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Bacterial vaginosis – diagnostic dilemma and implications

Bakterijska vaginoza – dijagnostičke dileme i implikacije

Sonja Atanasievska*, Dane Nenadić†, Slaviša Stanković‡, Vesna Protić-Djokić*, Elizabeta Ristanović*

Military Medical Academy, *Institute of Microbiology, †Center for Gynecology and Human Reproduction, Belgrade, Serbia; ‡University of Belgrade, Faculty of Biology, Belgrade, Serbia

Abstract

Background/Aim. Bacterial vaginosis (BV) is one of the most common microbial dysbiosis, characterized by a decrease of *Lactobacillus spp.* with an increase of other anaerobic bacteria species [*Gardnerella (G.) vaginalis, Atopobium (A.) vaginae, Prevotella spp, Mobiluncus spp*, etc.] causing serious gynecological and obstetric complications. Therefore, it is particularly important to have accurate and reliable diagnostic standards. The aim of this study was to compare the results of various diagnostic methods for detecting BV, such as Amsel, Nugent, and Ison and Hay criteria, as well as multiplex quantitative real-time polymerase chain reaction (mqRT-PCR) test. **Methods.** This study involved vaginal swabs from 235 patients of reproductive age. Nugent criteria were used as the 'gold standard' compared with Amsel and Ison/Hay criteria as well as mqRT-PCR test based on the detection and quantification of *G. vaginalis, A. vaginae*, *Lactobacillus spp.*, and total concentration of bacterial DNA. The *kappa* coefficient was employed to measure agreement between tests. **Results.** Our analysis demonstrated excellent agreement between Ison/Hay criteria and Nugent scores (*kappa* = 0.95), good agreement between Amsel and Nugent criteria (*kappa* = 0.78), while between Nugent criteria and mqRT-PCR test agreement was moderate (*kappa* = 0.59). Total agreements of Ison/Hay, Amsel, and mqRT-PCR against Nugent scores were 94.9%, 90.2%, and 74%, respectively. Nugent methods classified the highest number of intermediate patients - 60 (25.2%). The largest number of BV patients was detected by the mqRT-PCR method, while the largest number of healthy patients was detected by Amsel criteria. **Conclusion.** The mqRT-PCR is the best choice for BV diagnosis because it is more efficient at differentiating patients with intermediate results. Compared to Amsel and Nugent methods that group patients into 2 or 3 categories, the mqRT-PCR method recognizes other conditions of vaginal flora important for correct diagnoses and application of better therapeutic approaches, as well as preventing possible clinical consequences of this dysbiosis.

Key words:
diagnostic techniques, obstetrical and gynecological; microscopy; vaginal diseases; vaginal smears; vaginosis, bacterial.

Apstrakt

Uvod/Cilj. Bakterijska vaginoza (BV) je jedna od najčešćih vaginalnih disbioza, koja se karakteriše smanjenjem broja bakterija roda *Lactobacillus spp.* i povećanjem broja različitih anaerobnih bakterija [*Gardnerella (G.) vaginalis, Atopobium (A.) vaginae, Prevotella spp, Mobiluncus spp*, i dr]. S obzirom na to da BV može izazvati veliki broj ginekoloških i akušerskih komplikacija, važno je definisati tačne i pouzdane metode za njenu dijagnostiku. Cilj rada bio je da se uporede rezultati različitih dijagnostičkih metoda za detekciju BV kao što su Amsel-ovi, Nugent-ovi, Ison-ovi i Hay-ovi kriterijumi, kao i multipleks kvantitativni test *real-time polymerase chain reaction* (mkRT-PCR). **Metode.** Studijom je obuhvaćeno 235 uzoraka vaginalnih briseva pacijentkinja u reporoduktivnom periodu. Kao zlatni standard korišćeni su Nugent-ovi kriterijumi čiji su rezultati upoređeni sa Amsel-ovim, kriterijumima Ison-a i Hay-a, kao i mkRT-PCR testom, koji se bazira na detekciji i kvantifikaciji *G. vaginalis, A. vaginae*, *Lactobacillus spp.* i ukupnoj koncentraciji DNK prisutnih vrsta bakterija. *Kappa* koeficijent korišćen je kako bi se utvrdio stepen saglasnosti između korišćenih metoda. **Rezultati.** Analizom su utvrđeni sledeći stepeni saglasnosti: odličan između kriterijuma Ison/Hay-a i Nugent-a (*kappa* = 0,95), dobar između Amsel-ovih i Nugent-ovih kriterijuma (*kappa* = 0,78) i umeren izmedju kriterijuma Nugent-a i mkRT-PCR testa (*kappa* = 0,59). Ukupan stepen saglasnosti kriterijuma Ison/Hay-a, Amsel-a i mkRT-PCR testa sa Nugent-ovom metodom je iznosio 94,9%, 90,2% i 74%, redom. Nugent-ovom metodom otkriven je najveći broj intermedijarnih nalaza

