hand hygiene directly.

Other studied variables included mode of delivery, gestational age, Apgar score, resuscitation at birth, birth weight and head circumference, neonatal symptoms, type of feeding, isolation practices, length of stay, laboratory data, and follow-up. All neonates underwent SARS-CoV-2 real-time polymerase chain reaction (RT-PCR) with NP/OP swabs in the first 24 hours (h), and at 48 h of life, SARS-CoV-2 RT-PCR in umbilical cord blood or neonatal serum in the first 12h of life and SARS-CoV-2 anti-nucleocapsid antibodies in umbilical cord blood or neonatal serum in the first 24 h of life, repeated between one and three months of age. If asymptomatic, newborns were discharged between 36-48 h after vaginal deliveries and 48-72 h after cesarean deliveries. In addition, instructions to reduce the risk of perinatal infection transmission were reinforced.

Follow-up data variables after discharge included post-discharge family member (mother or other), presence of signs and symptoms of SARS-CoV-2 infection in the neonate, time of the first appointment, time of the second appointment, symptoms in mothers after discharge, type of feeding at the first appointment, type of feeding at the second appointment, time of first appointment with the Family Doctor/Pediatrician, provision of medical and/or emergency services care to the neonate, diagnosis at the emergency room, RT-PCR SARS-CoV-2 testing for other reasons after discharge, need of hospitalization, cerebral ultrasound outcomes, SARS-CoV-2 anti-nucleocapsid

antibodies collected between one to three months. We also evaluated medical care support related to breastfeeding or another type of feeding, baby weight gain, umbilical stump care and use of personal protective equipment (PPE) when handling the newborn.

Clinical specimens were obtained following CDC guidelines [12]. NP and OP swabs were inserted into a sterile viral transport medium tube. The serum was collected in a serum separator tube and centrifuged in accordance with CDC guidelines. All specimens were kept refrigerated at +4°C. Viral RNA was extracted by bioMérieux NucliSENS easyMag automated system from 200 μL clinical samples and eluted in 50 μL. The Shanghai Fosun Long March Medical Science Co., Ltd. 2019-Novel Coronavirus (2019-nCoV) RT-PCR detection Kit targeting the Orf1ab gene (specific for SARS-CoV-2) and E and N genes (targeting lineage B-betacoronavirus) was used following the manufacturer's recommendations. The assay included a heterologous amplification system (internal positive control) to identify possible RT-PCR inhibition and to confirm the integrity of the kit's reagents. Blood samples were analyzed using Elecsys® Anti-SARS-CoV-2 (Roche Diagnostics), an electrochemiluminescence immunoassay for the qualitative detection of antibodies (including IgG) against SARS-CoV-2 in human serum and plasma, using a recombinant protein representing the nucleocapsid (N) antigen in a dual antigen sandwich assay format, which favours the detection of high-affinity antibodies against SARS-CoV-2. All laboratory tests were performed in the same laboratory. According to their presence or absence, SARS-CoV-2 anti-nucleocapsid antibodies were described as positive or negative. Antibody titer was also evaluated.

A follow-up program was established as part of routine clinical care for all newborns from mothers with active perinatal SARS-CoV-2 infection, including medical evaluation (first contact through a phone call after discharge and subsequent medical attendance), laboratory evaluation (SARS-CoV-2 anti-nucleocapsid antibodies collected between one to three months of age) and cerebral ultrasound to all these newborns.

Descriptive and bivariate statistical analysis was performed using SPSS 25®, and differences were considered significant at $p < 0.05$. Continuous variables did not show a normal distribution using skewness and kurtosis tests. Therefore, results are presented in the median and interquartile range. Non-parametric Mann-Whitney test and Wilcoxon signed-ranks test were used to compare medians, and the Chi-square test was used to verify the association between two categorical variables.

All mothers gave informed consent for inclusion before participating in the study. The study was conducted in accordance with the Declaration of Helsinki. The ethics committee of the institution approved the study.

Results

Characterization of mothers with SARS-CoV-2 and newborns

During the study period, there were 3138 deliveries, and 73 neonates (2.3%) were born from mothers with SARS-CoV-2 infection. Table 1 characterizes SARS-CoV-2-positive mothers and their neonates. We had three cases of perinatal transmission. Case 1 was a 34-week premature newborn from a positive mother with severe COVID-19 disease. The newborn developed severe COVID-19 and tested positive for SARS-CoV-2 RT-PCR in NP/OP swabs and neonatal blood collected within the first 12 hours of birth – classified as congenital infection [13]. Case 2 was a 36-week preterm. The mother had mild COVID-19. The newborn was asymptomatic and tested positive for SARS-CoV-2 RT-PCR in NF/OP swabs, and neonatal blood collected within the first 12 hours of birth– was classified as congenital infection. Case 3 was a 38-week newborn from a positive mother with moderate COVID-19. This newborn was asymptomatic and tested positive for SARS-CoV-2 RT-PCR in NP/OP swabs in the first 24h and 48h of life, with a negative RT-PCR SARS-CoV-2 in cord blood and blood– classified as intrapartum acquired neonatal infection.

