Prevalence, Risk Factors, and Serotype Distribution of Group B Streptococcus Colonization in HIV-Infected Pregnant Women Living in Belgium: A Prospective Cohort Study

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Background. Group B streptococcus (GBS) infection is a leading cause of severe neonatal infection. Maternal GBS carriage during pregnancy is the main risk factor for both early-onset and late-onset GBS disease. High incidence of GBS infection has been reported in HIV-exposed but -uninfected infants (HEU). We aimed to determine the prevalence, characteristics, and risk factors for GBS colonization in HIV-infected and HIV-uninfected pregnant women living in Belgium.

Methods. Between January 1, 2011, and December 31, 2013, HIV-infected (n = 125) and -uninfected (n = 120) pregnant women had recto-vaginal swabs at 35–37 weeks of gestation and at delivery for GBS detection. Demographic, obstetrical, and HIV infection–related data were prospectively collected. GBS capsular serotyping was performed on a limited number of samples (33 from HIV-infected and 16 from HIV-uninfected pregnant women).

Results. There was no significant difference in the GBS colonization rate between HIV-infected and -uninfected pregnant women (29.6% vs 24.2%, respectively). HIV-infected women were more frequently colonized by serotype III (36.4% vs 12.5%), and the majority of serotype III strains belonged to the hypervirulent clone ST-17. Exclusively trivalent vaccine serotypes (Ia, Ib, and III) were found in 57.6% and 75% of HIV-infected and -uninfected women, respectively, whereas the hexavalent vaccine serotypes (Ia, Ib, II, III, IV, and V) were found in 97% and 100%, respectively.

Conclusions. HIV-infected and -uninfected pregnant women living in Belgium have a similar GBS colonization rate. A trend to a higher colonization rate with serotype III was found in HIV-infected women, and those serotype III strains belong predominantly to the hypervirulent clone ST17.

Keywords. HIV infection; multilocus sequence typing; pregnancy; serotype; Streptococcus agalactiae.

Mother-to-child transmission of HIV infection is decreasing worldwide thanks to the control of maternal infection by administration of combined active antiretroviral therapy (cART), associated in some cases with elective cesarean delivery and complete avoidance of breastfeeding (in industrialized countries). As a consequence, the number of HIV-exposed but -uninfected (HEU) children is rising, particularly in Sub-Saharan Africa, where HIV prevalence in pregnant women remains the highest [1]. Although not infected with HIV, it is now well established that HEU infants are affected by exposure to HIV and antiretroviral drugs during fetal life [2]. Epidemiological studies from both high-income and resource-limited countries have reported an increased risk of morbidity and mortality mainly of infectious origin for HEU infants as compared with HIV-unexposed (HU) infants [3–5]. This increased susceptibility to infectious diseases is likely related to multiple factors, including exposure to maternal co-infections, poor socioeconomic conditions, prematurity, low birth weight, lack of or suboptimal breastfeeding, and immune alterations, including decreased transfer of maternal antibodies and immune activation [2, 5, 6].

Group B streptococcus (GBS) or Streptococcus agalactiae is a commensal gram-positive coccus whose reservoir is the gastrointestinal tract, colonizing 10%–40% of healthy adults. GBS can be classified on the basis of their capsular polysaccharides (CPS), allowing the identification of 10 distinct serotypes (Ia, Ib, and II to IX). GBS is a leading
cause of neonatal severe sepsis and meningitis worldwide and is associated with a high mortality rate [7, 8]. The clinical spectrum of neonatal GBS sepsis is usually divided into early-onset disease (EOD) occurring during the first 6 days of life and late-onset disease (LOD) occurring between 7 and 90 days of life. Maternal GBS colonization is a major risk factor for both EOD and LOD [7]. Transmission of the bacteria can occur vertically just before birth, during delivery, or horizontally during the neonatal period [7]. Systematic screening in late pregnancy and antibiotic prophylaxis during labor in colonized pregnant women have dramatically reduced the incidence of EOD but have had no effect on LOD incidence [9].

