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CHAPTER 4

Developing an algorithm for the diagnosis of abnormal vaginal discharge in a Dutch clinical setting: a pilot study

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ABSTRACT

Abnormal vaginal discharge may be caused by bacterial vaginosis, vulvovaginal candidiasis, trichomoniasis and/or aerobic vaginitis. For the development of a diagnostic algorithm, tree-based classification analysis was performed on symptoms, signs and bedside test results of 56 patients, and laboratory tests (culture, Nugent score, qPCRs) were compared. Amplicon sequencing of the 16S rRNA gene was used as reference test for bacterial vaginosis and aerobic vaginitis, culture for vulvovaginal candidiasis and qPCR for trichomoniasis. For bacterial vaginosis, the best diagnostic algorithm was to screen at the bedside with a pH and odour test and if positive, to confirm by qPCR (sensitivity 94%; specificity 97%) rather than Nugent score (sensitivity of 59%; specificity 97%; p=0.031). The analysis for the other infections was less conclusive due to the low number of patients with these infections. The developed algorithm is sensitive, specific and reduces the need for laboratory tests in 50% of the patients.

INTRODUCTION

Abnormal vaginal discharge is the most common gynaecological reason why women of reproductive age consult their general practitioner (1). Abnormal vaginal discharge may be caused by (i) bacterial vaginosis (BV; 22-50% of cases); (ii) vulvovaginal candidiasis (VVC; 17-39% of cases); (iii) trichomoniasis (4-35% of cases), (iv) aerobic vaginitis (AV; 7-12% of cases) or mixed infection (<5% of cases in the western world) (2-5). For 24-40% of the patients with abnormal vaginal discharge no cause can be found (6-8). BV and AV are both polymicrobial syndromes characterized by a shift from *Lactobacillus*-dominated vaginal microbiota to a dysbiotic microbiota dominated by anaerobes or aerobes, respectively. VVC is a fungal infection, commonly caused by *Candida albicans*, whereas trichomoniasis is a sexually transmitted infection (STI) caused by *Trichomonas vaginalis* (TV). The presence of both BV and VVC is the most common mixed infection (3). These infections are associated with a number of adverse sequelae in obstetrics and gynaecology, including increased susceptibility to sexually transmitted infections and preterm birth (9).

Misdiagnosis has been hypothesised to be the main cause for up to 40% of the patients to return to their physician with persistent symptoms after treatment (3, 5, 10, 11). Alternative reasons for therapeutic failure may be incomplete eradication of pathogens during treatment, antimicrobial or antifungal resistance, the emergence of VVC after antibiotic treatment of BV, or a STI (re)infection from an untreated or new partner (12-16).

The 2018 European International Union against Sexually Transmitted Infections (IUSTI) World Health Organisation (WHO) guideline on the management of vaginal discharge recommends diagnosing BV, VVC, TV and AV using clinical symptoms, clinical signs and bedside tests, supported by laboratory test findings (17). However, no diagnostic algorithm is proposed but instead all options are presented (**Table 1**). For BV, Gram-stained microscopy (Nugent score) (18, 19) as well as CE-IVD marked quantitative real-time PCRs (qPCRs) assays are recommended as laboratory tests (20-24).

The aim of this pilot study was to develop an algorithm to diagnose women with abnormal vaginal discharge. The first step was to determine which combination of clinical symptoms, clinical signs and bedside test results were the strongest associated with BV, VVC, TV, AV and mixed infection. The second step was to determine the best performing laboratory tests for confirmation of the diagnosis. In retrospect, it was determined whether implementation of the algorithm would have reduced the number of patients that returned to their physician with persistent symptoms due to misdiagnosis.

Table 1. Overview of clinical symptoms and signs, bedside tests results, and available laboratory tests (17)

	Bacterial vaginosis	**Vulvovaginal candidiasis**	***Trichomonas vaginalis***	**Aerobic vaginitis**
Clinical symptoms	• Malodorous discharge (fishy odour)	• Vulval itching • Vulval soreness/ irritation • Dyspareunia	• Malodorous discharge • Vulval itching • Vulval soreness/ irritation • Dysuria • Rarely lower abdominal discomfort	• Vulval soreness/ irritation • Dyspareunia
Clinical signs	• Thin white homogenous discharge coating walls of vagina and vestibule[1]	• Curdy discharge • Vulval erythema and oedema	• Yellow-green discharge • Vulval/vaginal erythema and oedema • Cervical erythema 'Strawberry cervix'	• Purulent discharge • Vaginal erythema and oedema • Vaginal ulceration
Bedside tests • Vaginal pH • Amine odour test • Wet-mount microscopy	 • >4.5[1] • Positive[1] • Clue cells[1]	 • ≤4.5 • Negative • Pseudohyphae	 • >4.5 • Positive • Flagellated protozoa	 • >4.5 • Negative • Aerobic vaginitis score[2]
Laboratory tests	• Gram-stained microscopy (Nugent score[3] or Hay Ison criteria[4]) • CE-IVD marked assays	• Culture of *Candida* spp.	• CE-IVD marked qPCR	• Culture of aerobic bacteria such as *S. agalactiae, S. aureus* and *E. coli*

[1]Amsel's clinical criteria for diagnosis of bacterial vaginosis (25).

