SARS-CoV-2 Seroprevalence Among Parturient Women

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Abstract

SARS-CoV-2 has led to a pandemic of respiratory and multisystem disease, named COVID-19. Limited data are available for pregnant women affected by COVID-19. Serological tests, particularly those that provide quantitative information, are critically important to determine exposure and immunity to SARS-CoV-2 within both individuals and populations. Here, we completed SARS-CoV-2 serological testing of 237 parturient women at two centers in Philadelphia from April 4 to April 15, 2020. Using an assay with a 1.0% false positive rate, we show that 14/237 (5.9%) of parturient women possessed IgG and/or IgM SARS-CoV-2-specific antibodies. We found significant racial differences, with an 11.2% seropositive rate in black women and a 1.5% seropositive rate in women of other races. Seropositive women who received nasopharyngeal (NP) SARS-CoV-2 PCR (polymerase chain reaction) testing were all found to be positive. Continued serologic surveillance among pregnant women may inform perinatal clinical practices and can potentially be used to estimate seroprevalence within the community.

Authors Dustin D. Flannery and Sigrid Gouma contributed equally to this work.

Main

SARS-CoV-2 can cause severe disease in adult populations, particularly in those with underlying health conditions. SARS-CoV-2 serological tests are important for determining immunity within individuals and populations; however many commercial tests have high false positive rates and therefore cannot be used to accurately estimate seroprevalence in populations with relatively low levels of exposures. Serological tests are especially important for vulnerable populations such as pregnant women because immune status has implications for management of both the pregnant woman and the newborn.

Admission for delivery is one of the few instances in which otherwise healthy individuals are interacting with the medical system during the current shelter- in-place and provide an opportunity for population surveillance of SARS-CoV-2 serology.

We performed a prospective cohort study of pregnant women presenting for delivery from April 4 to April 15, 2020 at two academic birth hospitals in Philadelphia, Pennsylvania. Both hospitals are active clinical and research centers affiliated with the University of Pennsylvania, and combined represent 50% of live births in Philadelphia. Discarded maternal sera from delivery admission were collected, deidentified, and tested by ELISA for SARS-CoV-2 IgG and IgM antibodies to the spike receptor binding domain (RBD) antigen. Symptomatic pregnant women and those with known risk factors underwent SARS-CoV-2 NP nucleic acid PCR testing from April 4-12, 2020; universal PCR testing was initiated for all pregnant women presenting for delivery between April 13-15, 2020. Of 289 women who delivered during the study period, 237 (82%) had available discarded serum specimens and were included in the analysis. Most serum specimens (198/237, 84%) were derived from women living in areas within or immediately bordering the city of Philadelphia. Demographics and clinical characteristics of the women are shown in Table 1.
We validated our serological assay by testing serum samples collected from 834 individuals in the Penn Medicine Biobank prior to the pandemic in 2019 and 15 individuals who recovered from confirmed COVID-19 infections in 2020 (Figure 1a-b). All 15 serum samples from COVID-19 recovered donors contained high, but variable, levels of SARS-CoV-2 IgG (Figure 1a) and 10 of 15 samples contained detectable levels of SARS-CoV-2 IgM (Figure 1b). Conversely, only 5 of 834 samples collected before the pandemic contained SARS-CoV-2 IgG and only 4 of 834 samples contained SARS-CoV-2 IgM. Together, this indicates that there is an overall false positive rate ~1.0% (9/834) in our serological assay.

Consistent with our initial validation experiments, we found that only 1 of 140 samples collected from pregnant women before the pandemic (from 2009-2012) possessed IgG or IgM SARS-CoV-2 antibodies (Figure 1c-d). In contrast, we found that 14 of 237 (5.9%) pregnant women presenting for delivery from April 4 to April 15, 2020 possessed IgG or IgM SARS-CoV-2 antibodies (Figure 1c-d; p = 0.01 comparing samples from pre-pandemic and pandemic pregnant women). We identified 7 women with both SARS-CoV-2 IgG and IgM, 5 women with only SARS-CoV-2 IgG, and 2 women with only SARS-CoV-2 IgM (Extended Data Table 1). We found variable SARS-CoV-2 antibody levels in samples from these women (Figure 1c-d), similar to what we found in samples from individuals recovering from confirmed SARS-CoV-2 infections (Figure 1a-b). Notably, 11.2% of samples from black women were seropositive, whereas only 1.5% of samples from women of other races were seropositive (Table 1; p=0.003 comparing seropositivity of black women and women of other races).

