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Is Multiple Site Colonization with *Candida spp.* Related to Inadequate Response to Individualised Fluconazole Maintenance Therapy in Women with Recurrent *Candida* Vulvo-vaginitis?

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Running title: Multiple site *Candida* and maintenance therapy

Abstract

Objective. Although most women on fluconazole maintenance therapy for recurrent vulvovaginal candidosis experience a substantial improvement in quality of life, some do not respond to therapy. Is candidal colonization of extragenital sites related to suboptimal response to maintenance therapy?

Patients and Methods. Women included in a multi-centre follow up study(ReCiDiF) were evaluated for clinical signs and presence of yeasts in nose, mouth, anus, perineum and urine. *Candida* was diagnosed by positive microscopy, confirmed by positive culture or PCR. After treatment, women were divided into groups according to their response to a fluconazole maintenance regimen (optimal, sub-optimal and non-responders).

Results. The most frequent extra-vaginal *Candida* spp. were detected in urine (79.5%), perineum (78.6%), and anus (56.4%). Carriers of *Candida* in the mouth were more likely to have it in the anus (OR 3.2; 95%CI 1.4-7.7). Colonization in anus (OR 3.3; 95%CI 1.3 – 8.1) or in multiple extra-vaginal sites (OR 3.0; CI95% 1.2 – 7.4) were related to non-response to therapy. Candidal carriage in the anus did not increase anal and perianal symptoms.

Conclusion. Women with anal carriage and multiple site candidal colonization are less likely to respond to individualised decreasing dose fluconazole therapy.

Introduction

Candida is an opportunistic microorganism [1] which colonises oestrogenised vaginal mucosa [2]. The majority of women will develop vulvovaginal symptoms of candidosis at least once during their lives.. At least one in ten of them have recurrent disease, defined as more than 3 symptomatic episodes per year [2]. RVVC (recurrent vulvovaginal candidosis) has negative economic, physical, social and psychological impact on women and significantly impairs their quality of life.[3] Fluconazole maintenance therapy is effective in managing symptoms in most patients. [4 5 2] However, some women do not respond to these regimens, [4 5] creating a substantial challenge for both patient and clinician.

Little is known about the factors predicting non-response to fluconazole maintenance therapy. Mannose Binding Lectin 2 codon 54 gene polymorphism has been detected more frequently in women suffering from RVVC than in controls [6]. Unexpectedly, polymorphism of the B allele was associated with a better response to fluconazole maintenance therapy than the presence of wild type (A-) allele, [6] Also, non-*albicans* species, such as *C. glabrata, C. holmii, and Saccharomyces cerevisiae*, all species with limited sensitivity to fluconazole, were more often identified in vaginal cultures of non-responders. [4, 7] Finally, longer duration, higher severity of disease and a personal or familial history of atopic diseases such as eczema, were also linked to increased failure to respond to maintenance therapy (in press). In contrast, although some women with RVVC have decreased glucose tolerance, [1]glucose levels in blood and vagina were not related to non-response to therapy. [7] Thus clinicians need to identify more factors to predict non-response to fluconazole maintenance, to improve patient satisfaction and reduce treatment costs.

The presence of a reservoir of *Candida* spp. is linked to symptomatic recurrence of disease. Migration to the vagina of *Candida* from the rectum [2 8] and through salivary transmission during oral sex [9] may occur in a sexually active couple. Due to its anti-mycotic effects on the whole body, systemic (oral) therapy with fluconazole has the potential to be more effective than topical regimes for RVVC in those patients. Despite this, some women fail to respond as expected to oral suppressive therapy. In this report, we present our data of women with RVVC participating in the ReCiDif trial to determine if the presence of extra-vaginal candidal colonization contributes to treatment failure.

Material and methods

At recruitment for a multi-centre open-label follow up study, 117 patients with a history of at least 4 episodes of vulvo-vaginal candidosis per year, current clinical vulvovaginitis and positive candidal isolation from the vagina were tested for the presence of the yeast in other body sites. Following an induction dose of fluconazole 200mg given three times in the first week, women received individualized, reducing fluconazole maintenance treatment and were followed up for 1 to 2 years during therapy. [4] After induction treatment, and after each maintenance phase (designed to reduce dose levels), patients were checked for symptoms and the presence of *Candida* spp. by microscopy and culture, as outlined in our previous paper.[4] The study was approved by the central Ethics Committee of the University of Leuven, Belgium (ML1860), and by the local Ethics Committees of each participating centre: All patients signed informed consent before being enrolled.

