HIV-1 Is Associated With Lower Group B Streptococcus Capsular and Surface-Protein IgG Antibody Levels and Reduced Transplacental Antibody Transfer in Pregnant Women

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Background. Human immunodeficiency virus (HIV)-exposed infants are at increased risk of invasive Group B Streptococcus (GBS) disease; however, the reason for this increased susceptibility has not been characterized.

Methods. We compared GBS capsular and surface-protein maternal immunoglobin G antibody concentrations and cord-maternal ratios between HIV-infected and HIV-uninfected mother-newborn dyads.

Results. Median capsular antibody concentrations (µg/mL) were lower in HIV-infected than HIV-uninfected women for serotypes Ib (P = .033) and V (P = .040); and for pilus island (PI)-1 (P = .016), PI-2a (P = .015), PI-2b (P = .015), and fibrinogen-binding protein A (P < .001). For serotypes Ia and III, cord-maternal ratios were 37.4% (P < .001) and 32.5% (P = .027) lower in HIV-infected compared to HIV-uninfected mother-newborn dyads. The adjusted odds of having capsular antibody concentration ≥ 2 µg/mL when comparing HIV-infected to -uninfected women were 0.33 (95% confidence interval [CI], 0.15–0.75) and 0.34 (95% CI, 0.12–1.00) for serotypes Ia and III, respectively. Antibody levels and cord-maternal ratios were independent of CD4+ lymphocyte counts or HIV-1 viral load.

Conclusions. The lower GBS antibody concentrations and reduced transplacental antibody transfer in HIV-infected women, which likely contribute to their infants being at heightened susceptibility for invasive GBS disease, could possibly be mitigated by vaccination with a GBS conjugate vaccine currently under clinical development.

Keywords. antibody; Group B Streptococcus; HIV; immunity; Streptococcus agalactiae; transplacental transfer.

Group B Streptococcus (GBS) is a leading cause of sepsis and meningitis in newborns and young infants [1, 2]. A meta-analysis of studies undertaken from 2000 to 2010 reported the highest incidence of invasive GBS disease to be in low-middle income countries from Eastern and Southern Africa [3–7]. Maternal and newborn GBS serotype-specific capsular antibody has been associated with protection against homotypic serotype invasive GBS disease in infants [8]. Furthermore, GBS surface proteins which facilitate adherence to host epithelium such as pilus island (PI) PI-1, PI-2a, PI-2b; fibrinogen-binding protein A (FbsA); and GBS immunogenic bacterial adhesin (BibA) have been shown to be immunogenic, and induce antibodies in animal-model studies that improved survival following systemic GBS inoculation challenges [9–11].

Although maternal human immunodeficiency virus (HIV) infection is not associated with higher prevalence of recto-vaginal GBS colonization during pregnancy or at birth [12–16], a greater risk of invasive GBS disease has been reported in HIV-exposed compared
to HIV-unexposed infants [17, 18]. The basis for the increased susceptibility to invasive GBS disease in HIV-exposed infants remains to be ascertained and could include maternal HIV infection being associated with lower concentrations of protective GBS antibodies or impaired transplacental antibody transfer [19].

The aim of this study was to determine the effect of maternal HIV infection on immunoglobin G (IgG) serotype-specific (Ia, Ib, III, and V) capsular antibody and select GBS surface-protein (PI-1, PI-2a, PI-2b, BibA, and FbsA) antibody concentrations in the mother and transplacental transfer to their newborns.

METHODS

We undertook a cross-sectional study of pregnant women delivering at Chris Hani Baragwanath Academic Hospital from January to July 2013. This tertiary-level care hospital serves the black-African community of Soweto and surrounding areas. Pregnant women in this region deliver either at this hospital (approximately 22 000 births annually) or at the midwife-obstetric units (approximately 9500 births annually) [20].

