Accuracy of Nugent's Score and Each Amsel's Criteria in the Diagnosis of Bacterial Vaginosis

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Objective : To determine the diagnostic accuracy of Nugent's score and each Amsel's criterion in the diagnosis of bacteria vaginosis (BV), considering Amsel's criteria as the gold standard.
Design : Cross-sectional, descriptive study (diagnostic test)
Setting : Family planning clinic, Siriraj Hospital, Mahidol University
Subjects : A total of 217 women who attended the Family Planning Clinic at Siriraj Hospital between August and December 2003
Method : Pelvic examination was performed on each participant. Samples of vaginal discharge was tested for BV infection using both Amsel's criteria and Nugent's score. Interpretation was made blinded without knowledge of each test result. Using Amsel's criteria as a gold standard, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of Nugent's score and each of Amsel's criteria were estimated.
Results : Considering Amsel's criteria as the gold standard, Nugent's score showed a sensitivity of 65.6% (95%CI 46.8%, 80.8%), specificity of 97.3% (95%CI 93.5%, 99.0%), positive predictive value (PPV) of 80.8% (95%CI 60.0%, 92.7%), negative predictive value (NPV) of 94.2% (95%CI 89.7%, 96.9%) and accuracy of 92.6% (95%CI 88.1%, 95.6%). Both vaginal pH and whiff test demonstrated 100% sensitivity. However, vaginal pH showed lower specificity than the whiff test (58.9% and 97.3% respectively).
Conclusion : Nugent's score might not be suitable to use as a screening test for diagnosis of BV due to its low sensitivity. The whiff test is the best clinical criteria of Amsel's criterion in the diagnosis of BV due to its high sensitivity and specivity.

Keywords : Bacteria vaginosis, Diagnosis, Amsel's criteria, Nugent's score

Bacterial vaginosis (BV) is a disorder of the vaginal ecosystem characterized by a shift in the vaginal flora from the normally predominant *Lactobacillus* to one dominated by a mixed flora including *Gardnerella vaginalis* and *Mobiluncus, Provotella, Bacteriodes,* and *Mycoplasma species*[1]. *Lactobacillus* is normal flora in the vagina that produces an acidic medium via hydrogen peroxide (H_2O_2), which transforms glycogen in vaginal epithelium to lactic acid. The acidic medium produced by *Lactobacilli* suppresses the growth of other microorganisms[2].

BV is the most common cause of abnormal vaginal discharge in adult women. The prevalence of BV ranged between 10-30% in different populations all over the world[3]. About half of the women with BV, diagnosed by Amsel's criteria, are symptom free. Accurate diagnosis of BV is important as associated with adverse pregnancy outcome, pelvic inflammatory disease (PID), chorioamnionitis, and endometritis. Symptomatic women should be diagnosed and treated to alleviate their discomfort and to decrease these serious sequelae[1]. Postoperative infections, including post-abortion PID, post-hysterectomy cuff cellulitis and post-caesarian endomyometritis, have been shown to be associated with asymptomatic BV[4]. Moreover, preoperative antibiotic prophylaxis that covers BV-associated flora can reduce these complications[5].

The diagnosis of BV is generally by using Amsel's criteria[6]. This encompasses fulfilling three

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of the following four criteria: presence of homogeneous vaginal discharge, pH > 4.5, positive amine odor test, and presence of clue cells on vaginal wet smear[7]. Another proposed diagnostic criteria for BV is Nugent's score. It uses Gram stain to detect the shift of normal vaginal flora to other microorganism. Scoring is based on the number of lactobacilli (gram-positive rod), and other microorganisms (gram-negative to gram-variable bacilli, and gram-negative curved bacilli)[8] as demonstrated in Table 1[9].

The purpose of the present study was to determine the diagnostic accuracy of Nugent's score and each of Amsel's criteria in the diagnosis of BV, considering Amsel's criteria as the gold standard.