Data collected included demographics, medical history, sexual behaviour, clinical symptoms, and physical examination findings. Severity scores (1 to 4) of clinical signs of vulvo-vaginitis were recorded [2]. Wet mount smears, swabs for culture and/or polymerase chain reaction (PCR, in case of positive microscopy findings but negative culture) were collected before initiation of treatment. Swabs for culture were taken from vagina, perineum, anus, nose and mouth. Additionally, 50 ml of urine was collected and cultured for the presence of *Candida* spp. Wet mount smears were investigated using phase contrast microscopy at 400 times magnification according to the Femicare classification[10]. The diagnosis of *Candida* vulvo-vaginitis was confirmed by positive vaginal culture, or by positive PCR in case of culture negative, microscopy positive findings. PCR results of the 11 cases with microscopy positive, but culture negative, findings showed 7 cases with *C. albicans* only, 2 cases with *C. albicans* and a non-*albicans* co-infection (one with *C. glabrata* and *C. Parapsilosis*, and one with *C. parapsilosis*) and 2 non-albicans infections (*C. krusei*, and *C. glabrata*). Inclusion and exclusion criteria, the treatment protocol and patient characteristics have been published elsewhere. [4]

At the end of the trial, patients were divided into three groups: optimal responders (o-R), who had no relapses during treatment, suboptimal responders (s-R), who in whom we needed to prolong certain phases of the regimen because of clinical or subclinical relapse, and non-responders (non-R), who failed to respond more than once to the same treatment level and thereby discontinued the trial. As tests and questionnaires were collected at inclusion, and response groups were divided at the end of study, no bias towards any potential risk factors could have been introduced.

Data were analyzed using SPSS version 23.0 (SPSS Inc., Chicago, IL, USA) and reported in numbers (percentages) and mean ± standard deviation (SD). For comparison of proportions we

calculated z-test (adjusted p-value Bonferroni method when comparing column proportions), χ^2 or Fisher exact for small groups. The Kruskal-Wallis test was performed to analyze the three groups: o-R, s-R and non-R. Mann-Whitney U test was used to compare two groups (o-R+s-R combined versus non-R). Odds ratios were calculated with 95% confidence intervals. Binary logistic regression analysis was used to assess independent risk factors for non-response to therapy. A *p*-value less than 0.05 was considered significant.

Results

The mean age of women in this study was 34 years (SD ±10.4, range 19-66), a small minority (9/117, 7.7%) being older than 49 years (pre-menopausal or on hormonal therapy). Caucasian was the dominant ethnic background (98.9%). Extravaginal *Candida* spp. were detected most frequently from urine (79.49%), perineum (78.6%), and anus (56.4%) (**Table 1**). The nose and mouth were colonized in a much smaller proportion of patients. The majority (96.6%) of women were infected with *C. albicans*.

The prevalence of urinal, nasal, oral, anal and perineal candidal colonization between the o-R, s-R and non-R is reported in **Table 2 , top** The proportion of positive cultures in urine, nose, mouth and perineum did not differ between the three groups. However, anal candidal carriage was related to poor response to therapy (OR 3.3; 95%CI 1.3 – 8.1; p = 0.01). Despite the association of oral *Candida* with anal colonization (OR 3.2; 95%CI 1.4-7.7), the former was not, by itself, a risk factor for non-response (p=0.4). Also oral colonization was not associated with perineal (OR 2.36; 95%CI 0.7-7.6), urinary (OR 2.8; 95%CI 0.9-8.9) or nasal colonization (OR 0.96; 95%CI 0.9-1.0). The 3 patients with nasal colonization all responded optimally to therapy (p=0.05).

In twelve patients (10.3 %) no extra-vaginal *Candida* was detected. The other patients carried *Candida* in various body sites: 15 had one, 22 two, 43 three and 25 four positive locations (12.8%, 18.8 %, 36.8 % and 21.4 %, respectively). No patient had *Candida* detected in all five sites. Compared to patients with few or no extra-vaginal site positive, patients with multiple site colonization (3 or 4 sites positive) were more likely to be poor responders to fluconazole maintenance therapy (OR 3.0; 95%CI 1.2 – 7.4) **(Table 2, bottom**).