Table 1. Characteristics and clinical care of mothers with SARS-CoV-2 and newborns.

Maternal and Delivery Characteristics (n=73)	
Maternal symptoms	
Asymptomatic n (%)	60 (82.2)
Mild symptoms n (%)	11 (15.1)
Moderate symptoms n (%)	1 (1.4)
Severe symptoms n (%)	1 (1.4)
Mode of delivery	
Vaginal n (%)	44 (60.3)
Cesarean section† n (%)	29 (39.7)
Newborn Characteristics (n=73)	
Gestational age (weeks)	
Median (IQR)	39 (2)
Minimum-Maximum	28-42
Sex	
Male n (%)	38 (52.1)
Female n (%)	35 (47.9)
Birth weight (gram)	
Median (IQR)	3240 (752)
Minimum-Maximum	720-4370
Birth weight classification	
Appropriate for gestational age n (%)	61 (83.6)
Small for gestational age n (%)	9 (12.3)
Symmetric/Asymmetric n (%)	4 (44.4) / 5 (56.6)
Large for gestational age n (%)	3 (4.1)
Birth head circumference	
Median (IQR) cm	34 (2)
Minimum-Maximum cm	24-36.5
Apgar score 1´	
Median (IQR)	9 (1)
Minimum-Maximum	3-10
Apgar score 5´	
Median (IQR)	10 (1)
Minimum-Maximum	7-10
Apgar score 10´	
Median (IQR)	10 (0)
Minimum-Maximum	8-10
Complications in neonatal period	
Yes n (%)	16 (21.9)
Respiratory distress‡ n (%)	9 (12.3)
Transient tachypnea of the newborn n (%)	3 (4.1)
Hyaline membrane disease n (%)	2 (2.7)
Bronchopulmonary dysplasia n (%)	4 (5.5)
Neonatal indirect hyperbilirubinemia n (%)	6 (8.2)
Sepsis n (%)	5 (6.8)
Anemia n (%)	5 (6.8)
Periventricular-intraventricular hemorrhage n (%)	2 (2.7)
No n (%)	57 (78.1)
Newborn SARS-CoV-2 infection	
Perinatal transmission n (%)	3 (4)
Congenital infection n (%)	2 (3)
Intrapartum acquired neonatal infection n (%)	1 (1)
Hospitalization Characteristics (n=73)	
Room-in total	
Yes n (%)	33 (45.2)

Hospital stay

The median gestational age in asymptomatic mothers was 39 weeks, and 38 weeks in symptomatic mothers (p=0.159, Mann-Whitney test). The prematurity rate in infected mothers was higher (12%, n=8 of 73 neonates) than in non-infected mothers (6%, n=184 of 3065 neonates) (p=0.017, Chi-square test). The most frequent complication in neonates was respiratory distress, and 12% (n=9) of these neonates required supplemental oxygen [4% (n=3) invasive mechanical ventilation, 4% (n=3) non-invasive mechanical ventilation, 1% (n=1) high flow nasal cannula].

Thirteen percent (n=5) of the mother-newborn dyads were roomed-in in period A and 80% (n=20) in period B. In period A, 18% (n=7) of mother-newborn dyad separations were due to the newborn's clinical condition, and the remaining cases

No n (%)	40 (54.8)
Room-in Period A	
Yes n (%)	5 (13)
No n (%)	33 (87)
Room-in Period B	
Yes n (%)	28 (80)
No n (%)	7 (20)
Breast milk	
Yes n (%)	49 (67.1)
No n (%)	24 (32.9)
Breast milk period A	
Yes n (%)	14 (37)
No n (%)	24 (63)
Breast milk period B	
Yes n (%)	35 (100)
No n (%)	0
Length of stay	
Median (IQR) days	3 (3)
Minimum-Maximum days	1-83
Neonate's laboratory results	
SARS-CoV-2 by upper respiratory swab (RT-PCR) in the first 24 hours of life	68
Positive n (%)	3 (4.4)
Negative n (%)	64 (94.1)
Inconclusive n (%)	1 (1.5)
SARS-CoV-2 by upper respiratory swab (RT-PCR) at 48 hours	68
Positive n (%)	2 (2.9)
Negative n (%)	66 (97.1)
SARS-CoV-2 by RT-PCR in blood in the first 12 hours of life	65
Positive n (%)	3 (4.6)
Negative n (%)	52 (80)
Missing n (%)	10 (15.4)

Legend: IQR – Interquartile range.

