Original Investigation



Assessment of Maternal and Neonatal Cord Blood SARS-CoV-2 Antibodies and Placental Transfer Ratios

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(1) Cite (C) Permissions (A) Metrics (E) Comments





JAMA Pediatr

Published Online: January 29, 2021

2021;175;(6):594-600.

doi:10.1001/jamapediatrics.2021.0038



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tion between maternal and neonatal severe acute respiratory syndrome specific antibody concentrations?

SARS-CoV-2 IgG antibodies were transferred across the placenta in 72 of 83 eropositive, and cord blood IgG concentrations were directly associated with ions, whereas IgM antibodies were not detected in any cord blood sera. d with time elapsed from maternal infection to delivery and not associated ction.

ntal transfer of SARS-CoV-2 IgG antibodies supports the potential for to provide peopatal protection from SARS-CoV-2 infection





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ed antibodies are a key element of neonatal immunity. Understanding the ly responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-and subsequent transplacental antibody transfer can inform neonatal rnal vaccination strategies.

Objective To assess the association between maternal and neonatal SARS-CoV-2-specific antibody concentrations.

Design, Setting, and Participants This cohort study took place at Pennsylvania Hospital in Philadelphia, Pennsylvania. A total of 1714 women delivered at the study site between April 9 and August 8, 2020. Maternal and cord blood sera were available for antibody measurement for 1471 mother/newborn dyads.

Exposures SARS-CoV-2.

Main Outcomes and Measures IgG and IgM antibodies to the receptor-binding domain of the SARS-CoV-2 spike protein were measured by enzyme-linked immunosorbent assay. Antibody concentrations and transplacental transfer ratios were analyzed in combination with demographic and clinical data.

Results The study cohort consisted of 1714 parturient women, with median (interquartile range) age of 32 (28-35) years, of whom 450 (26.3%) identified as Black/non-Hispanic, 879 (51.3%) as White/non-Hispanic, 203 (11.8%) as Hispanic, 126 (7.3%) as Asian, and 56 (3.3%) as other race/ethnicity. Among 1471 mother/newborn dyads for which matched sera were available, SARS-CoV-2 IgG and/or IgM antibodies were detected in 83 of 1471 women (6%; 95% CI, 5%-7%) at the time of delivery, and IgG was detected in cord blood from 72 of 83 newborns (87%; 95% CI, 78%-93%). IgM was not detected in any cord blood specimen, and antibodies were not detected in any infant born to a seronegative mother. Eleven infants born to seropositive mothers were seronegative: 5 of 11 (45%) were born to mothers with IgM antibody

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porn to mothers with significantly lower IgG concentrations compared with of seropositive infants. Cord blood IgG concentrations were positively concentrations (r=0.886; P<.001). Placental transfer ratios more than 1.0 with asymptomatic SARS-CoV-2 infections as well as those with mild, virus disease 2019. Transfer ratios increased with increasing time between 1 delivery.

n this cohort study, maternal IgG antibodies to SARS-CoV-2 were transferred nptomatic as well as symptomatic infection during pregnancy. Cord blood lated with maternal antibody concentrations and with duration between y. Our findings demonstrate the potential for maternally derived SARS-CoV-2



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ection is primarily dependent on neonatal innate immune responses and entally acquired antibodies. The extent to which maternal antibodies re acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection during is important for understanding potential neonatal protection from VID-19) and for developing appropriate maternal vaccination strategies when

effective vaccines are widely available. To date and to our knowledge, studies of transplacental transfer of maternal SARS-CoV-2-specific antibodies to newborns are limited to case reports and small case series of women with symptomatic infection.¹⁻³

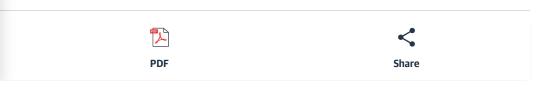
Our center has previously reported on the prevalence of antibodies to SARS-CoV-2 among women presenting for delivery at 2 large birth centers in Philadelphia, Pennsylvania. In that study, we validated a SARS-CoV-2 spike protein receptor-binding domain (RBD) serological test using samples of prepandemic sera from nonpregnant and pregnant patients, as well as sera from COVID-19-recovered donors. We then used this validated assay to test sera routinely collected from parturient women on admission for delivery. Among 1293 samples collected from April 4 to June 3, 2020, we found that 80 parturient women (6.2%) possessed IgG and/or IgM SARS-CoV-2-specific antibodies at a level above assay background. We observed race/ethnicity differences in seroprevalence, with higher rates in Black/non-Hispanic and Hispanic/Latino women. In the current study, we use this assay to test sera from parturient women and cord blood collected from April 9 to August 8, 2020, at one of our birth centers to measure the incidence, efficiency, and dynamics of placental transfer of maternal antibodies to the newborn.

