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Self-diagnosis of Vulvovaginal Candidiasis is Poor - A Comparison of Diagnostic Methods Introducing β-Glucan as a Complement

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Abstract

Aim: Self-diagnosis of Vulvovaginal Candidiasis (VVC) may result in misuse of over-the counter (OTC) antifungals. In this study the accuracy of self-diagnosis, clinical diagnosis, and laboratory diagnostic methods, including vaginal smear microscopy and a new method for the diagnosis of VVC (β-glucan), were compared using positive yeast culture as gold standard for diagnosis of VVC.

Methods: Women with self-diagnosed VVC (n=88), intending to buy OTC antifungals, were recruited from pharmacies and health care providers. A clinical examination was performed including vaginal samples for quantitative culturing of yeast, for β-glucan determination and vaginal smear microscopy (VSM).

Results: In all symptomatic women, 66% were culture-positive for yeast, 20% had bacterial vaginosis (BV) (12% concurrent with VVC), and 25% were not diagnosable. The sensitivity and specificity for diagnosis of VVC were similar for β-glucan (77% and 97%) and VSM (67% and 97%, respectively), while the sensitivity was low for clinical examination (40%). The sensitivity of VVC diagnosed by analysis of β-glucan was equal to gynecological examination combined with VSM.

Conclusion: The accuracy of self-diagnosis of VVC is poor. To reduce misdiagnosis women should be offered complementary diagnostic methods. For correct diagnosis analysis of β-glucan or a combination of clinical examination and laboratory VSM is recommended. In cases of therapy resistance vaginal yeast cell culture is recommended. A future rapid bedside test of β-glucan would be useful avoiding misdiagnosis.

Keywords: *Candida albicans*; Vulvovaginal candidiasis; Bacterial vaginosis; β-glucan; Vaginal smear microscopy; Self-diagnosis; Over-the-counter antifungals

Abbreviations: VVC: Vulvovaginal Candidiasis; OTC: Over-the Counter; VSM: Vaginal Smear Microscopy

Introduction

Vulvovaginal candidiasis (VVC) affects about three-quarters of fertile women during their lives, and about half of these women will develop another mycosis episode. The most common pathogen is *Candida albicans*, which account for approximately 80% of cases [1].

Recurrent or chronic VVC will develop in 5-8% of women [2,3]. VVC is considered chronic when at least four specific episodes unrelated to antibiotic therapy occur within a year [4]. Although predisposing risk factors are mostly unidentifiable, known aetiologies of recurrent VVC include treatment-resistant *Candida* species other than *Candida albicans*, frequent antibiotic therapy, contraceptive use, immunosuppression, sexual activity, and hyperglycaemia [1,4].

Thus, clinical evaluation of recurrent episodes is essential. Self-diagnosing women may ignore other causes or concurrent infections.

A correct diagnosis of vaginitis is dependent upon patients´ information, clinical findings and interpretation of vaginal specimen laboratory analyses. The clinical signs and symptoms of VVC are not always evident, and may be correlated with the vaginal yeast load [5]. Thus, gynecological examination with or without VSM may result in too few VVC diagnoses [6]. However, also over-diagnosis of VVC based on clinical examination and/or VSM has been reported [7]. Classical acute candidiasis usually begins with itching followed by burning. A white to yellowish granular scentless discharge is often but-especially in the early stages-not always present. Gynecological examination generally reveals florid or patchy redness of the vaginal wall and portio, and occasionally punctiform or patchy whitish exudates, which if wiped off may leave erosions or bleeding areas. However, all these signs and symptoms may not be present, contributing to the difficulty in diagnosing VVC.