The total severity score of vulvovaginitis was not related to the presence of *Candida* in any extravaginal site, but vulvar oedema and vaginal erosion scores were clearly associated with detection of extragenital *Candida*: women with perineal or multiple site candidal colonization had higher scores of vaginal erosion (both p=0.045) and had more pronounced vulvar oedema (p= 0.01, and p=0.005, respectively, **Table 3**).

Detection of *Candida* in urine was more frequent in patients with perineal culture-positives (OR 13.1; 95%CI 4.5-38.1) and the most prevalent species was *C. albicans* at 68.4% (n=80). The risk of detecting *Candida*-positive cultures both in perineum and urine increased with increasing severity of vulvar oedema (**Table 4**). However, neither colonization of both sites alone (OR 2.2; 95%CI 0.82-6.0), nor in co-existence of vulvar oedema (OR 2.0; 95%CI 0.82-4.8) was associated with an inferior response to the fluconazole maintenance regimen.

Candidal colonization in the anus was not associated with an increase in anal symptoms: anally colonised women were not more likely to have anal fissures (OR 0.9; 95%CI 0.41-2.1), anal itching (OR 1.0; 95%CI 0.39-2.5), haemorrhoids (OR 1.9; 95%CI 0.72-4.8), diarrhoea (OR 2.3; 95%CI 0.43-11.81), constipation (OR 1.7; 95%CI 0.66-4.5) or abdominal bloating (OR 1.4; 95%CI 0.51-3.9) than un-colonised women (**Table 5**). Perineal *Candida* colonization was associated with anal itching (OR 9.8; 95%CI 1.3-76.9) and constipation (OR 4.7; 95%CI 1.0-21.6), but not with

diarrhoea (OR 2.3; 95%CI 0.27-19.7), haemorrhoids (OR 3.0; 95%CI 0.81-11.0), anal fissures (OR 1.5; 95%CI 0.50-4.5) or abdominal bloating (OR 2.0; 95%CI 0.53-7.5). Details of an association between anal candidal colonization and sexual behaviour will be discussed in a separate report.

Discussion

We found that detection of *Candida* spp. in more than two extra-vaginal locations at the start of treatment was predictive of a poor response to maintenance therapy for RVVC, particularly when present in the perineum and/or anus. Although candidal colonization in the anus was associated with oral carriage, the latter was not itself a risk factor for non-response, most likely due to low numbers of women with oral colonization. Detection of *Candida* on the perineum was associated with its presence in urine, with vaginal oedema, anal itching and constipation. However, in multivariant analysis, only anal carriage of *Candida* was independently associated with poor response to maintenance therapy.

By and large, the most prevalent species in vaginal and extra-vaginal sites was *C. albicans*. The ability of *C. albicans* to form hyphae, is considered to be related to the severity of the symptoms, casting doubt by some authors on whether non-*albicans* species without hyphae formation can cause symptomatic vaginitis[11,12]. On the other hand, in women RVVC, a higher prevalence of non-*albicans* is found,[13] particularly when on fluconazole maintenance treatment[4].

Multiple site colonization (i.e. at more than 2 extra-genital sites) with *Candida sp*. was observed in over a half the cases and was clearly identified as a risk factor for poor response to fluconazole maintenance therapy. According to Chong et al, *C. albicans* strains associated with recurrent infections are more diverse than strains which cause sporadic infections. Researchers

hypothesized that the former may represent more virulent subtypes.[14] Also RVVC was found to be related to numerous human genetic mutations, leading to defective regulation of the IL-17 and C-type lectin pathways.[15] These defects were responsible for downregulation of the immune response to *Candida* and associated with RVVC.[15]

Despite detection of *Candida* in perineum, urine, mouth and nose in the majority of patients with RVVC, only anal colonization was predictive of non-response to treatment. The association of anal with oral candidal colonization, seems to support theories suggesting the gastrointestinal tract serves as a reservoir for *Candida* in RVVC cases. Other researchers have found that carriage of rectal *Candida* was higher during episodes of vulvo-vaginitis than during periods of remission.[8] Dynamic transmission of yeast by autoinoculation has been documented,[16] emphasizing the potential importance of extra-vaginal carriage, and justifying the use of oral fluconazole maintenance therapy to reduce recurrence rates in women with RVVC. [4 5]