In 2010, we reported a higher incidence of invasive group B streptococcal (GBS) infections in HEU infants compared with the general neonatal population [10], especially LOD. Similar findings were later reported in cohorts in France and South Africa [11, 12]. Various studies performed in both high-income and resource-limited countries have found similar rates of GBS colonization in pregnant HIV-infected and -uninfected women (reviewed in [13]). The increased susceptibility of HEU infants to severe GBS infection probably results from multiple factors, such as prematurity, lack of breastfeeding, lower maternal antibody transfer, and higher level of innate immune activation associated with enhanced immunopathology [5, 13–15]. Recent studies performed in Sub-Saharan Africa indicate that HIV-infected pregnant women might be preferentially colonized by serotype III strains, including the hypervirulent clone ST-17 [14, 16]. However, no study has been performed to date in women living in Europe. Maternal immunization represents a promising strategy to prevent GBS diseases in early life [17], and HIV-pregnant women represent a target group of interest. Data are thus needed regarding the serotype distribution in this at-risk population.

The present study was performed to prospectively characterize GBS colonization in a cohort of HIV-infected and -uninfected pregnant women recruited and followed in a hospital in Belgium.

METHODS

Study Population

The Elikya study is a birth cohort study that was conducted at the Saint-Pierre University Hospital, Brussels, with enrollment of newborns between January 2011 and December 2013, aiming to compare clinical, microbiological, and immunological features of HEU and HU infants. HIV-infected and -uninfected pregnant women were recruited during pregnancy as described previously [5]. Demographics and obstetrical data along with data related to HIV infection (viral load and current CD4 cell count during pregnancy, nadir CD4 cell count) were collected prospectively. The study was approved by the local ethics committee (CHU Saint-Pierre), and written informed consent was obtained at enrollment for all subjects.

Sample Collection and GBS Culture

Recto-vaginal swabs were collected routinely between 35 and 37 weeks during antenatal consultation, as recommended by Belgian guidelines [18], and at delivery in the context of the present study. Samples were placed into Amies transport medium (Copan Italia, Brescia, Italy) and were inoculated and incubated overnight in Todd-Hewitt Broth (Oxoid Ltd, Basingstoke, UK). Beginning in 2015, Lim broth was used. The broth was further subcultured in GBS Differential Agar/Granada Medium (BD GmbH, Heidelberg, Germany). The identification of all types of presumptive GBS colonies was confirmed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Isolated strains were preserved at –80°C in sterile skimmed milk without glycerol for further serotyping at the GBS National Reference Center (CHU Liège, Belgium). Women with a positive GBS culture at 35–37 weeks and/or delivery were considered colonized during pregnancy. Thirty-one samples were obtained in the original Elikya cohort, and 18 samples were obtained from a second cohort recruited between November 2015 and December 2017 (16 and 2 from HIV-infected and -uninfected women, respectively). Among the samples collected during the Elikya study, 3 strains were collected from newborns at birth (2 HEU and 1 HU) instead of their respective mothers.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing (AST) was performed routinely for the following antibiotics: penicillin, vancomycin, clindamycin, erythromycin, and fluoroquinolones (ciprofloxacin or levofloxacin). AST was performed using the disk diffusion test (Kirby-Bauer) using Clinical and Laboratory Standards Institute breakpoints.

Capsular Serotyping

Serotyping of CPS was performed using the modified Strep-B-Latex method (Strep-B-Latex kit, Statens Serum Institut, Copenhagen, Denmark) as previously described [19]. Identification of the GBS CPS serotype is based on the agglutination of latex beads that display CPS serotype-specific IgGs. In case of double agglutination with different serotypes of antiserum, the strains were subcultured and further characterized by serotyping. For some strains, the amount of capsule produced was not sufficient for characterization with an agglutination method; therefore, some isolates were “not typable” (NT).