Over the course of this study, NP swabs from 75 women were tested by SARS-CoV-2 PCR. We found that 7 of 7 seropositive women who were NP tested had a SARS-CoV-2 positive PCR result, whereas only 1 of 68 seronegative women who were NP tested had a SARS-CoV-2 positive PCR result (Table 1; p <0.0001). Although these data suggest that serological tests may be valuable for identifying active or recent infections, further study will be required to address this question since NP and serum samples were collected at variable times (0-12 days apart).

Large-scale serology testing is critical for estimating how many individuals have been infected during the COVID-19 pandemic. Due to widely-imposed social distancing requirements, and to recent decreases in on-site, discretionary medical care, it is currently difficult to collect serum for population-wide serological testing. The vast majority of pregnant women, however, continue to have multiple interactions with the medical system for prenatal care and for delivery during this pandemic, and therefore represent a unique population to assess SARS-CoV-2 immunity within a community. Our data suggest that ~4.9% (5.9% minus 1.0% false positive rate) of parturient women in Philadelphia from April 4 to April 15, 2020 were previously exposed to SARS-CoV-2. The immune status of pregnant women in this study may not fully represent the general population of Philadelphia, Pennsylvania; the study hospitals are high-risk centers and may over- represent pregnant women in the greater Philadelphia area most impacted by COVID-19. However, it is interesting to extrapolate these findings to estimate the potential number of cases in the population. Nearly 1.6 million people live in Philadelphia, and if seroprevalence of parturient women is similar to the general population, then roughly 78,400 people would be expected to have been infected with SARS-CoV-2 by April 15. As of April 15, there were 7,441 confirmed cases of COVID-19 in
Philadelphia and 222 of these individuals died of the disease. Our data not only suggest that there might be over 10 times as many exposed individuals as those with confirmed infection, but the case-fatality ratio based on these estimates would be ~0.30%. These general population estimates need to be interpreted with caution until studies directly comparing parturient women and the general population are completed to confirm the validity of such estimates. For example, parturient women may not represent individuals of different ages within the general population and women and men might mount different antibody responses upon infection with SARS-CoV-2. Further, in some instances deaths due to COVID-19 may be attributed to other causes, impacting current population estimates of infection-attributable mortality.

Prior perinatal COVID-19 studies have primarily focused on virus detection (i.e. nucleic acid testing) in pregnant women and have not evaluated immunity. Two published studies to date have assessed SARS-CoV-2 serology in pregnant women with active disease. A study of 6 parturient women in Wuhan, China with confirmed COVID-19 found all 6 women had elevated levels of SARS-CoV-2 IgG and IgM. A case report from Peru detailed a symptomatic pregnant woman with positive PCR testing and negative serology at presentation, who developed severe respiratory failure necessitating delivery; her IgM and IgG turned positive 4 days after delivery (9 days after symptom onset). Beyond describing individual response to infection, SARS-CoV-2 serological testing among pregnant women will be increasingly important for perinatal disease risk management, as well as for optimizing vaccine strategies when vaccines become available.

The strengths of our study include the large cohort of parturient women with available serum for testing, the inclusion of women with PCR-confirmed COVID-19 infection as well as those who were uninfected/untested, and comparison to a pre-pandemic cohort. We will continue similar studies to monitor SARS-CoV-2 antibody levels in parturient women as social distancing relaxes in Philadelphia and other parts of the United States. Additional studies will be needed to address the impact of maternal infection on neonatal immunity, and to determine those factors (including socioeconomic and environmental differences) that may contribute to our observed racial disparities in maternal virus exposure.

**Methods**

**Serum samples from parturient women**

All pregnant women at the two hospitals (Pennsylvania Hospital and Hospital of the University of Pennsylvania) have blood drawn for rapid plasma reagin (screening for syphilis per CDC guidelines) testing as part of routine clinical care on admission to the hospital for delivery. Residual serum from this testing was obtained from the clinical laboratory at the time it was otherwise to be discarded. Demographic data were collected from review of electronic medical records. Race was self-reported. ICD-10 CM diagnosis codes O24, E08-E13, Z79.4 were used to capture Type 1 diabetes, Type 2 diabetes and gestational diabetes; codes O10, O11, O13-O16, I10-I13, I15 were used to capture hypertensive disorders,
gestational hypertension and pre-eclampsia; and code J45 was used to capture any history of asthma before or during pregnancy. To ensure these codes correctly captured patient condition, we manually reviewed all records with any ICD diagnosis of diabetes, hypertension or asthma; and reviewed a random sampling of 50/130 (38%) of records without any identified ICD codes for these conditions. Serum sample identification numbers were assigned at random. The Institutional Review Board at the University of Pennsylvania approved this study with waiver of consent.