Methods

This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline. The institutional review board of the University of Pennsylvania approved this study as minimal risk that could not practicably be performed without waiver of consent.

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elivered at Pennsylvania Hospital in Philadelphia between April 9, 2020, and d in the study. During this period, women were routinely screened for SARS-ymerase chain reaction (NP-PCR) testing when admitted to the hospital for pregnancy due to SARS-CoV-2 exposure or COVID-19 symptoms. Women asma reagin at the time of delivery for routine syphilis screening, and pred for blood type and Coombs testing as clinically needed. Residual m from this testing was collected for study purposes at the time it would hospital laboratories. Sera were fully deidentified prior to antibody



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rum sample deidentification, demographic and clinical data, including prior to delivery, were collected from review of electronic medical as defined per definitions provided by the US Centers for Disease matic: no history of COVID-19 symptoms at time of delivery or on r_____ mild disease: symptoms that do not include shortness of breath or

radiographic evidence of pneumonia, with normal oxygenation; and (3) moderate to critical disease: symptoms that include shortness of breath or radiographic evidence of pneumonia, with or without administration of supplemental oxygen, noninvasive respiratory support, or mechanical ventilation. Race and ethnicity were abstracted from documentation, which is typically self-reported at the time of admission. Prepregnancy body mass index was abstracted from documentation in the medical record or from the patient's self-reported entry in birth registration. *International Statistical Classification of Diseases* and Related Health Problems, Tenth Revision diagnosis codes O24, E08 to E13, and Z79.4 were used to identify type 1 diabetes, type 2 diabetes, and gestational diabetes, and codes O10, O11, O13 to O16, I10 to 113, and 115 were used to identify hypertensive disorders, gestational hypertension, and preeclampsia. The accuracy of using these codes has been validated with medical record review. 4 Preterm delivery was defined as less than 37 weeks' gestation, and term was defined as 37 or more weeks' gestation. Only the first twin from each pair was included in all analyses.

Antibody Measurement

Sera were tested using a validated enzyme-linked immunosorbent assay with plates coated with recombinant SARS-CoV-2 spike protein RBD. The RBD protein was produced in 293F cells using plasmid provided by Mt Sinai Hospital in New York, New York, and purified by nickel-nitrilotriacetic acid resin (Qiagen). This assay was validated using samples from COVID-19-recovered donors and samples collected prior to the COVID-19 pandemic, as previously described. Briefly, coated enzyme-linked immunosorbent assav plates (Immulon 4 HBX; Thermo Fisher Scientific) were stored overnight at 4 °C, then washed the

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nits/mL relative to CR3022. Plasmids to express CR3022 were provided by

n duplicate at a 1:50 dilution. Samples with an IgG and/or IgM concentration

ation (0.20 arbitrary units/mL) were repeated in at least a 7-point dilution

series to obtain quantitative results. Samples with IgG and/or IgM concentrations more than 0.48 arbitrary

series to obtain quantitative results. Samples with IgG and/or IgM concentrations more than 0.48 arbitrary units/mL were considered seropositive. Samples with IgG and/or IgM concentrations below this cutoff were assigned a value of 0.24 arbitrary units/mL for statistical analysis.

Statistical Analyses

All antibody concentrations were \log_2 -transformed for analysis and geometric mean concentrations with 95% CIs were reported unless stated otherwise. Transfer ratio was calculated as infant IgG concentration divided by maternal IgG concentration. Correlations between (1) maternal and infant IgG concentrations and (2) transfer ratio and days between NP-PCR testing and delivery were reported using the Pearson correlation coefficient. Standard descriptive analyses, including Fisher exact test, unpaired t test, analysis of variance, Mann-Whitney U test, and Kruskal-Wallis test, were used as appropriate to compare demographics, clinical characteristics, timing, and reason for maternal NP-PCR testing, antibody concentrations, and transfer ratios between analytic groups in Table 1 and Table 2. Statistical significance was set at P<.05. Stata version 16 (StataCorp) and Prism version 8 (GraphPad) were used for analyses.

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Table 1. Maternal Illness Severity and Results of NP-PCR Testing and Antibody ConcentrationsMaternal Illness Severity and Results of NP-PCR Testing and Antibody Concentrations

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%; 95% CI, 5%-7%) were SARS-CoV-2 IgG- and/or IgM-seropositive. Twentypreviously identified as seropositive in our prior seroprevalence study.

