Comparison between Nugent’s and Hay/Ison scoring criteria for the diagnosis of Bacterial Vaginosis in WASP® prepared vaginal samples

Abstract

**Background:** The aim of this study was to compare two different Bacterial Vaginosis diagnosis criteria, Nugent’s score system and Hay/Ison criterion, by using Gram-stained vaginal smears.

**Materials and findings:** Gram-stained smears were prepared with 10 µl and 30 µl of 100 vaginal samples, collected in ESwab®, by the WASP® automatic system. All smears were examined at 1000X magnification and assessed using both Nugent and Hay/Ison criteria. In addition, the presence of clue cells was recorded. All the slides were assessed by three independent expert readers in a blinded manner. Considering the 10 µl smears, Nugent’s scoring results were: 90 patients with normal vaginal flora, five with intermediate flora and five with Bacterial Vaginosis. Instead, Hay/Ison criterion results were: 83 patients had Grade I, 12 Grade II and 5 Grade III. About the 30 µl ESwab® smears, the Nugent’s scoring results were: 92 patients with normal vaginal flora, three intermediate flora and five with Bacterial Vaginosis. In contrast, the results obtained by using the Hay/Ison criterion were: 84 patients had Grade I, 12 had Grade II and 4 had Grade III. Clue cells were detected in all the Bacterial Vaginosis cases.

**Conclusions:** The Hay/Ison criterion is a credible alternative to the Nugent’s score system for the diagnosis of Bacterial Vaginosis. Furthermore, the combination of WASP® automatic system and the Hay/Ison criterion can represent a fast and reliable workflow, especially in those laboratories where the request of vaginal culture tests is extremely high.

**Keywords:** Bacterial Vaginosis • Hay/Ison criterion • Nugent’s scoring system • WASP®, ESwab®, Gardnerella vaginalis

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Abbreviations: BV: Bacterial Vaginosis, PPV: predictive positive value, PNV: predictive negative value.

Introduction

Bacterial Vaginosis (BV) is the most common cause of vaginal discharge worldwide. It affects 20–30% of women in reproductive age attending sexually transmitted infection, and its prevalence can be as high as 50-60% in high-risk populations (e.g., those who practice commercial sex work) [1,2].

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BV is characterized by a change in the complex vaginal flora, in which a mixed microbial flora (Mobiluncus spp., Gardnerella vaginalis, Atopobium spp., Prevotella spp., Sneathia spp., etc.) [3,4] replaces the normal occurring Lactobacillus spp. (Lactobacillus jensenii, Lactobacillus gasseri, Lactobacillus iners and Lactobacillus crispatus) [5]. Even if many women with BV are asymptomatic, the pattern of BV symptoms varies from abundant and smelly discharge to a moderate increase of white discharge, which appears homogenous with a low viscosity, and sometimes coats the vaginal mucosa [6].

The exact pathogenesis of the disease remains unclear. In fact, different hypothesis have been considered to find out the principal trigger of the BV; one of them is the racial and societal differences, in which African American women have a higher probability to contract BV than European women because of their different vaginal pH [7]. Another hypothesis for the BV pathogenesis sees the G. vaginalis as the key-stone pathogen, which through its metabolic pathways and ability to form a biofilm, lowers the reduction-oxidation potential in the vaginal microbiome and then causes a marked decrease in lactobacilli and an increase in BV-associated bacteria [8].

The diagnosis of BV has been performed for many years by using clinical methods such as the Amsel’s criteria; this score is based on the presence of any three of the following four criteria: an elevated vaginal pH (>4.5), the presence of grayish discharge that adheres to the vaginal wall, a fishy odor (when vaginal secretions are mixed with 10% KOH on a slide), and the presence of clue cells represent more than 20% of the total cell population [6]. Unfortunately, Amsel’s criteria showed some limitations mainly because they were based on clinical signs that are neither quantifiable nor reproducible, so they were largely replaced by the Nugent’s score. This criterion consists of the calculation of the microbial flora by counting the microorganisms directly in the Gram-stained smears of vaginal fluid and by checking the presence of the Clue Cells: epithelial cells covered with bacteria. Nowadays, the Nugent’s criterion is the gold standard for the diagnosis of BV because it allows a more precise assessment of alteration in vaginal flora [9].

Nevertheless, the evaluation of vaginal smears and then the diagnosis of BV by using the Nugent’s score is strongly related to the reader’s expertise, which might have affected the result [10]. Therefore, in 2002, Hay and Ison described an easier score, which consists of the characterization of the vaginal flora into three different categories: Grade I (normal), Grade II (intermediate) and Grade III (BV) [11]. The assessment of the vaginal smear by this criterion depends on the relative ratio between the amount of Lactobacillus spp. and Gardnerella vaginalis.

The aim of this study was to compare the most used criteria for the diagnosis of BV, Nugent’s score system and the Ison/Hay criterion, in vaginal smears prepared using WASP automation.

