GYNECOLOGY

Vulvovaginal candidiasis: histologic lesions are primarily polymicrobial and invasive and do not contain biofilms

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BACKGROUND: The recent demonstration of a vaginal biofilm in bacterial vaginosis and its postulated importance in the pathogenesis of recurrent bacterial vaginosis, including relative resistance to therapy, has led to the hypothesis that biofilms are crucial for the development of vulvovaginal candidiasis. The histopathology and microbial architecture of vulvovaginal candidiasis have not been previously defined; neither has Candida, containing biofilm been reported in situ. The present study aimed at clarifying the histopathology of vulvovaginal candidiasis including the presence or absence of vaginal biofilm.

STUDY DESIGN: In a cross-sectional study, vaginal tissue biopsies were obtained from 35 women with clinically, microscopically, and culture-proven vulvovaginal candidiasis and compared with specimens obtained from 25 healthy women and 30 women with active bacterial vaginosis. Vaginal Candida infection was visualized using fluorescent in situ hybridization with ribosomal gene—based probes.

RESULTS: Candida microorganisms were confirmed in 26 of 35 biopsies obtained from women with vulvovaginal candidiasis; however, Candida containing biofilm were not detected in any of the cases. Histopathological lesions were exclusively invasive and accompanied by co-invasion with *Gardnerella* or *Lactobacillus* species organisms.

CONCLUSION: Histopathological lesions of vulvovaginal candidiasis are primarily invasive in nature and polymicrobial and do not resemble biofilms. The clinical significance of Candida tissue invasion is unknown.

Key words: *Atopobium*, biofilm, *Candida*, *Gardnerella*, *Lactobacillus crispatus* and *iners*, polymicrobial invasion, vulvovaginal candidiasis

Vulvovaginal candidiasis (VVC), specifically its recurrent form, is a highly problematic and a common clinical therapeutic challenge.[1] In clinical practice the diagnosis is mainly based on clinical signs and symptoms and a typical picture apparent on wet mount saline and 10% KOH microscopy.

Candida by culture or polymerase chain reaction validates clinical findings but is not routinely obtained or justified. Approximately 10—20% of asymptomatic healthy women harbor culturable *Candida* sp. and other yeasts in the vagina.[1,2]

The pathogenesis of acute VVC is currently thought to reflect a microbiome imbalance or dysbiosis in the vagina as well as an abnormal host mucosal immune response to the *Candida* organism. The reasons for and factors involved in the development of dysbiosis are poorly understood. The explanation as to why yeast that normally asymptomatically colonize the vagina and can cause symptoms and inflammation is controversial.[3,4]

A more recent hypothesis includes a switch from unstructured planktonic yeast growth to biofilm formation that facilitates transition from saprophytic to pathogenic yeast behavior.[5—9] Potentially, vaginal biofilm could explain acute sporadic VVC or be more relevant in recurrent VVC (RVVC) as a vaginal reservoir for yeast organisms following antifungal therapy and explain vaginal recolonization. Indeed, planktonic and biofilm forms of *Candida* seem to be different entities. *Candida*-biofilms have been demonstrated in a variety of experimental conditions in vitro as well as on prosthetic surfaces and endovascular and urethral catheters in vivo.

As a result of growing interest in the topic, numerous investigations have been dedicated to unraveling factors responsible for the development of *Candida* biofilms.[10—12] However, despite the widespread belief and broad acceptance of the possibility that *Candida* biofilms are a critical factor in VVC pathogenesis, we could find no publication that actually demonstrates a microscopic picture of the *Candida* biofilm on the vaginal surface. The only publication claiming *Candida albicans* forms biofilms on the vaginal mucosa exclusively includes pictures of smears from vagina without vaginal epithelium.[13] Confirmation of the presence of *Candida* mucosal biofilm in vivo is thus lacking.

Accordingly, we investigated the histopathology of VVC using fluorescent in situ hybridization (FISH). Biopsies from healthy women, women with bacterial vaginosis (BV), and women with VVC were comparatively investigated using FISH probes specific for fungi and bacteria.

