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Serum and breastmilk SARS-CoV-2 specific antibodies following BNT162b2 vaccine: prolonged protection from SARS-CoV-2 in newborns and older children

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ABSTRACT

Objectives: Vaccination is the best strategy against COVID-19. We aimed to determine antibodies against SARS-CoV-2 in breastmilk and serum of mothers vaccinated with the mRNA vaccine.
Methods: This prospective study included 18 lactating women vaccinated with the BNT162b2 vaccine. Serum and breastmilk were collected before the first dose (T0), at the second dose (T1), 3 weeks after the second dose (T2), and 6 months after the first dose (T3). Serum anti-SARS-CoV-2 Spike (S) Immunoglobulin G (IgG) and Immunoglobulin A (IgA) were measured using a semi-quantitative enzyme-linked immunosorbent assay (ELISA) and secretory antibody (s) IgG and IgA in breastmilk using quantitative analysis.
Results: We detected serum anti-S IgG and IgA in all women after vaccination. Specific IgG and IgA were higher at T1, T2, and T3 compared with T0 (P <0.0001). Higher antibody levels were observed at T2 and lower values at T3 versus T2 (P = 0.007). After 6 months, all patients had serum IgG, but three of 18 (16%) had serum IgA. In breastmilk, sIgA was present at T1 and T2 and decreased after 6 months at T3 (P = 0.002). Breastmilk sIgG levels increased at T1 and T2 and peaked at T3 (P = 0.008).
Conclusion: Secretory antibodies were transmitted through breastmilk until 6 months after anti-COVID-19 mRNA vaccination. Protection of the newborn through breastfeeding needs to be addressed.

Introduction

Since December 2019, a novel SARS-CoV-2 causing COVID-19 has been spreading worldwide (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020). The large number of patients requiring hospitalization and the high lethality rates of COVID-19 caused excessive stress on healthcare facilities. Because of the urgent need for preventive strategies, several vaccines have been speeding through the experimental phases. In December 2020, new mRNA vaccines were approved (Polack et al., 2020; Walter et al., 2022; Woodworth et al., 2021). The BNT162b2 vaccine was the first mRNA vaccine distributed worldwide and was administered to high-risk persons first (Polack et al., 2020).

Breastfeeding women were not included in phase II/III studies. However, hypothetic damage of liposomal RNA in human breastmilk was not demonstrated. No evidence supporting harmful effects concerning lactation was observed during animal studies (Golan et al., 2021). Very soon, many international and national societies have recommended discussing the risks and benefits of the vaccination for breastfeeding women at higher risk of exposure to the virus as healthcare professionals and offer them vaccination (Bartick et al., 2021; European Medicines Agency, 2020; Joint Committee on Vaccination and Immunisation, 2021; Hare and Womersley, 2020). In addition, preliminary data indicated that after SARS-Cov-2 infection, antibodies against SARS-CoV-2 might be present in breastmilk (Cervia et al., 2021; Fox et al., 2020), and 90%

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of those are secretory IgA with neutralizing activity, suggesting the same finding in women receiving a vaccination. Following these recommendations, at San Matteo University Hospital in Pavia, we decided to offer vaccination with the BNT162b2 mRNA vaccine to breastfeeding healthcare workers and collect breastmilk and serum to demonstrate specific antibody secretion in breastmilk after vaccination and to compare serum and secretory immune response.

Methods

Subjects enrolled

Between January 13, 2021, and March 1, 2021, at Fondazione IRCCS Policlinic San Matteo, 18 breastfeeding healthcare workers (median age 34 years, range 29-41) at different months postpartum (median 11 months; range 1-36) receiving the first dose of SARS-CoV-2 vaccine (BNT162b2) were enrolled. Serum and breastmilk samples were collected before the vaccination (T0), at the second dose (T1), at 3 weeks after the second dose (T2), and 6 months after the first dose (T3) for evaluation of SARS-CoV-2 antibody-specific response. Women with previous SARS-CoV-2 infection, confirmed through RT-PCR from nasopharyngeal swabs or serum anti-Nucleocapsid Protein (N) IgG, were excluded from the study. Ethical approval was obtained from the Medical Ethics Committee of the Policlinico San Matteo University Hospital, and written informed consent was obtained from all participants. Participants were asked to collect about 3 ml of milk. After centrifugation at 3000 rpm for 10 minutes, sera and milk were stored at −80°C until analyses were performed.

