Transplacental transfer of SARS-CoV-2 antibodies in recovered and BNT162b2-vaccinated patients

OBJECTIVE: Neonates have been found to be more susceptible to severe SARS-CoV-2 infection. Data regarding the transfer of anti-SARS-CoV-2 antibodies to the neonate of vaccinated women is limited, including only 3 studies concerning late third-trimester vaccination. The objective of this study was to assess the transplacental transfer of anti-SARS-CoV-2 antibodies in women vaccinated with the BNT162b2 vaccine during the second and third trimester.

STUDY DESIGN: A total of 40 parturients with singleton term pregnancies were recruited. Samples were collected from maternal and cord blood. Both maternal and neonatal samples were analyzed for anti-nucleocapsid (anti-N) and anti-spike (anti-S) antibodies. The study was approved by the local institutional review board (number 0055-21-AAA) and written informed consent was obtained from all participants.

RESULTS: Of the 40 women recruited, 28 were vaccinated with 2 doses of the BNT162b2 vaccine and 12 were COVID-19-convalescents (Supplemental Table 1). Median interval between COVID-19 diagnosis and delivery in the recovered group was 20.6 weeks (interquartile range [IQR], 17.6–36.9), whereas the median interval between second vaccine and delivery in the vaccinated group was 11.1 weeks (IQR, 9.3–15). Two women in the vaccinated group were anti-N-positive, suggesting past unknown infection (Supplemental Table 2).

Overall, maternal anti-S antibody levels were significantly higher in the vaccinated group than in the recovered group (145, IQR, 113–202 vs 41, IQR, 19–95 AU/mL, respectively; \(P<.008\)), as were neonatal anti-S antibody levels (216, IQR, 155–316 vs 64, IQR, 23–219 AU/mL, respectively; \(P<.026\)). Neonatal antibody levels were significantly higher than maternal levels in both groups (185, IQR, 85–316 vs 131, IQR, 59–198; \(P<.001\)). There was no significant difference in the neonatal to maternal anti-S ratio between the groups (Table).

There was a significant correlation between maternal and neonatal anti-S antibody levels (\(r=0.922, P<.001\)). However, there was no correlation between maternal anti-S levels and the neonatal to maternal anti-S ratio, nor between maternal anti-S levels and the interval to delivery. Moreover, the lack of correlation between maternal anti-S levels and the interval to delivery was also apparent when assessing the vaccinated and the recovered groups separately (Supplemental Table 3).

Regarding factors that may affect transplacental anti-S antibody transfer, using a linear regression model (Figure), we found that only birthweight had a significant positive impact on the neonatal to maternal anti-S ratio (\(\beta\)-coefficient, 0.509; 95% confidence interval, 0.032–0.986; \(P=.037\)).

CONCLUSION: This study assessed the maternal to neonatal transfer of SARS-CoV-2 antibodies in women vaccinated during the second trimester. Previously published studies assessed the transfer of anti-SARS-CoV-2 antibodies in women vaccinated in the late third trimester, in some cases without completing the vaccination course (ie, 1 week after the second dose). The fact that vaccinations were given close to delivery may explain the low neonatal to maternal antibody ratio found in all these studies. By contrast, our study found a high neonatal to maternal antibody ratio, similar to that found in studies on other vaccines.

The median gestational week at second dose was 26 (24–30 weeks; IQR, 14–34), enabling us to show that despite the long interval between vaccination and delivery, a high level of antibodies was maintained. We did not find an association between vaccination-to-delivery or infection-to-delivery interval and antibody levels. This can be explained by the relatively short time frame of our study and the fact that the study’s population comprised young, healthy women. Birthweight was found to affect transplacental antibody transfer. This is in accordance with previous data showing a correlation between birthweight and antibody transfer. We could not assess the correlation between gestational age and

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Vaccinated (28)</th>
<th>Recovered (12)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal anti-S (AU/mL)</td>
<td>145 (113–202)</td>
<td>41 (19–95)</td>
<td>.008</td>
</tr>
<tr>
<td>Neonatal anti-S (AU/mL)</td>
<td>216 (155–316)</td>
<td>64 (23–219)</td>
<td>.026</td>
</tr>
<tr>
<td>Neonatal/maternal anti-S ratio</td>
<td>1.48 (1.18–1.82)</td>
<td>1.35 (1.19–1.84)</td>
<td>.919</td>
</tr>
</tbody>
</table>

Anti-S antibody levels in maternal and neonatal serum. Data are presented as median (interquartile range). \(P\) values were calculated using the Mann–Whitney \(U\) test.

antibody transfer because all cases in this study were term deliveries.

Our study has a few limitations. The small sample size may have prevented us from detecting a time-dependent decrease in antibody levels or other factors that may have affected transplacental antibody transfer in addition to birthweight. Differences between the vaccinated and recovered groups may have been a source of bias. The risk of residual bias cannot be eliminated, although the use of multivariate analyses reduces that risk. We have no data regarding vaccines other than the BNT162b2, which was used exclusively in Israel. Lastly, waning of antibody titers in neonates remains yet to be determined.

Neonatal SARS-CoV-2 antibody levels were higher than maternal levels in both vaccinated and recovered patients. Antibody levels in newborns of vaccinated mothers were 3.4-fold higher than those in newborns of recovered mothers. Given that the protection afforded to neonates is based solely on maternal antibody transfer, vaccination of women during pregnancy might have an impact on the protection of their newborns.