**Serum samples from individuals fully recovered from COVID-19**

Samples from subjects who had recovered from laboratory confirmed SARS-CoV-2 infection and COVID-19 were obtained at the University of Pennsylvania. Subjects were consented and samples obtained after laboratory confirmed COVID-19 diagnosis and >14 days since resolution of symptoms. The Institutional Review Board at the University of Pennsylvania approved this study.

**Pre-pandemic human serum samples**

To validate our serological assay, serum samples from 834 adults (19-89 years old; 52% females) were collected via the Penn Medicine Biobank (PMBB) between October and December of 2019, prior to the COVID-19 pandemic. PMBB routinely consents individuals visiting the University of Pennsylvania healthcare system and obtains and stores biospecimens. Banked serum samples obtained from pregnant women from 2009-2012 were also utilized as pre-pandemic controls. For these banked samples, maternal serum was collected during the third trimester of pregnancy as part of an IRB-approved study. From this study, 140 samples were randomly selected from women who delivered at term (average gestational age at time of sample collection was 33.8 weeks, 80% black women).

**Enzyme-linked immunosorbent assay (ELISA)**

ELISAs were completed using plates coated with the receptor binding domain (RBD) of the SARS-CoV-2 spike protein. Plasmids for expressing this protein were provided by Florian Krammer (Mt. Sinai). SARS-CoV-2 RBD proteins were produced in 293F cells and purified using Ni-NTA resin (Qiagen). The supernatant was incubated for 2 hours with Ni-NTA resin at room temperature before the Ni-NTA resin was collected using gravity flow columns and the protein was eluted. After buffer exchange into PBS, the purified protein was stored in aliquots at -80°C. ELISA plates (Immulon 4 HBX, Thermo Scientific) were coated overnight at 4°C with 50 μL per well of PBS or a 2 mg/mL recombinant protein diluted in PBS. The next day, ELISA plates were washed 3 times with PBS containing 0.1% Tween-20 (PBS-T) and blocked for 1 hour with PBS-T supplemented with 3% non-fat milk powder. Prior to testing in ELISA, serum samples were heat-inactivated at 56°C for 1 hour. Serum samples were serially diluted in 2-fold in 96-well round-bottom plates in PBS-T supplemented with 1% non-fat milk powder (dilution buffer), starting at a 1:50 dilution. Next, ELISA plates were washed 3 times with PBS-T and 50 μL serum dilution was added to each well. Plates were incubated for 2 hours at room temperature using a plate mixer. Plates were washed again 3 times with PBS-T before 50 μL of goat anti-human IgG-HRP (Jackson ImmunoResearch Laboratories) (1:5,000) or goat anti-human IgM-HRP (SouthernBiotech) (1:1,000) secondary antibodies.
were added. After 1 hour incubation at room temperature using a plate mixer, plates were washed 3 times with PBS-T and 50 mL SureBlue TMB substrate (KPL) was added to each well. Five minutes later, 25 mL of 250 mM hydrochloric acid was added to each well to stop the reaction. Plates were read at an optical density (OD) of 450 nm using the SpectraMax 190 microplate reader (Molecular Devices). A spike-specific monoclonal antibody (CR3022) was included on each plate as a control, and serum antibody levels were reported as ug/mL amounts relative to the CR3022 monoclonal antibody. Plasmids to express the CR3022 monoclonal antibody were provided by Ian Wilson (Scripps). All samples were first tested in duplicate at a 1:50 serum dilution. Samples with an IgG and/or IgM concentration above the lower limit of detection (0.2 ug/mL) were repeated in at least a 7-point dilution series to obtain quantitative results.

**Establishment of a ELISA cutoff to distinguish seropositive versus seronegative**

We used results from the 2019 cohort (Figure 1a) to set ELISA cutoffs for seropositivity and seronegativity. Over the course of establishing our serological assay, we identified rare individuals who possessed pre-pandemic SARS-CoV-2 cross-reactive serum antibodies. Most of these individuals possessed very low levels of cross-reactive SARS-CoV-2 antibodies. We found that ~1% of samples from the pre-pandemic 2019 cohort had IgG and/or IgM levels of >0.48mg/mL, which was subsequently used as the cutoff for defining seropositivity in the 2020 cohort.