Correspondence to: Sonja Atanasievska, Military Medical Academy, Belgrade, Serbia, Crnotravska 17, 11 000 Belgrade, Serbia.
E-mail: sonja.atanasievska@gmail.com

– 60 (25,2%). Najveći broj bolesnica sa BV detekovano je mkRT-PCR metodom, dok je Amsel-ovim kriterijumima utvrđen najveći broj normalnih nalaza. **Zaključak.** Najbolji izbor testa za dijagnostiku BV je mkRT-PCR, jer je efikasniji u diferenciranju bolesnica sa intermedijarnim nalazom. U poređenju sa Amsel-ovim i Nugent-ovim kriterijumima pomoću kojih se bolesnice razvrstavaju u 2 i 3 kategorije, mkRT-PCR metod prepoznaje i druga stanja vaginalne flore, koja su važna za tačnu dijagnostiku i primenu boljeg terapijskog pristupa u lečenju BV, kao i prevenciji njenih mogućih posledica.

Ključne reči:
dijagnostičke tehnike, akušerstvo i ginekologija; mikroskopija; vagina, bolesti; vaginalni brisevi; vaginoza, bakterijska.

Introduction

Even though bacterial vaginosis (BV) is the most frequent and the most well-researched dysbiosis, it still presents as an enigmatic imbalance of vaginal microflora of unknown etiology [1]. The characteristics of BV include a reduced number of *Lactobacillus (L.) spp.* and an increase of other anaerobic bacteria [2]. It is identified by a lack of inflammatory symptoms and a signature metabolic pattern which includes an increased pH of the vagina (pH >4.5) and the presence of bioamine that causes a foul-smelling 'fishy' vaginal odor [3, 4]. BV may cause serious gynecologic and obstetric complications where 50% of patients are asymptomatic [5, 6].

Even though the application of molecular methods in recent decades has brought many improvements in understanding the vaginal microbiome, diagnostic criteria such as the Amsel criteria originally published in the American Journal of Medicine in 1983 and Nugent criteria from 1991 have remained the 'gold standard' of clinical and research approaches to BV diagnosis. Increasingly, researchers have revealed the flaws of these standards since the application of Amsel criteria divides patients into only one of two groups (normal and BV), while Nugent criteria introduce a third (intermediate) group which represents the state between normal and BV [7–9]. The first researchers who highlighted the existence of other states of vaginal microflora were Ison and Hay, who added two more classes to Nugent's existing three groups – predominant cocci (orig. Grade IV) and cases where epithelial cells are visible without bacterial forms (orig. Grade 0) [10].

A great number of bacteria may be detected not only in women with vaginal infections but also in healthy patients. Only with the application of multiplex quantitative real-time polymerase chain reaction (mqRT-PCR) test it is possible to confirm the quantitative relations between certain bacteria, which is crucial to enable us to differentiate between dysbiosis and eubiosis [11]. Previous research has confirmed that *Gardnerella G. vaginalis* and *Atopobium (A.) vaginae* are present in normal vaginal microflora. However, the high concentration of these bacteria is an important marker in BV diagnostics [12]. RT-PCR test is based on the detection and quantification of *G. vaginalis, A. vaginae, L. spp.*, and total concentration of bacterial DNA to differentiate between six groups of patients, including those with insufficient DNA copies and with vaginal flora of unspecified etiology.

Considering that BV is quite challenging to diagnose due to its complex polymicrobial nature, the aim of this study was to compare the results obtained by various detection methods such as Amsel, Nugent, and Ison and Hay criteria as well as mqRT-PCR.

