Antibody Testing of Infants Born to Asymptomatic COVID-19 Positive Mothers

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Abstract

Importance and Contribution of this Study: *Variability exists in the passage of SARS-COV-2 IgG from asymptomatic COVID-19 mothers to their newly born infants.*

Awareness of SARS-COV-2 IgG may contribute to the management of asymptomatic RT PCR COVID-19 positive pregnant women, their newborns, and future vaccination practices.

Objective: *Characterize COVID testing results of asymptomatic COVID-19 positive pregnant women and their infants. Our assumption/hypothesis maintained that all infants born to asymptomatic COVID-19 positive mothers would have detectable SARS-CoV-2 specific IgG.*

Study Design: *Retrospective chart review. Clinical demographics/COVID-19 testing of maternal/infant dyads were reviewed/collected for reporting purposes.*

Setting: *Center for Women and Infants (CWI), University of Louisville Hospital, Louisville, KY*

Participants: *Asymptomatic COVID-19 positive pregnant women/infant dyads admitted to the CWI between June 2020 to February 2021.*

Results: *36 COVID-19 positive asymptomatic mother/37 infant dyads (one set of twins) reviewed. 38% of the mother/infant dyads were positive for SARS-CoV-2 IgG, while 27% of mother/infant dyads were negative for IgG. A COVID-19 positive mother of twins was IgG negative, but both twins were positive. Two mothers in this study group had developed significant COVID-19 disease at 28w4d gestation and 34w0d gestation. Both required intensive care but recovered, and their pregnancies were maintained until 37w4d and 39w3d gestation, respectively. By the time of delivery, both mothers had negative COVID-19 RT PCR testing, but both infants were positive for SARS-CoV-2 IgG antibodies. COVID-19 RT PCR testing on both of these infants at 24 and 48 hours of age was negative.*

Conclusion: *SARS-CoV-2 IgG is passively transferred to the infant during pregnancy of asymptomatic positive COVID-19 mothers however appears variable and/or possibly based on the ability of IgG detection with current testing. Further investigation of the immune system's response to the SARS-CoV-2 virus during pregnancy can direct future management/treatment during pregnancy, especially in the wake of vaccination for the virus during pregnancy and emerging variants.*

Key Words: Pregnancy, Newborn, COVID-19, SARS-CoV-2 IgG, Immunity, Placenta

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Introduction:

Coronavirus is one of the largest members of the RNA viruses shown to cause diseases in humans and animals. The latest member of the coronaviruses is the COVID-19 which can result in severe acute respiratory syndrome. One area still developing is the antibody response to the virus during pregnancy and subsequently by the newborn infant. The current standard to diagnose COVID-19 involves identifying genetic material in specimens obtained via nasal swabs using reverse transcription-polymerase chain reaction (RT PCR). There has also been support to determine the production of SARS-Co-2 as a means to evaluate a previous or more recent infection.(1,2,3) We describe the detection of SARS-CoV-2 IgG of asymptomatic recent or previous COVID-19 positive pregnant women at the time of delivery. Our hypothesis or presumption was that all infants born to asymptomatic COVID-19 positive mothers would have SARS-CoV-2I specific IgG.

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Methods:

All patients admitted, including pregnant women presenting for delivery to the University of Louisville Hospital, Louisville, KY, from April 2020, have been tested by RT-PCR for the SARS-CoV-2 virus using emergency authorized COVID-19 tests marketed by four manufacturers (Roche, Becton Dickenson, Cepheid, and DiaSorin). The specific test utilized was dependent on reagent availability based on government-mandated allocation. Starting in June 2020, several pregnant women admitted to Labor & Delivery who tested positive for COVID-19 also underwent COVID-19 antibody testing. All infants born to COVID-19 positive mothers underwent nasopharyngeal COVID-19 PCR testing at 24 and 48 hours of age. Starting in June 2020, the majority of infants born to mothers positive for COVID-19 also underwent COVID-19 antibody testing. Data were retrospectively reviewed of these mother/infant dyads who delivered between June 2020 to February 2021. IRB approval for the study was granted both by the University of Louisville and the University of Louisville Hospital.

Collection and PCR testing for COVID-19:

CDC COVID-19 Guidelines were followed for RT PCR COVID-19 collection and testing using synthetic fiber swabs with plastic shafts. Specimens were placed immediately in sterile tubes with 2-3 ml of viral transport media (UTM, Universal Transport Media by Han-Chag medical).

COVID-19 antibody testing:

For antibody testing, the SARS-CoV-2 IgG assay was utilized on

the Architect 2000 instrument (Abbott Laboratories, Abbott Park, IL) under Emergency Use Authorization by the FDA. Serum or plasma samples were submitted for the standard of care IgG testing. The process involves a chemiluminescent microparticle immunoassay whereby the amount of SARS-CoV-2 IgG antibodies in the sample is related to the RLU detected by the system optics. Samples with RLU amounts greater than the cut-off value are reported as positive for SARS-CoV-2 IgG.