**Methods**

**Study population**

We collected 100 vaginal samples from reproductive age women, who came to the University Hospital of ‘Tor Vergata’ for a vaginal culture test. Every specimen was collected by using ESwab® liquid Amies system (Copan Italia, Brescia, Italy), which represents an optimal liquid medium to conserve and transport the typical bacterial populations present in BV and normal vaginal flora [12,13].

**Slides preparation and microscope examination**

The WASP® automatic system was used for the preparation of vaginal smears. Every ESwab® tube was inserted into the WASP® automatic system; afterwards, the WASP® collected 10 µl of liquid Amies and smeared it on a glass slide. Similarly, a second glass slide was prepared by using an amount of 30 µl from each ESwab® tube.

All the slides were air-dried and, subsequently, Gram-stained by PREVI Color Gram (bioMerieux, Inc., Durham, NC, USA), following the instructions of the manufacturer. The efficiency, reliability and reproducibility of the staining technique were checked by inserting a control slide in each run: BD BBL™ Gram slide (Becton, Dickinson & Company, Sparks, MD, USA). Afterwards, few drops of oil immersion were applied onto the slides and were then examined using the optical microscope Nikon Eclipse E600 (Nikon Instruments Europe BV, Amsterdam, Netherlands, EU) at 1000X magnification. Before the final assessment, the observation of all smears of the vaginal samples was performed by three independent readers in a blinded manner.

**Interpretative criteria of vaginal smears**

Two interpretative criteria were used for the evaluation of the vaginal flora in both 10 µl and 30 µl gram-stained vaginal smears: Nugent’s grading score and Hay/Ison criterion. The Nugent’s grading score is based on the classification of the vaginal flora by using a scale from 0 up to 10; moreover, the presence or absence of Clue Cells contributes to the diagnosis of a possible BV. The Nugent’s grading score is based on the classification of Scale’s value ranges between 4-6 in the presence of Clue Cells.
Cells or >7 are considered BV, while scores from 0 to 3 and 4 to 6 (without Clue Cells) are considered normal and intermediate, respectively (Table S1, A) [14]. The scale’s value is obtained by the addition of three different scores; in turn, every score represents the amount of a specific bacterial morphotype assessed by the reader through the optical microscope (Table S1, B). The microorganisms in the vaginal smears, counted by the reader, are: Lactobacillus spp. (large Gram-positive rods), Gardnerella/Bacteroides (small Gram-negative rods) and Mobiluncus spp. (curved Gram-variable rods).

In contrast to the Nugent’s score, the Hay/Ison criterion classifies the vaginal flora in three different grades: grade I (normal flora), describes the presence of Lactobacillus spp. morphotype only; grade II (intermediate flora), reduced number of Lactobacillus spp. morphotype and similar amount of mixed bacterial morphotypes; grade III (bacterial vaginosis), few or absent Lactobacillus morphotype and abundant presence of mixed bacterial morphotypes (Table S2). Moreover, the presence of the Clue Cells is considered as well.

**Statistical analysis**

Vaginal smear results were used to calculate sensitivity, specificity, Cohen’s kappa, predictive positive value (PPV) and predictive negative value (PNV) of the Hay/Ison criterion by considering the Nugent’s score system as gold standard.

**Results**

**Comparison of Nugent’s and Hay/Ison criteria results in 10 µl ESwab vaginal samples**

Considering Gram-stained smears prepared by using 10 µl of ESwab vaginal samples, 91 out of 100 patients (91%) had concordant results with different criteria, Nugent’s scoring system and Hay/Ison criterion. 82 (82%) patients were assessed as Normal (Nugent’s score) and Grade I (Hay/Ison criterion), four (4%) as Intermediate/Grade II and five (5%) were diagnosed as BV with both criteria (Table S3, A). All BV cases were characterized by the presence of Clue Cells; specifically, one of the five patients resulted to be Intermediate by using the Nugent’s scoring system, although the presence of Clue Cells allowed the final assessment of this patient as a BV.

The nine remaining patients had discordant results between the Nugent’s and the Hay/Ison criteria: eight (8%) patients were assessed as Normal (Nugent’s score) and Grade II (Hay/Ison criterion), one patient was classified as Intermediate (Nugent’s score) and Grade I (Hay/Ison criterion) (Table S3, A).

Sensitivity, specificity, PPV and PNV were calculated in two different scenarios: the first one considered Intermediate and Grade II scores as non-BV; conversely, the second scenario considered Intermediate and Grade II scores as BV (Table S3, B). For the evaluation of concordance between Hay/Ison criterion and Nugent’s score system in both scenarios, it was calculated Cohen’s Kappa. In accordance with the interpretative guidelines, the Kappa index was 1.00 (0.81-1.00=excellent; Table S3, B); the Kappa index in the second one was 0.62 (0.61-0.80=good; Table S3, B).

**Comparison of Nugent’s and Hay/Ison criteria results in 30 µl ESwab vaginal samples**

Similar results were obtained by reading Gram-stained smears prepared with 30 µl of ESwab vaginal samples. 89 out of 100 patients (89%) had concordant results with Nugent’s scoring system and Hay/Ison criterion: 83 (83%) were assessed as Normal/Grade I, two (2%) Intermediate/Grade II and four BV/Grade III with the presence of Clue Cells (Table S4 A). Similarly to the 10 µl vaginal samples one of these four patients was assessed as BV because of the Clue Cells, despite resulting Intermediate by using the Nugent’s scoring system.