FIGURE
Linear regression assessing the factors affecting the neonatal to maternal anti-S ratio

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REFERENCES
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SUPPLEMENTAL MATERIAL

Study protocol
This was a single center cross-sectional study conducted between May 2021 and July 2021. Healthy, pregnant women, admitted to the labor ward for delivery were assessed for possible inclusion to the study. Inclusion criteria were: singleton pregnancy; 37 to 42 completed gestational weeks; and either completion of the second dose of the BNT162b2 vaccine at least 1 week before delivery in the study group, or past documentation of COVID-19, including a positive SARS-CoV-2 polymerase chain reaction (PCR) result, and following the Israeli criteria for termination of quarantine (10 days after onset of disease in mild-moderate COVID-19, and 20 days in severe disease). Exclusion criteria were: known major malformation; an estimated fetal weight below the fifth centile; or lack of informed consent.

After obtaining written consent, maternal samples were collected. Umbilical cord blood was collected immediately after delivery. Anti-nucleocapsid (anti-N) antibody assays using the qualitative Elecsys anti-SARS-CoV-2 assay (Cobas, Roche Diagnostics, Switzerland) were performed to verify past infection in the recovered group and lack of infection in the vaccinated group. The test has a sensitivity of 89% and a specificity of 100%. Anti-spike (anti-S) antibody levels were tested with LIAISON SARS-CoV-2 S1/S2 Immunoglobulin G assay (DiaSorin, Saluggia, Italy). The test targets both the S1 and S2 subunits of the spike protein of the virus. The assay has a sensitivity and specificity of 97.4% and 98.5%, respectively. The cutoff value for a positive result being \( \geq 15 \) AU/mL, borderline between 12 and 14.9 AU/mL and negative \(< 12 \) AU/mL, according to manufacturer specifications.

To analyze the effect of time on antibody levels and transfer, we recorded the time interval from either the positive SARS-CoV-2 PCR in recovered patients or from the second vaccine dose to delivery in vaccinated patients. A total of 32 cases were calculated to be sufficient to identify a 20% difference in antibody levels. To account for possible drop-outs, 40 women were recruited. Normality of distribution was assessed using Q-Q plots. Categorical variables were expressed as number (percent) and continuous variables were expressed as mean (standard deviation) or median (interquartile range) according to distribution. Chi-square tests and Fisher exact tests were used for comparison of categorical variables. Comparisons of continuous variables were conducted using Mann—Whitney U test or Student t test, as appropriate. Correlations were performed using Spearman correlation and linear regression was utilized to assess variables affecting transplacental antibody transfer. Statistical analyses were conducted using the IBM Statistical Package for the Social Sciences (IBM SPSS v.20; IBM Corp, Armonk, NY).

The study was approved by the local institutional review board (approval number 0055-21-AAA) and written informed consent was obtained from all participants.

<table>
<thead>
<tr>
<th>Demographic and clinical data</th>
<th>Vaccinated (28)</th>
<th>Recovered (12)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>30.4±5.2</td>
<td>26±4.3</td>
<td>.014</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.4±3.4</td>
<td>30.1±3.9</td>
<td>.257</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>5 (17.9%)</td>
<td>4 (33.3%)</td>
<td>.411</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>39±1.1</td>
<td>39.9±1</td>
<td>.027</td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>11 (39.3%)</td>
<td>2 (16.7%)</td>
<td>.271</td>
</tr>
<tr>
<td>Interval to delivery (d)</td>
<td>11.1 (9.3—15)</td>
<td>20.6 (17.6—36.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Birthweight</td>
<td>3258±436</td>
<td>3650±465</td>
<td>.015</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>14/14</td>
<td>7/5</td>
<td>.629</td>
</tr>
<tr>
<td>Low 1-min Apgar</td>
<td>0</td>
<td>1 (8.3%)</td>
<td>.3</td>
</tr>
<tr>
<td>Low 5-min Apgar</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Continuous variables are represented as mean±standard deviation or median (interquartile range) according to distribution. Categorical variables are represented as number (percentage). P values were calculated using the t test, Mann—Whitney, or Fisher exact test, as appropriate. BMI, body mass index.

a BMI data were missing in 10 cases; b Days to delivery were calculated from date of COVID-19 diagnosis to date of delivery in the recovered group and from date of second vaccination to date of delivery in the vaccinated group; c Low 1- or 5-minute Apgar score was defined as a score <7.

### SUPPLEMENTAL TABLE 3
**Correlation of maternal anti-spike levels**

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Correlation coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal anti-S</td>
<td>0.922</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Anti-S ratio</td>
<td>0.268</td>
<td>.094</td>
</tr>
<tr>
<td>Days to delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>-0.277</td>
<td>.087</td>
</tr>
<tr>
<td>Recovered</td>
<td>0.014</td>
<td>.966</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>-0.136</td>
<td>.500</td>
</tr>
</tbody>
</table>

Spearman correlations between maternal anti-S levels to neonatal levels ratio, neonatal to maternal anti-S ratio, and time from exposure to either the virus causing COVID-19 or vaccine to delivery.

Anti-S, anti-spike.