[2]Aerobic vaginitis (AV) score combines information about *Lactobacillus* morphotypes, epithelial disruption and inflammation (26).

[3]Nugent score is based on the quantitative assessment of *Lactobacillus*, *Gardnerella* and *Mobiluncus* morphotypes (18).

[4]Hay Ison criteria is a simpler version of the Nugent score (19).

MATERIALS AND METHODS

Collection of samples and clinical data

All procedures performed were in accordance with the ethical standards of the local ethics board (METC Zuidwest Holland, The Hague, The Netherlands) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (No. 14-099; date of approval 16 January 2015). Written informed consent was obtained from all individual participants included in the study.

Sixty-four premenopausal women with complaints of abnormal vaginal discharge (increase in volume, 'thick or cheesy' in consistency, malodorous, itchy causing irritation, and/or a different colour from the norm of that woman) visiting the Gynaecology outpatient

clinic of the Haaglanden Medical Centre (The Hague, The Netherlands) between January and July 2015 were recruited for this study. At visit 1, gynaecological examination and a standardised interview with respect to clinical symptoms and signs were performed (**Table 1**). Vaginal secretions were collected for bedside and laboratory tests. Patients that did not complete the interview/gynaecological examination or had an indeterminate result for a bedside/laboratory test were excluded from the analysis. Amplicon sequencing of the 16S ribosomal RNA (rRNA) gene was used as reference test for BV/AV, culture for VVC, and qPCR for TV. Therapy was initiated according to routine hospital practice: treatment was initiated immediately if the clinical symptoms and signs were obvious, but if the clinical diagnosis was uncertain, treatment was postponed awaiting the culture results. Patients were treated according to the European guideline (27). A follow-up visit was scheduled approximately four weeks after visit 1. During this visit, clinical data and sample collection was repeated.

Bedside tests

Three bedside tests i.e., pH test, amine odour test and wet-mount microscopy, were performed by the physician. The pH test and amine odour test are part of Amsel's clinical criteria (25). pH of vaginal secretions was determined using pH indicator strips with a pH range from 4.0 to 7.5 (Johnson Test Papers, Oldbury, UK). A microscopic slide of vaginal secretions was prepared for detection of a fishy odour after addition of 10% potassium hydroxide (KOH). Another microscopic slide was prepared for detection of clue cells, pseudohyphae, and flagellated protozoa by wet-mount microscopy. The AV score was not determined (26).

Laboratory tests

Gram-stained microscopic slides were analysed to determine the Nugent score (18). Briefly, a score was generated by assessing the ratio of *Lactobacillus*, *Gardnerella vaginalis*, and *Mobiluncus* morphotypes. A score of 0-3 (normal) and 4-6 (intermediate) were interpreted as BV negative, and a score of 7-10 as BV positive. Poor quality slides were classified as indeterminate.

Culture of *G. vaginalis* for the diagnosis of BV was performed in the routine laboratory setting using vaginal secretions obtained with eSwabs as described previously (24). For the culture of yeasts, eSwabs were inoculated on Brilliance™ *Candida* Agar (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and incubated at 35°C in ambient air. Subcultures of *Candida* spp. were prepared for species identification by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) technology (Bruker corporation, Billerica, USA). Aerobic culture for the diagnosis of AV was not performed.

For molecular methods, vaginal secretions were obtained with an eSwab. DNA isolation and microbiota analysis were performed as described previously (24). Briefly, DNA was extracted with the MagNA pure 96 (Roche Diagnostics, Basel, Switzerland). V3-V4 amplicons

of the 16S rRNA gene were sequenced with the MiSeq desktop sequencer and analysed with MiSeq Reporter software (Illumina, San Diego, USA). Based on the microbiota profiles, samples were categorised as normal vaginal microbiota (>47% relative abundance of *Lactobacillus*), microbiota associated with BV (≤47% relative abundance of *Lactobacillus* and mainly anaerobes) or as microbiota associated with AV (≤47% relative abundance of *Lactobacillus* and mainly aerobes). The extracted DNA was also used for the following CE-IVD marked qPCRs: AmpliSens® Florocenosis/Bacterial vaginosis-FRT PCR kit (henceforth referred to as BV qPCR; InterLabService, Moscow, Russia) which uses relative concentration of *Lactobacillus* spp., *Gardnerella vaginalis*, *Atopobium vaginae* and total bacteria to diagnose BV, AmpliSens® Florocenosis/*Candida*-FRT PCR kit (henceforth referred to as VVC qPCR; InterLabService) targeting *Candida albicans*, *Candida glabrata* and *Candida krusei*, *Trichomonas vaginalis* real-time PCR assay (Diagenode Diagnostics, Seraing, Belgium) and the Cobas 4800 CT/NG v2.0 test (Roche Diagnostics) and the *Mycoplasma genitalium* real-time PCR assay (Diagenode) for detection of other STIs. For diagnosis of BV, only the AmpliSens BV assay was included since we previously showed that this was the best CE-IVD marked qPCR available for the diagnosis of BV (24). All qPCRs were performed according to the manufacturer's instructions using a LightCycler 480 or Cobas 4800 Instrument (Roche Diagnostics).

