SUPPLEMENTAL DATA

APPENDIX 1. PATIENT ENROLLMENT and SAMPLE COLLECTION PROCESSES

APPENDIX 2. LABORATORY ASSAY METHODS

**(1) ELISA (Enzyme-linked Immuno-sorbent Assay) for the Detection of SARS-COV-2 Antibodies**

The levels of SARS-CoV-2 IgG, IgA and IgM antibodies present in maternal and cord blood were assessed using an enzyme linked immunosorbent assay (ELISA) developed in collaboration with the Emory Medical Lab.(15) The assay utilizes recombinant 6x receptor-binding domain (RBD) of the SARS-CoV-2 Wuhan-Hu-1 (NR52309; GenBank MN908947); Horse Radish Peroxidase ( HRP)-conjugated anti human IgG (Jackson), IgA (Southern Biotech), or IgM (Invitrogen) secondaries were used. Detection of the HRP conjugates was achieved using o-phenylenediamine dihydrochloride (OPD). 1M HCl was used to stop the reaction. Optical density readings were performed at 492 nm using a synergy BIOTEK reader. Log endpoint titers were calculated by imputation after sigmoidal fitting using GraphPad Prism software under clinically validated OD cutoffs (0.2 for IgG, 0.15 for IgA, and 0.35 for IgM), determined relative to pre-pandemic negative controls and to convalescent plasma. Antibody end dilution titers were expressed as log10 (value).

**(2)** **SARS-CoV-2 Spike (S) Pseudovirus Neutralization Assays**

The SARS-CoV-2 neutralizing activity in maternal and cord blood was quantified using an assay developed by Crawford and colleagues.(16) The assays utilizes HIV-based SARS-CoV-2 S baring pseudoviral particles, encoded by a plasmid that contains a 21 amino acid intracellular domain truncation mutant of the SARS-CoV-2 spike protein (delta 21 spike) based on the strain Wuhan-Hu-1 GenBank NC\_045512.(17) This pseudoviral particle was then co-transfected with a lentiviral backbone construct that uses a CMV reporter to express the ZsGreen and firefly luciferase reporters (BEI catalog number NR-52516) in HEK293T cells for 72 hours. The supernatant was then collected and purified before transduced into ACE-2 expressing 293T target cells; these cells were then aliquoted and stored at -80 C for use in pseudovirus neutralization assays.

To measure neutralizing activity in plasma, a 5-fold dilution series was prepared for each sample and incubated with a standard amount of the SARS-CoV-2 pseudovirus. Pseudovirus-antibody complexes were then added to 1E5 adherent ACE-2 transduced HEK293T cells in a 96 well plate, centrifuged for 30 minutes and then incubated for 24 hours and at 37C under 5% CO2. Pseudovirus infection and inhibition of infection (neutralization) were quantified using the Promega Bright-Glo Luciferase Assay System and the luminometer fiber on a synergy BIOTEK plate reader. The 50% inhibitory concentration/dilution (IC50) for each plasma sample tested was determined by normalizing the luminescence signal in each sample dilution to the maximum signal in a pseudovirus alone control. IC50 log dilutions were then calculated by imputation after sigmoidal fitting of each neutralization curve using GraphPad Prism.

APPENDIX 3. **SARS-CoV-2 antibody and neutralization titers.** A-C) Antibody class-specific serological testing for SARS-CoV-2 receptor binding domain (RBD) IgG, IgA and IgM. Each curve represents a serial dilution of an individual serum or plasma sample, including control pooled pre-pandemic (green) and convalescent plasma (red). (D) Lentiviral pseudovirus reporter assay for the detection of neutralizing antibodies against SARS-CoV-2 spike protein. (E) Shown are neutralization curves normalized to maximum infectivity (pseudovirus alone), from which 50% inhibitory plasma dilutions were extrapolated for each sample by sigmoidal fitting.