Materials and Methods

Patients

The candidiasis group consisted of 35 randomly selected premenopausal women with confirmed vulvovaginal candidiasis (aged 19—37 years, mean 27 years), 25 women from Guangzhou, China, and 10 women from the

Cite this article as: Swidsinski A, Guschin A, Tang Q, et al. Vulvovaginal candidiasis: histologic lesions are primarily polymicrobial and invasive and do not contain biofilms. Am J Obstet Gynecol 2018;xxx:xx-xx.

0002-9378/$36.00

https://doi.org/10.1016/j.ajog.2018.10.023

AJOG at a Glance

Why was this study conducted?

Biofilms are hypothesized as crucial for the development of vulvovaginal candidiasis (VVC). We investigated vaginal biopsies from 35 women with VVC using fluorescent in situ hybridization and compared with specimens from healthy women and women with bacterial vaginosis.

Key findings

Contiguous *Candida* adherence was not detected in any of the cases or in controls. Histopathological lesions in 26 of 35 biopsies from VVC were exclusively invasive and accompanied by bacterial co-invasion. Lactobacilli (including *L. iners* and *L. crispatus)*, *Gardnerella*, and *Atopobium* were most frequently co-invading.

What does this add to what is known?

Polymicrobial mucosal invasion is an unrecognized feature of *Candida* vaginitis. Our results do not support lactobacilli being beneficial or protective. Different from bacterial vaginosis, we found no biofilm elements in vaginal biopsies obtained from women with VVC.

Friedrichhain Hospital in Berlin, Germany. Five women from Berlin and 8 from China had RVVC, and all others had sporadic VVC.

The diagnosis was based on the clinical appearance and microscopic evaluation of smears and culture. None of the women received antifungal treatment for 2 months prior to the investigation. In the candidiasis group, FISH-performing researches were not blinded to group diagnosis, but the investigators were not aware of individual data including results of culture, swab investigations, and clinical course.

Material from paraffin-embedded biopsies from 25 healthy women (aged 20–35 years, mean 26.4 years) investigated for routine care and 30 women with bacterial vaginosis (aged 18–40 years, mean 27.2 years) served as controls. All women in control groups were premenopausal. These materials had been preserved from previous studies on bacterial vaginosis (BV) and described.[14,15]

Biopsies of about 3–5 mm diameter were taken from the middle side wall of the vagina with biopsy forceps (No. ER 058 R; Schubert, Aesculap, Tuttlingen, Germany) and fixated with modified nonaqueous Carnoy solution (6/1/2 volume ethanol/glacial acetic acid/chloroform). The fixated materials could be stored in Carnoy at room temperature for up to 6 months. Usually the time used was convenient for the investigated subject and laboratory staff. Carnoy-fixated material was processed and embedded into paraffin blocks using standard techniques.

Four-micrometer-thick sections were placed on Super Frost plus slides (R. Langenbrinck, Emmendingen, Germany). Sections of vaginal biopsies were hybridized with ribosomal RNA–based FISH probes specific for all bacteria, *Gardnerella* cluster, *Atopobium* cluster, lactobacilli, *Lactobacillus iners* and *Lactobacillus crispatus*, all yeasts, and *Candida albicans* (Table 1).

All hybridizations were performed at 50°C using a protocol described previously.[16]

A Nikon e600 fluorescence microscope was used. The images were photodocumented with a Nikon DXM 1200F color camera and software (Nikon, Tokyo, Japan).

The study was reviewed and approved by the Institutional Review Board of Jinan University.

Results

Hybridizations signals positive for yeasts were detected in biopsies from 26 of 35 women with VVC/RVVC. No yeasts were observed in the healthy group and the BV group, regardless of the protocol used (Figure 1).

Within the candidiasis group, signals were positive for *Candida albicans* in 18 women, and 8 further samples were negative for *Candida albicans* but were hybridized with the universal for most yeasts PF2 probe (Table 2 and Figures 2–6). The histological patterns of candidiasis were identical in both sporadic as recurrent VVC.