Methods

This study included 235 patients of reproductive age with or without symptoms of vaginal infections. Recruitment and testing were undertaken from November 2018 to December 2019. Exclusion criteria included those with a recent history (< 2 weeks) of antibiotic use. Examination of patients and sample collection were performed at the Center for Gynecology and Human Reproduction, while sample testing was conducted at the Institute of Microbiology, the Military Medical Academy (MMA), Belgrade, Republic of Serbia. All participants gave their written consent to participate in this research. The research has been approved by the MMA Ethics Committee from December 25, 2018.

Four vaginal swabs (FLOQ Swabs, COPAN) were taken from each patient to enable further microbiological and clinical investigation. BV diagnoses were made based on existing microscopic and clinical criteria (Amsel, Nugent, Ison/Hay) but also using mqRT-PCR. Clinical examination identified the amount, consistency, and color of vaginal discharge. Vaginal pH was determined by indicator papers (Merck, pH from 4.0 to 7.0). Testing with 10% potassium hydroxide (KOH) was performed by adding a drop of 10% KOH to the swab taken from the vaginal side wall to identify the 'fishy' vaginal odor. Clue cells were identified using a wet mount slide. When using Amsel criteria, a positive diagnosis was made if 3 out of the 4 following criteria were confirmed: discharge in a homogenous grey/white color, a vaginal pH greater than 4.5, a positive test with 10% KOH of a 'fishy' vaginal odor, and the presence of clue cells. One of the swabs taken from the vaginal sidewall was used for the microscopic examination of a Gram-stained smear. Smears were examined by a Solaris biological microscope with a magnification of ×1,000. Samples of Gram-stained vaginal smears were evaluated and classified by Nugent and Ison/Hay criteria. Nugent criteria use a scoring system based on microscopic examination of Gram-stained swabs to confirm the presence and relation of Gram-positive rods (*L.*), Gram-negative and Gram-variable rods, and cocci (*G. vaginalis*, *Bacteroides spp.*) and curved Gram-negative rods (*Mobiluncus spp.*). The scoring system divides vaginal smears into three groups: normal flora (0-3 score), intermediate flora (4-6 score), and BV (7-10 score). Ison/Hay criteria classify swabs into five categories: Grade 0 (vaginal

smear without bacteria); Grade 1 (normal flora – dominance of Gram-positive rods); Grade 2 (intermediate flora – presence of Gram-positive and Gram-negative *coccobacilli*); Grade 3 (BV, dominance of Gram-variable rods and *coccobacilli* or curved Gram-positive rods); Grade 4 (dominance of Gram-positive cocci only).

Upon sampling, the swabs of the vaginal sidewalls used for the mqRT-PCR were stored in a transport medium and kept at -20 °C until DNA extraction (DNA-sorb-AM, AmpliSens). mqRT-PCR (AmpliSensFlorocenosis/Bacterial vaginosis-FRT) was used to detect and quantify *G. vaginalis, A. vaginae, L. spp.*, and the total number of bacteria. The ratio coefficients (RC), RC1 = log (Lac DNA) – log (Gv + Av DNA), RC2 = log (Bac DNA) – log (Lac DNA), and RC3 = log (Bac DNA) – log (Gv + Av DNA) were determined by the mutual relations between those bacteria. Based on coefficients, the patients were grouped into 6 categories: normal vaginal flora (RC1 > 1, *L. spp.* is the dominant flora); intermediate flora ($0.5 \leq RC1 \leq 1$, the same number of *L. spp.* and aerobic bacteria); BV (RC1 < 0.5, dominant *G. vaginalis* and *A. vaginae*); vaginal flora of non-specific etiology (RC2 > 1, RC3 > 2, any RC1 value, a small concentration of *L. spp*, but also *G. vaginalis, A. vaginae*); bacterial load decrease (RC > 1 but the total amount of bacteria DNA is less than 10^6 copies/mL and greater than 10^5 copies/mL); bacterial load insufficient for analysis (total amount of bacteria DNA is less than 10^5 copies/mL). All PCR reactions were performed on the PCR instrument (Sa-Cycler 96, Sacace, Biotechnologies).