Capsular Gene Typing by Polymerase Chain Reaction

In addition to serotyping, a genotyping method was performed to detect genes coding for the CPS to confirm all determined serotypes and to characterize NT isolates. Genomic DNA was extracted using the Maxwell 16 SEV cell kit (Promega, Leiden, the Netherlands). Approximately 1 ng of DNA was used in the polymerase chain reactions (PCRs) with primers and conditions as described by Poyart et al. [20]. For the determination of the serotype IX, an additional PCR was done as previously described [21]. The amplicons were analyzed by running 2% agarose gel electrophoresis, and the presence of DNA fragments with the same size as those of controls with known serotypes was used to establish the capsular gene type.
Multilocus Sequence Typing for Serotype III Strains

Multilocus sequence typing (MLST) was performed to further characterize serotype III isolates. Genomic DNA was extracted using the Maxwell 16 SEV cell kit (Promega). Approximately 1 ng of DNA was used in the PCRs. Briefly, fragments were amplified with Taq polymerase (Applied Biosystems, Foster City, CA). Thermal PCR cycles were as follows: 94°C for 1 minute for the initial denaturation step, followed by 35 cycles of denaturation at 94°C for 1 minute, hybridization at 57°C for 1 minute, and elongation at 72°C for 90 seconds. A final extension at 72°C for 7 minutes was then applied. Primers used in this study are as described on the MLST GBS database (https://pubmlst.org/sagalactiae/). The amplified fragments were then purified and sequenced with the BigDye terminator method (Applied Biosystems, Foster City, CA). The nucleotide sequences were determined with an ABI prism sequencer (Applied Biosystems, Foster City, CA) and analyzed by the Sequence scanner, V1.0, software. Alleles for 7 loci were assigned on the MLST website (https://pubmlst.org/sagalactiae/), and each isolate was defined by the corresponding sequence type (ST).

Statistical Analysis

Proportions were compared using the chi-square and Fisher exact tests. Continuous data were compared using the Wilcoxon rank-sum test. Statistical analyses were performed with R using the Gmisc package [22].

RESULTS

Population Characteristics and Prevalence of GBS Carriage According to HIV Status

Characteristics of HIV-infected and -uninfected pregnant women recruited in the study are summarized in Table 1. HIV-infected women were slightly but significantly older and were more frequently multiparous and more frequently of Sub-Saharan African origin than HIV-uninfected women. Gestational age at delivery and birth weight were lower in HEU as compared with HU newborns, reflecting the higher proportion of preterm births. There was no statistically significant difference in the GBS overall carriage during pregnancy between the 2 groups (29.6% vs 24.2%, \( P = .39 \)), even when the subanalysis was restricted to Sub-Saharan African women (30.7% vs 29.3%, \( P = 1 \)). Persistent colonization was defined by a positive GBS swab at delivery in women with a positive swab at 35–37 weeks and was observed in 6/23 (26.1%) HIV-infected women and 6/20 HIV-uninfected women (30%). The median durations between the 2 samples were 23 days and 25.5 days, respectively.

<table>
<thead>
<tr>
<th>Table 1. Population Characteristics and Prevalence of GBS Infection According to HIV Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at delivery, mean (SD), y</td>
</tr>
<tr>
<td>Sub-Saharan Africa origin, No. (%)</td>
</tr>
<tr>
<td>BMI before or early pregnancy, median (IQR), kg/m²</td>
</tr>
<tr>
<td>Diabetes before pregnancy, No. (%)</td>
</tr>
<tr>
<td>First pregnancy, No. (%)</td>
</tr>
<tr>
<td>Gestational age, median (IQR), wk</td>
</tr>
<tr>
<td>Cesarean section, No. (%)</td>
</tr>
<tr>
<td>Birth weight, mean (SD), g</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; GBS, group B streptococcus; IQR, interquartile range.
fluoroquinolones (levofloxacin or ciprofloxacin) was found between HIV-infected and -uninfected women.