We found no yeast in the biopsies of 9 women from the VVC group (5 from China and 3 from Berlin). This could

TABLE 1
Applied FISH probes

Probe name	Sequence 5′–3′	Target organism	RNA values
Eub 338	GCT GCC TCC CGT AGG AGT	Most bacteria	16S rRNA
Gard 662	CCA CCG TTA CAC CGC GAA	*Gardnerella cluster*	16S rRNA
Liner23-2	CTG CTC ACC TAS TTT CCG G	*Lactobacillus iners*	23S rRNA
Lab158	GGT ATT AGC A(T/C)C TGT TTC CA	*Lactobacillus* sp *Enterococcus* sp	16S rRNA
Lcrisp16-1	CGT CAT TAC CGA AGT AAA TCT GT	*Lactobacillus crispatus*	16S rRNA
Ato291	GGT CGG TCT CTC AAC CC	*Atopobium* cluster	16S rRNA
Fungi			
Caal	GCC AAG GCT TAT ACT CGC T	*Candida albicans*	18S rRNA
PF2	CTC TGG CTT CAC CCT ATT C	Most yeasts	18S rRNA

rRNA, ribosomal RNA.
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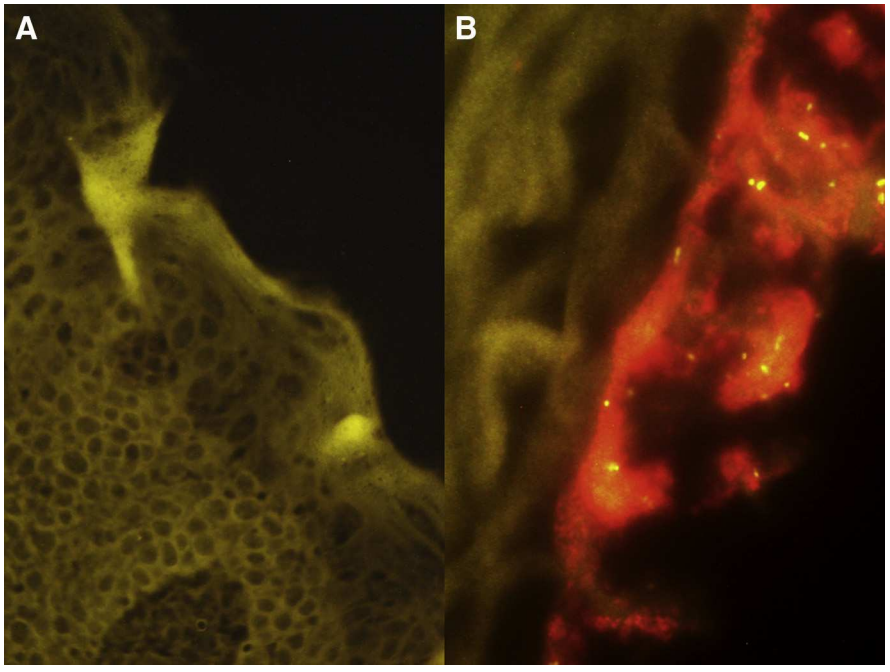
FIGURE 1
Mucosa and biofilms



A, Biopsy from a healthy woman, Eub 338 Cy3 × 400, (all bacteria, *yellow fluorescence*). The surface of the healthy vaginal epithelium is free from adherent bacteria. **B,** Biopsy from a woman with bacterial vaginosis, multicolor hybridization × 1000, Lab Cy3 (lactobacilli, *yellow fluorescence*) and Gard Cy5 (*Gardnerella*, *dark red*). Bacteria build a prolific, highly concentrated biofilm on the surface of the vaginal epithelium. There is no infiltration of the epithelium.

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be due to the nonuniform fungal invasion over the epithelial surface, with some regions remaining uninvolved as demonstrated in Figure 3 (a1−a3).

All fungal cells detected by hybridization were mainly invasive, with variable hyphae penetrating more or less deeply into the epithelial surface of the biopsy (Figures 2−6) and leaving some of the biopsy areas completely free (Figure 3 [a1−a3]. The invasive character of the infection was especially well seen when fungal fluorescence hybridization was 4′,6′-diamino-2-phenylindole (DAPI) counterstained, revealing all eukaryotic cells (Figures 2−6).