The HIV-1 sero-prevalence among pregnant women in this setting was 28.4% during the study period [20]. The provision of antiretroviral therapy (ART) to prevent mother-to-child transmission of HIV has been detailed elsewhere [21, 22]. Briefly, following routine confirmation of HIV infection in the pregnant women, a CD4⁺ lymphocyte count is measured, which at the time if >350 cells/µL zidovudine (AZT) was provided until delivery. Pregnant women with a CD4⁺ count ≤350 cells/µL or World Health Organization clinical stage 3 or 4 were initiated on triple ART. From April 2013, all HIV-infected pregnant women irrespective of CD4⁺ lymphocyte count were initiated on triple ART [22].

The study sample size was calculated based on the assumption that the antibody transfer rate is normally distributed with a standard deviation of approximately 0.5. We also assumed a transplacental antibody transfer ratio of 1.0 in HIV-uninfected mother-newborn dyads [23, 24]. A sample of 79 HIV-infected and 79 HIV-uninfected pregnant women was required to detect at least 20% difference in transplacental transfer ratio between HIV-exposed compared to HIV-unexposed newborns with 80% power and α < 0.05.

Study staff enrolled women in the labor and delivery wards during normal working hours from Monday to Friday. Inclusion criteria were: an infant birth weight ≥2500 grams, known maternal HIV status during pregnancy, and willingness to participate in the study. Gestational age was estimated using the following hierarchy of methods: antenatal ultrasound examination before 24 completed gestational weeks, the Ballard score done within 24 hours of birth, a reliable history of the last menstrual period, an antenatal sonar done at ≥24 weeks, or the fundal symphysis height (centimeters) examination during labor. Cord blood was taken at the time of birth and maternal blood within 12 hours of delivery from enrolled participants. Cord blood was withdrawn using a needleled syringe from the umbilical vessels. Blood samples were allowed to clot at room temperature and transported to the Respiratory and Meningeal Pathogens Research Unit within 4–6 hours for processing and storage. The blood was stored at 2°C–8°C if not processed immediately for a maximum period of 24 hours. Blood was centrifuged for 5 minutes at a 3220 relative centrifugal force and the serum then aliquoted and stored at −70°C. Serum samples were thawed and analyzed in batches. Newborns were not tested for HIV-1 infection immediately after delivery.

The Luminex fluorosence based microbead ismortosorbent assay was used to measure IgG antibodies to capsular serotypes Ia, Ib, III, and V, and to surface-proteins PI-1, PI-2a, PI-2b, BibA, and FbsA. Capsular and PI protein antigens were kindly provided by Novartis Vaccines and Diagnostics (Italy), while BibA and FbsA protein antigens were provided by Valneva Austria GmbH. Capsular polysaccharides were coupled to the microsphere beads (Bio-Rad, Hercules, California) with the crosslinking agent 4-(4,6 dimethoxy[1,3,5](triazin-2-yl)-4-methyl-morpholinum (DMTMM) and protein antigens were coupled to beads with a 2-step carbodiimide reaction [25, 26]. Polygam (purified pooled commercial gammaglobulin; National Bioproducts, South Africa) was used as reference serum and calibrated with standard capsular serotype-specific GBS reference serum kindly provided by Prof Carol J. Baker. For protein-specific antigen antibody determination, reference serum was assigned arbitrary units (AU) of 10 000 AU/mL. Bead fluorescence was read with the Bio-Plex 200 instrument using Bio-Plex Manager 5.0 software (Bio-Rad, Texas). Details are described in the Supplementary Appendix.

Serum capsular IgG was reported in micrograms per milliliter (µg/mL) with a lower limit of detection of 0.0008, 0.002, 0.004, and 0.016 µg/mL for serotypes Ia, Ib, III, and V, respectively; while protein-specific IgG was reported in AU per milliliter (AU/mL) with a lower limit of detection of 41, 110, 46, 6, and 19 per AU/mL for Pil-1, Pil-2a, Pil-2b, BibA, and FbsA, respectively. Samples below these limits were assigned a value of half the lower limit of detection for statistical analysis.