Until the past two decades, treatment of VVC remained entirely within gynecologists, but since then antifungal products have been prescription free and available to OTC and thus an easy access for women for self-treatment. The rapid escalation in the sale of these products may imply that women are using them inappropriately. In fact, a history of recurrent VVC has been shown to be associated with symptoms such as vestibulitis and dyspareunia (vulva pain syndrome) [8]. Ferris et al. showed that errant use of OTC antifungals is among self-diagnosed symptomatic women, since almost every second

woman did not have VVC as confirmed by culturing or VSM [9]. In addition, every third woman with a confirmed VVC also had a concurrent bacterial vaginosis.

Antifungal agents can effectively treat mucosal candidiasis, however resistant strains are not uncommon among women colonized or infected with Candida [10-12]. In recent years a change in epidemiological trends has been observed. The incidence of VVC appears to increase and presents a high probability to recur [13] and a significant increase in infections caused by non-*albicans* species of candida has been stated, the most common of which is *Candida glabrata* [14].

The purpose of the study was to investigate the frequency of VVC among self-diagnosed symptomatic women, who were going to purchase OTC antifungal products for treatment of their assumed VVC, using yeast-positive culture as gold standard. Furthermore, we wanted to assess the accuracy by which clinical examination and laboratory methods (VSM, quantification of a biomarker of fungi [1-3]-β-D glucan) agreed with yeast-culture positive VVC, and whether the vaginal fungal load affected the clinical and laboratory diagnoses of VVC.

Methods

Study population

Fertile women, who were self-diagnosed with VVC and either contacted pharmacy or gynecological clinic (midwife) for advice to buy an OTC product for medical treatment, were admitted to a gynecologist the very same day for evaluation and culture sampling. Women were informed about the study and signed the approved consent form. Patients were excluded from the study if they had their menstrual period, if they had taken antimicrobial agents during the preceding 4 weeks, if they had had intercourse during the previous 24 hours. Demographic information was obtained and a questionnaire completed. The survey included questions about symptoms, earlier diseases, parity, miscarriage, contraceptives, history of vaginitis and other personal characteristics (Table 1).

Characteristics	Number of women (%)
Number of women, n=92	
Mean age, years	31 (29-34)*
Women who answered n=63	
Marital status	
single	12 (19%)
co-habitating/married	51 (81%)
Women who answered n=87	
Established male sexual Partnership	63 (72%)
Women who answered n=81	
Job status	
Employed	48 (59%)
Students	28 (35%)
Unemployed	5 (6.2%)
Women who answered n=77	
Contraceptives	
No	37 (48%)
Oral contraceptives	31 (40%)
IUD-Cu	4 (5.2%)
IUD-L	5 (6.5%)
Women who answered n=92	
History of prior genital infection	
Human papilloma virus	4 (4.4%)
Bacterial vaginosis	11 (12%)
Chlamydia	7 (7.6%)
Genital herpes	5 (5.4%)
Cervical intraneoplasia	6 (6.5%)
Women who answered n=84	
Urinary infection	47 (56%)
Women who answered n=68	
Earlier candida episodes	
0	1 (1.5%)
1-3[1]	24 (35%)
5-10	21 (31%)
>10	22 (32%)
*95% confidence interval of the mean. [1]None had 4 episodes of Candida.	

Table 1: Characteristics of women entering the study by self-diagnosis and purchase of antifungal at the pharmacy.

Clinical examination and specimen collection

Specimens were obtained during speculum examination of the vagina. A dry sterile speculum was inserted before any other vaginal examination was performed. Care was taken to avoid contact with the external genitalia and other sources of contamination.