Occasionally single fungal cells or blastospores could be seen in slime-covering biopsies; however, a fungal biofilm embedded in its own extracellular matrix was never observed.

Fungal infiltration was always accompanied by co-invasion with bacterial components. Bacteria were either evenly distributed over the depth of the fungal invasion (typical for *Gardnerella* and some lactobacilli, 18 cases total) or concentrated at the forefront of the fungal invasion (*Lactobacillus iners* co-invasion, Figure 5 and 6, 8 cases total).

Co-invading bacteria were polymicrobial, representing a broad spectrum of vaginal microbiota. Lactobacilli, *Gardnerella*, and *Atopobium* were most frequently seen. *Gardnerella* was always associated either with considerable amounts of lactobacilli ranging from 10^8 to 10^{11} (80%) and/or *Atopobium* (60%). A high concentration of lactobacilli could accompany the fungal invasion in the absence of *Gardnerella* or *Atopobium*. (Figures 5 and 6).

Co-infiltrating lactobacilli were often but not exclusively represented by *Lactobacillus iners* (Table 1).

Comment

Many excellent reviews are dedicated to different aspects of *Candida* research.[2−5,7,8,12] Our perception of VVC is based only on interpretation of indirect indices: epidemiology of

TABLE 2
Histopathological findings

Variables	Superficial biofilms	Infiltration by fungi plus bacteria	Nonadherent microorganisms in slime only	*Candida*/ other fungi	Gard 662 *Gardnerella*	Ato291 *Atopobium*	Lab158 (without Gard or Ato)	*L. iners*
VVC (n = 35)	0	26[a]	2	18/8	10	6	19 (9)	11
BV (n = 30)	30	0	0	0	30	25	21 (0)	12
Healthy (n = 25)	1	0	9	0	3	0	9 (8)	3

Ato, *Atopobium*; *BV*, bacterial vaginosis; *FISH*, fluorescent in situ hybridization; *Gard*, *Gardnerella*; *VVC*, vulvovaginal candidiasis.

[a] The fungal invasion was always accompanied by bacterial coinfiltration as detected with the Eub 338 FISH probe, which represents all bacteria. Such a bacterial coinfiltration is characteristic for VVC and never occurred in healthy women or in BV. *Gardnerella*, even when highly concentrated, accompanied *Candida* in VVC but did not build a characteristic biofilm as seen in BV.