**GBS Serotype Distribution According to HIV Status**

In total, GBS serotypes were determined from cultures of positive swabs from 33 HIV-infected women and 16 HIV-uninfected women. Three-quarters of HIV-infected and HIV-uninfected women in whom GBS was serotyped were from Sub-Saharan Africa (75.8% vs 75.0%, \( P = 1 \)). One subject in the HIV-infected group was colonized by 2 strains (III and V). As shown in Table 4, serotype distribution was different between the 2 groups. Serotype Ia was found in 9/16 HIV-uninfected women (56.2%), whereas serotypes III and V were the 2 main serotypes identified in HIV-infected women (12/33 or 36.4% and 10/33 or 30.3%, respectively).

The proportion of exclusively trivalent vaccine serotypes was limited but similar in both groups, whereas hexavalent vaccine (GBS6) serotypes were found in all but 1 subject in the HIV-infected group.

**MLST of Serotype III**

The distribution of MLST of serotype III strains is depicted in Table 5. Most of the serotype III isolates were identified as sequence type 17 (ST 17), belonging to the hypervirulent clonal complex (CC) 17.

**DISCUSSION**

This is the first study prospectively assessing GBS colonization in HIV-infected pregnant women living in a high-income country. One strength of this study is that the majority of subjects were screened at 2 time points during pregnancy (between 35–37 weeks and at delivery). Indeed, intermittent carriage of GBS during pregnancy has been previously reported [23].

In accordance with several previous studies performed worldwide (reviewed in [13]), we show that the prevalence of GBS colonization was not different between HIV-infected and
Table 4. Capsular Serotype Distribution According to HIV Status

<table>
<thead>
<tr>
<th>HIV-Infected (n = 12), No. (%)</th>
<th>HIV-Uninfected (n = 33), No. (%)</th>
<th>PValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-Saharan Africa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newborn</td>
<td>2 (6.1)</td>
<td>1 (6.2)</td>
</tr>
<tr>
<td>Mother, delivery</td>
<td>6 (18.2)</td>
<td>4 (25.0)</td>
</tr>
<tr>
<td>Serotype Ia</td>
<td>5 (15.2)</td>
<td>9 (56.2)</td>
</tr>
<tr>
<td>II</td>
<td>2 (6.1)</td>
<td>1 (6.2)</td>
</tr>
<tr>
<td>III</td>
<td>3 (9.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>III-V</td>
<td>11 (33.3)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>IV</td>
<td>1 (3.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>V</td>
<td>9 (27.3)</td>
<td>3 (18.8)</td>
</tr>
<tr>
<td>VII</td>
<td>1 (3.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Serotypes III &amp; V</td>
<td>21 (63.6)</td>
<td>5 (31.2)</td>
</tr>
<tr>
<td>Exclusively trivalent vaccine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serotypes (II-Ia-Ib)</td>
<td>19 (57.6)</td>
<td>12 (75.0)</td>
</tr>
<tr>
<td>Hexavalent vaccine serotypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Ia-Ib-II-III-IV-V)</td>
<td>32 (97)</td>
<td>16 (100)</td>
</tr>
</tbody>
</table>

-Table 5. ST Clone Distribution Among Serotype III Strains According to HIV Status

<table>
<thead>
<tr>
<th>HIV-Infected (n = 12), No. (%)</th>
<th>HIV-Uninfected (n = 18), No. (%)</th>
<th>PValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>7 (58.3)</td>
<td>1 (50.0)</td>
</tr>
<tr>
<td>19</td>
<td>2 (16.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>335</td>
<td>1 (8.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>861</td>
<td>1 (8.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>1167</td>
<td>1 (8.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>1182</td>
<td>0 (0.0)</td>
<td>1 (50.0)</td>
</tr>
</tbody>
</table>

Abbreviation: ST, sequence type.

Mechanisms of this differential pattern of serotype distribution might be the consequence of different immune responses between HIV-infected and -uninfected women. Immunity to GBS is known to be serotype specific [27]. Notably, serotype III is known to be less immunogenic as compared with other serotypes [28]. Humoral immunity, that is, anticapsular antibody production, plays an important role in anti-GBS immunity [17], and HIV infection is associated with B-cell dysfunction that persists after ART initiation [29]. A study performed in South Africa in HIV-uninfected women highlighted the role of serotype-specific cellular immunity in the clearance of GBS colonization during pregnancy [30]. In the study by Dangor et al., quantitative differences between serotype-specific antibody concentrations were found for some serotypes but not serotype III [14]. Thus, despite a normal CD4 count, the functionality of serotype III GBS-specific CD4 response and antibody GBS-specific response might be qualitatively different in HIV-infected as compared with HIV-uninfected women, and that could impact serotype III clearance from the genital tract.