†*Of the 29 cesarean sections performed, six mothers had symptoms (one mother with severe symptoms, one mother with moderate symptoms and five mothers with mild symptoms). Only two mothers had COVID-19 pneumonia, which was why delivery was performed by caesarean section. All other cesarean sections were for other reasons.*

‡ *Only one 34-weeks premature neonate with congenital SARS-CoV-2 infection presented respiratory distress. Of the remaining eight newborns with respiratory distress and without SARS-CoV-2 perinatal disease, six neonates also presented sepsis (five neonates were premature and one was full-term) and the remaining two were term neonates.*

were due to hospital logistics. In period B, 20% (n=7) of mother-newborn dyads were separated due to a maternal or newborn clinical condition unrelated to neonatal SARS-CoV-2 infection. The neonate with intrapartum-acquired neonatal infection was born in period B, and the two neonates with congenital infection were born in period A.

Due to logistics and hospital policies, all newborns received breast milk in period B, but only 37% (n=14) of newborns were breastfed during period A.

The median length of stay was 3 days. A maximum stay of 83 days was verified in a preterm neonate of 28 weeks with no evidence of SARS-CoV-2 perinatal transmission.

In our cohort, we did not find differences between neonates breastfed or fed by formula regarding the severity of maternal symptoms (p=0.261, Chi-square test), length of stay (p=0.160, Chi-square test) and birth weight (p=0.161, Chi-square test).

Clinical follow-up after discharge

Post-discharge follow-up is described in table 2. Follow-up was achieved in 93% (n=68) of neonates, and none developed disease or clinical symptoms compatible with SARS-CoV-2 infection in the neonatal period.

All neonates were discharged with a first appointment scheduled. Forty-two (62%) neonates were evaluated in the first week after discharge and 54 (79%) were observed during the first 14 days after discharge. The three neonates with SARS-CoV-2 perinatal infection were observed at a median of 7 days after hospital discharge (minimum 4 days, maximum 12 days). The hospital appointment was the first medical evaluation in 80% (n=50) of the cases. One infant was observed in the hospital appointment at 90 days because he missed the first appointment scheduled for the first week of life. All mothers were in self-isolation and remained asymptomatic or with mild symptoms. However, they

Table 2. Follow-up after discharge characteristics

Post discharge follow-up – first appointment	
Yes n (%)	68 (93%)
No n (%)	5 (7%)
Post discharge follow-up – second appointment	
Yes n (%)	61 (84%)
No n (%)	12 (16%)
First appointment (number of days after discharge)	
Median (minimum – maximum)	6 (2-90)
Second medical appointment (number of days after first appointment)	30 (7-67)
Median (minimum – maximum)	
Breastmilk at first appointment	
Yes n (%)	46 (68)
No n (%)	22 (32)
Breastmilk at the second appointment	
Yes n (%)	37 (61)
No n (%)	24 (39)
Recurrence to Emergency department	
Yes n (%)	11 (16)
No n (%)	57 (84)
Diagnosis at the Emergency department	
Acute nasopharyngitis n (%)	5 (7.5)
Poor feeding/failure to thrive n (%)	3 (4)
Delayed umbilical stump drop n (%)	1 (1.5)
Newborn colic n (%)	1 (1.5)
Toxic erythema n (%)	1 (1.5)
Hospitalization	
Yes n (%)	0
No n (%)	68 (100)

Table 3. SARS-CoV-2 antibody titers

Laboratory Results	
Neonates tested for anti-nucleocapsid antibodies at first 24h of life (n)	69
Neonates with positive antibodies n (%)	38 (55.1)
Antibodies median titer (IQR)	9.7 (18.8)
Antibodies minimum titer - maximum titer	1.1-126.1
Neonates with negative antibodies n (%)	31 (44.9)
Neonates tested for anti-nucleocapsid antibodies at 1 to 3 months of life (n)	58
Neonates with positive antibodies n (%)	23 (39.7)
Antibodies median titer (IQR)	5.1 (12.6)
Antibodies minimum titer - maximum titer	1.3-107.6
Neonates with negative antibodies n (%)	35 (60.3)
Neonates with positive anti-nucleocapside antibodies at both 24h of life and 1 to 3 months of life (n)	20
Neonates with positive anti-nucleocapside antibodies at 24h of life and negative anti-nucleocapside antibodies at 1 to 3 months of life (n)	10

Legend: IQR – Interquartile range

were encouraged to use a face mask and to perform hand hygiene during newborn handling.