However, it does not explain why, according to our findings, systemic therapy failed to prevent *Candida* vulvo-vaginitis relapses in anal *Candida* carriers. One reason could be that the non-vaginal sites were more frequently infection by fluconazole insensitive, non-albicans species. However, according to our data (See Table 1) the frequency on non-albicans species in the extra-vaginal locations did not differ from those in the vagina. Another explanation could be that candidal colonization in the anus is not the cause of recurrent candidal infections, but rather an indicator of other underlying causes. The lack of association between anal and intestinal symptoms and anal colonization could be consistent with that hypothesis. Additionally, clinical features of some genetic mutations affecting the gastrointestinal system are in line with this.[17,18] For example, in women with STAT1 mutation, oral and perineal candidal infections cause the most troublesome symptoms, yet the small intestine is usually found to be free of *Candida.*[17] In patients carrying an

AIRE mutation suffering from gastrointestinal symptoms and express the presence of *Candida* in the gut,[17,18,19] auto-antibodies against tryptophan hydroxylase enzyme impair gastrointestinal function.[19,20]

Candidal colonization in urine was frequently found in our patients. This is inconsistent with the theory that the urine of healthy persons is supposed to be sterile. So we suspect that contamination with vulvar or perineal organisms could have occurred during urination. Detection of *Candida* in urine was indeed associated with its detection on the perineum and in women with pronounced vulvar oedema. This could explain the strong relation between urinary colonization and the degree of vulvar oedema we found. As we did not use catheterized urine, this finding could be associated with difficulty obtaining urine samples without contamination from vulva and perineum in women with various degrees of vulvar oedema.

The study has some limitations. First, it would also have been interesting to genotype vaginal and anal "couples of strains", in order to show that these strains were related, and to strengthen the relationship evidenced in this study between anal colonization and non-response to fluconazole therapy. However, such tests were not performed and strains are no longer available. Also, fluconazole sensitivity of the vaginal strains at study entry would have been interesting but were not performed. Further, due to insufficient number size, it was not feasible to examine the importance of low, middle or high level of colonization, missing out on quantitative assessment of the findings. Finally, it was a disadvantage that extra-site cultures were not tracked during the follow up visits, in order to explain better the reason for non-response over time. However, the study was not budgeted to perform multiple site cultures during 10 subsequent visits.

To our knowledge, this is the first study analysing the relation between extra-vaginal candidal colonization and response to fluconazole maintenance therapy. We demonstrated that, although multiple site culture positivity is related to non-response, only the presence of anal candidal colonization is strongly predictive of poor response to therapy. We also discovered that in depth analysis of anal or intestinal clinical symptoms cannot predict anal *Candida* carriage.

In conclusion, poor response to therapy is associated with candidal colonization at multiple sites, especially in the anus and perineum, prior to starting fluconazole treatment. Anal itching and constipation was associated with candidal colonization on the perineum, but not with response to the fluconazole maintenance regimen. Compared to RVVC women with negative anal cultures. women with positive anal cultures for *Candida* were 5 times more likely to fail to respond to maintenance therapy,

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Table 1. Prevalence of candidal colonization of the body sites according to culture results.

Localization N=117	*C. albicans*	*C. albicans* + *C. glabrata*	*C. glabrata*	*C. albicans* + *C tropicalis*	*C. parapsilosis*	Negative*	Not done
Vagina	100 (85.5%)	2 (1.7%)	1 (0.9%)	0	0	14 (12.0%)*	0
Urine	90 (76.9%)	2 (1.7%)	1 (0.9%)	0	0	22 (18.8%)	2 (1.7%)
Nose	2 (1.7 %)	0	0	0	1 (0.9%)	114 (97.4%)	0
Mouth	35 (29.9%)	1 (0.9 %)	0	0	0	81 (69.2%)	0
Perineum	90 (76.9%)	2 (1.7 %)	0	0	0	25 (21.4%)	0
Anus	63 (53.8 %)	0	2 (1.7 %)	1 (0.9 %)	0	51 (43.6%)	0

**Candida* was unequivocally detected on fresh mount microscopy on two occasions in 2 cases or by PCR in the other cases (C. *albicans* 8 (6.8%); C. *albicans+C. glabrata+C. parapsilosis* 1 (0.9%); *C.albicans+C.parapsilosis* 1 (0.9%); *C. glabrata* 1 (0.9%); *C. krusei* 1 (0.9%).