Material and Method

The subjects were women who attended the Family Planning Clinic at Siriraj Hospital between August and December 2003. A total of 217 women who agreed to participate were enrolled in the study. Exclusion criteria included menstruating or having vaginal bleeding, pregnancy, using any vaginal suppository drugs, previously diagnosed with human immunodeficiency virus (HIV) infection, having visible vaginal or cervical mass suspected cancer, and within six weeks of post-abortion or postpartum.

After informed consents were obtained, pelvic examination was performed. An un-lubricated speculum was inserted into the vagina and the characteristic of the vaginal discharge was evaluated by fellow or residents at Family Planning Clinic at Siriraj Hospital. Samples of the vaginal discharge were obtained with two dry cotton-wool tipped swabs from vaginal fornices. One of the swabs was pressed briefly against an indicator paper (Merck, Germany: range 4.0-6.0) to measure the pH, then this swab was mixed into two drops of normal saline solution in the glass tube. One drop of the mixture was dropped onto the glass slide then a cover slip was placed over the mixture. The mixture was examined in a phase-contrast microscope at 400x magnification to identify clue cells. The other swab was smeared onto the glass slide, then this swab was mixed into two drops of 10% potassium hydroxide solution for amine testing. The composite clinical diagnosis was defined as presence of at least three of the following: homogeneous vaginal discharge, pH > 4.5, positive amine test, and presence of clue cell. The smear on the glass slide was air-dried. Gram stained was independently examined under the microscope under oil immersion at 1000x magnification by one experienced microbiologist at Family Planning

Table 1. Nugent's scoring system [6]

Score	Organism morphotype per high power field		
	Lactobacillus (parallel-sided, Gram-positive rods)	Gardnerella/Bacteriodes (tiny, Gram-variable coccobacilli and rounded, pleomorphic, Gram-negative rods with vacuoles)	Mobiluncus (curved, Gram-negative rods)
0	> 30	0	0
1	5-30	< 1	1-5
2	1-4	1-4	> 5
3	< 1	5-30	
4	0	> 30	

Clinic. Nugent's score was assigned as shown in Table 1. A score of ≥ 7 indicated BV infection[6]. Interpretation was made blindly, without knowledge of the diagnosis by Amsel's criteria.

Descriptive statistics were used to describe the patient's characteristics using mean, standard deviation, number percentage, and proportion as appropriate. Using Amsel's criteria as a gold standard, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of Nugent's score and each of Amsel's criteria were estimated.

Results

A total of 217 women were enrolled. Table 2 shows the characteristics of the women. The mean age was 34.8 ± 9.9 years. The majority of the women were married (96.8%) with 1-2 children (89.4%). Approximately 40% were employees and 89% graduated with less than Bachelor degree. Hormonal and IUD were the two most common contraceptive methods used by these women (46.6% and 38.2% respectively).

Table 3 shows the reported symptoms of the women. The most common symptoms were abnormal vaginal discharge and pelvic pain (36.4% each), and 38% reported no symptom.

BV was diagnosed by Amsel's criteria in 32 (14.7%) and by Nugent's score in 26 (12%) of 217 women as shown in Table 4.

Table 4 shows the comparison between Nugent's score and Amsel's criteria for diagnosis of BV. Considering Amsel's criteria as the gold standard, Nugent's score showed a sensitivity of 65.6% (95% CI 46.8%, 80.8%), specificity of 97.3% (95% CI 93.5%, 99.0%), positive predictive value (PPV) of 80.8% (95% CI 60.0%, 92.7%), negative predictive value (NPV) of 94.2% (95% CI 89.7%, 96.9%) and accuracy of 92.6% (95% CI 88.1%,

Table 2. Demographic characteristics (n=217)

Characteristics	n (%)
Age (mean±SD)	34.8±9.9 years
Status	
• Single	5 (2.3%)
• Married	210 (96.8%)
• Widowed/divorced	2 (0.9%)
Occupation	
• Housewife	66 (30.4%)
• Own business	45 (20.7%)
• Bureaucrat/official	14 (6.5%)
• Employee	87 (40.1%)
• Others	5 (2.3%)
Education	
• Elementary or lower	102 (47.0%)
• High school	89 (41.0%)
• Bachelor or higher	26 (12.0%)
Parity	
• 0	6 (2.8%)
• 1	96 (44.2%)
• 2	98 (45.2%)
• > 3	17 (7.8%)
Current contraceptive use	
• IUD	83 (38.2%)
• Hormonal	101 (46.6%)
• Male condom	5 (2.3%)
• Conventional	28 (12.9%)