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previously identified as seropositive were NP-PCR tested except for 1 who declined ro

Figure 1. Study Flow Diagram



SARS-CoV-2 indicates severe acute respiratory syndrome coronavirus 2.

^aIncludes 29 sets of twins; only the first twin is included in all analyses.

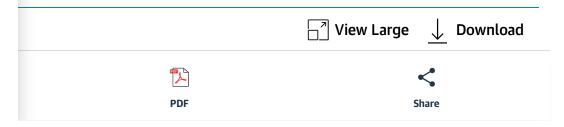
bIncludes 21 sets of twins.

There was a positive correlation between SARS-CoV-2 IgG concentrations in cord and maternal sera (r = 0.886; P<.001; **Figure 2**A). SARS-CoV-2 IgM antibodies were not detectable in any of the 72 infants who were seropositive. Of the 11 women who were seropositive with infants who were seronegative, 5 women

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I, and the remaining 6 women had significantly lower geometric mean IgG in the 72 women with seropositive infants (1.27 vs 5.22 arbitrary units/mL; *P* ly concentrations were not significantly different in women with seronegative in with seropositive infants (0.64 vs 0.81 arbitrary units/mL; *P*=.57; **Figure** ion between severity of maternal infection, maternal IgG concentration, and (1). Women with moderate or critical illness had higher IgG and IgM orn to these women had higher IgG concentrations, but these differences int.



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rnal and Neonatal Cord Sera Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Concentrations

centrations in sera from seropositive women and matched cord blood from s) and seronegative (n = 11; open circles) infants. IgG concentrations in cord blood

positively corrected with maternal IgG concentrations (r=0.886; P<.001). B, IgM concentrations in sera from seropositive women with seropositive (n=72; filled circles) and seronegative (n=11; open circle) infants. Horizontal lines represent geometric mean titers and error bars indicate the 95% CI (P=.57 using an unpaired t test on \log_2 -transformed IgM concentrations). In panels A and B, the horizontal dashed line indicates 0.48 arbitrary units/mL, which was the cutoff used to distinguish positive vs negative samples. Samples that were below this cutoff were assigned an antibody concentration of 0.24 arbitrary units/mL. C, Association of duration in days from nasopharyngeal polymerase chain reaction (NP-PCR) test to delivery with transplacental antibody transfer. Transfer ratio of IgG antibodies from mother to infant (n=26 matched mother-infant dyads) is positively correlated with days from NP-PCR test to delivery (r=0.620; P<.001).

Transfer ratios were not different among infants born to mothers with asymptomatic or symptomatic illness (<u>Table 1</u>). Excluding asymptomatic women whose onset of exposure or infection cannot be reliably determined, we used the timing of symptom-prompted viral testing as a surrogate for onset of infection. We assessed the association between transfer ratio and onset of maternal infection among a subset of 26 women with mild, moderate, or critical COVID-19 illness, who had a positive NP-PCR test result prior to delivery, and who delivered at term gestation. We observed a positive correlation between transfer ratio and increasing time between NP-PCR testing and delivery (r=0.620; P<.001; <u>Figure 2</u>C). We further explored the association of maternal, fetal, and newborn characteristics with the infant's serostatus among all mothers who were seropositive (n=83) and with incremental transfer ratio among mothers who were

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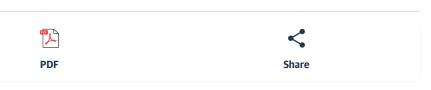
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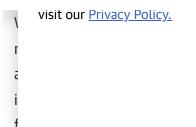
<u>e 2</u> and <u>Table 3</u>). We found no association between maternal demographic eristics and cord seropositive status (<u>Table 2</u>). The geometric mean transfer ring preterm (<37 weeks; n=8) vs term (≥37 weeks; n=70) gestation 3-1.21] vs 0.96 [95% CI, 0.81-1.13]; P=.41).

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ss Transfer Ratio Categories Among IgG-Seropositive Women (n=78)

oss Transfer Ratio Categories Among IgG-Seropositive Women (n=78)





tibody directed to the RBD of the SARS-CoV-2 spike protein in 1471 ingle birth center in Philadelphia from April to August 2020 and detected IgG 72 cord blood sera. Determination of correlates of maternal and neonatal a for SARS-CoV-2 research in maternal-child health domains, 6 and our the dynamics of maternally derived, potentially protective neonatal immunity.