Table 2. *Candida* colonized non-vaginal body sites according to response groups

Site	**Optimal responders** **N=38**	**Suboptimal responders** **N=46**	**Poor responders** **N=33**	**p-value[§]**	**p-value [§§]**
Urine	30 (78.9%)	35 (79.5%)*	28 (84.8%)	0.78	0.49
Nose	3 (7.9%)	0	0	0.053	0.56
Mouth	11 (28.9 %)	13 (28.3%)	12 (36.4%)	0.71	0.41
Anus	21 (55.3 %)	20 (43.5 %)	25 (75.8%)	0.**02**	0.**01**
Perineum	27 (71.1 %)	36 (78.3 %)	29 (87.9 %)	0.23	0.14
0-2 sites	18 (47.4%)	23 (50.0%)	8 (24.2%)	0.05	0.02
3-4 sites	20 (52.6)	23 (50.0%)	25 (75.8%)		

§ p-value for trend.

§§ p-value between the poor responders and responding group (optimal + suboptimal).

* urine culture was not done for two patients.

Table 3. Association of clinical severity scores of vulvo-vaginitis with the finding of *Candida* at various extra-vaginal sites (n=117).

Clinical signs	Man Witney U *p*-value of risk of positive *Candida* culture					
	Anus	Perineum	Urine	Mouth	Nose	Multiple sites
Vulvar erythema	0.42	0.55	0.84	0.74	0.58	0.47
Vulvar pruritus	0.27	0.98	0.56	0.35	0.79	0.66
Vulvar excoriation	0.25	0.51	0.83	0.85	0.96	0.43
Vulvar oedema	0.05	**0.01**	**0.02**	0.39	0.10	**0.005**
Vaginal erosion	0.08	**0.045**	0.48	0.32	0.82	**0.048**
Vaginal erythema	0.93	0.08	0.84	0.57	0.61	0.66
Total severity score	0.09	0.07	0.67	0.89	0.68	0.08

Table 4. Association of perineum and urine candidal detection with severity scores of vulvar oedema

Severity score of vulvar oedema	Both perineum and urine *Candida*-positive	OR	CI95%		*p*-value
			Lower	Upper	
1	11/21 (52.38%)	5.5	0.71	42.6	0.15
2	30/48 (62.5%)	5.0	1.20	20.9	0.02
3	35/40 (87.5%)	36.0	5.21	248.66	0.00
4	6/6 (100%)	-	-	-	-

Table 5. Prevalence of possible risk factors for extravaginal candidal colonization in anus and multiple sites.

Condition	Anus colonization			Colonization of multiple sites		
	Positive	Negative	p-value	3-4	0-2	p-value
Dermatological conditions						
Atopic disease	24/66 (36.4%)	16/51 (31.4%)	0.57	27/68 (39.7%)	13/49 (26.5%)	0.14
Psoriasis	3/51 (5.9%)	3/66 (4.5%)	1.0	4/68 (5.9%)	2/49 (4.1%)	1.00
Eczema	10/51 (21.6%)	11/66 (15.2%)	0.47	11/68 (16.2 %)	10/49 (20.4%)	0.56
Anal-intestinal symptoms						
Anus fissure	15/59 (25.4%)	12/43 (27.9%)	0.78	14/59 (23.7%)	13/43 (30.2%)	0.46
Anus itching	14/59 (23.7%)	10/42 (23.8%)	0.99	15/59 (25.4%)	9/42 (21.4%)	0.64
Diarrhoea	6/59 (10.2%)	2/42 (4.8%)	0.46	6/59 (10.2%)	2/42 (4.8%)	0.46
Obstipation	17/59 (28.8%)	8/42 (19.0%)	0.26	17/59 (28.8%)	8/42 (19.0%)	0.26
Abdominal bloating	13/59 (22.0%)	7/42 (16.7%)	0.51	13/59 (22.0%)	7/42 (16.7%)	0.51
Haemorrhoids	18/59 (30.5%)	8/42 (19.0%)	0.19	18/59 (30.5%)	8/42 (19.0%)	0.19

Reference List

(1) Donders GG, Prenen H, Verbeke G, Reybrouck R. Impaired tolerance for glucose in women with recurrent vaginal candidiasis. Am J Obstet Gynecol 2002; 187(4):989-993.