For analytical specificity of each GBS antigen-microsphere set, reference serum was incubated at 1:100 dilutions with each GBS antigen and incubated at 37°C for 2 hours. The specificity was recorded as the difference in reactivity between the absorbed and unabsorbed serum samples in a multiplex assay. Homologous inhibition was >90% for all capsular polysaccharide and protein antigens with the exception of serotype V (88%) and Fbs-A protein (32%). Heterologous inhibition across antigens was <15%; except for serotype Ib, which was inhibited by 31% with serotype Ia, and for serotype V, which was inhibited by 17% with serotype III.

In HIV-infected women, CD4⁺ lymphocyte counts measured during pregnancy were recorded and maternal blood obtained at the time of delivery was tested for HIV-1 RNA viral load.
using the real-time polymerase chain reaction COBAS Ampli-
Prep/COBAS TaqMan HIV-1 Test, version 2.0 (Roche COBAS;
Roche Molecular Systems, Branchburg, New Jersey), which has
a lower limit of detection of 20 copies per milliliter, with values
below this being assigned an arbitrary value of 20.

Maternal GBS colonization was assessed at delivery by per-
forming separate lower vaginal and rectal swabs. Rayon-
tipped swabs were used for sampling, which was placed into
Amies transport medium without charcoal (Medical Wire
Equipment Co Ltd Cat: MW170, UK) and transported to
the laboratory for processing. The laboratory methods of
GBS identification and serotyping on vaginal and rectal
swabs have been described [27].

Data Analysis
Maternal and cord blood IgG antibody concentrations were mea-
sured, and cord-blood-to-maternal ratio calculated to compare
the efficiency of transplacental antibody transfer between HIV-
exposed and HIV-unexposed newborns. Demographic charac-
teristics were compared between HIV-uninfected and HIV-infected
mother-newborn dyads using χ² or Fisher’s exact test for propor-
tions; while the Mann–Whitney test was used to compare the me-
dians. Antibody concentrations remained nonparametric after
log transformation; thus, median concentrations are reported.

Median maternal antibody concentrations were compared
between HIV-uninfected and HIV-infected women at delivery
and cord blood antibody concentrations between HIV-
exposed and HIV-exposed newborns using the Mann–
Whitney test. Using quantile regression, we further compared
median maternal antibody concentrations, cord blood anti-
body concentrations, and cord-maternal ratios between HIV-
uninfected and HIV-infected women, and adjusted for overall
colonization, colonizing serotype for homotypic capsular anti-
bodies, maternal age, and parity. We also compared the propor-
tions of HIV-infected and -uninfected women with capsular
antibody concentrations above various thresholds proposed to
be protective against invasive GBS disease in their infants [8].
In HIV-infected women, CD4+ T-lymphocyte counts and
HIV-1 viral load was correlated with maternal antibody concen-
trations and cord-maternal ratios using Spearman’s test. Fur-
thermore, we compared maternal antibody concentrations and
cord-maternal ratios at varying CD4+ lymphocyte counts and
HIV-1 viral load thresholds using the Mann–Whitney test.

Data were analyzed using STATA version 13.1 (College Station,
Texas) and GraphPad Prism version 6.05 for Windows (Graph-
Pad Software, La Jolla, California). Two-tailed P values < .05 were
considered statistically significant. Written informed consent
was obtained from the women at time of study enrollment. The study
was approved by the University of Witwatersrand Human Re-
search Ethics Committee (HREC number: M120905) and regis-
tered as an observational study on the South African National
Clinical Trial Register (DOH-27-0113-4310).