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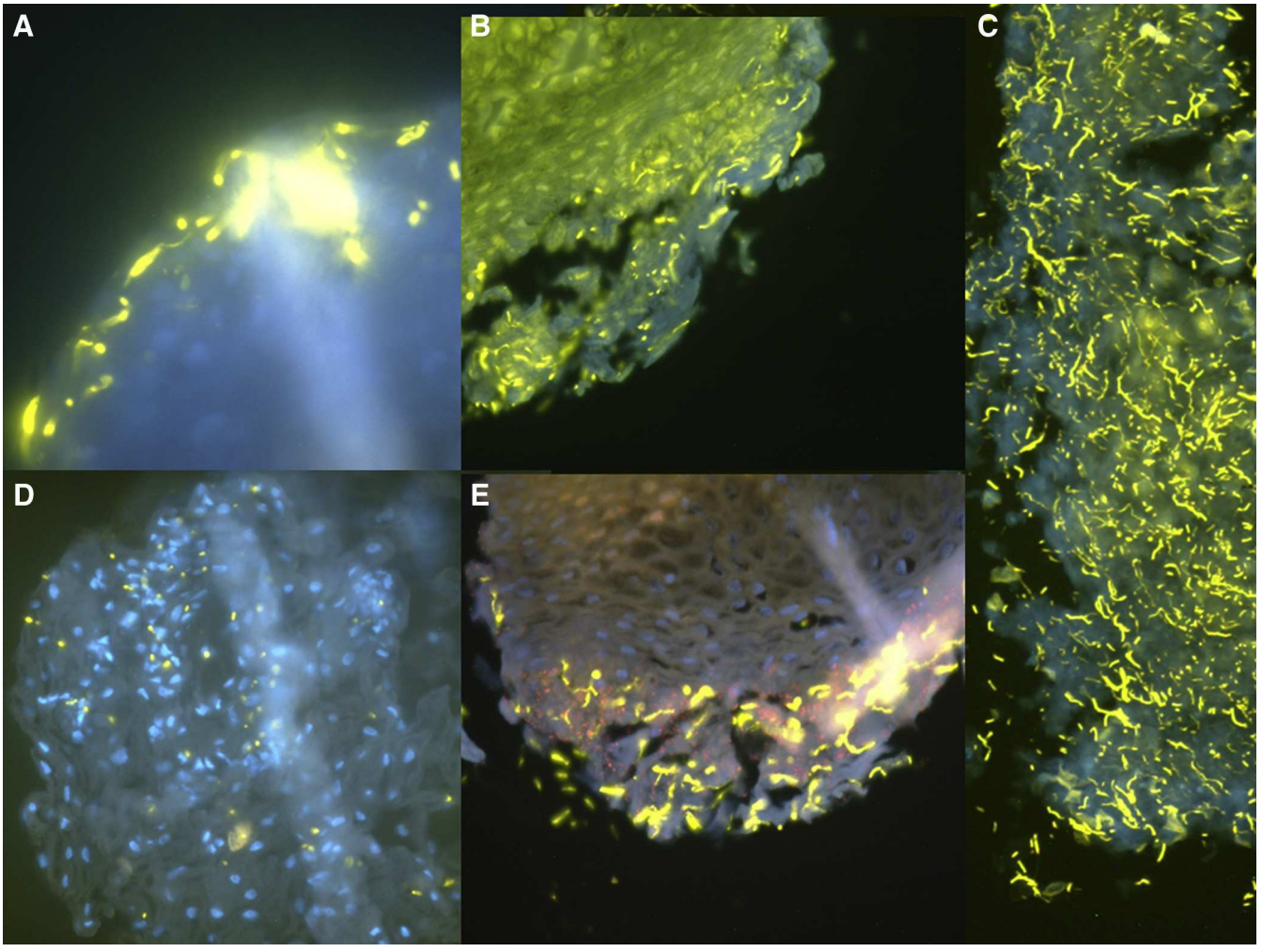
FIGURE 2
Examples of *Candida* invasion



Biopsies from 5 women with vulvovaginal Candidiasis Caal Cy3 (*Candida*, *yellow fluorescence*) demonstrating different grades of infiltration of the vaginal epithelium by *Candida* without adjacent adherent biofilms or components. The infiltration is particularly well seen by counterstaining of the vaginal tissues with DAPI (*blue fluorescence* of the human tissue). Magnification, ×1000 **(A)**; ×400 **(B, D, and E)**; ×100 **(C)**.

DAPI, 4′,6′-diamino-2-phenylindole.