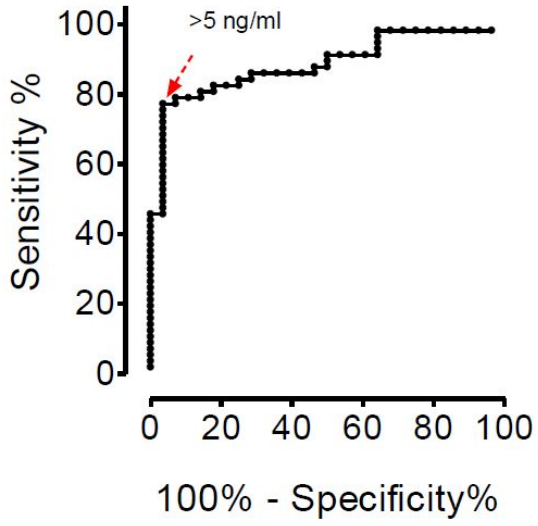
Two cotton-tipped swabs were taken from the posterior vault at different sites and immediately placed in 1 ml of sterile water each. A third swab was taken from yet another different site in the same region and immediately placed into transport medium. A fourth swab was taken from the skin in the introitus of the vagina and immediately put into transport medium. Vaginal pH was measured on the speculum by use of pH strips with a range of 4.0-7.0 (Merck).

The clinical diagnosis of VVC was based on erythema of the vaginal mucosa together with swelling and an adherent yellow-white grainy discharge. Women not diagnosable were ruled as not having atrophic vaginitis, irritant dermatitis, cervicitis, acute salpingitis, or diabetic pruritus. Two experienced gynecologists performed the clinical examinations.

Laboratory diagnosis of VVC and BV

Upon arrival to the research laboratory (Department of Infectious Diseases/Clinical Bacteriology, Sahlgrenska Academy, University of Gothenburg), the two swabs in transport medium were transferred to tubes containing 1 ml of 0.03 M NaCl. These two swabs and the two in sterile water were vortexed for 1 minute. The swabs were discarded and all samples, except one (vaginal fluid in sterile water), were serially diluted in 0.03 M NaCl. The samples and their dilutions were cultured on Sabouraud agar plates for 1-2 days at 37°C. The remaining tube with the water-suspension was aliquoted and stored frozen for chlamydia PCR and β-glucan analyses. The laboratory diagnosis of VVC was defined as a positive fungal culture in any of the three swab specimens. Yeast colonies were identified according to morphological characteristics and biochemical properties, such as ability to produce chlamydoconidia, and colour formation on chromagar Candida Medium (BD). Vaginal smears were Gram stained and examined for BV according to the criteria by Nugent et al. [15]. The very same slide was examined at both 100- and 1000 x magnification for yeast cells and pseudo hyphae. Presence of yeast cells and/or pseudo hyphae was defined as VSM based diagnosis of VVC. VSM was performed by two of the authors with coded smears (BA, IMB).

β-glucan determination



Suppl Figure 1: ROC curve of β-glucan. The area under the ROC curve, AUR, was 0.884 with a 95% CI of 0.813 – 0.956 and a p value of <0.0001. At the cut off value >5 ng/ml (5.1 ng/ml) the sensitivity was 77% and the specificity 96%. The ROC curve was based on women with positive candida culture (n=56), and as controls those with negative culture results (n=28).

The β-glucan concentration in vaginal secretion was determined using the end-point Glucatell kit (Associates of Cape Code Incorporated, MA, US). All of the glass ware used was heated to 180°C overnight to inactivate any contaminants. The frozen samples were thawed and diluted 1:100, and 1:1000 in glucan-free water. The assay was run according to the manufacturer's manual, including the azo-coupling procedure for increasing the sensitivity of the assay. The absorbance of the samples was read at 560 nm, and a standard curve was plotted. The β-glucan concentration was determined for each vaginal sample run in duplicate.

A positive β-glucan value for the diagnosis of VVC was defined as ≥ 5 ng/ml. This cut off value was retrieved by making a Receiver Operating Characteristic (ROC) curve, using the women with positive yeast culture as the gold standard and those without as control (Supplementary Figure S1).

Statistics

Analyses of contingency tables for sensitivity, specificity, positive predictive value, and negative predictive value were performed by using GraphPad Prism statistical software package. A ROC curve was used for defining an optimal cut off value for β-glucan diagnosed VVC with the same software package as above. Correlations were calculated by using Spearman rank correlation test.