Frequency of sexual intercourse has also been associated with a higher rate of acquisition of specific GBS serotypes and might play a role in the differential pattern of serotype distribution between HIV-infected and -uninfected women [31]. We could not test this hypothesis as this parameter was not recorded in our study. A recent meta-analysis has shown that important variations of serotype distribution exist worldwide [32]. It is unlikely that ethnicity would play a role in the distinct serotype distribution in our observation as the proportion of women of Sub-Saharan African origin was the same in the HIV-infected and -uninfected pregnant women included. However, regional variations in the distribution of serotypes have been reported between the middle, western, southern, and eastern parts of Sub-Saharan Africa [32]. We could not assess such variability due to the limited sample size.

We characterized serotype III strains using MLST and found that the majority of the strains belong to the hypervirulent clone ST17. Interestingly, a recent study performed on almost 8000 pregnant women in Kenya found an association between HIV infection and colonization by clonal complex ST17 strains [16]. In the latter study, co-trimoxazole use was associated with colonization with CC17 strains, suggesting that antibiotic use, more frequently found in HIV-infected pregnant women [33], might contribute to the selection of more virulent strains. The
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Potential conflicts of interest. All authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


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Author contributions. N.D. wrote the first draft of the manuscript and performed the statistical analyses. T.G., A.M., J.L., C.A., and M.C. designed the study. P.B. provided clinical care to the patients. Y.M. and L.B. collected and processed the GBS strains. R.S. and P.M. performed GBS strain analyses. All authors edited, reviewed, and approved the final manuscript.

This relatively higher frequency of serotype III, including the hypervirulent clone ST17, found in HIV-infected women has potential implications for the health of their HEU infants. Serotype III is associated with more persistent colonization [27], which is associated with higher risk of newborn infection [23], in particular with LOD [35]. Higher carriage of this strain could explain the higher incidence of GBS LOD reported in our institution and in South Africa [10, 12].

The current preventive strategy for GBS neonatal severe diseases (bacteremia, pneumonia, and meningitis) recommended in different European countries [9, 18] is based on the screening of GBS carriage in late pregnancy combined with antibiotic prophylaxis during labor in carriers. This strategy significantly decreases the incidence of EOD but not LOD [7]. GBS immunization during pregnancy and the subsequent production of maternal antcapsular antibodies that are transplacentally transferred would provide protection against both EOD and LOD. A phase II nonrandomized trial using the trivalent vaccine (serotypes Ia, Ib, and III conjugated to CRM197) performed in HIV-infected and HIV-uninfected pregnant women found lower geometric mean concentrations of GBS serotype-specific antibody in HIV-infected mothers and their newborns [36]. Importantly, this difference in antibody level was found in women with low (<350/mm³) and high (>350/mm³) CD4 cell counts. Our results regarding serotype distribution indicate that such a vaccine would cover 57.6% and 73.3% of HIV-infected and -uninfected women, respectively. Coverage with the hexavalent GBS vaccine (GBS6) targeting serotypes Ia, Ib, II, III, IV, and V would be much higher: 97% and 100% in HIV-infected and -uninfected women, respectively.

In conclusion, in this cohort of pregnant women of mostly Sub-Saharan African origin who were living in Europe, HIV infection was not associated with increased GBS carriage during pregnancy but with a trend to a higher proportion of colonization with serotype III GBS strains, including the hypervirulent clone ST17. This could contribute to the higher risk of GBS sepsis observed in HEU infants. Further studies are needed to decipher interactions between GBS serotype-specific immunity and HIV infection and how it would be impacted by maternal immunization, with the aim to reduce carriage in pregnancy and neonatal GBS sepsis.