Nugent criteria were used as the 'gold standard'. To be able to compare the results statistically, Ison/Hay and mqRT-PCR results were divided into three groups (normal, intermediate, and BV). The Ison/Hay criteria were modified from 5 categories to 3, where Grade 0 was merged with Grade 1, and Grade 4 was merged with Grade 5. The results of the modified mqRT-PCR were divided into 3 categories. Groups 'vaginal flora of non-specified etiology' and 'bacterial load insufficient for analysis' were merged into an intermediate group. The result, 'bacterial load decreased', was considered a normal finding.

To enable comparison of all these criteria and because Amsel criteria have only 2 groups, all 4 methods were divided into 2 groups: non-BV and BV. Normal and intermediate results of vaginal swabs (Nugent and Ison/Hay criteria) were classified as a non-BV group. In relation to PCR criteria, all results which were non-BV (normal, intermediate, 'bacterial load insufficient for analysis' and 'vaginal flora of non-specified etiology') were grouped into one non-BV group.

The *kappa* coefficient was used to measure the agreement between tests. *Kappa* values were interpreted according to Altman [13]. All data were processed using the software package IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA).

Results

The samples of all 235 patients were tested successfully against all four criteria for BV diagnosis. The average age of patients was 29.39 (± 6.685). An overview of all results obtained through Amsel, Nugent, Ison/Hay criteria, and mqRT-PCR test are presented in Table 1. A comparison of the results between different methods (Amsel, Ison/Hay criteria, and mqRT-PCR) against the Nugent 'gold standard' criteria is presented in Table 2.

The measure of agreement between Amsel and Nugent criteria was reported to be good (*kappa* = 0.78). The total agreement of these two criteria was 90.2%. The sensitivity of Amsel criteria in our study was 82.05%, while the specificity was 94.27%.

The agreement between the modified Ison/Hay criteria and the Nugent criteria is excellent, with a *kappa* coefficient of 0.95, while the total agreement is 94.9%. Ten patients with Grade 0 and Grade 4 were included in the intermediate

Table 1

Overview of results using different methods for diagnosis of bacterial vaginosis (BV)

Methods	N	INT	BV	Grade 0/ bacterial load insufficient for analysis	Grade 4	Vaginal flora of non-specified etiology	Bacterial load decreased
Amsel	162 (68.9)	-	73 (31)	-	-	-	-
Nugent	97 (41.3)	60 (25.5)	78 (33.1)	-	-	-	-
Ison/Hay	95 (40.4)	50 (21.3)	76 (32.3)	10(4.2)	4 (1.7)	-	-
mq RT-PCR	114 (48)	9 (3.8)	90 (38)	10 (4.2)	-	5 (2.1)	7 (2.9)

N – normal flora; INT – intermediate flora; mqRT-PCR – multiplex quantitative real-time polymerase chain reaction. Results are presented as n (%)

Table 2

Comparison of different methods (Amsel, Ison/Hay criteria, and mqRT-PCR) against Nugent criteria

	Nugent				Nugent					Nugent			
Amsel	Non-BV	BV	Total	Ison/Hay	N	INT	BV	Total	mqRT-PCR	N	INT	BV	Total
Non-BV	148	14	162	N	96	9	0	105	N	90	28	3	121
BV	9	64	73	INT	1	49	0	50	INT	5	14	5	24
Total	157	78	235	BV	0	2	78	80	BV	2	18	70	90
				Total	97	60	78	235	Total	97	60	78	235

N – normal flora; INT – intermediate flora; BV – bacterial vaginosis; Non-BV – normal and intermediate results; mqRT-PCR – multiplex quantitative real-time polymerase chain reaction.

under the Nugent criteria. The specificity of the Ison/Hay criteria is 100%, while the sensitivity is 98.73%. Our research showed that the biggest disagreement between these two criteria was in the intermediate group. Nine patients with intermediate results in Nugent criteria were assessed as healthy under Ison/Hay criteria, while 2 intermediate patients were classified as BV.