Results

Of the 92 enrolled women in the study, culturing was missed in four samples, and additional five smears (wet mounts) and four vaginal fluid samples were missed (Figure 1).

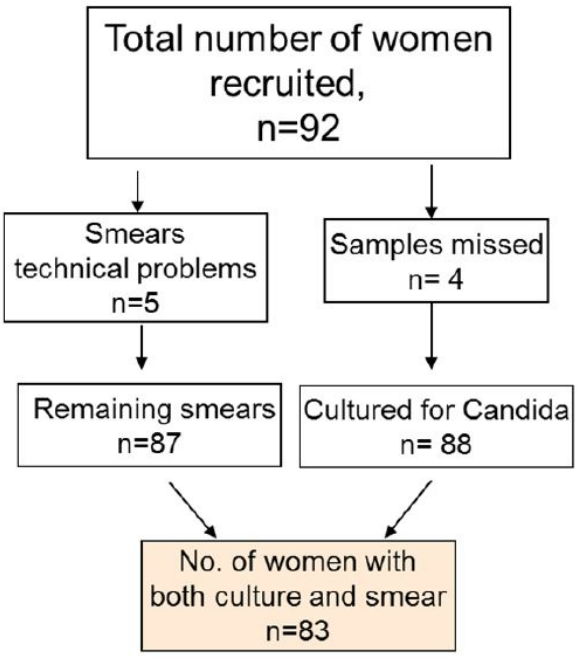


Figure 1: Number of women entering the study, and samples available for the various analyses.

Twenty-four women did not answer the question about the frequency of earlier candida episodes. The characteristics of women entering the study by self-diagnosis and purchase of antifungal at the pharmacy are shown in Table 1. The number of women with clinical diagnosis of VVC was 28% (25/88), BV 20% (17/87) according to Nugent scoring, and women with no diagnosis 54% (45/83), i.e. neither clinical VVC nor BV. By the clinical examination other causes than BV

and VVC were excluded (such as vulvar lichen sclerosus, lichen planus, atrophic vaginitis, and diabetic pruritus). None of the women was tested positive for chlamydia.

Culture proven VVC

Culture proven VVC was found in 66% (58/88) of the women, and all isolated yeast cultures were *C. albicans* except for one, which was *C. glabrata*. The clinical examination agreed with culture proven VVC in 40% of the women (23/58). Furthermore, 12% (6/53) of the culture positive women had a concurrent BV. Taken together, the diagnosis of VVC (based on culturing) and BV were found in 75% of women self-diagnosed with VVC, leaving 25% of them without a diagnosis (21/83).

Symptoms in women with positive yeast culture

Since the women self-diagnosed their complaint, some typical symptoms associated with VVC were included in the questionnaire. The most common symptom was itching (81%), followed by smarting/burning complaint (73%) and discharge (69%), (Table 2). The frequency of itching did not differ between culture-positive and culture-negative women. However, more culture-positive women experienced smarting/pain than culture-negative women (p=0.040). Although swelling only occurred in a minority of the women it was significantly more common among culture-positive than culture-negative women (p=0.044). Thus, the symptoms listed in Table 2 are weak markers for VVC.

Symptom	Number of women (%)		
	Total number	Culture positive#	Culture negative
	n=86†	n=57	n=29
itching	70 (81)	49 (86)	21 (72)
smarting pain/burning	63 (73)	46 (81)	17 (59)*
swelling	24 (28)	20 (35)	4 (14)¤
discharge	59 (69)	41 (72)	18 (62)
dryness	21 (24)	15 (26)	6 (21)
dysuria	20 (23)	15 (26)	5 (17)

†Two questionnaires not complete.

#A positive culture was defined as ≥ 10 CFU.

*Fisher´s exact test, p=0.0397

¤Fisher´s exact test, p=0.0442

Table 2: Frequency of vaginitis symptoms in self-diagnosed women with positive# or negative vaginal yeast culture.