Table 3. Symptoms (n=217)

Symptoms*	n (%)
Asymptomatic	84 (38.7%)
Abnormal vaginal discharge	79 (36.4%)
Foul smelly discharge	35 (16.1%)
Vaginal itching	42 (19.4%)
Vaginal irritation	28 (12.9%)
Dyspareunia	43 (19.8%)
Pelvic pain	79 (36.4%)

* each woman may have more than one symptom

Table 4. Comparison between Nugent's score and Amsel's criteria for diagnosis of BV

	Amsel's criteria		
	Positive for BV n (%)	Negative for BV n (%)	Total n (%)
Nugent's score ≥ 7	21(65.6)	5(2.7)	26(12.0)
Nugent's score < 7	11(34.3)	180(97.3)	191(88.0)
Total	32(100.00)	185(100.00)	217(100.00)

95.6%) as shown in Table 5. Table 5 also shows the diagnostic value of each of Amsel's criteria. Both vagi-

Table 5. Diagnostic value of Nugent's score and each criterion of Amsel's criteria

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Nugent's score	56.6	97.3	80.8	94.2	92.6
Homogeneous discharge	59.5	91.9	62.5	96.0	89.9
pH	100	58.9	29.7	100	65.0
Whiff test	100	97.3	86.5	100	97.7
Presence of clue cell	71.9	97.3	82.1	95.2	93.5

nal pH and whiff test demonstrated 100% sensitivity. However vaginal pH showed a lower specificity than the whiff test (58.9% and 97.3% respectively).

Discussion

Bacterial vaginosis (BV) is often misdiagnosed using clinical criteria because the components are subjective and dependent on the acuity of the clinician and available equipment. The results of the study by Nugent et al[9] indicated that criteria for diagnosis of BV using Gram stain can be reproduced reliably by different centers and microbiologists. Summary score can assess the degree of alteration in vaginal flora and allow for standardized interpretation based on bacterial morphotypes identified.

Both clinical and the Gram stain criteria are acceptable methods of diagnosis of BV, however, subtle differences are apparent when these methods are compared to each other. Enrique et al[10] showed that sensitivity and specificity of the Nugent's score compared to the Amsel's criteria were 97% and 98%, respectively. Schwebke et al[11] showed that vaginal Gram stain (Nugent's score) was more sensitive than Amsel's criteria for diagnosis of BV.

However, in the present study the sensitivity and specificity of the Nugent's score were 65.6% and 97.3%, respectively. Lower sensitivity of Nugent's score in the present study might be explained by the subjective interpretation and technical error although the authors used only one experienced microbiologist to interprete the slides. Biases were minimized by blinding Nugent's score interpretation and scoring was given strictly based on the criteria (Table 1).

Considering the different parameters of Amsel's criteria, the diagnosis of BV by vaginal pH > 4.5 and positive amine odor test demonstrated the best sensitivity of 100% that might be used as a screening test for BV. The whiff test showed a sensitivity of 100 %, specificity of 97.3%, PPV of 86.5%, NPV of 100%,

and accuracy of 97.7%, which seemed to be the best of Amsel's criteria for diagnosis of BV. Previous study[10] showed that the sensitivity and specificity for each of Amsel's criteria were 86% and 45% for pH > 4.5, 81% and 99% for the whiff test, 92% and 97% for the presence of clue cells. Similar results were observed in the present study as well.