Antibody response

Anti-SARS-CoV-2 Spike (S) serum IgG and IgA antibodies were measured by a semi-quantitative enzyme-linked immunosorbent assay (ELISA) (Euroimmun, Luebeck, Germany) at different time points, according to the manufacturer's instructions. Results were expressed as a ratio concerning an internal calibrator (RU/ml).

Secretory antibody (sIgG and sIgA) quantification in breastmilk samples was performed with the same kit (Euroimmun). We used a standard curve obtained from a breastmilk pool derived from women previously infected with SARS-CoV-2. We defined that 100 AU/ml of specific sIgA (IgA-AU/ml) and 100 AU/ml of specific sIgG (IgG-AU/ml) were contained in this pool.

Statistical analysis

Patient characteristics and COVID-19 symptoms were expressed as mean with SD or median with interquartile range depending on their distribution. Comparisons of antibody levels at T1, T2, and T3 with respect to T0 were performed fitting generalized equation models with first order autocorrelation to take into account the clustered nature of the data.

Results

The demographic and clinical characteristics of 18 lactating women and their newborns are listed in Table 1.

Table 1
Demographic and clinical characteristics of 18 lactating women and their newborns

Age median ± SD at delivery	34,0 (± 4,5)
(minimum, maximum) years	29-45
Caucasian n (%)	18/18 (100%)
Co-morbidities n (%)	3/18 (16%)
Thyroid disorders	3/18 (16%)
Delivery data	
Gestational age at delivery median ± SD (Range)	39,5 (1,4)
Delivery at term	18/18
Cesarean delivery	4/18 (22%)
Vaginal delivery	14/18 (78%)
Infant and breastfeeding data	
Median newborn age at 1st vaccination	11,5 (SD 8,5 1-36)
SGA	1/18
Newborn's side effects after vaccination	None
Mother's side effects post-vaccination n (%)	
Minor side effects (n. person)	5/8
Myalgia	3/18
Headache	3/18
Local pain	1/18
Arm lymph node swelling	1/18
Fever	1/18
Skin rash	1/18
Tiredness	-1/18
Arthralgia	1/18
Arm numbness	1/18
Major side effects	None

Antibody response elicited by BNT162b2 vaccine

Serum

As listed in Table 2, the evaluation of serum anti-S antibodies showed that serum IgG was significantly higher at T1, T2, and T3 compared with T0 (median 5.9 RU/ml, range 0.3-8.2; median 7.6 RU/ml, range 6.3-8.3 and median 5.0 RU/ml, range 2.8-5.8 respectively; P <0.0001 for all). Higher levels were observed at T2 compared with T1 (P <0.02), whereas T3 values were significantly lower than T2 values (P <0.001). At T1, one subject (5.8%) showed specific IgG levels under the cut-off that became positive after the second dose administration. A more homogeneous distribution of antibody values was observed at T2 and T3 compared with T1 (Figure 1).

Serum anti-S IgA values were significantly higher at T1, T2, and T3 compared with T0 (median 3.3 RU/ml, range 0.2-7.5; median 5.0 RU/ml, range 1.6-7.4 and median 3.1 RU/ml, range 0.6-8.0, respectively; P <0.0001 for all). As observed for specific IgG, serum-specific IgA higher levels were observed at T2 compared with T1 (P <0.001). In contrast, significantly lower values were observed at T3 compared with T2 (P = 0.007). For serum IgA, scattered values at each time point were observed (Figure 1). At T3, three samples (17.6%) showed serum anti-S IgA levels under the cut-off value. The same subjects showed low antibody levels even at T1 and T2 (Table 3).

Breastmilk

Breastmilk sIgG levels increased at T1 (median 15 IgG-AU/ml, range 15-95), T2 (median 28 AU/ml, range 15-117), and T3 (median 114 AU/ml, range 82-156) (Figure 1, Figure 1 Supplement) compared with T0 (all values <15 IgG-AU/ml), reaching significantly higher levels at T3 (P = 0.008). sIgA levels were significantly higher at T1 (median 422 IgA-AU/ml, range 8-1500) and T2 (median 565 IgA-AU/ml, range 47-1500) compared with T0 (median 80 IgA-AU/ml, range 8-468) (P <0.0001, for all), whereas at T3 (median 155 IgA-AU/ml, range 44.5-350), levels decreased compared with T0. As well as for SARS-CoV-2 IgG and IgA serum response, breastmilk sIgA was induced after the first vaccine dose, reaching higher levels 3 weeks after complete vaccination. However, 6 months after the second dose, we observed a significant decrease (P = 0.002) (Figure 1, Figure 1 Supplement).