Assessment of accuracy by clinical examination, microscopic examination of vaginal smears, and β-glucan determination for diagnosis of VVC, using positive yeast culture as gold standard

Since the clinical examination resulted in 28% VVC diagnoses among the self-diagnosed women, and in only 40% of those that were culture-positive, we wanted to assess whether the diagnosis would be more accurate by VSM or by determining the level of the fungal marker, β-glucan, in the vaginal samples. The results of the evaluation of vaginal smears and β-glucan analysis were compared with culturing results. The sensitivity of clinical VVC was low (Table 3). The findings of yeast cells/pseudo hyphae in vaginal smears were more accurate than clinical examinations for diagnosis of VVC, although the combination of clinical and VSM showed a higher sensitivity. β-glucan determination showed the overall highest performance values (Table 3). The sensitivities of VSM and β-glucan determination were significantly higher than that of clinical examination (p=0.0029 and p<0.0001 respectively, Fisher´s exact test). The combination of clinical examination and VSM gave a similar sensitivity as β-glucan determination. Three women, who were clinically diagnosed as VVC, were negative for yeast culture, VSM and β-glucan.

Diagnosis of VVC	Sensitivity	Specificity	PPV	NPV
	% (N/total N)	% (N/total N)	%	%
Clinical examination¤ (n=88)	40 (23/58)	90 (27/30)	89	44
VSM (n=83)	67 (37/54)	97 (28/29)	97	61
Combined: (n=84) (clinical exam/VSM)	76 (41/54)	87 (26/30)	91	67
β-glucan# (n=84[a])	77 (43/56)	97 (27/28)	98	68

*Sensitivity, specificity, and positive and negative predictive values (PPV, NPV) were calculated for clinical examination, microscopy (VSM), the combination of both, and β-glucan quantification to compare their performances in diagnosis of VVC. Positive Candida culture was used as gold standard, and the prevalence of VVC was 58 out of 88 women (66%) in the study group of women with self-diagnosis.

¤Clinical examination was performed by speculum examination of the vaginal mucous membrane, vaginal discharge, palpation of vagina, uterus and adnexa. This was combined with test of pH on vaginal fluid and sniff test, and wet smear microscopy.

#A positive β-glucan level was defined as ≥ 5 ng per ml, based on the ROC curve (area under the ROC curve was 0.884 with a 95% CI of 0.813 – 0.956, and a p value of <0.0001.

[a]Four vaginal fluid samples were missing for the β-glucan analysis (two from each of the groups of women with or without a positive yeast culture.

Table 3: Diagnostic performance of clinical¤ and microscopic examination of vaginal smear (VSM), the combination of both, and β-glucan quantification for diagnosis of VVC*.

Effects of the vaginal yeast cell load on the diagnosis of VVC, as determined by clinical examination, VSM, or β-glucan measurements

We investigated whether the discrepancy between positive yeast culture and diagnosis as assessed by clinical examination, VSM, or β-glucan determination could be related to the vaginal yeast cell load. The concentration of vaginal yeast cells varied from tenfold to 10^7 colony-forming units (CFU) per sample with a geometric mean of 8×10^4 CFU (Figure 2a). Almost 90% of the culture positive women carried between 10^3- 10^6 CFU of yeast cells per vaginal sample. The frequency of clinical VVC, which reached its peak value (50%) at a yeast CFU concentration between ≥ 10^3 to <10^4, did not correlate with the yeast load (Figure 2b). Neither was a correlation established between the fungal load and the frequency of swelling or smarting/burning. The frequency of microscopy-based VVC correlated significantly with the concentration of yeast CFU (spearman r=0.68, p<0.0001), although yeast cells at CFU numbers below 1000 were not

observable by microscopy. β-glucan (cut-off value 5 ng/ml, suppl Figure S1) was better than VSM or clinical examination over the range of ≥ 10^2 – <10^4 CFU per ml. However, at ≥ 10^6 CFU per ml and more, the number of women with positive β-glucan decreased somewhat (Spearman r=0.62, p<0.0001). Thus, microscopy-based diagnosis of VVC appeared to be the most consistent diagnostic tool over all concentration ranges of yeast CFU, except at the concentration range ≥ 10 -<10^3 CFU per sample, where the β-glucan appeared somewhat better.