RESULTS
Of the 320 women screened, 70 refused consent and 76 failed to
meet the inclusion criteria. We therefore enrolled 174 mother-
newborn dyads, 10 of whom were subsequently excluded (includ-
ing 9 dyads where the newborn gestational age was ≤36 weeks,
and 1 dyad in whom maternal blood was taken >12 hours follow-
delivery). Thus, 164 mother-newborn dyads were analyzed,
including 81 HIV-uninfected and 83 HIV-infected women, all
of whom had singleton births. Except for HIV-infected women
being older (median 30.7 vs 26.0 years; P = .006), they were oth-
erwise similar in demographic characteristics compared to HIV-
uninfected women (Table 1). Among the 83 HIV-infected
women at the time of delivery, 36 (43.4%) were on triple ART,
46 (55.4%) on AZT only, and 1 (1.2%) had not received any
ART. The median duration on triple ART from initiation to de-

delivery was 13.4 weeks (range, 1.4–44) and 17.1 weeks (range,
2.4–42.7) for women on AZT only. Overall, 49 (29.9%) of 164
women were colonized with GBS; colonization rates were similar
in HIV-uninfected (27.2%) and HIV-infected (32.5%) women
(Table 1). The commonest colonizing serotype was Ia (59.1%
of all serotypes) in HIV-uninfected women and III (40.7% of se-
rotypes) in HIV-infected women (Table 1).

All women had detectable antibody levels to all 4 GBS ser-
types, although cord blood antibody levels were not detected in
2 samples for serotype Ia and in 5 samples each for serotypes Ib,
III, and V. Regarding surface-protein antibodies, only 1 woman
had undetectable antibody levels to PI-2a. For cord blood sam-
plies, antibody levels were undetectable on 2 samples for BibA,
5 samples for PI-1 and PI-2b, and 6 samples for PI-2a. The final analysis included all samples, as results were
similar when the above samples were excluded from the analysis
(data not shown).

Maternal HIV Infection Status and Capsular Antibodies
Median capsular antibody concentrations (µg/mL) were lower in
HIV-infected than HIV-uninfected women for serotypes Ib (0.06
vs 0.09; P = .033) and V (0.40 vs 0.59; P = .040); similar trends
were observed for serotype Ia (0.13 vs 0.36; P = .077), but this
difference was not significant (Figure 1A–D, Supplementary
Table 1). Median cord blood capsular antibody concentrations
(for all serotypes) were significantly lower in HIV-exposed
than in HIV-unexposed newborns; the respective antibody
concentrations (µg/mL) for serotypes Ia, Ib, III, and V were
0.07 versus 0.26 (P = .005), 0.07 versus 0.15 (P = .013), 0.15 versus
0.25 (P = .005), and 0.34 versus 0.57 (P = .004) (Figure 1A–D,
Supplementary Table 1).

After adjusting for confounding factors, we compared maternal
antibody concentrations between HIV-infected and -uninfected
women at multiple percentiles using quantile regression analysis.
Significant differences in antibody concentrations for serotypes Ia,
III, and V between HIV-infected and -uninfected women were
found at higher percentiles (above 65th), suggesting that HIV-infected women also tended to have lower antibody concentrations that HIV-uninfected at higher percentiles (Supplementary Table 2). Corroborating this, we demonstrated that a lower proportion of HIV-infected women had capsular antibody concentrations above thresholds of $\geq 1 \mu g / m L$ and $\geq 2 \mu g/mL$ for serotypes Ia, III, and V (Table 2). Using multivariate analysis, with an antibody concentration of $<0.5 \mu g/mL$ as a referent, the adjusted odds of having capsular antibody concentration $\geq 2 \mu g/mL$ in HIV-infected compared to uninfected women were 0.33 (95% confidence interval [CI], .15–.75; $P = .008$), 0.34 (95% CI, .12–1.00; $P = .049$), and 0.50 (95% CI, 0.16–1.54; $P = .228$) for serotypes Ia, III, and V, respectively (Table 2).