**Statistical methods**

Standard descriptive analyses using $\chi^2$ test, Fisher’s exact test, and Mann-Whitney test as appropriate, compared the demographic and clinical characteristics between the seropositive and seronegative groups. Statistical significance was set at p-value <0.05. Statistical analyses were performed using Stata version 14 (StataCorp, College Station, TX) and Prism version 8 (GraphPad Software).

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>COVID-19</td>
<td>coronavirus disease 2019</td>
</tr>
<tr>
<td>NP</td>
<td>nasopharyngeal</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>severe acute respiratory syndrome coronavirus 2</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
</tbody>
</table>

**Declarations**

**Data availability**
All serological data are included in the manuscript. Additional clinical data is available upon reasonable request.

Acknowledgments:

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Author Contributions:

DDF conceptualized and designed the study, collected data, drafted the initial manuscript, and revised the manuscript.

SG led the serological experiments, collected data, and revised the manuscript.

SM conceptualized and designed the study, designed the data collection instruments, carried out the analyses, and revised the manuscript.

MBD designed the data collection instruments, collected data, carried out the analyses, and revised the manuscript.

MRP collected data and revised the manuscript.

ECW collected data and revised the manuscript.

JSG conceptualized and designed the study, and revised the manuscript.

CPA completed serological assays, analyzed data, and revised the manuscript.

MJB completed serological assays, analyzed data, and revised the manuscript.

MW completed serological assays, analyzed data, and revised the manuscript.

ECG completed serological assays, analyzed data, and revised the manuscript.

EMA completed serological assays, analyzed data, and revised the manuscript.

ARG obtained and proceeded samples from recovered donors.

JK obtained and proceeded samples from recovered donors.
NH obtained and proceeded samples from recovered donors.
AP obtained and proceeded samples from recovered donors.
JD obtained and proceeded samples from recovered donors.
OK designed and established recovered donor cohort.
DM processed and characterized samples from recovered donors.
AB oversaw acquisition, processing, and characterization of samples from recovered donors.
LAV designed and established recovered donor cohort.
JW supervised recruitment of participants in PMBB and identification of samples for serology testing.
AV analyzed demographic data of PMBB participants.
RL provided samples for the pre-pandemic pregnant controls.
JSM provided statistical advice, performed statistical analyses, and revised the paper.
DJR provided input on the use of PMBB controls and revised the manuscript.
MAE provided input and samples for the pre-pandemic pregnant controls and revised the manuscript.
EJW designed, established, and oversaw healthy donor cohort studies and made revisions to the manuscript.
KMP conceptualized and designed the study, coordinated and supervised data collection, and revised the manuscript.
SEH conceptualized and designed the study, coordinated and supervised serological studies, and revised the manuscript.

**Competing interests:**

SEH has received consultancy fee from Sanofi Pasteur, Lumen, Novavax, and Merck for work unrelated to this report. EJW is a member of the Parker Institute for Cancer Immunotherapy. EJW has consulting agreements with and/or is on the scientific advisory board for Merck, Roche, Pieris, Elstar, and Surface Oncology. EJW is a founder of Surface Oncology and Arsenal Biosciences. EJW has a patent licensing agreement on the PD-1 pathway with Roche/Genentech. All other authors declare no competing interests related to this work.

**Additional information:** Correspondence and requests for materials should be addressed to hensley@pennmedicine.upenn.edu and karen.puopolo@pennmedicine.upenn.edu. Reprints and
permissions information is available at www.nature.com/reprints.