Availability of data and materials

Sequencing data are available in the NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) repository with the accession number PRJNA524112.

Statistical analysis

For statistical analysis the software package SPSS (IBM, Chicago, IL, USA) version 25 was used. First, univariate analysis was performed to determine which symptoms, signs and bedside test results were associated with BV, VVC, TV, AV and mixed infection using the chi-squared test. Subsequently, a diagnostic algorithm to distinguish between BV, VVC, TV, AV and mixed infection was developed by building a tree-based classification model using CHAID (Chi-Squared Automatic Interaction Detection). Sensitivity and specificity of different tests were compared using the McNemar test.

RESULTS

Population characteristics

Sixty-four women complaining of abnormal vaginal discharge were recruited. The mean age of these patients was 34 years (range 18-52 years), 19 (30%) were pregnant and the majority of the patients were of European origin (**Supplementary Table S1**). Eight patients

did not complete the interview/gynaecological examination or had an indeterminate result for the pH test, leaving 56 patients for further analysis. These 56 patients were categorised as BV positive (n = 17), VVC positive (n = 7), AV positive (n = 5), mixed infection (BV and VVC; n = 3) or BV, VVC and AV negative (n = 24) using microbiota analysis as the reference test for BV and AV, and culture of *Candida* spp. for VVC. None of the patients was positive for TV according to the qPCR assay.

Determination of the best diagnostic algorithm

Step 1: screening based on clinical symptoms and signs, and bedside test results. To determine which combination of symptoms, signs and bedside test results were strongest associated with BV, VVC, AV and mixed infection, first univariate analyses were performed using microbiota analysis and yeast culture as reference test (**Supplementary Table S2**). **Table 2** summarises the data of the variables that were statistically significant associated with the different entities. A vaginal pH > 4.5 was most strongly indicative for BV, AV and mixed infection. Also, malodorous discharge, positive amine odour test, and detection of clue cells by wet-mount microscopy were significantly associated with BV. For VVC, curdy discharge and detection of pseudohyphae by wet-mount microscopy were the strongest predictors. For AV, the strongest predictors were lower abdominal discomfort and vulval/vaginal erythema and oedema. The latter was also significantly associated with mixed infection. Comparable results were obtained using the BV and VVC qPCRs as reference tests (**Supplementary Table S3**).

Subsequently, a tree-based classification analysis was performed with all variables that were significant associated with the different entities (**Supplementary Figure S4**). This multivariate analysis showed that a vaginal pH test, the amine odour test and the presence of lower abdominal discomfort was the best combination to distinguish between BV, VVC, AV and mixed infection. The presence of curdy discharge or vulval/vaginal erythema and oedema, and the detection of clue cells or pseudohyphae by wet-mount microscopy were not of added value. This screening step is the first part of the diagnostic algorithm (**Figure 1: step 1**).

Step 2: confirmation of the diagnosis using laboratory tests. According to the European guideline, the diagnosis based on the bedside tests **(Figure 1: step 1**) should be confirmed by laboratory tests (**Figure 1: step 2**). For BV, both the Nugent score and the BV qPCR are suggested as confirmation test. Bedside tests followed by qPCR as confirmation test resulted in a sensitivity of 94%, while using the Nugent score as confirmation test yielded a sensitivity of 59% (p = 0.031, McNemar test). Specificity of both was 97%. This implies that by using the Nugent score instead of the qPCR the diagnosis BV would have been missed for six patients (35%). As further note, our proposed algorithm showed significant better performance than the routine diagnostic approach of the local hospital based on clinical symptoms, signs and culture of

G. vaginalis, which is still applied in many hospitals and among general practitioners (data not shown).

For VVC, both culture and qPCR were evaluated as confirmation test. The VVC qPCR confirmed all 10 VVC culture positive samples (8 *C. albicans*, 1 *C. glabrata*, 1 *C. krusei*; mean 23.66 Ct; range 19.10-32.35 Ct), and identified three additional positive samples with a slightly higher mean Ct value of 29.55 (2 *C. albicans*, 1 *C. krusei*; 21.65-35.90 Ct). Screening followed by yeast culture or qPCR as confirmation test resulted in both cases in a sensitivity of 71% and specificity ≥ 96% (**Figure 1: step 2**).