Overall, median cord-maternal ratios for capsular antibody ranged between 75% to 119% in HIV-uninfected mother-newborn dyads and 47% to 93% among HIV-infected mother-newborn dyads (Table 3). In the multivariate model, after adjusting for overall colonization, serotype-specific colonization, maternal age, and parity, the cord-maternal ratio was 37.4% ($P < .001$) and 32.5% ($P = .027$) lower for serotypes Ia and III in HIV-infected compared to HIV-uninfected mother-newborn dyads (Table 3). Two infants born to HIV-infected women developed late-onset GBS meningitis from serotypes Ia and III at 19 and 22 days of age, and among whom their mothers antibody concentrations were 0.08 and 0.12 for the homotypic serotypes and the transplacental ratio was 0.14 and 0.69, respectively.

### Maternal HIV Infection Status and Surface-Protein Antibodies

As compared to HIV-uninfected women, HIV-infected women had lower median antibody concentrations (AU/mL) against surface-protein PI-1 (549 vs 1020; $P = .016$), PI-2a (1130 vs 1972; $P = .015$), PI-2b (611 vs 1072; $P = .015$), and FbsA (1444 vs 2169; $P < .001$), but not significantly so for BibA (3829 vs 4790; $P = .236$) (Figure 2A–E, Supplementary Table 1). Cord blood median surface-protein antibody concentrations were lower in HIV-exposed compared to HIV-unexposed newborns for PI-1 (502 vs 1177; $P = .039$), PI-2b (478 vs 865; $P = .024$), and FbsA (1717 vs 2758; $P = .010$) (Figure 2A–E, Supplementary Table 1). The median cord-maternal ratios (range, 76%–126%) were similar for all antibodies directed against surface-proteins between HIV-uninfected and HIV-infected mother-newborn dyads (Table 3).

### Effect of HIV Viral Load and CD4+ Lymphocyte Count on GBS Antibody in HIV-infected Women

In HIV-infected women, 71 of 83 (85.5%) had a CD4+ lymphocyte count measured within 6 months before delivery with a median CD4+ lymphocyte count of 423 cells/µL (range, 46–1268). The median HIV-1 viral load in 79/83 (95.2%) participants was 96 copies/mL (range, 20–146 055) and undetectable
in 28 of the 79 (35.4%) samples. There was no correlation between CD4+ lymphocyte counts and maternal antibody concentrations or between CD4+ lymphocyte counts and cord-maternal ratios for any of the 9 measured antibodies. Furthermore, median maternal antibody concentrations and cord-maternal ratios were similar when stratified by different thresholds of CD4+ lymphocyte counts (Supplementary Tables 3 and 4). Similarly, there was no correlation between maternal HIV-1 viral load and maternal antibody concentration or cord-maternal ratios for any of the 9 measured antibodies (Supplementary Tables 3 and 4).

**DISCUSSION**

The findings from our study suggest that the possible mechanisms for the increased susceptibility to invasive GBS disease...
in HIV-exposed infants may relate to lower maternal capsular and surface-protein antibody concentrations, and inefficient transplacental transfer of capsular antibody to the fetus of HIV-infected women [28–30]. HIV-infected women had lower GBS capsular antibody concentrations than their HIV-uninfected counterparts, and notably a lower proportion of

Table 2. Proportion of HIV-infected and HIV-uninfected Women With Capsular Antibody Concentrations (µg/mL) Above Different Thresholds