References


**Tables**

*Table 1. Demographics and Clinical Characteristics of Pregnant Women Cohort from April, 2020*
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (n = 237)</th>
<th>Seropositive1 (n = 14)</th>
<th>Seronegative (n = 223)</th>
<th>p-value2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years), median (IQR)</td>
<td>31 (27, 35)</td>
<td>27.5 (22, 32)</td>
<td>32 (27, 35)</td>
<td>0.02</td>
</tr>
<tr>
<td>Race3, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black/Non-Hispanic</td>
<td>98 (42.8)</td>
<td>11 (84.6)</td>
<td>87 (40.3)</td>
<td>0.003</td>
</tr>
<tr>
<td>White/Non-Hispanic</td>
<td>85 (37.1)</td>
<td>1 (7.7)</td>
<td>84 (38.9)</td>
<td>0.04</td>
</tr>
<tr>
<td>Asian</td>
<td>20 (8.7)</td>
<td>1 (7.7)</td>
<td>19 (8.8)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>16 (7.0)</td>
<td>0</td>
<td>16 (7.4)</td>
<td>0.61</td>
</tr>
<tr>
<td>Other</td>
<td>10 (4.4)</td>
<td>0</td>
<td>10 (4.6)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Health Insurance (public payor), n (%)</td>
<td>106 (44.7)</td>
<td>7 (50.0)</td>
<td>99 (44.4)</td>
<td>0.68</td>
</tr>
<tr>
<td>Pre-pregnancy BMI4, n (%)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight (25.0 to &lt;30.0)</td>
<td>57 (24.4)</td>
<td>2 (15.4)</td>
<td>55 (24.9)</td>
<td>0.74</td>
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<tr>
<td>Obese (≥30.0)</td>
<td>59 (25.2)</td>
<td>6 (46.2)</td>
<td>53 (24.0)</td>
<td>0.07</td>
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<tr>
<td>Positive GBS result5, n (%)</td>
<td>73 (30.8)</td>
<td>4 (28.6)</td>
<td>69 (30.9)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Diabetes6, n (%)</td>
<td>17 (7.2)</td>
<td>4 (28.6)</td>
<td>13 (5.8)</td>
<td>0.01</td>
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<tr>
<td>Hypertension6, n (%)</td>
<td>76 (32.1)</td>
<td>8 (57.1)</td>
<td>68 (30.5)</td>
<td>0.04</td>
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<tr>
<td>Asthma6, n (%)</td>
<td>46 (19.4)</td>
<td>4 (28.6)</td>
<td>42 (18.8)</td>
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<tr>
<td>Cesarean delivery, n (%)</td>
<td>80 (33.8)</td>
<td>7 (50.0)</td>
<td>73 (32.7)</td>
<td>0.19</td>
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<tr>
<td>Gestational age at delivery, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.40</td>
</tr>
<tr>
<td>&lt;37 weeks</td>
<td>31 (13.1)</td>
<td>3 (21.4)</td>
<td>28 (12.6)</td>
<td></td>
</tr>
<tr>
<td>≥37 weeks</td>
<td>206 (86.9)</td>
<td>11 (78.6)</td>
<td>195 (87.4)</td>
<td></td>
</tr>
<tr>
<td>SARS-CoV-2 PCR tested7, n (%)</td>
<td>75 (31.7)</td>
<td>7 (50.0)</td>
<td>68 (30.5)</td>
<td>0.13</td>
</tr>
<tr>
<td>Positive, n (% tested)</td>
<td>8/75 (10.7)</td>
<td>7/7 (100.0)</td>
<td>1/68 (1.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Liveborn infant status, n (%)</td>
<td>231 (97.5)</td>
<td>14 (100.0)</td>
<td>217 (97.3)</td>
<td>&gt;0.99</td>
</tr>
</tbody>
</table>

Footnotes: 1Seropositivity based on either IgG or IgM level >0.48 µg/mL. 2Difference in maternal age was tested using Mann-Whitney U test, differences in proportion for all other characteristics were tested using χ² tests or Fisher’s exact tests as appropriate at each level of the characteristic (e.g. Black women compared to non-Black women). 3Race missing for 1 seropositive and 7 seronegative mothers. 4Pre-pregnancy BMI missing for 1 seropositive and 2 seronegative mothers; pre-pregnancy BMI obtained from self-reported entry in birth registration or pre-pregnancy BMI documented in the medical records. 5Reflects the result of the last known GBS testing for mother obtained prior to delivery. 6Diagnosis based on delivery admission ICD-10 CM diagnosis codes for diabetes (O24, E08-E13, Z79.4), hypertension (O10, O11, O13-O16, I10-I11, I13, I15) and asthma (J45). 7Testing period includes between 7 days before delivery admission to 7 days after discharge. BMI, body mass index; GBS, group B *Streptococcus*, IQR, interquartile range; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Figures
Serum SARS-CoV-2 antibody levels in COVID-19 pandemic and pre-pandemic individuals. (a-b) Relative concentrations of SARS-CoV-2 IgG (a) and IgM (b) in serum collected before the COVID-19 pandemic (n=834) and serum collected from COVID-19 recovered donors (n=15). (c-d) Relative concentrations of SARS-CoV-2 IgG (c) and IgM (d) in serum collected from pregnant women from 2009-2012 (n=140) and from April 4-15, 2020 (n=237). Dashed lines indicate 0.48 µg/mL, which was used to distinguish positive versus negative samples (see Methods).

Figure 1
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ExtendeddataTable1.pdf