The results of the comparison of the modified mqRT-PCR test and Nugent score are shown in Table 2. The agreement between these two criteria was moderate (*kappa* coefficient = 0.59), while the total agreement was 74%. The sensitivity of the PCR criteria was 89.74%, and the specificity was 87.26%. Out of the 10 patients whose bacterial load was insufficient for analysis under Nugent criteria, 6 were in the intermediate group, 2 were diagnosed with BV, and the other 2 had a normal vaginal smear. Vaginal flora of unknown etiology was detected by mqRT-PCR in 5 patients, while the Nugent criteria showed 2 patients with normal tests, 2 diagnosed with BV, and 1 with an intermediate result. Out of seven patients assessed as 'bacterial load decreased' under Nugent criteria, 2 have been classified as normal and 5 as having intermediate results.

A comparison of methods divided into three groups is shown in Figure 1. The use of Nugent criteria produced the highest number of intermediate results, 60 (25.5%), while the modified mqRT-PCR method detected only 24 (10.2%). Comparatively, the mqRT-PCR test detected the largest number of healthy patients and BV diagnoses.

To enable the comparison of various diagnostic criteria, all four methods were divided into non-BV and BV groups (Figure 2). The highest number of BV cases was detected by the mqRT-PCR method – 90 (38.2%), while the greatest number of non-BV patients were detected by Amsel criteria – 162 (68.9%).

Discussion

This study aimed to compare the application of different methods in the diagnosis of BV. As shown in Table 1, Amsel and Nugent criteria may define 2 and 3 groups, respectively, whereas according to Ison and Hay criteria and mqRT-PCR test, the 'other conditions' of vaginal flora have been recognized. Considering that the Nugent criteria are the most frequently used as the 'gold

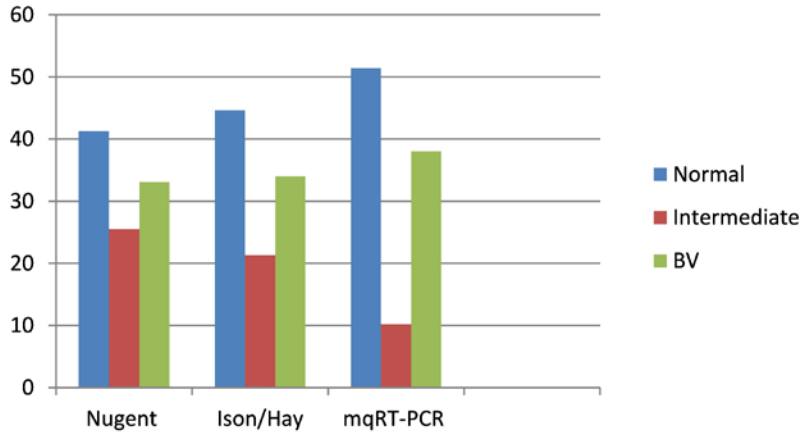

Fig. 1 – Comparison of methods divided into 3 groups (normal flora, intermediate flora, BV – bacterial vaginosis). mqRT-PCR – multiplex quantitative real-time polymerase chain reaction.

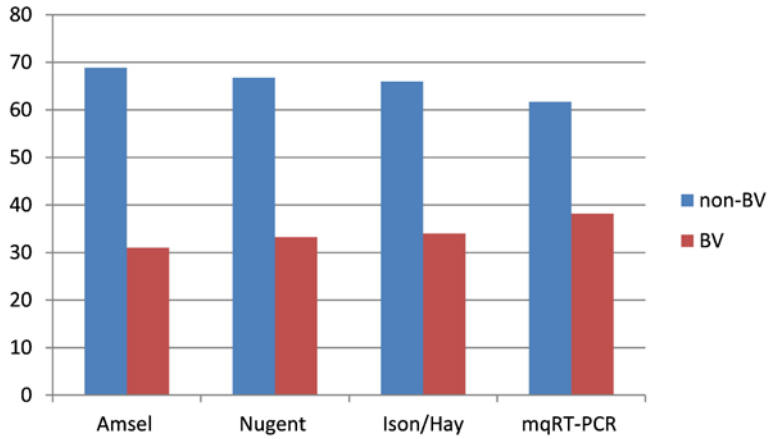

Fig. 2 – Comparison of criteria divided into 2 groups [nonbacterial vaginosis (non-BV) – normal and intermediate results of vaginal swabs and bacterial vaginosis (BV)]. mqRT-PCR – multiplex quantitative real-time polymerase chain reaction.

standard' in literature, we compared other methods for BV diagnosis with the Nugent one.

