The Genital Microbiome in Studies of Bacterial Vaginosis

Jeanne Marrazzo, MD, MPH
University of Washington

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DISCUSSION

• Bacterial vaginosis (BV)
  – Clinical importance
    • Implications for STI/HIV transmission; reproductive health
  – Traditional guidance for treatment trials
    • Endpoint definition; FDA guidance

• Implications of microbiome approach for BV
  - Endpoint definition
  - Pathogenesis & natural history
    - Sexual behavior (BVAB transmission)
Benefits of an Optimal Vaginal Environment

- Protection from clinical BV, PID, other pathogens
  - HIV (60% increase), chlamydia, GC, trichomoniasis
- Optimal birth outcomes (short-term)
  - Normal birth weight
  - Normal timing of delivery
  - Fewer pregnancy-associated infections
- Optimal health outcomes (long-term)
  - Transfer of maternal microbiota to infant
    - Lower rates of autoimmune diseases (asthma), metabolic disorders (Dominguez-Bello 2010; Torrazza 2011; Neu 2011)
    - Mediated by rapid colonization of skin, gut, genital tract with maternal microbiota

Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns

Maria G. Dominguez-Bello, Elizabeth K. Costello, Monica Contreras, Magda Magris, Glida Hidalgo, Noah Fierer, and Rob Knight
Gram Stain (Nugent score) & Clinical Criteria (Amsel’s) Define BV & Cure

**Nugent = 0**
- pH < 4.7
- "Normal" discharge
- No odor with KOH
- No clue cells

**Nugent 7 - 10**
- pH ≥ 4.7
- Homogenous discharge
- Amine odor with KOH
- Clue cells

**Amsel’s Criteria**
Vaginal Fluid Gram Stain (Nugent Criteria)

- Gram positive rods
  - “Lactobacillus types”
  - 0-3
- Gram negative or Gram variable rods or coccobacilli
  - “Gardnerella or Bacteroides types”
  - Curved Gram negative rods
  - “Mobiluncus types” 7-10
- 4-6

Advantages
- Simple sample collection
- Efficient storage and transport
- Standardized scale for interpretation

Widely Used Diagnostic Tool – Gold Standard for BV Diagnosis

BV TREATMENT TRIALS:
Proposed Timeline & Terminology

NIH Workshop on BV, 2008

Assess for early treatment failure
- BV- early cure
  - Assess for late treatment failure
    - BV+ relapse
    - BV- late cure
    - BV+ persistence

Day 0 → Day 7-10 → Day 30-40

BV treatment
MTZ or clindamycin

Marrazzo STD 2010
FDA Guidance for BV Trials

- BV definition at enrollment:
  - All 4 Amsel criteria plus confirmation by Nugent score (≥7)
  - NOTE: symptoms not required
- 1998: clinical cure = absence of all Amsel criteria; therapeutic cure = clinical cure plus Nugent score <4 at 21-30 days after starting treatment
- On review in 2012, definition of clinical cure was modified to exclude Amsel’s pH criteria
FDA Guidance for BV Trials

• BV definition at enrollment:
  – All 4 Amsel criteria plus confirmation by Nugent score (≥7)
  – NOTE: symptoms not required

• Clinical cure:
  – Absence of KOH odor, clue cells, abnormal discharge
  – Symptom resolution (additional; not well standardized)
Are These Relevant in the Era of Molecular Approaches?

- **BV definition at enrollment:**
  - All 4 Amsel criteria plus confirmation by Nugent score (≥7)
  - NOTE: symptoms not required

- **Clinical cure:**
  - Absence of KOH odor, clue cells, abnormal discharge
  - Symptom resolution (additional; not well standardized)
**Key Questions**

- **Nugent Criteria:** Do bacterial species other than those previously described (*Lactobacillus, Gardnerella, Bacteroides, Mobiluncus*) contribute to bacterial morphotypes observed by Gram stain?

- **Amsel Criteria:** Do all BV-associated bacteria contribute equally to these signs?
  - Quantity? Identity?
“Although current data suggest that there are important differences between women diagnosed with BV with or without the presence of Mobiluncus morphotypes, it is currently unknown whether these variations can be directly attributed to Mobiluncus species or to some other unknown organisms for which these Gram stain findings are a marker.”
More Than Meets the Eye: Associations of Vaginal Bacteria with Gram Stain Morphotypes Using Molecular Phylogenetic Analysis

Sujatha Srinivasan¹, Martin T. Morgan², Congzhou Liu¹, Frederick A. Matsen², Noah G. Hoffman³, Tina L. Fiedler¹, Kathy J. Agnew⁴, Jeanne M. Marrazzo⁵, David N. Fredricks¹,⁵,⁶*
Women with BV
Broad-Range PCR and Pyrosequencing