Discussion

SARS-CoV-2 infections in a newborn may be acquired in two different ways: less common but potentially serious by vertical transmission, as shown by many authors that have gathered evidence of SARS-CoV-2 RNA on the placenta, amniotic fluid, and

Table 2
Antibody response elicited by BNT162b2 vaccine in serum and breastmilk over time

Time from vaccine somministration	T0(1st dose)	T1(2nd dose)	T2(3 weeks after 2nd dose)	T3(6 months after 1st dose)
Serum anti-S IgG (Median RU/ml, range)	<0,8 RU/ml	5.9 (0.3-8.2)	7.6 (6.3-8.3)	5.0 (2.8-5.8)
Serum anti-S IgA (Median RU/ml, range)	<0,8 RU/ml	3.3 (0.2-7.5);	5.0 (1.6-7.4)	3.1 (0.6-8.0)
Breastmilk anti-S sIgG (Median AU/ml, range)	<15 IgG-AU/ml	15 (15-95)	28 (15-117)	114 (82-156)
Breastmilk anti-S sIgA (Median AU/ml, range)	<15 IgG-AU/ml	422 (8-1500)	565 (47-1500)	80 (8-468)

AU = arbitrary units ; IgA = Immunoglobulin A; IgG = Immunoglobulin G; RU = relative units.