Our research indicates that the 'gold standard' criterion should avoid an intermediate group. Nugent score takes into consideration only three bacterial morphotypes, which makes it difficult to comprehensively and accurately describe the diversity and complexity of the vaginal microflora. The criteria do well to describe patients with healthy or BV flora, but those with intermediate results are overlooked in most studies, remaining undefined from the point of epidemiology, clinical studies, and therapy. With the application of Nugent criteria, a significant number of women with potentially different vaginal flora (aerobic vaginitis, coinfection, mixed infection) would remain undetected. In our study, 60 (25.5%) patients were classified as intermediate by Nugent criteria which was the highest of any of the methods evaluated here.

Our results revealed the best agreement between Nugent and Ison/Hay criteria (94.9%), noting that due to our diagnostic verification, Ison/Hay criteria had been reduced to three groups. Modified Ison/Hay criteria essentially reflect Nugent classification, while the score description uses qualitative assessment for the presence of certain bacterial morphotypes instead of bacterial counts. The difference between these two methods would have been greater if we had not reduced the number of groups. Ison and Hay highlighted the fact that the microscopic results of vaginal discharge are more complex than those assessed by Nugent criteria which define only three cell morphotypes. However, the introduction of new groups is limited by the fact that the new methodology must 'survive' comparison with the gold standard, hindering the widespread implementation of these new methods.

The results of the comparison of Nugent and Ison/Hay criteria in our study are similar to the results from the original work of Ison/Hay, where the agreement of these two criteria was very good (*kappa* coefficient = 0.91) [10]. In their study, Chawla et al. [14] also compared these two criteria. However, their approach to statistical analysis by merging the intermediate group with normal results yielded a *kappa* coefficient of 0.83. Comparatively, merging the intermediate group with BV provided better agreement compared to the Nugent criteria (*kappa* coefficient = 0.9).

Amsel criteria are still widely employed in both research and scientific setting and are often used as the gold standard alongside Nugent criteria, according to the literature. We consider that the main flaw of this method is the classification of patients into only 2 groups despite various broad-spectrum disturbances of vaginal microflora. In our research, the agreement between these two methods is good (90.2%). Because of only two groups in Amsel criteria, Nugent criteria were reduced to BV and non-BV, which includes patients with healthy and intermediate vaginal flora. Mohammadzadeh et al. [15] similarly evaluated the agreement between Amsel and Nugent criteria, with results similar to our current study (*kappa* coefficient = 0.8). In comparison, Mahajan et al. [16] reported the agreement between these two criteria to be moderate, with a markedly lower efficiency score (*kappa* = 0.58).

Perhaps the best illustration of the flaws and problems linked to two 'gold standards' are presented in two independent studies by Sha et al. [17] and Amit et al. [18]. Based on Nugent criteria, Sha et al. [17] diagnosed BV in 203 patients, while only 75 were diagnosed using Amsel criteria. Therefore, 128 women were presented with false-negative results compared to the Nugent criteria. On the contrary, Amit et al. [18] diagnosed BV using Amsel criteria in 145 patients, while 79 were diagnosed with the BV using Nugent criteria. Therefore, 66 women were presented with false positive results. It is highly unlikely that the knowledge and skills of these two groups of researchers differ so much to obtain almost completely contradicting results. What seems like a more logical and plausible explanation is that these opposing diagnoses are the result of imperfections and flaws in current diagnostic criteria.

Accurate interpretation for Nugent scoring requires previous experience and skills in microscopy. In addition, the evaluation of the microscopic results by the Nugent method can be difficult as there are no defined criteria for differentiation of the three basic morphotypes in the scoring system. The biggest disagreements are related to issues such as what morphotypes to consider as Gram-positive rods, i.e., counting them as *L.*, what are the differences between cocci and short rods, and how to identify morphotypes bacteria such as *G. vaginalis* and *Prevotella* as they can vary in morphology from round to elongated (conditional to the coloring aspects) [19]. Evaluation of microscopic samples also highlights the difficulty of differentiating *G. vaginalis* and *L. iners*. *G. vaginalis* is a Gram-variable, i.e., it can be color stained as 'Gram-positive' or 'Gram-negative', while *L. iners* is difficult to identify when stained as it will present as 'Gram-negative'. That is because *L. iners* more often takes the morphological shape of *coccobacilli* than *bacilli*. The current research in microbiology indicates that these two microorganisms can be detected in healthy women as well as in those with BV (50–90%), which can lead to incorrect conclusions in categorization by Nugent score [20, 21]. Considering all these shortcomings, it is clear why the results of different studies and research teams differ to such a great extent.