Table 2. Clinical symptoms, clinical signs and bedside tests associated with BV, VVC, AV and mixed infection

	Microbiota analysis as reference test for BV (n = 17)		**Culture of *Candida* spp. as reference test for VVC (n = 7)**		**Microbiota analysis as reference test for AV (n = 5)**		**Mixed infection (positive for BV and VVC; n = 3)**	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Clinical symptoms								
• Malodorous discharge	**88%**	**49%**	43%	35%	60%	37%	67%	38%
• Lower abdominal discomfort	59%	44%	43%	41%	**100%**	**47%**	0%	40%
Clinical signs								
• Curdy discharge	6%	69%	**57%**	**82%**	0%	75%	33%	77%
• Vulval/vaginal erythema and oedema	12%	77%	29%	82%	**60%**	**84%**	**67%**	**83%**
Bedside test results								
• Vaginal pH > 4.5	**94%**	**62%**	29%	41%	**100%**	**49%**	100%	47%
• Positive amine odour test	**94%**	**69%**	29%	47%	20%	47%	33%	49%
• Detection of clue cells by wet-mount microscopy	**77%**	**56%**	NA	NA	NA	NA	100%	49%
• Detection of pseudohyphae by wet-mount microscopy	NA	NA	**57%**	**84%**	NA	NA	67%	81%

AV: aerobic vaginitis; BV: bacterial vaginosis; NA: not applicable; VVC: vulvovaginal candidiasis.
The bold test characteristics indicate which variables were statically significantly ($p < 0.05$) positive associated per infection using the chi-squared test.

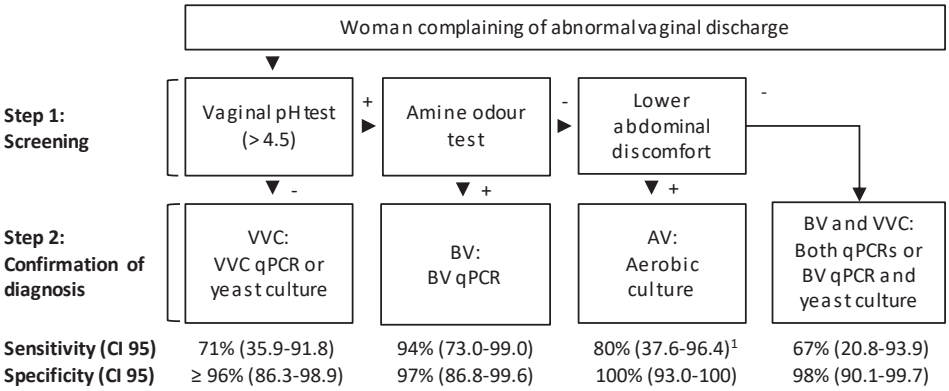

Figure 1. Best algorithm based on clinical symptoms, bedside and laboratory tests. BV: bacterial vaginosis; VVC: vulvovaginal candidiasis; AV: aerobic vaginitis; BV and VVC: mixed infection. [1]Sensitivity and specificity are calculated based on the screening results since data of aerobic culture is lacking.

For mixed infection (BV and VVC), screening followed by either both qPCRs or the combination BV qPCR and yeast culture resulted in a sensitivity of 67% and specificity of 98%. Performing standard both qPCRs or the combination BV qPCR and yeast culture would result in a sensitivity of 100% for the diagnosis of BV, VVC and mixed infection. For AV, no CE-marked qPCR is yet available leaving aerobic culture as the only confirmation test, which was not performed in this study.

Evaluation of algorithm

In retrospect, it was determined whether implementation of the algorithm as depicted in **Figure 1** would have reduced the number of patients that returned to their physician with persistent symptoms due to misdiagnosis. Eight of the 56 included patients failed to attend both visits. Of the remaining 48 patients, 27 (56%) patients returned at visit 2 with persistent symptoms. For each of these patients, microbiota (BV and AV), culture (VVC) and qPCR (VVC and STIs) data of both visits were compared (**Figure 2**). Based on this comparison, more insight in the possible cause of the persistent symptoms could be obtained. Eight (30%) of the 27 patients were misdiagnosed at the first visit (*red*). This number would have been three (11%) if our proposed algorithm was used. Treatment failure (*orange*) or the emergence of a (different) infection (*purple*) were responsible for the persistent symptoms in another eight (30%) patients. No cause was found for 10 (37%) patients (*green*) of which seven had already negative reference test results at their first visit. One pregnant patient (4%) was BV positive at her first visit but was not treated for BV as she delivered before the test results became available (*blue*). She remained BV positive after giving birth. None of the patients were positive for a STI at both visits.