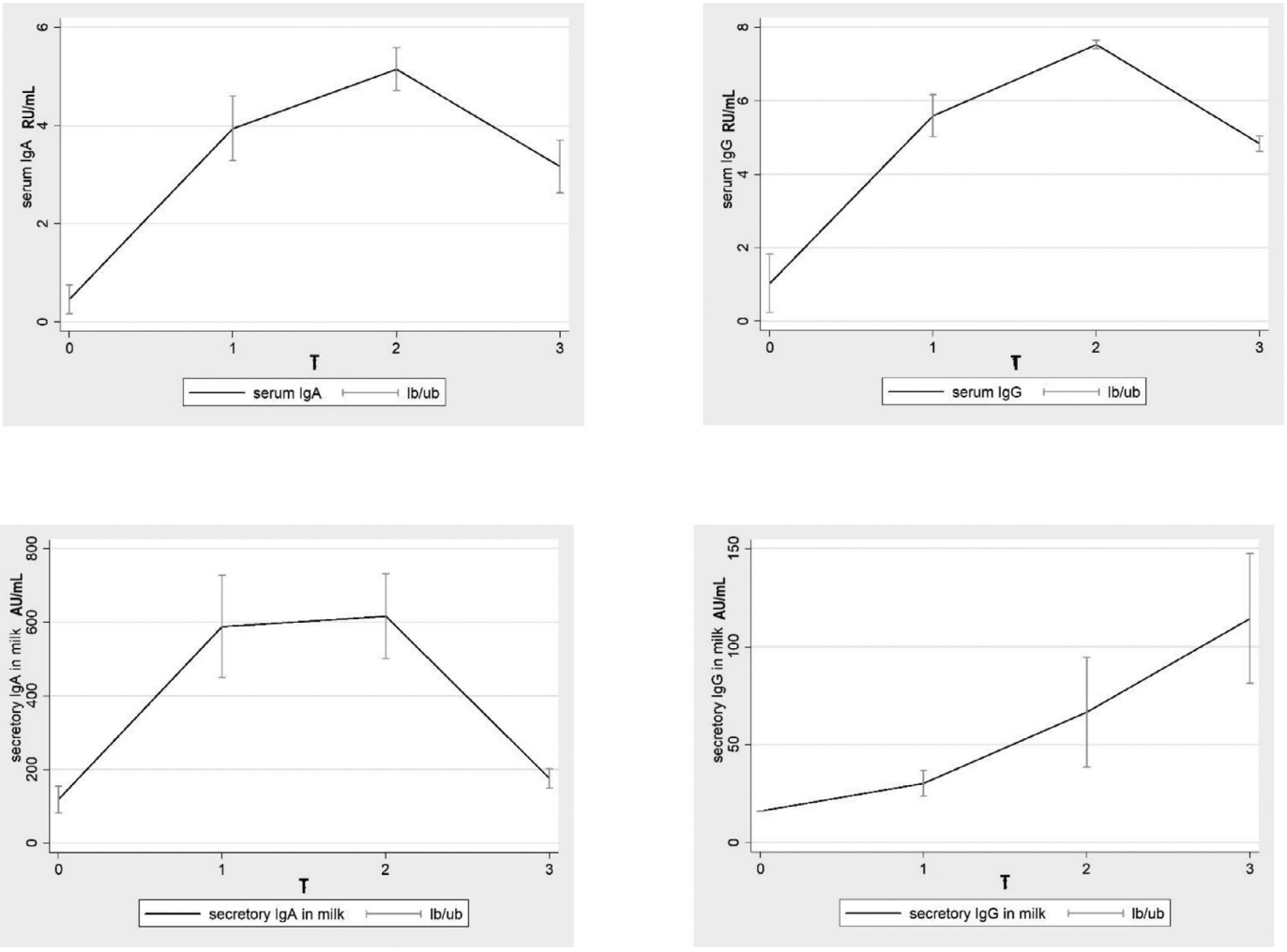

Figure 1. Serum IgA and IgG, secretory breastmilk IgA and IgG
AU = arbitrary units ; IgA = Immunoglobulin A; IgG = Immunoglobulin G; RU – **relative units**

Table 3
Serum IgA levels in the three patients with negative levels at T3

Subject code	T1 (RU/ml)	T2 (RU/ml)	T3 (RU/ml)
#77	1.40	1.60	0.60
#106	1.90	5.10	0.90
#145	0.70	2.20	0.90

cord blood (Kotlyar et al., 2021; Patanè et al., 2020); and more frequently after delivery through large respiratory droplets containing the SARS-CoV-2 virus from affected mothers (Liguoro et al., 2021).

Maternal transmission of SARS-CoV-2 through breastfeeding was not demonstrated (Centeno-Tablante et al, 2021). When SARS-CoV-2 was found in the breastmilk of affected women, it was not possible to culture it (Krogstad et al., 2022).

Conversely, much evidence exists on the benefit of human breastmilk in protecting feeding babies from infections such as influenza and respiratory viruses (WHO Collaborative Study Team on the role of breastfeeding on the prevention of infant mortality, 2000; Goldman, 1993).

It is well known that secretory IgA represents the primary protective component of human breastmilk, blocking pathogen entry on the mucosal surface of lactating infants (WHO Collaborative Study Team on the role of breastfeeding on the prevention of infant mortality, 2000; Goldman, 1993). Anti-SARS-CoV-2 immunity was demonstrated mainly in serum and breastmilk of women recovered from SARS-CoV-2 infection (Fox et al., 2020) and, as observed in our study, in women vaccinated with anti-COVID-19 mRNA vaccines (Baird et al., 2021; Golan et al., 2021; Gonçalves et al., 2021; Perl et al., 2021). A previous study reported data where vaccinated breastfeeding women had an immune re-

sponse characterized by a significant increase in sIgA and sIgG after the second dose (Perl et al., 2021). However, our results are in accordance with sIgA but not for sIgG.

For sIgA, this is proof of the presence of a booster effect for mucosal immunity, and this effect may theoretically be replicated with additional doses. The confirmation that sIgA represents the first response of the immune system to SARS-CoV-2 (Sterlin et al., 2021), followed by IgG, and that breastfeeding women express robust immune response as reported for non-lactating women (Cassaniti et al., 2022).

Our study has shown that after 6 months, sIgA antibody levels significantly decrease. However, this deflection appears to strongly affect women with the weakest initial sIgA response. A possible explanation for the sIgA decline is that the intramuscular route of administering the vaccine cannot trigger a strong mucosal response that lasts over time. Moreover, in this case, we cannot exclude a selective IgA deficiency (Sterlin et al., 2021).