From hospital discharge until the first appointment, there was no significant reduction in the number of breastfed newborns (p=0.9471, Chi-Square test), as well during the time between the first and second appointments (p=0.4079, Chi-Square test). In addition, seven (20%) mothers reported concerns about feeding difficulties and the baby's weight gain.

Eleven neonates went to the Emergency Room (ER), and five presented with acute nasopharyngitis. These five neonates had a median of 14 days of life, and three underwent SARS-CoV-2 RT-PCR in NF/OF swabs, all with negative results. One of these was neonate case 3 (SARS-CoV-2 asymptomatic perinatal infection). In addition, three neonates were observed with poor feeding and failure to thrive, all were exclusively breastfed, and two started infant formula supplementation after medical evaluation.

Laboratory results - SARS-CoV-2 antibodies kinetics

SARS-CoV-2 antibodies kinetics are described in Table 3. Anti-nucleocapsid SARS-CoV-2 total antibodies were evaluated in the first 24 hours of life and reevaluated at the second appointment (between one and three months). We found a significant decrease in anti-nucleocapsid antibodies titer since birth (p<0.001, Wilcoxon signed-ranks test). Exceptionally, we found an increase of anti-nucleocapsid antibodies titer in four neonates. Three of these neonates had perinatal SARS-CoV-2 infection with no detectable antibodies at 24h of life. One became positive on day 5 (index 31.6), another on day 4 (index 20.3) and the other on day 41 (index 9.4). The fourth neonate did not have neonatal SARS-CoV-2 infection and was always asymptomatic. He re-

Table 4. Comparison between groups with positive and negative antibodies at the first 24 hours of life.

	Anti-nucleocapsid antibodies at the first 24 hours of life (n=69)		Statistical test
	Group 1 Positive (n=38)	Group 2 Negative (n=31)	
Maternal symptoms			
Asymptomatic n (%)	34 (89.5)	23 (74.2)	p=0.096
Symptoms n (%)	4 (10.5)	8 (25.8)	Chi-square
Time between maternal positive test and delivery (days)			
Median (IQR)	1 (2)	1 (4)	p=0.763
Minimum-Maximum	0-14	2-12	Mann-Whitney
Gestational age (weeks)			
Median (IQR)	39 (2)	39 (2)	p=0.926
Minimum-Maximum	28-41	28-42	Mann-Whitney
Perinatal SARS-CoV-2 infection			
Yes (3) n (%)	0 (0)	3 (9.7)	NA
No (66) n (%)	38 (100)	28 (90.3)	
Newborn complications			
Yes (15) n (%)	8 (21.6)	7 (23.3)	P=0.867
No (52) n (%)	29 (78.4)	23 (76.7)	Chi-square
Length of stay (days)			p=0.104
Median (IQR)	3 (3)	3 (4)	Mann-Whitney
Minimum-Maximum	1-83	1-45	

Legend: NA - Non applicable

Table 5 – Mother and newborn characteristics (CUS)

	Abnormal CUS	Normal CUS	Statistical test
Maternal symptoms			
Asymptomatic n (%)	19 (79.1)	36 (81.8)	
Mild symptoms n (%)	4 (16.7)	6 (13.6)	p=0.790, Chi-Square test
Moderate symptoms n (%)	0	1 (2.3)	
Severe symptoms n (%)	1 (4.2)	1 (2.3)	
Neonate Gestational Age (weeks)			
Median (IQR)	38 (5)	39 (2)	p=0.008, Mann-Whitney
Minimum-Maximum	28-41	34-42	Test
Neonate Birth weight (gram)			
Median (IQR)	2812.5 (997)	3327.5 (678)	p=0.005, Mann-Whitney
Minimum-Maximum	720-4222	2565-4370	Test
Premature neonates			NA
n (%)	9 (13.2)*	3 (4.4)	
Term neonates			NA
n (%)	15 (22.1)**	41 (60.3)	

Legend: IQR – Interquartile range; NA - Non applicable

** Abnormal findings in the 9 premature neonates: diffuse white matter injury (n=4), grade 1 intraventricular hemorrhage (n=2), lenticulostriate vasculopathy (n=2), subependymal cysts (n=2). Case 1, table 1 (34 weeks gestational age) presented lenticulostriate vasculopathy and subependymal cysts. Case 2, table 1 (36 weeks gestational age) presented lenticulostriate vasculopathy.*

*** Abnormal findings in the 15 term neonates: grade 1 intraventricular hemorrhage (n=2), diffuse white matter injury (n=1), lenticulostriate vasculopathy (n=7), thalamostriate vasculopathy (n=6) subependymal cysts (n=3), choroid plexus cyst (n=2).*

vealed positive antibodies at birth (index 23.2) and at day 14 (index 24.5), with an increase in anti-nucleocapsid antibodies titer.