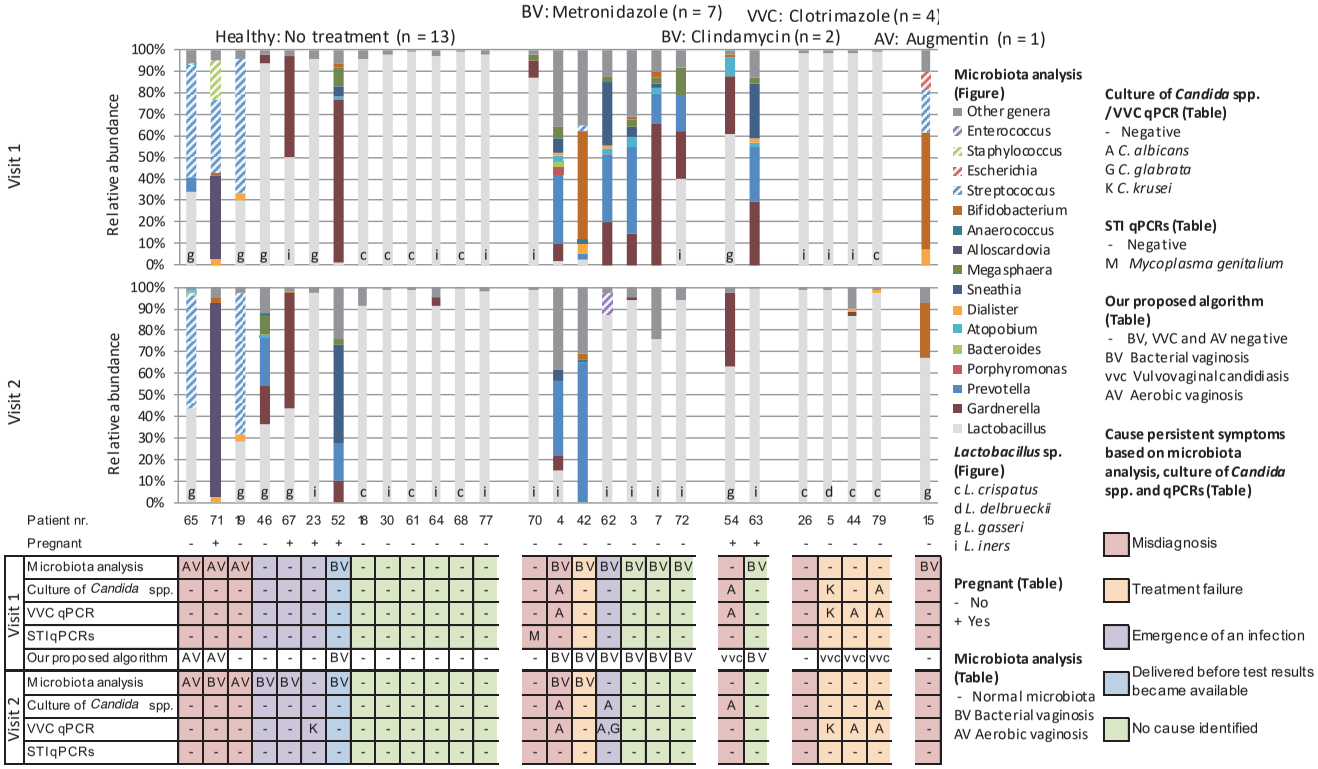

Figure 2. Retrospective evaluation of the proposed algorithm using data from 27 patients with persistent symptoms. Current treatment was initiated based on the routine diagnostic approach of the hospital using clinical symptoms, signs and culture results. Patients diagnosed with BV were treated with metronidazole or clindamycin in case of pregnancy or lactation. Patients diagnosed with VVC were treated with clotrimazole. One patient was treated with amoxicillin-clavulanic acid for a vaginal *Escherichia* coli infection. For each patient, cause of persistent symptoms was determined by comparison of data obtained during both visits. Misdiagnosis was defined as a disagreement between the test outcomes at visit 1 and the current treatment decision (*red*), treatment failure as an agreement between the positive test outcomes of both visits and the current treatment decision (*orange*), and emergence of an infection as a disagreement between the positive test outcomes of both visits (*purple*). No cause was identified when the patient was not or correctly treated and all test results of visit 2 were negative (*green*). One pregnant patient was BV positive at her first visit but was not treated for BV as she delivered before the test results became available (*blue*).

DISCUSSION

To the best of our knowledge, this is the first study developing an algorithm to diagnose women with BV, VVC, AV or mixed infection based on the clinical symptoms, clinical signs, bedside and laboratory tests as described in the European guideline. Microbiota analysis was used as reference test for BV and AV, and culture for VVC. The results of this study suggest that with a simple algorithm BV can be identified with a high degree of certainty, and the need of laboratory tests to be performed and the number of patients returning to the physician with persistent symptoms can be reduced significantly.

This study showed that none of the clinical symptoms or signs can differentiate between BV, VVC, AV and mixed infection, whereas the combination of two bedside tests (pH and amine odour test) turned out to be of diagnostic value to differentiate between BV or AV and other entities (step 1). In line with previous reports, an elevated vaginal pH was indicative for patients with a dysbiotic vaginal microbiota (26, 28-30). The amine odour test was required to differentiate between BV (BV is more likely when test positive) and AV (AV is less likely when test positive). Patients with a mixed infection of BV and VVC had an elevated pH and a negative amine odour test. The presence or absence of lower abdominal discomfort was found to differentiate between AV and mixed infection (BV and VVC positive). Patients with a normal vaginal pH had most likely VVC. However, both observations of mixed infection associated with negative amine odour test and lower abdominal discomfort differentiating AV from mixed infection were based on a low number of positive samples and should be confirmed in a larger population.