<table>
<thead>
<tr>
<th>Antibody Concentration</th>
<th>HIV-infected n = 83</th>
<th>HIV-uninfected n = 81</th>
<th>aOR (95% CI)a</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.5</td>
<td>59 (71.1)</td>
<td>46 (66.8)</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>≥0.5</td>
<td>24 (28.9)</td>
<td>35 (43.2)</td>
<td>0.44 (.22–.89)</td>
<td>.021</td>
</tr>
<tr>
<td>≥1</td>
<td>17 (20.5)</td>
<td>30 (37.0)</td>
<td>0.37 (.16–.72)</td>
<td>.005</td>
</tr>
<tr>
<td>≥2</td>
<td>14 (16.9)</td>
<td>26 (32.1)</td>
<td>0.33 (.15–.75)</td>
<td>.008</td>
</tr>
<tr>
<td>Ib</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.5</td>
<td>72 (86.7)</td>
<td>72 (88.9)</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>≥0.5</td>
<td>11 (13.3)</td>
<td>9 (11.1)</td>
<td>1.34 (.51–3.52)</td>
<td>.550</td>
</tr>
<tr>
<td>≥1</td>
<td>7 (8.4)</td>
<td>4 (4.9)</td>
<td>2.11 (.57–7.78)</td>
<td>.261</td>
</tr>
<tr>
<td>≥2</td>
<td>3 (3.6)</td>
<td>2 (2.5)</td>
<td>1.95 (.30–12.59)</td>
<td>.482</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.5</td>
<td>64 (77.1)</td>
<td>55 (67.9)</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>≥0.5</td>
<td>19 (22.9)</td>
<td>26 (32.1)</td>
<td>0.48 (.23–1.02)</td>
<td>.058</td>
</tr>
<tr>
<td>≥1</td>
<td>10 (12.1)</td>
<td>17 (21.0)</td>
<td>0.37 (.14–.95)</td>
<td>.038</td>
</tr>
<tr>
<td>≥2</td>
<td>7 (8.4)</td>
<td>14 (17.3)</td>
<td>0.34 (.12–1.00)</td>
<td>.049</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.5</td>
<td>49 (59.0)</td>
<td>37 (45.7)</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>≥0.5</td>
<td>34 (41.0)</td>
<td>44 (54.3)</td>
<td>0.58 (.30–1.11)</td>
<td>.099</td>
</tr>
<tr>
<td>≥1</td>
<td>14 (16.9)</td>
<td>23 (28.4)</td>
<td>0.46 (.21–1.03)</td>
<td>.059</td>
</tr>
<tr>
<td>≥2</td>
<td>6 (7.2)</td>
<td>10 (12.3)</td>
<td>0.50 (.16–1.54)</td>
<td>.228</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus.

a Adjusted odds ratio (OR) (95% CI)-calculated OR with 95% confidence of disease using logistic regression (adjusted for parity, maternal age and serotype-specific colonization).

Table 3. Transplacental Antibody Transfer (Cord to Maternal Blood Ratio) Between HIV-uninfected and HIV-infected Mother-newborn Dyads

<table>
<thead>
<tr>
<th>Capsular serotypes</th>
<th>HIV-uninfected Mother-newborn Dyads Median CMR (IQR) b n = 81</th>
<th>HIV-infected Mother-newborn Dyads Median CMR (IQR) n = 83</th>
<th>Reduction, % c</th>
<th>P Value d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>0.749 (0.562–1.021)</td>
<td>0.649 (0.322–0.754)</td>
<td>37.4</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Ib</td>
<td>1.187 (0.730–1.959)</td>
<td>0.930 (0.593–1.574)</td>
<td>21.7</td>
<td>.493</td>
</tr>
<tr>
<td>III</td>
<td>0.902 (0.605–1.229)</td>
<td>0.609 (0.407–0.976)</td>
<td>32.5</td>
<td>.027</td>
</tr>
<tr>
<td>V</td>
<td>0.954 (0.677–1.310)</td>
<td>0.825 (0.543–1.158)</td>
<td>13.5</td>
<td>.084</td>
</tr>
<tr>
<td>Surface-proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI-1</td>
<td>1.056 (0.835–1.453)</td>
<td>0.948 (0.669–1.431)</td>
<td>10.2</td>
<td>.379</td>
</tr>
<tr>
<td>PI-2a</td>
<td>0.904 (0.545–1.317)</td>
<td>1.262 (0.613–3.000)</td>
<td>NR</td>
<td>.213</td>
</tr>
<tr>
<td>PI-2b</td>
<td>1.006 (0.598–1.588)</td>
<td>0.904 (0.562–1.521)</td>
<td>10.1</td>
<td>.500</td>
</tr>
<tr>
<td>BlbA</td>
<td>0.860 (0.687–1.139)</td>
<td>0.759 (0.539–1.126)</td>
<td>11.7</td>
<td>.207</td>
</tr>
<tr>
<td>FbsA</td>
<td>0.964 (0.601–1.695)</td>
<td>1.159 (0.454–2.347)</td>
<td>NR</td>
<td>.385</td>
</tr>
</tbody>
</table>