Neonates with negative antibodies at one to three months had significantly lower antibodies at 24h of life compared with neonates with positive antibodies at one to three months, which reported higher antibodies at 24 hours of life (p<0.001, Wilcoxon signed-ranks test).

Comparison between groups with positive and negative antibodies at the first 24h of life

Table 4 compares groups with positive (group 1) and negative (group 2) antibodies at the first 24 hours of life. The antibodies titer was not associated with maternal symptoms, the time between a maternal positive test and delivery, length of stay or newborn complications.

Cerebral ultrasound evaluation

As part of our protocol follow-up, sixty-eight newborns (93%) underwent cerebral ultrasound (CUS) evaluation in the first month of life (table 5). There were no statistically significant differences in CUS results of neonates born to symptomatic and asymptomatic mothers (p=0.790, Chi-Square test). Twelve neonates (17.6%) were premature, and 82.4% (n=56) were term. Most premature neonates had abnormal CUS, and most term neonates had normal CUS (p= 0.003, Fisher's Exact Test).

Follow-up after discharge

We described our experience following neonates born to mothers with SARS-CoV-2 infection after discharge, considering our hospital COVID-19 policies and measures.

During the study period, there were 3138 deliveries, and 2.3% (n=73) of neonates were from SARS-CoV-2 positive mothers. This incidence rate was similar to that of other Portuguese studies that reported an incidence rate of 1.6% (23 in 1438 deliveries) and 4.6% (81 in 1755 deliveries) of incidence of neonates born to SARS-CoV-2 positive mothers [14,15].

Hospitalization and maternal and neonate characteristics Most mothers (82%, n=60) were asymptomatic and diagnosed at admission. Twelve percent (n=9 of 73) of neonates were born preterm, which is twice the rate of the one found in non-infected mothers (6%, n=184 of 3065), statistically significant (p=0.017). These findings are described in the literature and suggest that although pregnant women do not seem to suffer from a more severe disease than the general population, SARS-CoV-2 infection in pregnancy might be related to an increased risk of preterm birth, mainly due to a maternal condition [14-19].

In our cohort, a C-section was performed in 40% (n=29 of 73) of the deliveries, which was higher than in the non-infected women group (29%, n=889 of 3065), p=0.06. Separation of mothers and infants during the postpartum period negatively affects maternal-infant bonding and breastfeeding [20]. However, based on the evidence, rooming-in and skin-to-skin contact appear safe with droplet and hygiene precautions [21,22]. Unfortunately, for logistical reasons, we could not accommodate mothers and newborns until January 2021. Furthermore, since we achieved conditions to room-in (period B), we have seen the benefits for the mother-newborn dyad, including on breastfeeding rate (100%, n=35). In any case, we reinforce that the room-in and room-out variables present differences in incidence, which may interfere with these results.

We also know that the approach to SARS-CoV-2-positive pregnant women in our country was different from centre to centre at the beginning of the pandemic. A Portuguese study that evaluated 24 different hospitals between April and May 2020 reported that 61% of neonates were separated from their mothers, and 70% were not breastfed.[23] There are also literature reports that describe breastfeeding rates that go as low as 17% and as high as 86% [16,19,24]. In our sample, breast milk was given to 67% (n=49) of the neonates. Although we considered that some measures applied throughout the pandemic in our hospital, such as not allowing skin-to-skin contact at birth and mother-newborn separation, may have harmed the outcomes such as breastfeeding, we also considered that encouraging mothers to extract breast milk through a milk pump might have had a positive impact on breastfeeding.

Maternal self-report by phone also allowed us to have a good follow-up and avoid unnecessary in-person visits that would place patients and healthcare professionals at risk of SARS-CoV-2 exposure. Therefore, telemedicine was seen as a pragmatic solution. The first phone call by a physician was performed at a median of 6 days after hospital discharge. This median agrees with the follow-up recommendations for a neonate from a positive SARS-CoV-2 mother [25,26].

After discharge, we followed 93% of neonates (n=68), and none was hospitalized or had SARS-CoV-2 documented infection. Most mothers (67.1%, n=49 of 73) were exclusively breastfeeding during hospitalization. After discharge, exclusive breastfeeding was found in 68% (n=46 of 68) of the mothers during

the first appointment and 61% (n=37 of 61) during the second appointment. Our protocol follow-up was important to promote and support breastfeeding mothers.

