Maternal immune profiles of abnormal prenatal ultrasonographic findings following SARS-CoV-2 infection

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Background
Background

- Clinical spectrum of SARS-CoV-2 infection in pregnancy is variable\(^1\)
  - Asymptomatic to severe disease
- Possible increased risk of adverse pregnancy and neonatal outcomes with SARS-CoV-2 infection in pregnancy
  - Fetal death, preterm birth, preeclampsia, low birthweight infants, emergency cesarean birth\(^2\)
  - Greatest risk for severe or critical disease\(^3\)

1. Allotey et al, BMJ 2020
3. Metz et al, Obstet Gynecol 2021
Background

- **Dysregulated immune response** induced by SARS-CoV-2 suggested as the primary driver for severe disease and associated adverse outcomes\(^1\)

- Changes in maternal immunity following infections may be associated with adverse perinatal outcomes
  - Limited data on the immunological consequences following SARS-CoV-2 infection in pregnancy

1. Del Valle et al, Nat Med 2020
Background

• Pregnancies recovering from COVID-19 → at least one ultrasonographic assessment after symptom resolution suggested
  • Evaluate for fetal growth and morphologic changes
• Potential effects of SARS-CoV-2 infection on prenatal imaging and its association with pregnancy outcomes is not well-described
Objective

• To characterize the maternal immune profiles following SARS-CoV-2 infection in pregnancy

• To evaluate the correlation of COVID-19 induced alteration of maternal immunity with prenatal ultrasonographic abnormalities
Methods
Study Design

• Prospective observational cohort study
• Single quaternary care center
• Pregnancies with SARS-CoV-2
  • Diagnosed by NP RT-PCR between March 2020 and February 2021
• Healthy pregnant controls concurrently recruited
  • Confirmed negative SARS-CoV-2 by NP RT-PCR (universal screening on admission to Labor & Delivery)
Procedures and Sample Collection

• Peripheral blood specimens
  • Collected at two time points:
    1. Initial diagnosis (time of enrollment)
    2. Time of delivery (admission to Labor & Delivery)
  • Isolated sera subjected to high-throughput Next Generation Sequencing-based proteomics profiling
    • Olink Explore 1536 panel (PEA technology + NGS) → detect 1472 proteins
Procedures and Sample Collection

• Serial prenatal ultrasonographic examination following infection
  • Standard fetal biometry
    • Biparietal diameter, head circumference, abdominal circumference, femur length
    • Estimated fetal weight (grams) by Hadlock formula
  • General morphological evaluation for signs of congenital infection
  • Amniotic fluid volume assessment
  • Placental thickness measurement
  • Doppler evaluation
    • Umbilical artery (UA)
    • Middle cerebral artery (MCA)
Data Analysis

- Categorized as **abnormal ultrasound** if any of the following were identified at least once:
  - Fetal growth restriction
  - Placentomegaly
  - Infection-associated structural anomaly
    - Intracranial calcifications, ventriculomegaly, microcephaly, cardiomegaly, intraabdominal calcifications, echogenic bowel, ascites, pleural effusion, pericardial effusion, hydrops
  - Abnormal UA or MCA Doppler measurement
    - UA Doppler – elevated S/D ratio, absent end diastolic flow, or reverse end diastolic flow
    - MCA Dopplers – elevated PSV (> 1.5 MoM)
  - Abnormal amniotic fluid volume
    - Oligohydramnios, polyhydramnios
  - Other structural anomaly
Comparison of sera proteome profiles

SARS-CoV-2 positive with abnormal ultrasound vs Gestational age-matched healthy controls
Statistical Analysis

- Normalized protein expression (NPX) values of proteins and samples
  - Compared against limits of detection (LOD) for each protein
  - Proteins with > 80% of NPX values lower than LOD excluded
- Gene expression Fold Change (FC) calculated using ΔΔCt Method
  - FC for each gene calculated as 2-ΔΔCt
- Maternal characteristics and obstetric outcomes compared by prenatal ultrasonographic findings
  - Chi-square or Fisher’s exact – categorical variables
  - T-test or Wilcoxon rank-sum test – continuous variables
- Analysis performed on R
Results
Study Population

93 SARS-CoV-2 positive pregnancies from March 2020-February 2021

Excluded: 21 without prenatal ultrasonographic exam

72 SARS-CoV-2 positive pregnancies with at least 1 prenatal ultrasonographic exam

41 (57%) Normal Ultrasound

31 (43%) Abnormal Ultrasound
Abnormal Ultrasonographic Findings

31 (43%) Abnormal Ultrasound

- Fetal Growth Restriction: 8
- Placentomegaly: 17
- Ascites: 1
- Hydrops: 1
- Echogenic Bowel: 2
- Oligohydramnios: 2
- Polyhydramnios: 2
- Abnormal Doppler (UA or MCA): 3

Fetal Growth Restriction and Placentomegaly are the most common findings.
### Maternal Demographics

No significant differences in maternal demographics or clinical characteristics in pregnancies with COVID-19 that did or did not have an abnormal ultrasound.
Obstetric and Neonatal Outcomes

Neonatal outcomes available for infants delivered before March 1, 2021 (N = 65)