Fluorescence Micrographs & Gram Stains of Vaginal Fluid Smears for 2 Women with Nugent Score = 10

<table>
<thead>
<tr>
<th></th>
<th>qPCR</th>
<th>FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mobiluncus DNA</strong></td>
<td>$2.5 \times 10^5$</td>
<td><strong>Mobiluncus cells</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$&lt; 1$</td>
</tr>
<tr>
<td><strong>BVAB1 DNA</strong></td>
<td>$2.4 \times 10^9$</td>
<td><strong>BVAB1 cells</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$661$</td>
</tr>
<tr>
<td><strong>Mobiluncus DNA</strong></td>
<td>$1.3 \times 10^7$</td>
<td><strong>FISH</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BVAB1 DNA</strong></td>
<td>$5.1 \times 10^8$</td>
<td></td>
</tr>
<tr>
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<td></td>
<td></td>
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<tr>
<td><strong>Mobiluncus cells</strong></td>
<td>145</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>BVAB1 cells</strong></td>
<td>908</td>
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</tbody>
</table>
Conclusions

• Separate lines of evidence suggest that curved GNR designated Mobiluncus morphotypes on Gram stain are more likely BVAB1 in our study population:
  – Broad-range PCR with pyrosequencing
  – Species-specific quantitative PCR.
  – Fluorescence in situ hybridization

• *Prevotella* and *Porphyromonas* spp. were significantly associated with the Bacteroides morphotype, whereas *Bacteroides* species were rare

• Implications:
  – Treatment studies monitor response by Gram stain
  – Natural history studies examine transitions in the microbiota by Gram stain
  – This needs evaluation!
Bacterial Taxa Associated with Amsel’s Criteria

- Leptotrichia amnionii & Eggerthella sp. associated with each criteria
- Stars denote bacteria present in >75% of women with BV
- Taxa in bold denote those associated with Amsel’s criteria as a composite unit

Srinivasan 2012
Cervicitis in Women Attending an STD Clinic: Association with Specific BVAB

<table>
<thead>
<tr>
<th>Presence Alone</th>
<th>Detection in the Cervix</th>
<th>Detection in the Vagina</th>
<th>Presence and Load‡</th>
<th>Detection in the Cervix</th>
<th>Detection in the Vagina</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aOR (95% CI)†</td>
<td>p-value*</td>
<td>aOR (95% CI)†</td>
<td>p-value*</td>
<td>aOR (95% CI)†</td>
</tr>
<tr>
<td>Mageeibacillus indolicus</td>
<td>4.38 (0.84–23.68)</td>
<td>0.086</td>
<td>2.93 (0.57–14.78)</td>
<td>0.232</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus jensenii</td>
<td>0.17 (0.02–0.89)</td>
<td>0.032</td>
<td>0.41 (0.06–2.17)</td>
<td>0.401</td>
<td></td>
</tr>
</tbody>
</table>

Gorgos , Sycuro (STD in press)
Cervicitis
Associated with friability

Gorgos, Sycuro
(STD in press)
BV & Increased HIV Acquisition

- Overall, increased risk of ~60% (Atashili 2008)
- Loss of H$_2$O$_2$ (directly virucidal)
- Activation of CD4 by alkaline pH
- Upregulation of cytokines that promote local HIV replication (TNF-alpha, IL-1 beta) & increased shedding
  - HIV shedding increased with intermediate flora or BV (Rebbapragada 2008; Coleman 2007; Sha 2005; Tanton 2011)
    - Not in all prospective studies (Wang 2001; Moreira 2009)
  - Successful BV treatment: decreases in IL-1 beta, IL-8, RANTES & activated CD4 T-cells at endocervix, including CCR5 and CD69 expression (Rebbapragada 2008)
# Bacterial Vaginosis Associated with Increased Risk of Female-to-Male HIV-1 Transmission: A Prospective Cohort Analysis among African Couples

Craig R. Cohen¹,², Jairam R. Lingappa³,⁴, Jared M. Baeten³,⁴,⁶, Musa O. Ngayo⁷, Carol A. Spiegel⁸, Ting Hong³, Deborah Donnell⁹, Connie Celum³,⁴,⁶, Saidi Kapiga¹⁰, Sinead Delany¹¹, Elizabeth A. Bukusi¹,³,⁵,⁷

<table>
<thead>
<tr>
<th>Model</th>
<th>HR</th>
<th>Adjusted HR*</th>
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</thead>
<tbody>
<tr>
<td>Primary analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-visit BV</td>
<td>3.62 (1.74-7.52)</td>
<td>3.06 (1.35-6.95)</td>
</tr>
<tr>
<td>Sensitivity Analyses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current visit BV</td>
<td>5.30 (2.21-12.74)</td>
<td>3.97 (1.67-9.43)</td>
</tr>
<tr>
<td>More severe BV status</td>
<td>7.19 (2.59-19.94)</td>
<td>6.98 (2.12-23.0)</td>
</tr>
</tbody>
</table>

*Fixed covariates: age, geographic region, partner HSV-2 status, circumcision, randomization assignment and STD; Time-dependent covariates: pregnancy, hormonal contraception, plasma HIV-1 RNA, unprotected sex with study partner, CD4 count, outside partners, no. of sex acts with study partner, genital ulcer disease.