Discordant results were observed in eleven patients: nine (9%) were assessed as Normal (Nugent’s score) and Grade II (Hay/Ison criterion), one (1%) as Intermediate (Nugent’s score) and Grade I (Hay/Ison criterion), and one patient was assessed as BV by Nugent’s and as Grade II by Hay/Ison (Table S4, A). The last one resulted to have BV, per the Nugent’s scoring system, because of the presence of Clue Cells combined with an Intermediate score.

Sensitivity, specificity, PPV and PNV were calculated in two different scenarios: the first one considered Intermediate and Grade II results as non-BV; the second scenario, considered Intermediate and Grade II scores as BV (Table S4, B). Finally, the Cohen’s Kappa was calculated to assess concordance between Hay/Ison and Nugent’s score criteria considering both scenarios. In accordance with the interpretative guidelines, the Kappa index was 0.88 (0.81-1.00=excellent; Table S4, B); the Kappa index in the second one was 0.53 (0.41-0.60=moderate; Table S4, B).

**Discussion**

The diagnosis of the Bacterial Vaginosis has seen a development of different criteria since 1980s, when Amsel et al. described for the first time clinical signs to diagnose BV; at the same time, Spiegel et al. [6,15] defined a scoring system for bacteria morphotype in gram-stained smears. This scoring system was later defined by Nugent et al. [9]. Later, in 1994, Ison et al.
validated a simpler method to interpret vaginal smears, which was updated in 2002 [11,16].

In this study, we compared two different interpretative criteria for the assessment of gram-stained ESwab® vaginal smears, Nugent’s score system and Hay/Ison criterion. For the first time, all smears were prepared by using 10 µl and 30 µl of ESwab® vaginal samples with the WASP® automation system, which prepared every smear directly from the ESewab® liquid Amies. Per our results, the WASP® allowed the correct processing of all vaginal ESwab® samples obtaining good quality of the smears with a rapid workflow; the good quality was also related to the correct storage of every sample, which was collected in a specific ESwab® tube.

Noteworthy is the different amount of Intermediate and Grade II scores observed in both 10 µl and 30 µl of ESwab® vaginal samples, assessed with both Nugent and Hay/Ison criteria. The number of ESwab® vaginal samples assessed as Grade II were higher than those assessed as Intermediate. The reason for this might be the lack of a clear cut-off in the Hay/Ison criterion, unlike the Nugent’s score system, which allows the precise evaluation by the decrease of Lactobacillus spp presence.

Previous studies have already proved the correlation between the presence of Clue Cells and Bacterial Vaginosis so we did consider the Clue Cells presence as an important element to diagnose Bacterial Vaginosis, when the Nugent’s score resulted to be Intermediate. In this way, when we saw epithelial cells coated with anaerobic Gram-variable coccobacilli Gardnerella vaginalis in an Intermediate score situation, we assessed that specific sample as BV [14].

Considering both Nugent and Hay/Ison criteria, the only discordant interpretative case regarded a gram-stained smear prepared by using 30 µl of ESwab® vaginal sample; it resulted to be a BV case by the Nugent’s score system and Grade II by the Hay/Ison criterion. Since the presence of Clue Cells is considered as one of the parameters to diagnose BV only in the Nugent’s score, this might be one of the causes of this discrepant result.

**Conclusion**

In conclusion, our study shows that the WASP® automatic system is a fast and reliable methodology for the preparation of smears for Gram staining using 10 µl and 30 µl of ESwab® vaginal samples. Even if the Nugent’s score system remains the gold standard to evaluate vaginal flora, the Hay/Ison criterion is a valuable alternative, above all in those laboratories where the request of vaginal culture tests is extremely high.

**Author’s Contributors**

Francesco Paolo Antonucci and Walter Mirandola had equally contributed to the study design and paper preparation.

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**Footnotes**

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**Executive summary**

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**Materials and findings:** Gram-stained smears were prepared with 10 µl and 30 µl of 100 vaginal samples, collected in ESwab®, by the WASP® automatic system. All smears were examined at 1000X magnification and assessed using both Nugent and Hay/Ison criteria. In addition, the presence of clue cells was recorded. All the slides were assessed by three independent expert readers in a blinded manner. Considering the 10 µl smears, Nugent’s scoring results were: 90 patients with normal vaginal flora, five with Intermediate flora and five with Bacterial Vaginosis. Instead, Hay/Ison criterion results were: 83 patients had Grade I, 12 Grade II and 5 Grade III. About the 30 µl ESwab® smears, the Nugent’s scoring results were: 92 patients with normal vaginal flora, three intermediate flora and five with Bacterial Vaginosis. In contrast, the results obtained by using the Hay/Ison criterion were: 84 patients had Grade I, 12 had Grade II and 4 had Grade III. Clue cells were detected in all the Bacterial Vaginosis cases.

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