<table>
<thead>
<tr>
<th></th>
<th>Abnormal Ultrasound N=29</th>
<th>Normal Ultrasound N=36</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mode of Delivery</strong></td>
<td></td>
<td></td>
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<tr>
<td>Vaginal birth</td>
<td>22 (75.86)</td>
<td>23 (63.89)</td>
<td>0.58</td>
</tr>
<tr>
<td>Cesarean birth</td>
<td>6 (20.69)</td>
<td>11 (30.56)</td>
<td></td>
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<tr>
<td>Operative vaginal birth</td>
<td>1 (3.45)</td>
<td>2 (5.56)</td>
<td></td>
</tr>
<tr>
<td><strong>Gestational age at birth (weeks), median (range)</strong></td>
<td>39 (21-41)</td>
<td>39 (27-41)</td>
<td>0.34</td>
</tr>
<tr>
<td>Preterm birth</td>
<td>6 (20.69)</td>
<td>5 (13.89)</td>
<td>0.74</td>
</tr>
<tr>
<td><strong>Birthweight (grams), median (range)</strong></td>
<td>2914 (309-4252)</td>
<td>3108 (939-4780)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Low birthweight</strong></td>
<td>10 (31.25)</td>
<td>2 (5.56)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Small for gestational age</td>
<td>5 (15.63)</td>
<td>1 (2.78)</td>
<td>0.09</td>
</tr>
<tr>
<td>NICU admission</td>
<td>6 (18.75)</td>
<td>27 (19.44)</td>
<td>1</td>
</tr>
<tr>
<td>Neonatal respiratory distress</td>
<td>8 (25)</td>
<td>5 (13.89)</td>
<td>0.17</td>
</tr>
<tr>
<td>Any neonatal complication</td>
<td>14 (43.75)</td>
<td>10 (27.78)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Abnormal ultrasound associated with lower birthweight and birthweight < 2500 g

UCLA Health | David Geffen School of Medicine
Placentomegaly vs Fetal Growth Restriction

Adverse Neonatal Outcome after COVID-19 in pregnancy

- Placentaomegaly: 3.6% vs 96.4%
- Fetal Growth Restriction: 18.2% vs 81.8%

p = 0.002
p < 0.001
Maternal Serum Samples

Abnormal Ultrasonographic Findings

- Placentomegaly (N = 17)
- Fetal Growth Restriction (N = 8)

vs

GA-Matched Controls

- Healthy Controls (N = 17)
Serum Proteomic Profiles: Abnormal Ultrasound after SARS-CoV-2 Infection

Placentomegaly

Fetal Growth Restriction

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Serum Proteomic Profiles: Placentomegaly

**Protein** | **Function**
--- | ---
**Upregulated**
IFNLR1 | Class II cytokine receptor, activation of JAK/STAT pathway
IL17F | Cytokine expressed by activated T cells, production of other cytokines, inhibits angiogenesis of endothelial cells
TNFRSF4 | TNF-receptor superfamily, activates NF-κB, suppresses apoptosis, role in CD4+ T cell response and T cell-dependent B-cell proliferation and differentiation
FGFR2 | Fibroblast growth factor receptor, mitogenesis and differentiation, bone growth

**Downregulated**
ADAMTS13 | Cleaves vWF multimers in plasma, controlling vWF-mediated platelet thrombus formation
IL32 | Proinflammatory cytokine, induces production of TNFα from macrophage cells
Serum Proteomic Profiles: Fetal Growth Restriction

Protein | Function
--- | ---
**Upregulated**
MMP3 | Matrix metalloproteinase family, breakdown of extracellular matrix in embryonic development, reproduction, and tissue remodeling
DPP4 | Dipeptidyl peptidase, glucose and insulin metabolism, immune regulation, functional receptor of MERS-CoV

**Downregulated**
IGFBP7 | Insulin-like growth factor-binding protein family, regulates IGF availability and modulates IGF binding to receptors, stimulates prostacyclin production and cell adhesion
Placentomegaly vs Fetal Growth Restriction

Placentomegaly

\[ 182 \]

\[ 4 \]

Fetal Growth Restriction

\[ 41 \]

Common genes between 2 ultrasound findings

<table>
<thead>
<tr>
<th>Gene</th>
<th>Placentomegaly</th>
<th>Fetal growth restriction</th>
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<tbody>
<tr>
<td>ERBB4</td>
<td>-2</td>
<td>4</td>
</tr>
<tr>
<td>TGFBR3</td>
<td>-2</td>
<td>2</td>
</tr>
<tr>
<td>NCAM1</td>
<td>-2</td>
<td>2</td>
</tr>
<tr>
<td>PLA2G7</td>
<td>-4</td>
<td>4</td>
</tr>
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Fold change (relative to Healthy)
Conclusion
Summary

- SARS-CoV-2 positive pregnancies with abnormal ultrasonographic findings had highly inflammatory immune profiles
- Placentomegaly and fetal growth restriction most common ultrasound finding
  - Adverse neonatal outcomes: ↑ fetal growth restriction, ↓ placentomegaly
- 182 cytokines significantly altered in placentomegaly
  - ↑ IFNLR1, IL17F, TFNFRSF4, FGFR2
  - ↓ ADAMTS13, IL32
- 41 cytokines significantly altered in fetal growth restriction
  - ↑ MMP3, DPP4
  - ↓ IGFB7
- Placentomegaly and fetal growth restriction both demonstrated dysregulated cytokine expression but with a distinct pattern
  - 4 overlapping cytokines but inverse responses - ERBB4, TGFBR3, NCAM1, PLA2G7
Strengths and Limitations

- **Strengths**
  - Quaternary care center - heterogenic population
  - Prospective cohort
  - Comprehensive specimen and data collection

- **Limitations**
  - Exploratory study
    - Does not propose conclusive underlying biologic mechanism (further investigation required)
  - Observational study
    - Confounding factors and reverse causality
Conclusions

• SARS-CoV-2 infected pregnancies that develop abnormal ultrasound findings have immune profiles consistent with a pronounced inflammatory response

• Placentomegaly and fetal growth restriction both have dysregulated but unique immune profiles
  • Insight into pathogenesis of COVID-19 and changes in maternal immunity after infection
  • Potential biomarkers to predict perinatal complications after SARS-CoV-2 infection in pregnancy
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