Therefore, no correlation between serum and breastmilk IgA was observed, probably because of the two sources of IgA in breastmilk (serum monomeric or mucosal polymeric from breast mucose-associated lymphoid tissue MALT) (Gonçalves et al., 2021; Gray et al., 2021; Perl et al., 2021). Despite a low level of sIgA in breastmilk over time, Gonçalves et al. have shown that repeated breastfeeding probably results in the accumulation of sIgA that retains a neutralization effect on SARS-CoV-2 (Gonçalves et al., 2021). The same authors have observed a similar neutralizing effect but to a lesser extent for breastmilk sIgG. Interestingly, our group observed a weak increase in breastmilk sIgG levels at 6 months but a decrease in sIgA levels simultaneously. We cannot compare quantification outcomes of breastmilk sIgG and sIgA because a semi-quantitative analysis for serum IgG and IgA evaluation was used. We observed the trend over time (Figure 1 Supplement).

The explanation for this finding may be that most studies reported a peak of IgG after the second dose. However, as in our study, the observation period is not long enough to observe the real expression of the curve (Charepe et al., 2021; Golan et al., 2021; Gonçalves et al., 2021). Moreover, other studies have observed that other factors may impact the sIgG levels: breastfeeding for longer than 6 months may increase the sIgG level over time, and this is a possible contribution to what we observed (Abuidhail et al., 2019; Charepe et al., 2021; Czosnykowska-ìukacka, 2020). Therefore, the small number of patients and the lack of observation after 6 months may limit the interpretation of this finding.

Notably, increasing evidence in other respiratory infections supports a more important role in neonatal immune response for IgG in breastmilk (Demers-Mathieu et al., 2021; Mazur et al., 2019). We suggest a similar role in vaccine-induced breastmilk immunity. The beneficial effects could last for months after anti-COVID-19 vaccination with mRNA formulations.

Newborns from SARS-CoV-2 infected mothers showed an increased admission rate to a neonatal unit (Allotey et al., 2020). Several cases of severe disease were reported in infants younger than 6 months (de Siqueira Alves Lopes et al., 2021). In this scenario, immune response persistence after 6 months may be particularly important in planning vaccination strategy in puerperium and pregnancy. Numerous studies have observed that certain vaccines induce changes in breastmilk composition, mainly if offered in late pregnancy (Hahn-Zoric et al., 1993; Schlaudecker et al., 2013; Shahid et al., 2002; Su et al., 2016). Therefore, many authors have already demonstrated IgG antibodies passage through the placenta in SARS-CoV-2 infected women during pregnancy or after mRNA vaccination (Beharier et al., 2021; Cassaniti et al., 2021). The favorable effect on newborns must be addressed. However, we believe vaccination during lactation or late pregnancy could confer protection thanks to the specific IgG transfer through the placenta, as already demonstrated and used for pertussis and influenza (Su et al., 2016), but also by adding mucosal protection through breastmilk sIgA and sIgG. If established with other studies, breastmilk antibodies may be beneficial in preventing SARS-CoV2 in newborns and infants not included in the vaccination program considering that additional vaccine doses could confer continuous protection.

We are aware that we have used a commercially available ELISA to detect SARS-CoV-2 specific serum IgA (sample dilution 1:100). We tested it also for sIgA quantification in saliva and breastmilk (sample dilution 1:5). We found it specific and reproducible; however, we will perform more investigations to standardize the assay for secretory antibodies.

Therefore, the restricted number of cases could represent a limitation of our study. However, the data clearly indicate that passive immunization in this setting is safe and possibly worthwhile.

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Ethical approval

This study is a prospective analysis approved by the Medical Ethics Committee of the Fondazione IRCCS Policlinico San Matteo, Pavia, Italy.

Author contributions

Alessandra Ricciardi Conceived and designed the analysis; Collected the data; Contributed data or analysis tools; Wrote the paper.Paola Zelini Contributed data or analysis tools; Performed the analysis; Wrote the paper.Irene Cassaniti Conceived and designed the analysis; Collected the data; Contributed data or analysis tools; Marta Colaneri: Contributed data or analysis tools; Performed the analysis; Raffaele Bruno Wrote the paper.Fausto Baldanti Wrote the paper.Maria Antonietta Avanzini Contributed data or analysis tools; Wrote the paper.Annalisa De Silvestri: Contributed data or analysis tools; Performed the analysis

Conflict of interests

The authors have no competing interests to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.06.055.

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