EDUCATIONAL COMMENTARY - CLUE CELL MORPHOLOGY: DIAGNOSTIC CONSIDERATIONS

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Learning Outcomes:
Upon completion of this exercise, the participant will be able to:

- Discuss clinical considerations of bacterial vaginosis as related to *Gardnerella vaginalis* infections.
- Identify clue cells based on classic morphology.
- Explain the rationale of current recommendations for clinical and laboratory testing to diagnose bacterial vaginosis, as well as the relevance of utilizing confirmatory Gram staining for clue cells.

Bacterial vaginosis (BV) is the most common female reproductive tract infection during the childbearing years. Clinical correlations and morphologic evidence of this infection are somewhat ambiguous, however. *Gardnerella vaginalis* may be a causative factor associated with BV. However, *G. vaginalis* may constitute normal vaginal flora in some women. Even in some women who may be asymptomatic, a low grade infection may be present. Morbidity is a consideration in bacterial vaginosis; if left untreated, pelvic inflammatory disease may ensue. Identification methodologies of bacterial vaginosis range from clinical features to laboratory-based tests. These may include Amsel’s criteria, Nugent’s score, and Gram-stain identification, as well as cytologic recognition, microbiologic, and molecular assays.

Clinical Considerations
Bacterial vaginosis is a clinical consideration for both asymptomatic and symptomatic females who are infected with a polymicrobial infection predominated by *G. vaginalis* as well as other anaerobic microorganisms. Vaginosis indicates that there is minimal inflammation of vaginal epithelium (nonspecific vaginitis).

The most common presenting symptom is an uncharacteristic vaginal discharge that is homogeneous in consistency and grayish white in color, and that will react positively with the amine test, emitting a foul, fishy odor. Other symptoms may include vaginal itching and irritation, dyspareunia, and pelvic pain. Clinical sequelae of untreated bacterial vaginosis include pelvic inflammatory disease, endometritis, and other complications related to reproductive health.

The clinical composite criteria for the diagnosis of BV were developed by Amsel and colleagues. The presence of at least 3 of the following 4 signs signifies the presence of *G. vaginalis* in 98% of cases.

1. A characteristic thin (nonviscous), homogeneous vaginal discharge of milky consistency; in the clinical setting, an increased adherence of this discharge to the vaginal wall may be noted.
2. Upon application of 10% potassium hydroxide (KOH) to the vaginal discharge, a fishy (trimethylamine) odor is liberated; this is referred to as the “whiff test” and is essentially the development of an alkalinization reaction.
3. Vaginal pH >4.5 (typically pH 5-6)
4. Presence of clue cells

Approximately half of the women with BV diagnoses by Amsel’s criteria are asymptomatic. In the pregnant female population, BV is associated with a range of adverse effects including preterm delivery of low-birthweight infants due to chorioamnionitis, episiotomy infections, postpartum bacteremia, post-caesarean endomyometritis, and post-abortion infection. There is also associated morbidity for the fetus. Additional research has found a causal association with the prevalence and acquisition of HIV in some female populations.

**Morphologic Criteria**

A “clue cell” is typically identified through direct, microscopic examination of a wet-mount smear from a vaginal discharge or secretion, which reveals squamous epithelial cells with cohesive organisms that are gram-negative to gram-variable coccobacilli (Image 1). Gram-stained vaginal discharge smears are the most reliable morphologic test in the diagnosis of BV and may be used to support clinical findings without further culture.

A relative absence of a ‘healthy’ ecosystem of the normal vaginal flora (gram-positive lactobacilli) is also noted. From this, Nugent and coworkers developed a rating system that utilizes the Gram stain to detect the shift from normal flora (*Lactobacillus* predominant) to other microbe patterns associated with BV (gram-negative coccobacilli).

Nugent’s score is based on the number of gram-positive rods in comparison with gram-negative to gram-variable bacilli (to include gram-negative curved bacilli, as well). The Table displays Nugent’s scoring system to be used in conjunction with microscopic evaluation of wet mounts at ×1000 magnification (oil immersion). A Nugent’s score of ≥7 indicates BV infection and likelihood to necessitate clinical management.