APPENDIX 4. REGRESSION ANALYSIS METHODS

A linear regression analysis was performed to analyze the relationship between log maternal RBD IgG titer (outcome) and the latency of maternal infection (natural logarithm days). Log days were used to help ensure adherence to the underlying assumptions of linear regression. The estimated slope for log maternal RBD IgG titer versus log latency days was significantly different from zero (p = 0.049).

**The following regression analyses likely have low statistical power:**

**Maternal RBD IgG Response**

Next a linear regression analysis was performed to analyze the relationship between log maternal RBD IgG titer (outcome) and the latency of maternal infection (loge days) and maternal infection status (symptomatic or asymptomatic).

We assumed that log maternal RBD IgG titer could be expressed as a linear function of the log latency of maternal infection, maternal infection status and the interaction between log latency of maternal infection and maternal infection status (the statistical interaction compares the slopes between women with symptomatic infection and women with asymptomatic infection). The slopes were not different (p = 0.50) and both estimated slopes did not differ from zero (p = 0.56 for women with asymptomatic infection and p= 0.46 for women with symptomatic infection). Therefore, a common slope linear regression analysis was performed for the relationship between log RBD IgG titer, and the log latency of maternal infection (loge days) and maternal infection status (symptomatic or asymptomatic). Th estimated slope was statistically zero (p = 0.72) and the latency adjusted log maternal RBD IgG titer was 2.249 for asymptomatic infection and 2.934 for symptomatic infection (p = 0.10).

The covariate-adjusted mean log maternal RBD IgG titer for each study group (2.249 for asymptomatic or 2.934 for symptomatic maternal infection status) was defined as the predicted outcome value obtained by evaluating the regression equation for each study group at the mean of the continuous predictor (log latency days) for the two study groups. This regression analysis is often referred to as analysis of covariance. Since log latency of maternal infection was not predictive of log maternal RBD IgG titer (p=0.72 for slope = 0 a two-sided , two-sample equal-variance t-test to compare log maternal RBD IgG titer between women with symptomatic infection versus asymptomatic infection was performed and resulted as follows:

Mean log maternal RBD IgG titer for asymptomatic infection = 2.1935 (95% confidence interval: 1.690 to 2.700).

Mean log maternal RBD IgG titer for symptomatic infection = 2.9824 (95% confidence interval: 2.6113 to 3.3534.

Mean difference: 0.7877, 95% confidence interval 0.1974 to 1.378), P value = 0.01

The 95% confidence interval implies that if the estimate for the observed mean difference is true and the study were run many more times with the same sample size, 95% of the time the estimated observed mean difference in log maternal RBD IgG titer will be between 0.1974 and 1.378.

**Relationship of maternal anti RBD IgG to Cord anti RBD IgG**

A linear regression analysis was performed to analyze the relationship between log cord RBD IgG titer (outcome) and the latency of maternal infection (natural logarithm days). The estimated slope for log cord RBD IgG titer versus log latency days was significantly different from zero (p = 0.037).

Next a linear regression analysis was performed to analyze the relationship between log cord RBD IgG titer (outcome) and the latency of maternal infection (loge days) and maternal infection status (symptomatic or asymptomatic).

We assumed that log cord RBD IgG titer could be expressed as a linear function of the log latency of maternal infection, maternal infection status and the interaction between log latency of maternal infection and maternal infection status (the statistical interaction compares the slopes between women with symptomatic infection and women with asymptomatic infection). The slopes were not different (p = 0.71) and both estimated slopes did not differ from zero (p = 0.87 for women with asymptomatic infection and p= 0.56 for women with symptomatic infection). Therefore, a common slope linear regression analysis was performed for the relationship between log cord RBD IgG titer, and the log latency of maternal infection (loge days) and maternal infection status (symptomatic or asymptomatic). Th estimated slope was statistically zero (p = 0.62) and the latency adjusted log cord RBD IgG titer was 1.629 for asymptomatic infection and 2.298 for symptomatic infection (p = 0.11).