Results:

From June 2020 to February 2021, charts of mothers and their newborn infants were reviewed whose admission or recent pre-admission screen was positive for SARS-CoV-2. There were 36 mother/37 infant dyads which included one set of twins. Delivery was by cesarian section in 14 (38%) of the cases. Eight infants (22%) required admission to the neonatal intensive care unit (NICU) unrelated to COVID-19.

One infant tested positive for SARS-CoV-2 by RT PCR at 24 and 48 hours. The infant was delivered by repeat cesarian section at 37w4d gestation with rupture of membranes at delivery. Pregnancy was complicated by oligohydramnios. The infant was allowed to bond with the mother in recovery using proper personal protective equipment and remained in an incubator in the mother's room until discharge. The mother and infant were both negative for SARS-CoV-2 IgG. Placenta pathology was reported as normal. The family was lost to follow-up, but our Pediatric Infectious Disease specialists, who are aware of all significant and/or hospitalized COVID-19 pediatric cases, have no record of this infant requiring subsequent hospitalization.

Table 1 is a summary of the SARS-CoV-2 IgG Maternal/Infant Dyads. Fourteen mother/infant dyads (38%) were positive for SARS-CoV-2 IgG, while ten dyads (27%) were negative for SARS-CoV-2 IgG. Five (14%) of the mothers tested positive for SARS-CoV-2 IgG while their infants were negative. The mother of the twins tested negative for SARS-CoV-2 IgG, while both twins were positive and confirmed on retesting. There were five mother/infant dyads (14%) with incomplete IgG testing.

Nasopharyngeal COVID-19 RT PCR testing of infants occurred at 24 and 48 hours of age. In the first 24 hours of life before IgG testing occurred, eight (50%) infants positive for IgG received their mother's breastmilk, and eight (50%) received formula. Infants NPO on admission to the NICU all tested negative for IgG.

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Discussion:

Unique to our study is the focus to characterize detection of SARS-CoV-2 IgG in asymptomatic positive COVID-19 pregnant women upon admission for delivery and of their newborn infant.

All of our mothers at the time of presentation were asymptomatic for COVID-19. Nineteen (53%) of the 36 mothers displayed SARS-CoV-2 IgG, thirteen (36%) were negative for IgG, and four (11%) were not tested for IgG. Of the 37 infants born to SARS-CoV-2 positive mothers, 14 (39%) were positive for SARS-CoV-2 IgG antibodies. Five (14%) infants whose mothers tested positive for IgG were negative for the SARS-CoV-2 specific IgG. Ten of the dyads (27%) revealed that both mother and infant were negative for SARS-CoV-2 IgG. Our initial hypothesis/assumption was that all infants born to asymptomatic COVID-19 mothers would have SARS-CoV-2 IgG antibodies. However, our results indicate that not all mothers had IgG to be passively transferred to the infant; some infants were negative for SARS-CoV-2 IgG when their mothers displayed IgG, as noted in the case of twins, the infants displayed IgG while their mother was negative for SARS-CoV-2

Table 1. Results on SARS-CoV-2 IgG Maternal/Infant Dyads

DYADS	N=36*
Maternal IgG +/Infant IgG +	14 (38%)
Maternal IgG -/Infant IgG -	10 (27%)
Maternal IgG +/Infant IgG -	5 (14%)
Maternal IgG -/Infant IgG +	2 (5%)*
INCOMPLETE TESTING	
Maternal IgG testing but no infant IgG testing	1 (3%)
No maternal IgG testing but infant IgG testing completed	4 (11%)

*Includes set of twins

IgG. P. Egerup et al. investigating the frequency of SARS-CoV-2 antibodies in their general population of parturient women, their infants, and partners, also found the variable presence of antibodies of infants born to mothers found to be COVID-19 positive. (15)

It has been reported that in adults, the median day from COVID-19 symptoms/infection until IgG seroconversion was approximately 13 days, while others similarly reported the median time for seroconversion of all isotypes (IgG, IgM, IgA) was at ~ 12days. (2,4) Likewise, in the Cochrane Review by JJ Deeks *et al.,* there was a low sensitivity of IgG seroconversion in the first week of symptoms, while the highest IgG values occurred by the third week after the onset of symptoms. (5) F. Zullo *et al.* demonstrated peak production of SARS-CoV-2 IgG at ~ 18 days (range 10-20 days) after the onset of symptoms. (6) They also indicated that repeat nasopharyngeal COVID-19 RT PCR testing was unlikely to be positive after 28 days from the onset of symptoms. Unlike patients described in these studies, our subjects, including infants, were asymptomatic with variable detectable IgG. The significance of our results and antibiotic production timing in asymptomatic SARS-CoV-2 positive pregnant women and their infants remains unknown but may be of interest for further investigation. A better understanding of the immune system's response to the SARS-CoV-2 virus and timing of antibody production could serve as Zullo *et al.* referred to as an "immunity passport" to address better how patients are managed, especially in relation to isolation and quarantine recommendations. (6)