According to Latorre G. et al. [27], lockdown and home confinement decreased exclusive breastfeeding. Pacheco F. et al. [28] also reported that COVID-19-positive mothers may face clinical impediments to breastfeeding. On the one hand, there is limited professional support during the first days after birth to help parents deal with negative experiences. In addition, social distancing measures impair the familial and social environment that supports the parents' journey. On the other hand, mothers previously not planning to breastfeed may have changed their plans because the pandemic increased their time at home and their awareness of the role of breastfeeding as a protective measure. Studies also suggest that telemedicine consultation during outbreaks, considering the risks of SARS-CoV-2 transmission, allowed healthcare workers to decrease the concern of families and reduce indiscriminate ER access. As described in the previous articles [27,28], telemedicine consultation made it possible to support most mothers. However, unfortunately, in 16% of cases, we could not prevent infants from going to the ER because we could not perform a physical examination during a telephone appointment. One of our study limitations was that we carried out medical appointments by phone and not by video call due to technical issues. Although all neonates remained asymptomatic on follow-up, as we did not perform repeated SARS-CoV-2 RT-PCR tests during the isolation period, we cannot ensure that perinatal transmission was not higher.

Laboratory results

To date, few articles in the literature explore antibody kinetics in the neonatal period. Studies suggest that SARS-CoV-2 antibodies are transferred from an infected mother to her neonate during the third trimester through the placenta [29-32]. In our sample, most infants (55.1%, n=38) had positive anti-nucleocapsid antibodies in the first 24 hours of life. Although most infants maintained positive antibodies for at least three months, we found a decrease in the anti-nucleocapsid antibodies' median titer from birth to 1-3 months of age (p=0.000, Wilcoxon signed-ranks test). Antibody titer tended to decrease over time, but as expected, it did not happen in the three neonates that presented perinatal SARS-CoV-2 infection, probably related to their antibody production in response to SARS-CoV-2 infection and not just transplacentally passive transference of maternal antibodies.

Further studies are needed to determine whether the mother's infection severity or time of infection influences the infant's antibody titer. A study by Mo H. et al. [28] revealed that neonates infected with SARS-CoV-2 during the second trimester of pregnancy had a longer duration of detectable IgG than neonates born to women infected during the third trimester.

We also point out that laboratory techniques for determining antibodies (index determination, in our center) are different between centres; therefore, it is difficult to compare different studies. We emphasize the importance of standardizing methodologies to facilitate the interpretation of results in the future.

Cerebral ultrasound

We found that most neonates with abnormal CUS had lower birthweight and lower gestational age, and prematurity can explain cerebral abnormal findings. On the other hand, in 22.1% (n=15) term neonates, abnormal cerebral findings were found, and we could not justify these findings with complications, low birth weight or maternal symptoms. Furthermore, we found no relation between mother symptoms and severity and CUS alterations.

We also reinforce that we found abnormal CUS in the two neonates with SARS-CoV-2 congenital infection. However, as we do not yet know the long-term impact of these cerebral abnormal findings, we believe that these infants should be followed up with long-term neurodevelopmental assessment.

To date, we did not find in the literature any study on ultrasound cerebral findings in neonates born to mothers with SARS-CoV-2 infection. Unfortunately, there are also few publications about the neurological effects of SARS-CoV-2 in neonates, and studies have small sample sizes. Yan K. et al. [33] describe cranial MRI changes, namely white matter changes, in four neonates with COVID-19. Engert V et al. [34] describe a case of a premature neonate with intracranial bleeding and periventricular leukomalacia as a potential consequence of post-COVID-19 hyperinflammation during pregnancy. Larger and longer-term multicentric studies are urgently needed to clarify the impact of SARS-CoV-2 virus infection on the CNS and neurodevelopment.

Limitations of our study include the small sample size, the fact that we did not discriminate IgM and IgG SARS-CoV-2 antibodies in all newborns and also the fact that we only included mothers with active perinatal SARS-CoV-2 infection (between 14 days prior to and up to 48 hours after delivery). Also, other possible implications could have been studied, such as the impact on cardiac function/structure, kidney or gastrointestinal affection. Long follow-up of these children is also needed. In the future, we would like to conduct a similar study to focus on the structural or functional role of SARS-CoV-2 in the CNS and its impact on an infant's neurodevelopment.

Conclusion

Although one of the risks of perinatal SARS-CoV-2 infection is prematurity due to maternal illness, the findings of this study suggest a limited impact of maternal, perinatal SARS-CoV-2 infection on early neonatal outcomes. We also found that rooming-in mothers and newborns can be safe, as transmission rates of SARS-CoV-2 seem to be low.

We reinforce the importance of following these infants after discharge. We believe this article may contribute to a better understanding SARS-CoV-2 infection in the neonatal period and its short-time consequences. Given the reduced number of neonates with perinatal SARS-CoV-2 infection, we also stress the importance of multicentric studies. More clinical and epidemiological studies with larger cohorts are needed in order to develop evidence-based clinical guidelines.