The covariate-adjusted mean log cord RBD IgG titer for each study group (1.629 for asymptomatic or 2.298 for symptomatic maternal infection status) was defined as the predicted outcome value obtained by evaluating the regression equation for each study group at the mean of the continuous predictor (log latency days) for the two study groups. This regression analysis is often referred to as analysis of covariance. Since log latency of maternal infection was not predictive of log cord RBD IgG titer (p=0.62 for slope = 0), we then performed a two-sided two-sample equal-variance t-test to compare log cord RBD IgG titer between women with symptomatic infection versus asymptomatic infection.

Mean log cord RBD IgG titer for asymptomatic infection = 1.554 (95% confidence interval: 1.006 to 2.102).

Mean log cord RBD IgG titer for symptomatic infection = 2.364 (95% confidence interval: 2.027 to 2.701.

Mean difference: 0.8101, 95% confidence interval 0.2105 to 1.4097, P value = 0.0098.

**Median**

Median log cord RBD IgG titer for asymptomatic infection = 1.83 (95% confidence interval: 0.59 to 2.18).

median log cord RBD IgG titer for symptomatic infection = 2.410 (95% confidence interval: 1.89 to 2.85

Median difference: 0.58, 95% confidence interval -0.301 to 1.461

APPENDIX 5. Patient disease severity, antibody, and antigen levels at delivery

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | RBD IgG (log EDT) | RBD IgM (log EDT) | Neonatal: Maternal Ratio | Neutralizing Ab (IC50) | Nucleopcapsid Antigen (pg/mL) |
| Patient | COVID-19 Disease Severity | Maternal IgG | CordIgG | Maternal IgM | CordIgM |  | Maternal | Cord | Maternal  | Cord |
| 1 | Moderate | 3.58 | 2.34 | 2.31 | 0 | 0.73 | 3.3 | 0 | 0.00 | 0 |
| 2 | Asymptomatic | 2.81 | 2.01 | 1.95 | 0 | 0.69 | 0 | 0 | 0.00 | 0 |
| 3 | Moderate | 3.34 | 3.11 | 2.9 | 1.48 | 0.70 | 3.18 | 0 | 0.00 | 0 |
| 4 | Mild | 2.37 | 1.61 | 1.88 | 0 | 0.85 | 2.75 | 3.46 | 0.00 | 0 |
| 5 | Mild | 3.23 | 2.62 | 2.18 | 0 | 0.96 | 2.18 | 0 | 0.00 | 0 |
| 6 | Mild | 1.9 | 1.39 | 2.17 | 0 | 0.85 | 0 | 0 | 9.18 | 0 |
| 7 | Moderate | 2.69 | 1.37 | 2.04 | 0 | 0.97 | 3.11 | 2.5 | 0.00 | 0 |
| 8 | Moderate | 1.7 | 0 | 2.89 | 0.4 | 0.82 | 0 | 0 | 0.00 | 0 |
| 9 | Mild | 3.24 | 2.67 | 2.13 | 0 | 0.42 | 0 | 2.39 | 0.00 | 0 |
| 10 | Asymptomatic | 0.63 | 0 | 3.17 | 0 | - | 0 | 0 | 0.00 | 0 |
| 11 | Mild | 3.91 | 2.85 | 1.31 | 0 | 0.68 | 3.68 | 0 | 0.00 | 0 |
| 12 | Asymptomatic | 0.31 | 1.76 | 2.84 | 0 | - | 0 | 0 | 2.43 | 0 |
| 13 | Mild | 3.69 | 2.13 | 1.77 | 0 | 0.77 | 2.54 | 0 | 0.00 | 0 |
| 14 | Asymptomatic | 2.93 | 2.41 | 2.88 | 0 | 0.60 | 3.34 | 0 | 0.00 | 0 |
| 15 | Asymptomatic | 2.14 | 2.76 | 2.56 | 0 | 1.00 | 3.31 | 0 | 0.00 | 0 |
| 16 | Mild | 2.86 | 1.67 | 2.87 | 0 | 0.84 | 2.92 | 0 | 0.00 | 0 |
| 17 | Asymptomatic | 2.88 | 3.61 | 1.94 | 0 | 0.96 | 2.85 | 2.32 | 0.00 | 0 |
| 18 | Mild | 2.1 | 1.18 | 1.32 | 0 | 0.80 | 2.5 | 0 | 0.00 | 0 |
| 19 | Severe | 4.19 | 1.89 | 2.52 | 0 | 0.86 | 4.4 | 2.67 | 0.00 | 0 |
| 20 | Asymptomatic | 1.18 | 1.83 | 0 | 0 | 1.00 | 3.3 | 0 | 0.00 | 0 |
| 21 | Mild | 2.54 | 2.89 | 2.91 | 0 | 0.74 | 3.29 | 0 | 0.00 | 0 |
| 22 | Asymptomatic | 2.72 | 1.53 | 2.19 | 0 | 0.67 | 2.71 | 0 | 0.00 | 0 |
| 23 | Asymptomatic | 2.81 | 2.18 | 2.33 | 0 | 1.03 | 3.01 | 2.44 | 0.00 | 0 |
| 24 | Asymptomatic | 2.45 | 0 | 2.28 | 0 | 0.62 | 2.88 | 0 | 0.00 | 0 |
| 25 | Asymptomatic | 2.67 | 3.1 | 2.47 | 0 | 0.82 | 2.81 | 0 | 0.00 | 0 |
| 26 | Asymptomatic | 2.98 | 2.56 | 3.28 | 0 | - | 2.13 | 0 | 0.00 | 0 |
| 27 | Mild | 3.52 | 2.93 | 2.62 | 0 | 0.88 | 3.07 | 3.07 | 0.00 | 0 |
| 28 | Asymptomatic | 3.13 | 1.95 | 2.47 | 0 | 0.82 | 0 | 0 | 0.00 | 0 |
| 29 | Mild | 3.33 | 0.59 | 0 | 3.08 | 0.88 | 3.05 | 0 | 0.49 | 0 |
| 30 | Asymptomatic | 1.93 | 2.01 | 1.49 | 0 | 1.01 | 0 | 1.85 | 0.00 | 18.15 |
| 31 | Asymptomatic | 1.35 | 2.6 | 2.25 | 0 | 0.44 | 0 | 0 | 11.90 | 0 |
| 32 | Critical | 2.51 | 1.95 | 2.24 | 0 | 0.80 | 2.24 | 0 | 1780.00 | 0 |