Our findings align with current evidence that suggests that although placental and neonatal SARS-CoV-2 transmission may occur, ¹⁻³ such events are not common, ⁷⁻¹¹ We did not detect IgM antibodies in any cord blood serum samples even in cases of critical maternal illness or preterm delivery, supporting that maternal-fetal SARS-CoV-2 transmission is rare.^{2,3} Of greater concern is the potential for newborns to be infected postnatally from contagious mothers or other household contacts. We found efficient transfer of lgG antibodies from women who were SARS-CoV-2 seropositive (transfer ratios ≥1.0 in 40 of 72 infants who were seropositive), and a positive correlation between maternal and cord antibody concentrations. Our findings are aligned with studies of vaccine-elicited antibodies to pertussis, rubella, hepatitis B, and influenza, where cord sera/maternal sera transfer ratios ranging from 0.8 to 1.7 have been observed. 12,13 Higher maternal antibody concentrations and a higher transfer ratio were associated with increasing duration between onset of maternal infection and time of delivery. Multiple other factors, such as antigenelicited IgG subclass, maternal infections, maternal immunodeficiency, placental pathology, and gestational age at birth, are known to affect transfer efficiency and will require further study for SARS-CoV-2.^{14,15} We did not observe a significant difference in transfer efficiency comparing infants born preterm (defined as <37 weeks' gestation), but this finding was likely affected by small numbers (n=9) of preterm infants, with the earliest born at 31 weeks' gestation. Further studies will be needed to define transplacental antibody dynamics at earlier gestational ages.

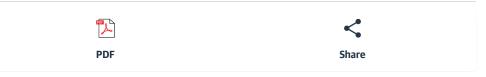
When vaccines are widely available, the optimal timing of maternal vaccination during pregnancy will need

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I factors including the time needed to ensure neonatal protection. The dy who were seropositive were asymptomatic, with uncertain timing of viral of women in our study whose onset of infection could be estimated by '-PCR testing, all cord sera were seropositive if the maternal NP-PCR testing delivery.

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clude a large cohort with access to available discarded specimens, allowing a g for delivery throughout the study period, as opposed to studies targeting identified during pregnancy or at the time of delivery. This study has several ite sample collection; small numbers of samples from preterm births; reliance



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were not able to study the sole association of gestational age with ibodies, nor can we rule out that SARS-CoV-2 infection itself at specific times ociated with the efficiency of transplacental antibody transfer.

Our findings demonstrate the potential for maternally derived antibodies to provide neonatal protection from SARS-CoV-2 infection and will help inform both neonatal management guidance and design of vaccine trials during pregnancy. Further studies are needed to determine if SARS-CoV-2 antibodies are protective against newborn infection; if so, at what concentration; and whether the transplacental kinetics of vaccine-elicited antibodies are similar to naturally acquired antibodies.

Article Information

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Accepted for Publication: January 7, 2021.

Published Online: January 29, 2021. doi:10.1001/jamapediatrics.2021.0038

Author Contributions: Dr Puopolo had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Flannery and Gouma contributed equally to this work.

Concent and design. Flannery Gouma, Dhudasia, Mukhopadhyay, Hensley, Puopolo. retation of data: All authors. We use cookies and other technologies to collect annery, Gouma, Dhudasia, Mukhopadhyay, Woodford, Arevalo. information about your use of our websites and ript for important intellectual content: Gouma, Dhudasia, Mukhopadhyay, online apps. Some of Morris, Weirick, McAllister, Bolton, Anderson, Goodwin, Hensley, Puopolo. these cannot be disabled. Unless you reject nonnecessary cookies, we Jouma, Dhudasia, Mukhopadhyay, Morris. may also share your information with thirdensley, Puopolo. party advertising and analytics partners who may serve you with targeted ads. . To learn **PDF** Share

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es: Dr Hensley reported consultancy fees from Sanofi Pasteur, Lumen,
unrelated to this study. Dr Puopolo reported grants from Children's Hospital
d for Excellence and US Centers for Disease Control and Prevention during
the conduct of the study. No other disclosures were reported.

Funding/Support: Funding for this study was provided in part by a Children's Hospital of Philadelphia Foerderer Grant for Excellence to Dr Puopolo.

Role of the Funder/Sponsor: The funder had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: We thank Jeffrey Lurie (Philadelphia Eagles), Joel Embiid (Philadelphia 76ers), Josh Harris (Philadelphia 76ers), and David Blitzer (Philadelphia 76ers) for philanthropic support that was used to establish the serological assays used in this study. We thank Florian Krammer, PhD (Mt. Sinai; no compensation provided), for providing the severe acute respiratory syndrome coronavirus 2 spike receptor-binding domain expression plasmids and Ian Wilson, DPhil (Scripps; no compensation provided), for providing plasmids to express monoclonal CR3022.

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