Abbreviation: HIV, human immunodeficiency virus.

a Cord to maternal ratio (CMR).
b Interquartile range (IQR).
c Reduction in cord to maternal ratio comparing HIV-infected and HIV-uninfected mother-newborn dyads; calculated as the cord to maternal ratio for HIV-infected/ HIV-uninfected women, subtracted from 1.
d Using quantile regression (adjusted for overall colonization, colonizing serotype for capsular antibodies, maternal age and parity).

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Figure 2. Tukey box-and-whisker plots comparing surface-protein antibody concentrations of Pil-1 (A), Pil-2a (B), Pil-2b (C), BibA (D), and FbsA (E) between HIV-uninfected and -infected mothers, and HIV-unexposed and -exposed newborns. The y-axis has been log10 scaled. For the box-and-whisker plots, the box represents the distance of the 25th and 75th percentiles with the median represented by the solid line within the box. The upper whisker represents 1.5 times the interquartile distance from the 75th centile, while the lower whisker represents 1.5 times the interquartile distance from the 25th centile. The dot symbols represent outliers above the upper whisker. Abbreviations: AU, arbitrary units; HIV, human immunodeficiency virus; Mother HIV+, HIV infected; Mother HIV−, HIV uninfected; Newborn HIV+, HIV exposed; Newborn HIV−, HIV unexposed.
HIV-infected women had capsular antibodies above the putative "protective" thresholds that has been reported to protect against invasive GBS disease in their infants [8]. The lower GBS antibody concentrations in HIV-infected women could represent waning of natural acquired antibody or reduced humoral immune responsiveness to recto-vaginal colonization, which likely induces the antibody responses (personal correspondence Gaurav Kwatra-manuscript under preparation). Additionally, reduced maternal exposure to GBS may also result in lesser antibody production to various serotype-specific epitopes [8]. This is supported by some studies that reported a lower prevalence of GBS colonization in HIV-infected women, including previously in our setting [15, 16], although this was not observed in the current study cohort.

The transplacental transfer of antibodies to serotypes Ia and III, which account for the majority (72%) of invasive GBS disease globally [3], was 37.4% and 32.5% lower in HIV-exposed compared to HIV-unexposed newborns, respectively. Additionally, maternal capsular antibody concentrations were lower in HIV-infected women compared to HIV-uninfected women for serotypes Ib and V, with a trend toward being lower for serotype Ia, but not for serotype III. Serotype III, which has the highest invasive potential, is the least immunogenic of all serotypes [31, 32] and this may explain why concentrations were similar in HIV-infected and -uninfected women. Furthermore, the trend toward higher colonization prevalence of serotype III in HIV-infected compared to HIV-uninfected women in our study may have contributed to similar serotype III antibody concentrations between the women.

We also measured antibody concentrations to select GBS surface-proteins, which induce antibody responses and could be possible vaccine targets. There is, however, a paucity of data on these GBS surface-protein antibody concentrations and no international reference standards exist. Thus, we can only report on the comparisons using in-house reference serum employed consistently across all samples. HIV-infected women had lower median concentrations for all GBS surface-proteins, although antibody differences to BibA were not significant. In addition, we observed that contrary to the capsular antibody transfer, the transfer of surface-protein antibodies from mother to fetus was more efficient, and similar between HIV-infected and HIV-uninfected mother-newborn dyads. This may occur because surface-protein antibodies, which are mainly subclass IgG1, are more efficiently transferred than capsular antibodies, which are predominantly of subclass IgG2 [33].