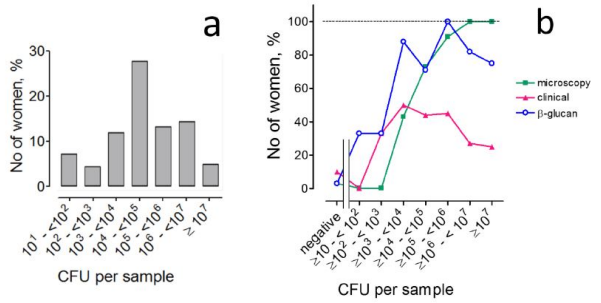


Figure 2: (a) Frequency of women related to the concentration of vaginal yeast cells (CFU) per sample (n=58). The major part of women carried a yeast load between 103 to < 107 CFU per ml.
(b) Distribution of women with clinical-, microscopy-, or β-glucan diagnosed VVC in relation to vaginal yeast cell load (CFU). A positive β-glucan was defined as ≥ 5 ng/ml (based on ROC curve, see supplementary Figure S1).

Discussion

In the present study, it was shown that 66% of women, who presumed they had VVC, and intended to purchase OTC antifungal products, actually had VVC confirmed by yeast cell culture. Thus every third woman (34%) is misdiagnosed and mistreated, and is in need of professional guidance. In addition, to evaluate the accuracy of self-diagnosis by women, we analyzed the accuracy by which clinical examination and laboratory methods, such as VSM and β-glucan determination, would identify VVC in the very same women. A clinical examination combined with VSM for diagnosis of VVC, showed a sensitivity of 76%, which is similar to the sensitivity of 77% for β-glucan alone. The conclusion can therefore be made that analysis of β-glucan as a future rapid bedside test at pharmacies and gynecological clinics would be optimal complements for diagnosis of VVC. The weakness of our study is that the sample size is small, and there is a risk of selection bias. On the other hand, the study objects were randomly recruited to the study.

Compared to our results, Ferris and colleagues found that 54% of women who bought OTC antifungals for presumed VVC had the infection, either pure or mixed with other infections (mainly BV) [9]. VVC in their symptomatic population was defined as the occurrence of pseudo hyphae by wet mount microscopy of vaginal smear or a positive fungal culture. Our result of 12% with VVC with coexisting BV was close to the corresponding 19% of Ferris et al. [9]. Our finding of every fourth woman not being diagnosable and every fifth with pure or mixed BV of the total group of women with presumed VVC is alarming in the aspect of self-treatment with antimycotics. Repetitive treatment with an OTC product would give the impression of numerous VVC infections, and incorrectly self-diagnosed VVC may overestimate the frequency due to the lack of effect on their complaint. Apart from the fact that these women will experience treatment failure, repeated treatments with antimycotics are associated with complications, i.e increased resistance against *Candida*, and hypersensitive mucosal membranes such as local vulva pain syndrome (vestibulitis, dyspareunia) [4,8,11,12,16,17]. Moreover, an unhealthy misdiagnosed vaginal microbiota places women at increased risk of both bacterial and viral sexually transmitted infections [18]. Vaginitis induced by non-*albicans* species is clinically indistinguishable from that caused by *C. albicans*; moreover such species are more often resistant to treatment. The incidence of VVC caused by non-*albicans* strains is thought to be increasing because of single dose azole treatment and the use of OTC [4]. About half of *C. glabrata* strains isolated from cases of recurrent VVC have shown reduced sensitivity to fluconazole compared with *C. albicans* [4,19]. In earlier reports, 15% of women with VVC were shown to have *C. glabrata* [4]. However, in our study we found only one woman with *C. glabrata* (1.1%), despite repetitive occurrences and treatment cycles of the presumed VVC.