Notably, staining for confirmation of “clue cells” serves as a criterion per Amsel’s and Nugent’s identification models. Gram staining of vaginal discharge secretions is an objective and rapid diagnostic indication (or test) for BV, as well as an inexpensive way to diagnose clue cells.

**Image 1.** Clue cells. Squamous epithelial cells cohesively covered by gram-negative to gram-variable coccobacilli (×1,000).
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Table. Nugent’s Scoring System to Compare Microbe Morphology and Gram-Stain Characteristics in Relation to Bacterial Vaginosis Infection.

<table>
<thead>
<tr>
<th>Score</th>
<th>Organism Morphology (HPF)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram-Positive Rods (Lactobacillus)</td>
</tr>
<tr>
<td>0</td>
<td>&gt;30</td>
</tr>
<tr>
<td>1</td>
<td>5-30</td>
</tr>
<tr>
<td>2</td>
<td>1-4</td>
</tr>
<tr>
<td>3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

HPF indicates high-power field.

Clue cells may also be recognized on Pap-stained specimens (from the vagina or cervix) in the typical agglomeration, highlighted by a dark-purplish punctate hue coating the cytoplasm of squamous cells (Image 2, A and B). A comparative image of normal epithelial cells without bacterial contamination is shown in Image 3. According to The Bethesda System for Reporting Cervical Cytology, the presence of clue cells and the related possibility of vaginal flora being replaced by mixed coccobacilli may be indicated as “bacteria morphologically consistent with shift in vaginal flora.” This descriptive diagnosis should be correlated clinically. For patients who have typical presenting symptoms of BV, this cytologic discovery may be consistent with BV. An added benefit of recognizing these “clues” on cytology specimens is that for those patients who are asymptomatic, yet may have an underlying infection, close clinical follow-up may be warranted.
Diagnostic Considerations
Microbiology cultures from vaginal discharge specimens of patients presenting with BV have identified anaerobic flora consisting not only of Gardnerella vaginosis, but Mycoplasma, Mobiluncus, Provotella, Peptostreptococcus, Fusobacterium, Porphyromonas, and Bacteriodes species as well. Microbiologic studies have revealed evidence that the vaginal discharge also has an increased concentration of succinate relative to lactate composition, as well as an increased presence of diamines putrescine and cadaverine.

Interestingly, there is a relative lack of the normal flora, Lactobacillus, which under physiologic conditions produces an acidic milieu that transforms glycogen (within the squamous cells’ cytoplasm) to lactic acid through hydrogen peroxide production. In turn, this lactic acid suppresses the growth of other organisms. Gardnerella and associated species in the setting of BV are able to predominate over this healthy normal flora. Bacterial vaginosis may bring with it a host of disease characteristics that may be asymptomatic or symptomatic in nature. However, diagnosis on the basis of clinical criteria is more practical than diagnosis through culture.

As a laboratory identification method, polymerase chain reaction (PCR) is extremely sensitive and powerful for bacterial quantification; yet the cost of PCR is typically not justified in the clinical laboratory setting for identification of organisms involved with BV.
Conclusion/Current Recommendations
Clue cells, as identified with the Gram-stain, are the single most useful criterion in the diagnosis of BV. Whether female patients are symptomatic or not, BV may carry serious clinical outcomes that may merit treatment. Clinicians and laboratorians alike should be familiar with established guidelines for the diagnosis and treatment of Gardnerella-induced BV.

Reference materials and suggested readings
Centers for Disease Control and Prevention. STD Facts – Bacterial Vaginosis. Available at:

Chaijareenont K. Accuracy of Nugent's score and each Amsel's criteria in the diagnosis of bacterial vaginosis.
J Med Assoc Thai. 2004;87:1270-1274


Sodhani P. Prevalence of bacterial vaginosis in a community setting and role of the pap smear in its detection.