The clinical tests should be followed by a confirmation test (step 2). Patients with an elevated pH and positive amine odour test should be tested for BV, normal pH for VVC, elevated pH, negative amine odour test and presence of lower abdominal discomfort for AV and elevated pH, negative amine odour test and absence of lower abdominal discomfort for BV and VVC. The BV qPCR performed significant better as confirmation tests for BV than the Nugent score. For the detection of *Candida* spp., the test characteristics of culture and the VVC qPCR were comparable. The advantage of performing the VVC qPCR, next to the BV qPCR, is the short turnaround time and the necessity of submitting one sample only. The reasons to perform the culture rather than the VVC qPCR are the probably lower costs, detection of all yeasts and the possibility to perform susceptibility testing. For AV, only aerobic culture is available as confirmation test.

Instead of the abovementioned algorithm one could choose to routinely perform both BV and VVC qPCRs and aerobic culture for detection of AV. To reduce the number of aerobic cultures, an alternative route would be to perform aerobic culture based on the outcome 'unspecified dysbiosis' by the BV qPCR since this result is indicative for the diagnosis of AV (24). Routinely performing BV and VVC qPCR (and AV culture) make bedside tests, which are time consuming and unpleasant to perform (31, 32), redundant and provide a better

diagnostic outcome but likely increase the laboratory costs. In our study population, the result of the bedside tests indicated no BV in approximately 50% of the patients, reducing the number of diagnostic assays to be performed and associated costs by the same percentage.

The best diagnostic approach to detect TV could not be determined. Our study population lacked TV positive patients and was at low risk for STIs. We suggest testing patients who are at risk for TV and other STIs with qPCR. However, the developed diagnostic algorithm may not extrapolate to regional or racial groups with high risk for TV and other STIs. Furthermore, performing a qPCR is only feasible in resource-rich settings.

This study also provides more insight in the cause of persistent symptoms. Misdiagnosis, treatment failure and emergence of a different infection after treatment were important causes of persistent symptoms. However, for approximately 40% of the patients experiencing (persistent) abnormal vaginal discharge no cause could be found. These patients probably have physiological discharge or may suffer from other conditions, such as cervicitis, mucoid ectopy, vulval dermatoses or allergic reactions. In agreement with our findings, others reported that for 24-40% of the patients with abnormal vaginal discharge no cause could be found (6-8). Implementation of the proposed algorithm might have reduced the number of patients that returned to their physician with persistent symptoms by approximately 20%.

A limitation of this study is the small study population. The analysis of the clinical symptoms, clinical signs and bedside test results for the diagnosis of VVC, TV, AV and mixed infection were less conclusive, since each group contained less than seven positive patients. A larger study population is required to validate the proposed algorithm. Another limitation is the lack of aerobic culture and AV score data. AV is a relatively newly recognised cause of vaginal discharge, which is the reason why Aerobic culture and AV score were not included in the study design (26). In a follow-up study, these methods should be included to confirm the redundancy of the AV score in the test algorithm and the utility of the aerobic culture as confirmation test. The advantage of this study is the development of an algorithm for BV, VVC, AV and mixed infection instead of a separate algorithm for each entity using microbiota analysis as reference test for BV and AV.

CONCLUSIONS

The best algorithm to diagnose BV is to screen at the bedside with a pH test and amine odour test, and if positive, to confirm by qPCR. This is a sensitive and specific approach, and in line with the 2018 European (IUSTI/WHO) guideline. Furthermore, application of this algorithm reduces the need for laboratory tests significantly and reduces the number of patients with misdiagnosis, leading to less patients returning to the physician after treatment.

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SUPPLEMENTAL APPENDIX

Supplementary Table S1. Population characteristics

Characteristics	Women with complaints of abnormal vaginal discharge (n = 64)
Age, mean (range)	34 (18-52)
Ethnicity, n (%)	
European	51 (80)
Latin-American	3 (5)
African	2 (3)
East Asian	2 (3)
South Asian	2 (3)
Middle Eastern	2 (3)
Mixed origin	2 (3)
Use of vaginal shower gel, n (%)	5 (8)
Sexually active, n (%)	55 (86)
Number of sexual partners in the past three months, mean (range)	1 (0-1)
Anticonception, n (%)	
No anticonception	38 (59)
Anticonception pill	12 (19)
Levonorgestrel intrauterine devices	9 (14)
Condom	4 (6)
Copper intrauterine devices	1 (2)
Pregnant, n (%)	19 (30)
Breast feeding, n (%)	3 (5)
First day of last menstrual period	At least 4 days ago[1]

[1]Menstrual bleeding results in an indeterminate vaginal pH test, causing exclusion of the patient from the study