In conclusion, Nugent's score might not be suitable to use as a screening test for the diagnosis of BV due to its low sensitivity but the advantage of diagnosis of BV by Nugent's score that the gram stain slide may be easily retrospective and used for multiple opinion to confirm the diagnosis of BV. However, the sensitivity of Nugent's score can be improved by standardization of the technique and a good training program for microbiologists. Both vaginal pH and the whiff test demonstrated 100% sensitivity but the whiff test showed a significantly higher specificity. Both criteria may be useful in screening and diagnosis of BV in some clinical studies with limited resources.

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ความแม่นยำถูกต้องของ Nugent's score และแต่ละเกณฑ์ของ Amsel's criteria ในการวินิจฉัย Bacterial Vaginosis

กันต์ธิดา ชัยจรีนนท์, กรกฎ ศิริมัย, ดิฐกานต์ บริบูรณ์หิรัญสาร, อรวรรณ คีรีวัฒน์

วัตถุประสงค์ *: เพื่อประเมินความถูกต้องแม่นยำของ Nugent's score และแต่ละเกณฑ์ของ Amsel's criteria ในการวินิจฉัย bacterial vaginosis เปรียบเทียบกับ Amsel's criteria ซึ่งเป็นการตรวจมาตรฐาน*

ชนิดของการวิจัย *: การวิจัยเชิงพรรณนา (cross-sectional study for diagnostic test)*

สถานที่ทำการวิจัย *: หน่วยวางแผนครอบครัว ภาควิชาสูติศาสตร์นรีเวชวิทยา คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล*

กลุ่มตัวอย่าง *: สตรีจำนวน 217 คน ที่มาตรวจที่คลินิกวางแผนครอบครัว โรงพยาบาลศิริราช ในช่วงเดือนสิงหาคม 2546 ถึง ธันวาคม 2546*

วิธีการศึกษา *: ทำการตรวจภายในผู้เข้าร่วมวิจัยทุกรายทำการเก็บตัวอย่าง เพื่อวินิจฉัยภาวะการติดเชื้อ Bacterial Vaginosis โดยใช้ Nugent's score ซึ่งเป็นวิธีการตรวจแบบใหม่ เปรียบเทียบกับ Amsel's criteria ซึ่งเป็นการตรวจมาตรฐาน (gold standard) โดยไม่ทราบผลการวินิจฉัยในแต่ละวิธีการ นำข้อมูลที่ได้มาวิเคราะห์ทางสถิติเพื่อหาความไว ความจำเพาะ และความถูกต้องแม่นยำในการวินิจฉัยการติดเชื้อ Bacterial Vaginosis โดยวิธี Nugent's score และแต่ละเกณฑ์ของ Amsel's criteria*

ผลการวิจัย *: การวินิจฉัยภาวะการติดเชื้อ Bacterial Vaginosis โดยใช้ Nugent's score มีความไวร้อยละ 65.6 (95% CI 46.8, 80.8) ความจำเพาะร้อยละ 97.3 (95% CI 93.5, 99.0) positive predictive value ร้อยละ 80.8 negative predictive value ร้อยละ 94.2 ความถูกต้องแม่นยำ ร้อยละ 92.6% (95%CI 88.1%, 95.6%) เมื่อเปรียบเทียบกับ Amsel's criteria ซึ่งเป็นวิธีการตรวจมาตรฐาน การวัดค่าความเป็นกรดด่างของช่องคลอดและ whiff test มีความไว ร้อยละ 100 แต่อย่างไรก็ตาม การวัดความเป็นกรดด่างของช่องคลอดมีความจำเพาะร้อยละ 58.9 ซึ่งต่ำกว่า whiff test ที่มีความจำเพาะร้อยละ 97.3*

สรุป *: Nugent's score ไม่เหมาะกับการเป็นการตรวจวินิจฉัยเพื่อคัดกรองโรค bacterial vaginosis เนื่องจากมีความไวในการวินิจฉัยโรคต่ำ เมื่อพิจารณาแต่ละเกณฑ์ของ Amsel's criteria ในการวินิจฉัย bacterial vaginosis พบว่า whiff test น่าจะเป็นเกณฑ์การวินิจฉัยที่ดีเนื่องจากมีความไวและความจำเพาะในการวินิจฉัยโรคสูง*