Ethics approval and informed consent

The ethics committee of the institution Centro Hospitalar Lisboa Ocidental, Lisbon, approved the study.

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Conflict of interest:

The authors declare no potential conflict of interest, real or perceived. There was no sponsor(s) in the study design; collection, analysis and interpretation of data; writing of the report; and the decision to submit the paper for publication.

Author's contribution:

Joana Moscoso, Inês Belo, Joana Ribeiro and Mónica Marçal: original draft conceptualization and design, manuscript writing. Mónica Marçal, Filipa Gamboa and Madalena Tuna: manuscript editing and review. All authors read and approved the final manuscript.

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References

1. Robaina-Castellanos GR, Riesgo-Rodríguez SC. Congenital and intrapartum SARS-CoV-2 infection in neonates: hypotheses, evidence and perspectives. MEDICC Rev. 2021 Jan;23(1):72–83.

2. Prakesh S, Diambomba Y, Acharya G, Morris S, Bitnun A. Classification system and case definition for SARS-CoV-2 infection in pregnant women, fetuses, and neonates. Acta Obstet Gynecol Scand. 2020 May; 99(5):565-568.

3. Gale, C, Quigley M, Placzek A, Knight M, Ladhani S, Draper S, et al. Characteristics and outcomes of neonatal SARS-CoV-2 infection in the UK: a prospective national cohort study using active surveillance. Lancet Child Adolesc Health 2021;5(2):113-121.

4. Wallace B, Chang D, Woodworth K, DeSisto C, Simenona R, Ko J, et al. Illness severity indicators in newborns by COVID-19 status in the United States, March–December 2020. J Perinatol 2021; Nov 2;1-8.

5. Goh XL, Low YF, Ng CH, Amin X, Ng Yvonne. Incidence of SARS-CoV-2 vertical transmission: a meta-analysis. Arch Dis Child Fetal Neonatal Ed. 2021;106:112-113.

6. Raschetti R, Vivanti AJ, Vauloup-Fellous C, Loi B, Benachi A, De Luca D. Synthesis and systematic review of reported neonatal SARS-CoV-2 infections. Nat Commun. 2020 Oct 15;11(1):5164.

7. Ryan L, Plötz FB, Van den Hoogen A, Latour J, Degtyareva M, Keuning M, et al. Neonates and COVID-19: state of the art. Pediatr Res 2022 Jan;91(2):432-439.

8. Norman M, Navér L, Söderling J, Ahlberg M, Askling H, Arronson B, et al. Association of Maternal SARS-CoV-2 Infection in Pregnancy With Neonatal Outcomes. JAMA. 2021 May 25;325(20):2076-2086.

9. McCarthy J, Liu D, Kaskel F. The Need for Life-Course Study of Children Born to Mothers With Prior COVID-19 Infection. JAMA Pediatr. 2021 Nov 1;175(11):1097-1098.

10. Priyanka M, Saini L, Einspieler C. Prediction of neurodevelopmental outcomes in SARS-CoV-2 infections. Pediatr Neurol. 2021 Jul;120:3.

11. Singer G, Evankovich D, Fisher K, et al. Coronavirus infections in the nervous system of children: a scoping review making the case for long-term neurodevelopmental surveillance. Pediatr Neurol. 2021 Apr;117:47-63.

12. COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Consulted at 6 december 2022. Available at https://www.covid19treatmentguidelines.nih.gov/.

13. Correia CR, Marçal M, Vieira F, Santos E, Novais C, Maria AT, et al. Congenital SARS-CoV-2 Infection in a Neonate With Severe Acute Respiratory Syndrome. Pediatr Infect Dis J.2020.Dec;39(12):e439-e443.

14. Centers for Disease Control and Prevention. Pregnant and Recently Pregnant People. Consulted at 6 december 2022. Available at: https://www.cdc.gov/coronavirus/2019-ncov/need-extra- precautions/pregnant-people.html

15. Narang K, Enninga EAL, Gunaratne MDSK, Ibirogba E, Trad A, Elrefaei A, et al. SARS-CoV-2 Infection and COVID-19 During Pregnancy: A Multidisciplinary Review. Mayo Clin Proc. 2020;95(8):1750-1765.

16. Moffat A, Dessie S, O'Leary K, Lumba R, Rhee S. Short-term outcomes of infants born to mothers with SARS-CoV-2 infection. J Matern Fetal Neonatal Med. 2022; 35 (25):8192-8198.