APPENDIX 6. Mean Differences in maternal and cord serologic response in symptomatic compared to asymptomatic infection

|  |  |  |  |
| --- | --- | --- | --- |
|   |   |   | Median Difference and 95% CI |
| Variable | Group | Median | **Median Difference** | Lower 95% | Upper 95% |
| Maternal Anti-RBD IgG | asymptomatic | 2.67 | 0.56 | 0.312 | 1.432 |
|   | symptomatic | 3.23 |  |  |  |
|   |   |  |  |  |  |
| Maternal Anti-RBD IgA | asymptomatic | 1.32 | 0.75 | 0.248 | 1.748 |
|   | symptomatic | 2.07 |  |  |  |
|   |   |  |  |  |  |
| Maternal Anti-RBD IgM | asymptomatic | 2.33 | 0.15 | 0.704 | 0.404 |
|   | symptomatic | 2.18 |  |  |  |
|   |   |  |  |  |  |
| Maternal Neutralizing Potency | asymptomatic | 2.71 | 0.04 | 1.446 | 1.526 |
|   | symptomatic | 2.75 |  |  |  |
|  |  |  |  |  |  |
| Cord Anti-RBD IgG | asymptomatic | 1.83 | 0.58 | 0.301 | 1.461 |
|  | symptomatic | 2.41 |  |  |  |

APPENDIX 7. Relative Anti-RBD IgG titer in maternal sera and matched cord blood in (A.) symptomatic vs asymptomatic paired samples and in (B.) ≤ or > 14 days between time of positive PCR and delivery. The *dot plots* depict relative IgG and IgM titer against SARS-CoV-2 the S-protein of the receptor binding domain. Data are represented as the log 10(value) end dilution titer. Significance was determined by Wilcoxon matched pairs signed rank tests. Differences were not statistically significant.