Our results are consistent with reports showing reduced transplacental transfer of maternal antibodies directed against epitopes of varicella (31% reduction), measles (35% reduction), pneumococcus (24%–30% reduction), *Haemophilus influenzae* type b (23% reduction), pertussis (40% reduction), and tetanus (27%–52% reduction) in HIV-infected compared to HIV-uninfected mother newborn dyads [28, 29, 34–36]. However, no difference in transplacental antibody transfer between HIV-infected and -uninfected women for pathogens such as herpes, some pneumococcal serotypes, and influenza has also been reported [29, 34, 37]. Transplacental IgG antibody transfer is thought to occur via an active transport mechanism utilizing neonatal Fc receptors found on the placenta [30, 33, 38]. The decrease in transplacental antibody transfer in HIV-infected women is thought to be as a consequence of maternal hypergammaglobulinemia, which saturates the neonatal Fc receptors [39]. Other reasons for the variation in transplacental antibody transfer may relate to differences in IgG subclass and mechanism of transfer of antibody (ie, active or passive transport) [33].

Although our study did not identify a significant association between CD4+ lymphocyte counts and HIV-1 viral loads on maternal antibody and cord-maternal ratios among HIV-infected women, the study was not powered (with a sample size of 79) to detect a significant relationship when the true correlation is between −0.35 and 0.35. Similarly, no association has been observed between maternal CD4+ lymphocyte counts and transplacental transfer of pneumococcal, *H. influenzae* type b, pertussis, and tetanus antibodies in HIV-infected women [28, 29], whereas a positive correlation with CD4+ lymphocyte counts and maternal antibody concentrations was reported to antibodies to pertussis, pneumococcus, and tetanus [28]. More recently, a large European cohort study reported an increased risk of bacterial infections in HIV-exposed infants, particularly in women with low CD4+ lymphocyte counts [40]. Most pregnant women in our setting had undetectable HIV-1 viral load and had immune reconstituted at the time of antibody sampling. A study conducted in Nairobi in HIV-infected women reported a 44% decrease of measles antibody transfer with every log_{10} increase in viral load, indicating that infants born to women with advanced maternal HIV infection may be at increased risk of disease due to reduced acquisition of maternal antibody concentrations [41].

Limitations of our study include that we did not match for age and colonization status in HIV-infected and -uninfected women; however, we adjusted for these factors in the multivariate analysis and findings remained consistent. Furthermore, we did not quantify the effect that cross-reactivity of serotype Ib with Ia (as previously documented by Brigsten et al [42]) may have had on the absolute antibody concentration for serotype Ib. The assay was, however, applied consistently to both HIV-infected and -uninfected dyads and hence is unlikely to alter the differences observed between HIV-infected and HIV-uninfected women in our study. Also, our study only measured IgG antibodies, while IgA antibodies may also be transplacentally transferred, and have been associated with protection against GBS invasive disease in animal model studies [11, 43]. Additionally, CD4+ lymphocyte counts were measured as part of standard-of-care at any time within 6 months (mean, 2.8 months) of delivery and the study was not specifically powered...
to address whether immunological status or HIV-1 viral load were associated with differences in maternal antibody or transplacental antibody transfer.

The lower GBS antibody concentrations and reduced transplacental antibody transfer in HIV-infected women, which places their infants at risk for invasive GBS disease, may be mitigated by maternal GBS vaccination. Furthermore, an investigational trivalent GBS polysaccharide-protein conjugate vaccine was found to be less immunogenic in HIV-infected than HIV-uninfected pregnant women [44]. Therefore, in HIV-burdened settings, maternal vaccination may require modified formulations or dosing schedules in HIV-infected women.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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**References**