Supplementary Table S2. Evaluation of clinical symptoms, clinical signs, and bedside test results for the diagnosis of bacterial vaginosis (n = 17), vulvovaginal candidiasis (n = 7), aerobic vaginitis (n = 5) and mixed infection (n = 3) using microbiota analysis and culture of *Candida* spp. as the reference tests

	Total number of positive women (n = 56)	Microbiota analysis as the reference test for BV			Culture of *Candida* spp. as the reference test for VVC			Microbiota analysis as the reference test for AV			Mixed infection (positive for BV and VVC)		
		Sensitivity (CI 95)	Specificity (CI 95)	p[1]	Sensitivity (CI 95)	Specificity (CI 95)	p[1]	Sensitivity (CI 95)	Specificity (CI 95)	p[1]	Sensitivity (CI 95)	Specificity (CI 95)	p[1]
Clinical symptoms													
Dyspareunia[2]	unknown	Could not be determined			Could not be determined			Could not be determined			Could not be determined		
Dysuria	0 (0%)	Not applicable			Not applicable			Not applicable			Not applicable		
Malodorous discharge	35 (63%)	88% (65.7-96.7)	49% (33.9-63.8)	0.009	43% (15.8-75.0)	35% (22.9-48.7)	0.251	60% (23.1-88.2)	37% (25.3-51.0)	0.904	67% (20.8-93.9)	38% (25.9-51.2)	0.878
Low abdominal discomfort	32 (57%)	59% (36.0-78.4)	44% (29.3-59.0)	0.867	43% (15.8-75.0)	41% (28.2-54.8)	0.414	100% (56.6-100)	47% (34.1-60.5)	0.042	0% (0.0-56.0)	40% (27.6-53.1)	0.040
Vulval itching	25 (45%)	35% (17.3-58.7)	51% (36.2-66.1)	0.353	71% (35.9-91.8)	59% (45.3-71.8)	0.128	40% (11.8-77.0)	55% (41.4-67.8)	0.827	67% (20.8-93.9)	57% (43.3-69.1)	0.430
Vulval soreness/irritation	19 (34%)	29% (13.3-53.1)	64% (48.4-77.3)	0.637	43% (15.8-75.0)	67% (53.4-78.8)	0.594	60% (23.1-88.2)	69% (55.0-79.7)	0.197	33% (6.2-879.2)	66% (52.6-77.3)	0.982
Clinical signs													
Curdy discharge	13 (23%)	6% (1.1-27.0)	69% (53.6-81.4)	0.043	57% (25.1-84.2)	82% (68.6-90.0)	0.023	0% (0.0-43.5)	75% (61.1-84.5)	0.198	33% (6.2-79.2)	77% (64.5-86.6)	0.670
Purulent discharge	0 (0%)	Not applicable			Not applicable			Not applicable			Not applicable		
Thin white homogenous discharge coating walls of vagina and vestibule[3]	18 (32%)	47% (26.2-69.0)	74% (58.9-85.4)	0.115	0% (0.0-35.4)	63% (49.3-75.3)	0.052	20% (3.6-62.5)	67% (53.0-78.0)	0.542	67% (20.8-93.9)	70% (56.5-80.5)	0.188
Vulval/vaginal erythema and oedema	11 (20%)	12% (3.3-34.3)	77% (61.7-87.4)	0.327	29% (8.2-64.1)	82% (68.6-90.0)	0.525	60% (23.1-88.2)	84% (72.0-91.8)	0.017	67% (20.8-93.9)	83% (70.8-90.8)	0.035
Yellow-green discharge	20 (36%)	41% (21.6-64.0)	67% (51.0-79.4)	0.573	43% (15.8-75.0)	65% (51.3-77.1)	0.673	40% (11.8-77.0)	65% (51.0-76.4)	0.834	0% (0.0-56.2)	62% (48.8-74.1)	0.184
Bedside test results													
Vaginal pH > 4.5[3]	31 (55%)	94% (73.0-99.0)	62% (45.9-75.1)	< 0.001	29% (8.2-64.1)	41% (28.2-54.8)	0.128	100% (56.6-100)	49% (35.9-62.3)	0.035	100% (43.9-100)	47% (34.4-60.3)	0.110
Positive amine odour test[3]	28 (50%)	94% (73.0-99.0)	69% (53.6-81.4)	< 0.001	29% (8.2-64.1)	47% (33.7-60.6)	0.225	20% (3.6-62.5)	47% (34.1-60.5)	0.160	33% (6.2-79.2)	49% (36.1-62.1)	0.553
Detection of clue cells by wet-mount microscopy[3]	30 (54%)	77% (52.7-90.4)	56% (41.0-70.7)	0.023	Not applicable			Not applicable			100% (43.9-100)	49% (36.1-62.1)	0.097
Detection of pseudohyphae by wet-mount microscopy	12 (21%)	Not applicable			57% (25.0-84.2)	84% (71.0-91.5)	0.014	Not applicable			67% (20.8-93.9)	81% (68.6-89.4)	0.050

AV: aerobic vaginitis; BV: bacterial vaginosis; CI: confidence interval; VVC: vulvaginal candidiasis.