Log$_{10}$ HIV RNA concentration in plasma and female genital secretions compared by vaginal flora category

<table>
<thead>
<tr>
<th>Vaginal Flora</th>
<th>Log$_{10}$ HIV Mean ± SD</th>
<th>P-Value vs. normal vaginal flora</th>
<th>P-Value* vs. normal vaginal flora</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genital HIV RNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal vaginal flora</td>
<td>3.04 ± 0.99</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Intermediate vaginal flora</td>
<td>3.25 ± 1.01</td>
<td>0.0035</td>
<td>0.058</td>
</tr>
<tr>
<td>BV</td>
<td>3.23 ± 0.99</td>
<td>0.0023</td>
<td>0.095</td>
</tr>
<tr>
<td><strong>Plasma HIV RNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal vaginal flora</td>
<td>3.81 ± 1.00</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Intermediate vaginal flora</td>
<td>3.96 ± 1.07</td>
<td>0.037</td>
<td>N/A</td>
</tr>
<tr>
<td>BV</td>
<td>3.99 ± 1.07</td>
<td>0.0056</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*After controlling for plasma HIV RNA

Modest increase: 0.2 log$_{10}$

Cohen PloS Med 2012
• Bacteria may activate Langerhans cells and CD4+ T-cells (Donoval, 2006; deJong 2009)
  • May involve direct stimulation by BVAB of relevant immune targets in male genitalia
  • BVAB / LB shared in male & female partners (Bukusi 2011; Gray 2009; Marrazzo 2009)
• Male circumcision changes microbiota of penis, and reduces women’s risk of subsequent BV (Price 2010; Gray 2008; Liu 2013)
Some Answers, but…

- BV is a dysbiotic syndrome associated with acquisition of complex vaginal bacterial communities that include many uncultivated species
  - Heterogeneity may explain differences in treatment outcomes, relapse rates, & incidence of adverse sequelae
  - This heterogeneity – ideally measured with comprehensive, complementary approaches to defining bacterial communities—should be defined in both treatment trials & natural history studies
  - We can’t neglect to study the behavioral & host factors involved
Acknowledgements:
UW-FHCRC Genital Health Study Team

• David Fredricks
• Kathy Ringwood
• Tina Fiedler
• Kathy Thomas
• Sujatha Srinavasan
• Congzhou Liu
• Kathy Agnew
• Nancy Dorn
• Dana Kubulis
• Dwyn Dithmer
• Laura Sycuro
Thank you!
• Production of sialidase (IgA destruction), glycosidase, volatile amines; IL-8 increase variable; ?SCFA (Mirmonsef 2012)

Mitchell, Marrazzo 2014
Association of bacteria with NGU

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopobium</td>
<td>8.3</td>
<td>7.8</td>
</tr>
<tr>
<td>BVAB2</td>
<td>4.5</td>
<td>1.0</td>
</tr>
<tr>
<td>BVAB3</td>
<td>1.3</td>
<td>0</td>
</tr>
<tr>
<td>Lepto/Sneath</td>
<td>15.3</td>
<td>5.9</td>
</tr>
<tr>
<td>Megasphaera</td>
<td>0.6</td>
<td>0</td>
</tr>
</tbody>
</table>

**OR (95% CI):**
- Atopobium: 1.1 (0.42-2.66), p=1.0
- BVAB2: 4.7 (0.57-39.05), p=0.15
- BVAB3: --
- Lepto/Sneath: 2.9 (1.1-7.4), p=0.03
- Megasphaera: --
Association of *Mobiluncus* morphotype with BVAB1 sequence reads

Curved Gram-negative rods visualized by Gram stain are more likely to be the uncultivated bacterium, BVAB-1, rather than the widely accepted *Mobiluncus* spp.
Women with BV
Species-Specific Quantitative PCR

16S rRNA gene copies/swab

<table>
<thead>
<tr>
<th>Species</th>
<th>Nugent 7-8</th>
<th>Nugent 9-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVAB1</td>
<td>P = 0.015</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Mobiluncus</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10E+00  10E+01  10E+02  10E+03  10E+04  10E+05  10E+06  10E+07  10E+08  10E+09  10E+10