17. Salvatore CM, Han J-Y, Acker KP, Tiwari P, Jin J, Brandler M, et al. Neonatal management and outcomes during the COVID-19 pandemic: an observation cohort study. Lancet Child Adolesc Heal. 2020 Oct;4(10):721-727.

18. Ferreira M, Garcia C, Barroso R. Caracteristicas de Recem-Nascidos Filhos de Maes com Infeccao por SARS-CoV-2 num Hospital Portugues. Acta Med Port. 2021; 34(10).

19. Lamba V, Lien J, Desai J, Talati A. Management and short-term outcomes of neonates born to mothers with active perinatal SARS-CoV-2 infection. BMC Pediatr. 2021. 13;21(1):400.

20. Bass JL, Gartley T, Kleinman R. World Health Organization Baby- Friendly Hospital Initiative Guideline and 2018 Implementation Guidance. JAMA Pediatr. 2019;173(1):93-94.

21. Congdon JL, Kair LR, Flaherman VJ, Wood K, LoFrumento M, Nwaobasi-Iwuh E, et al. Management and Early Outcomes of Neonates Born to Women with SARS-CoV-2 in 16 U.S Hospitals. Am J Perinat. 2021; 38 (6): 622-631.

22. Ronchi A, Pietrasanta C, Zavattoni M, Saruggia M, Schena F, Sinelli MT, et al. Evaluation of Rooming-in Practice for Neonates Born to Mothers With Severe Acute Respiratory Syndrome Coronavirus 2 Infection in Italy. JAMA Pediatr. 2021;175(3):260–266.

23. Jonet J, Condessa L, Limbert M, Roquette M, Tavares A, Cunha M. Was Rooming-In a Safe Approach to Newborns from SARS-Cov-2 positive mothers?. Port J Pediatr 2021;52:252-6.

24. Knight M, Bunch K, Vousden N, Morris E, Simpson N, Gale C, et al. Characteristics and outcomes of pregnant women admitted to hospital with confirmed SARS-CoV-2 infection in UK: national population based cohort study. BMJ. 2020;369:2107.

25. American Academy of Pediatrics. Management of infants born to mothers with suspected or confirmed COVID-19, 2020. Consulted at 6 december 2022. Available: https://www.aap.org/en/pages/2019-novel-coronavirus-covid-19-infections/clinical-guidance/faqs-management-of-infants-born-to-covid-19-mothers/

26. Direção Geral da Saúde. COVID-19: Cuidados ao Recém-Nascido na Maternidade. Orientação nº 026/2020 (Data 19/05/2020). Atualização 27/10/2021.

27. Latorre G, Martinelli D, Guida P, Masi E, Benedictis R, Maggio L. Impact of COVID-19 pandemic lockdown on exclusive breastfeeding in non-infected mothers. Int Breastfeed J. 2021 Apr 17;16(1):36.

28. Pacheco F, Sobral M, Guiomar R, Torre-Lucca A, Caparros-Gonzales R, Ganho-Ávila A. Breastfeeding during COVID-19: A Narrative Review of the Psychological Impact on Mothers. Behav Sci (Basel). 2021 Mar 14;11(3):34.

29. Ferretti S, Gatto A, Pansini V, Curatola A, Capossela L, Currò V, et al. Telephone consultation during Coronavirus outbreak in a Pediatric Emergency Department: methodological approach of a tertiary care center in a COVID-19 hospital setting. Eur Rev Med Pharmacol Sci. 2020 Nov;24(21):11440-11444.

30. Mo H, Wang M, Wang M, Han Y, Zhang Y, Hu K. Detectable antibodies against SARS-CoV-2 in newborns from mothers infected with COVID-19 at different gestational ages. Pediatr Neonatol. 2021 May;62(3):321-323.

31. Gee S, Chandiramani M, Seow J, Pollock E, Modestini C, Das A, et al. The legacy of maternal SARS-CoV-2 infection on the immunology of the neonate. Nat Immunol. 2021 Dec;22(12):1490-1502.

32. Bwire GM, Njiro BJ, Mwakawanga DL, Sebas D, Sunguya B. Possible vertical transmission and antibodies against SARS-CoV-2 among infants born to mothers with COVID-19: A living systematic review. J Med Virol. 2021 Mar;93(3):1361-136.

33. Yan K, Xiao FF, Jiang YW, Xiao T, Zhang D, Yuan W, et al. Effects of SARS-CoV-2 infection on neuroimaging and neurobehavior in neonates. World J Pediatr. 2021 Apr;17(2):171-179.

34. Engert V, Siauw C, Stock A. Severe Brain Damage in a Moderate Preterm Infant as Complication of Post-COVID-19 Response during Pregnancy. Neonatology. 2021;118(4):505-508.