[1]Chi-squared test was used for statistical comparison

[2]Not all women could confirm the presence or absence

[3]Amsel criteria for diagnosis of BV

Supplementary Table S3. Evaluation of clinical symptoms, clinical signs, and bedside test results for the diagnosis of bacterial vaginosis (n = 14), vulvovaginal candidiasis (n = 7) and mixed infection (n = 6) using the qPCRs as the reference tests

	Total number of positive women (n = 56)	BV qPCR as the reference test for BV[1]			VVC qPCR as the reference test for VVC			Mixed infection (positive BV and VVC qPCR)		
		Sensitivity (CI 95)	Specificity (CI 95)	p[2]	Sensitivity (CI 95)	Specificity (CI 95)	p[2]	Sensitivity (CI 95)	Specificity (CI 95)	p[2]
Clinical symptoms										
Dyspareunia[3]	unknown	Could not be determined			Could not be determined			Could not be determined		
Dysuria	0 (0%)	Not applicable			Not applicable			Not applicable		
Malodorous discharge	35 (63%)	86% (60.1-96.0)	45% (31.2-60.1)	0.038	43% (15.8-75.0)	35% (22.9-48.7)	0.251	83% (43.7-97.0)	40% (27.6-53.8)	0.265
Low abdominal discomfort	32 (57%)	71% (45.4-88.3)	48% (33.4-62.3)	0.212	71% (35.9-91.8)	45% (31.9-58.7)	0.414	17% (3.0-56.4)	38% (25.9-51.9)	0.034
Vulval itching	25 (45%)	43% (21.4-67.4)	55% (40.0-68.8)	0.877	57% (25.1-84.2)	57% (43.3-70.0)	0.477	50% (18.8-81.2)	56% (42.3-68.8)	0.780
Vulval soreness/irritation	19 (34%)	36% (16.3-61.2)	67% (51.6-79.0)	0.871	43% (15.8-75.0)	67% (53.4-78.8)	0.594	33% (9.7-70.0)	66% (52.2-77.6)	0.974
Clinical signs										
Curdy discharge	13 (23%)	7% (1.3-31.5)	71% (56.4-82.8)	0.100	57% (25.1-84.2)	82% (68.6-90.0)	0.023	33% (9.7-70.0)	78% (64.8-87.3)	0.534
Purulent discharge	0 (0%)	Not applicable			Not applicable			Not applicable		
Thin white homogenous discharge coating walls of vagina and vestibule[4]	18 (32%)	50% (26.8-73.2)	74% (58.9-84.7)	0.099	0% (0.0-35.4)	63% (49.3-75.3)	0.052	33% (9.7-70.0)	68% (54.2-79.2)	0.947
Vulval/vaginal erythema and oedema	11 (20%)	14% (4.0-39.9)	79% (64.1-88.3)	0.560	14% (2.6-51.3)	80% (66.4-88.5)	0.703	50% (18.8-81.2)	84% (71.5-91.7)	0.048
Yellow-green discharge	20 (36%)	29% (11.7-54.7)	62% (46.8-75.0)	0.520	43% (15.8-75.0)	65% (51.3-77.1)	0.673	17% (3.0-56.4)	62% (48.2-74.1)	0.303
Bedside tests										
Vaginal pH > 4.5[4]	31 (55%)	100% (78.5-100)	60% (44.5-73.0)	< 0.001	29% (8.2-64.1)	41% (28.2-54.8)	0.128	67% (30.0-90.3)	46% (33.0-59.6)	0.555
Positive amine odour test[4]	28 (50%)	100% (78.5-100)	67% (51.6-79.0)	< 0.001	43% (15.8-75.0)	49% (35.6-62.5)	0.686	17% (3.0-56.4)	46% (33.0-59.6)	0.084
Detection of clue cells by wet-mount microscopy[4]	30 (54%)	79% (52.4-92.4)	55% (40.0-68.8)	0.030	Not applicable			50% (18.8-81.2)	46% (33.0-59.6)	0.853
Detection of pseudohyphae by wet-mount microscopy	12 (21%)	Not applicable			43% (15.8-75.0)	82% (68.6-90.0)	0.140	67% (30.0-90.3)	84% (71.5-91.7)	0.004

AV: aerobic vaginitis; BV: bacterial vaginosis; CI: confidence interval; VVC: vulvaginal candidiasis.

[1]Categrory BV positive

[2]Chi-squared test was used for statistical comparison

[3]Not all women could confirm the presence or absence

[4]Amsel criteria for diagnosis of BV

Supplementary Table S4. Development of diagnostic algorithm to distinguish between bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), aerobic vaginitis (AV) and mixed infection by building a tree-based classification model using Chi-Squared Automatic Interaction Detection. Microbiota analysis and culture of *Candida* spp. were used as reference tests.