The symptomatology as well as the correct diagnosis of vaginal yeast infection has the highest level of inaccuracy among the vaginal infections [4,6,9]. Landers found that patients with a discharge and pruritus were more likely to have BV (31%) than yeast (23%), and equally likely to have no diagnosable infection (25%) [6]. In our study, pruritus, smarting pain/burning, and discharge were the most common symptoms in both yeast culture positive and negative women. Although smarting pain/burning and swelling were the only symptoms that were significantly increased in yeast culture positive women, still a majority of the culture negative women experienced smarting pain/burning, and 2/3 of the culture positive women experienced no swelling. Thus, "typical" symptoms of VVC are extremely weak per se. Jonson et al. found that 25% of young healthy women had some kind of lower genital tract complaint [20]. They also notified that itching was the most commonly described symptom and was associated with acetowhite patches. Andersson et al. postulated that the most specific symptom in VVC is pruritus, while this criterion correctly predicted VVC in only 38% of 60 patients [21]. In our study, pruritus was as common in culture negative as in culture positive women. There is also an association between pruritus and HPV infection, and some dermatological diseases (lichen sclerosus, eczema). Itching is thus of no discriminatory value regarding vaginal infections [22,23].

Interestingly, the β-glucan determination appeared to be the most sensitive method at low levels of yeast cells (10 - 10^3 of yeast cells), and the VSM at the highest levels (≥ 10^6 of yeast cells). Thus, these two methods seem to complement each other. The clinical diagnosis alone, on the other hand, identified at most 50% of VVC in the range of 10^3 – 10^5 yeast cells. Thus, below or above this range even less women were clinically diagnosed as VVC. This fact contribute to the low sensitivity found for clinical diagnosis. The variation in sensitivity depending on the yeast cell load and diagnosis method may partly explain differences between various reports, and emphasize the importance of using yeast culture as gold standard in investigations concerning VVC. The reason why the number of women with a positive β-glucan was somewhat reduced at 10^6 CFU per ml or more is not known. Several explanations for partly inhibitory effects of high amounts of yeast cells are plausible. One explanation could be that presence of excess amounts of β-glucan disturbs the binding to the protein participating in the activation of the proclotting enzyme system of the *Limulus* ameobocyte lysate [24].

Possibly, high numbers of yeast cells in vaginal fluids need to be diluted further or heat-inactivated, in order to counteract inhibitory activities on the assay system.

Every fourth woman out of the total number of women with symptoms was without a diagnosis. Cultivation-independent molecular methods may prove useful in assessing vaginal microorganisms for their role in lower genital complaints not being VVC or BV [3,25]. Thus, more in-office or OTC diagnostic tools are warranted to increase not only the accuracy of VVC diagnosis, but also to find other possible infectious agents in women with symptoms that resemble VVC vaginitis, although is neither Candida infection nor BV.

In summary, VVC diagnosed by the women themselves leads to significant numbers of misdiagnosis. Our recommendation is that women with recurrent symptoms of VVC should be offered gynecological examination combined with VSM and/or a test such as β-glucan. When failure with repeated treatments they should be further examined with vaginal culture for yeast identification. The biochemical method of analyzing β-glucan is presently not used for the diagnosis of VVC and has to our knowledge not been described in any clinical study evaluating VVC. The method could be developed into a rapid bedside test to achieve better accuracy in the diagnosis of VVC and to avoid inaccurate self-treatment.

Acknowledgment

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Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved 2011-10-06 (Dnr 611-11) by the Regional ethical committee.

Disclosure

The authors report no conflict of interest.

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