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Specific to pregnancy and the newborn, Flannery *et al.* measured maternal and cord blood SARS-CoV-2 IgG/IgM antibodies of asymptomatic (60%)/symptomatic (40%) positive COVID-19 RT PCR pregnant women at the time of delivery. Eight percent of their study population (N=83) had detectable COVID-19 antibodies, and of these patients, 87% of their infants had detectable COVID-19 IgG at the time of the delivery. In our study population, 59% of the COVID-19 RT PCR positive mothers had detectable COVID-19 IgG, but only 38% of their infants were positive for COVID-19 IgG. The discrepancy might be explained since 100% (versus 60%) of our mothers were asymptomatic at the time of delivery. Flannery *et al.* did note in their study that infants of mothers with low IgG levels were negative for IgG at birth. Possibly in our asymptomatic population, the maternal IgG levels were so low that placental transfer did not occur or could not be detectable by the qualitative assays we used for SARS-CoV-2 IgG. The other possible explanation could be that delivery occurred before the placental transfer of antibody could occur, which would support Flannery *et al.* findings that placental transfer ratios increased with the increased time between maternal infection and delivery.

Two of the mothers included in this descriptive study developed significant COVID-19 disease at 28w4d gestation and 34w0d gestation. Both required intensive care but recovered, and their pregnancies were maintained until 37w4d and 39w3d gestation, respectively. Both mothers had negative COVID-19 RT PCR testing prior to delivery, but both infants were positive for SARS-CoV-2 IgG antibodies indicating potential passive immunity for ~3 months. COVID-19 RT PCR testing on both of these infants at 24 and 48 hours of age were negative.

This presence would suggest that transplacental passive immunity from symptomatic COVID-19 stricken mothers to their newborn infant is maintained for a similar time period of 3 months as reported in the general population with symptomatic COVID-19 infection. Isho B et al. found in their general/asymptomatic + COVID-19 /non-pregnant study population that serum and saliva SARS-CoV-2 IgG antibodies were maintained for at least three months. (8) Iyer et al. found decay of IgM and IgA at 49 and 71 days respectively after the development of COVID-19 symptoms and slower decay of SARS-CoV-2 IgG.(4)

We had one infant that tested positive by SARS-CoV-2 RT PCR. Neither mother nor infant had SARS-CoV-2 IgM testing, but both were negative for SARS-CoV-2 IgG. The placenta pathology was read as normal, but because the infant was exposed to the mother after delivery, it was felt that vertical infection of the virus did not occur.

Although we did not specifically evaluate breast milk, we did look at feeding practices prior to the infant's 24 hour nasopharyngeal COVID-19 RT PCR and SARS-CoV-2 IgG testing. There were no significant differences in RT PCR and IgG results between those receiving mother's breast milk or formula. Pace *et al.* analyzed breast milk of COVID-19 positive mothers and found all to contain SARS-CoV-2 specific IgA and IgG. (13) Likewise, Demers-Mathieu V *et al.* concluded from their evaluation that SARS-CoV-2 antibodies in breastmilk could serve to provide passive immunity and protect from COVID-19 disease. (16) Centeno-Tablante *et al.* reviewed transmission of SARS-CoV-2 through breast milk and concluded there was no evidence to support the virus is transmitted via breast milk. (14) Specific viral antibody testing would appear to support breastfeeding as a means to contribute protection to the infant. With the advent of COVID-19 vaccination during pregnancy, the determination of passive immunity using viral-specific antibody testing could serve to further substantiate breast milk as a way to prolong protection to the infant.

There are significant limitations to this study. This was a retrospective study with incomplete SARS-CoV-2 IgG antibody testing, numbers are limited, and SARS-CoV-2 antibody testing was qualitative and not quantitative. Qualitative testing does provide more timely results, which is important when there is a need to make rapid clinical treatment decisions. For the purpose of a study, however, qualitative testing and the absence of SARS-CoV-2 antibody levels fail to provide true antibody decay or timing of each individual immune response to the virus.

"SARS-CoV-2 IgG is passively transferred to the infant during pregnancy of asymptomatic positive COVID-19 mothers however is not an absolute."

Conclusion:

Our descriptive study contributes information regarding SARS-COV-2 antibody detection during pregnancy of asymptomatic COVID-19 positive women presenting for delivery. SARS-CoV-2 IgG is passively transferred to the infant during pregnancy of asymptomatic positive COVID-19 mothers however is not an absolute. Further investigation of the immune system's response to

the SARS-CoV-2 virus during pregnancy can direct future management/treatment during pregnancy, especially in the wake of vaccination during pregnancy and emerging variants.

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