In the last decade, with the advent of molecular methods, it has been discovered that the qualitative and quantitative diversity of the vaginal microbiome is much more complex than previously considered based on microscopic analysis and culture testing [11, 22]. Multiplex PCR quantitative testing used in our research detected the presence of *L. spp.*, *G. Vaginalis*, and *A. vaginae*, as well as the total DNA concentration of bacteria. Based on the mutual relationship between these bacteria, all patients were divided into 6 categories. The results showed that the largest number of BV diagnoses – 90 (38.3%), were detected using the mqRT-PCR method. The intermediate group included 15 (6.4%) patients with vaginal flora of non-specified etiology, while 9 (3.8%) patients were presented as intermediate vaginal flora.

The biggest discrepancies in our research are identified between patients classified as the intermediate group.

Twenty-eight patients classified in the intermediate group by the Nugent score were healthy, while 18 women who were assessed as intermediate by the Nugent score were diagnosed with BV in mqRT-PCR testing. Compared to the Nugent score, mqRT-PCR testing detected more patients with healthy or BV vaginal flora. Our results show moderate agreement (74%) between these two methods. However, the intermediate results obtained by the mqRT-PCR method are more reliable, which gives this method a considerable selective advantage compared to the microscopic methods. Besides, the advantage of the mqRT-PCR test is in its objectivity, the potential to quantify bacteria, and detect imbalance of flora of unknown etiology, which is important for assessing the vaginal flora status. In addition, the possibility of detecting *A. vaginae* may be important for therapeutic approaches because of the resistance of this bacteria to metronidazole. The limitation of mqRT-PCR used in this study is that it detects the presence of only two of the most frequent anaerobic bacteria, as well as an inability to distinguish species within the *L. spp* group.

Similar to our research methodology, the study done by Van den Munckhof et al. [23] used the same mqRT-PCR test for the detection of BV (AmpliSensFlorocenosis/Bacterial vaginosis-FRT). The findings of this study pointed out that the mqRT-PCR test showed the biggest agreement with Amsel, Nugent, culture, and BD MAX Vaginal Panel, based on 16sRNA genomic sequencing for microbiota analysis. Compared to genomic sequencing, culture sensitivity was 29.9%, Amsel method sensitivity was 61.5%, Nugent criteria and BD MAX Vaginal Panel sensitivity were 63.9%, while the mqRT-PCR test showed a sensitivity of 80.6% [23]. In their study, Dhiman et al. [24] compared the Nugent score and RT-PCR for BV detection in 125 patients of reproductive age, and they achieved results of total agreement similar to our results (81.8%).

A limitation of the current study is our exclusion of the existence of other vaginal dysbiosis and our focus on the diagnosis of BV only. Future studies should aim to include a complete analysis of the vaginal microbiome to provide a more accurate and 'broader picture' of pathogenesis since the presence of other conditions of vaginal dysbioses, such as aerobic vaginitis and overgrowth of *Candida spp.*, can significantly influence the assessment of the vaginal microflora status.

Conclusion

Although Nugent criteria have been used in BV diagnostics for the past 30 years, the existence of the intermediate group raises questions about the applicability of this diagnostic tool in research and scientific setting. Our results report that Nugent and Ison/Hay criteria carry the greatest accordance, followed by Amsel criteria. The mqRT-PCR method was denoted as the least in agreement with Nugent criteria, attributed to stark differences in underlying methodology and molecular biological principles. Considering that the mqRT-PCR method is more efficient at differentiating patients with intermediate results compared to Nugent and Ison/Hay methods, we concluded that mqRT-PCR is the best choice for BV diagnoses. Compared to Amsel and Nugent methods that divide patients into 2 or 3 categories, the mqRT-PCR method recognizes other conditions of vaginal flora that are important for correct diagnoses and the application of better therapeutic approaches.

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