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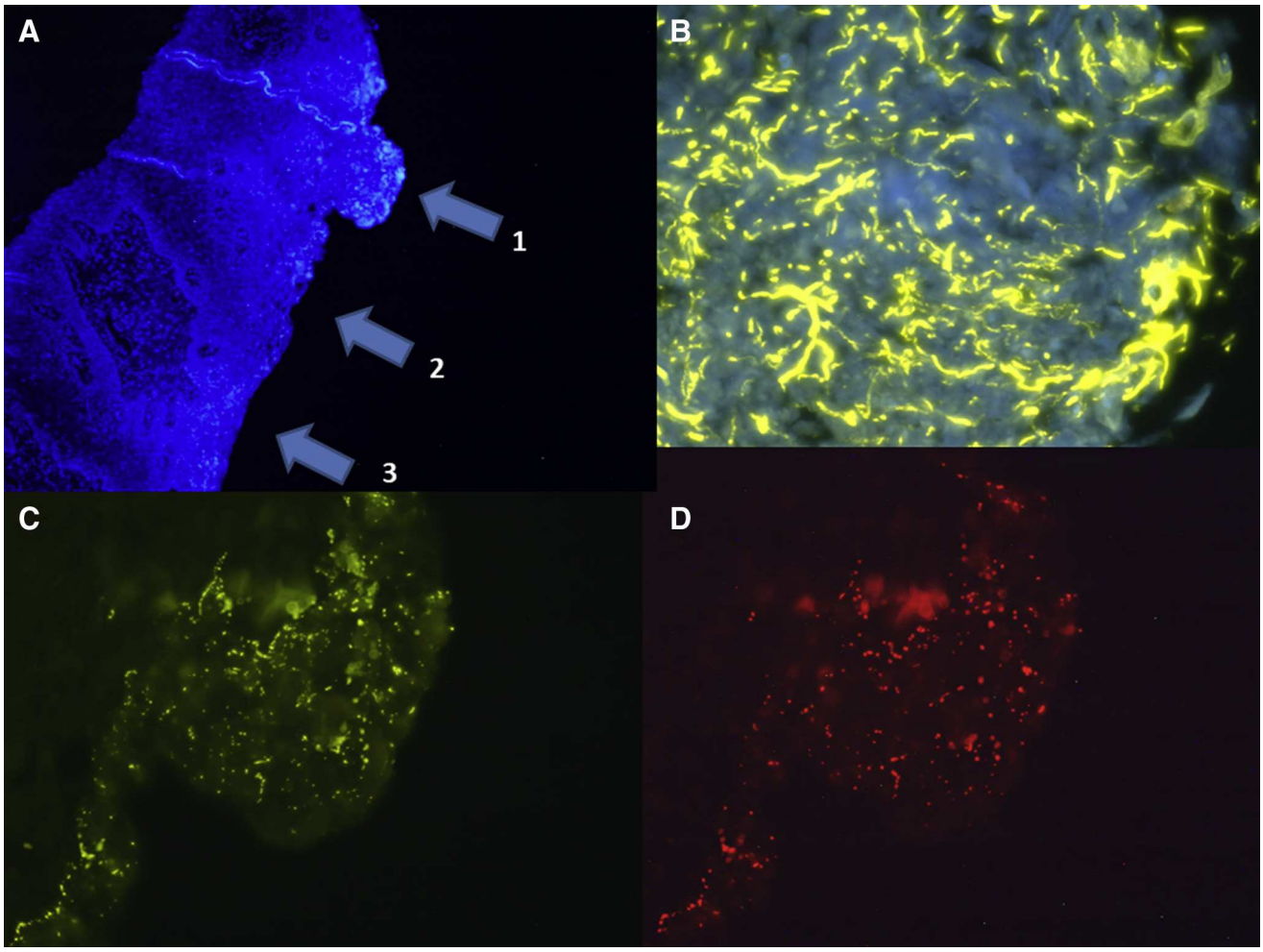
colonization and disease, macroscopic appearance of lesions, symptoms, investigation of vaginal smears using microscopy, culture or molecular genetic identification of microorganisms, and simulation of infection/colonization in vitro and in experimental animal models.

This is the first study that directly visualizes *Candida* microorganisms in vaginal biopsies, and the results substantially contradict widespread assumptions. We found no biofilm elements in vaginal biopsies obtained from women with VVC. Results were identical in women with acute sporadic or recurrent VVC.

This is in contrast to biofilms seen in bacterial vaginosis. Moreover, *Candida* cells on the surface were single, nonconfluent, and what is most important nonadherent to the vaginal epithelium. This cannot be explained by processing sample biases.

Carnoy solution, which we used for fixation of the biopsy samples, demonstrated its high efficiency in preserving biofilms and even bacteria-free slime on different mucosal surfaces.[15] However, in appropriate and representative specimens obtained in VVC, we were unable to detect any contiguous *Candida* layer on the mucosal or epithelial surface. It is possible that the cementing properties of the extracellular matrix, which is produced by *Candida*, is very low, unable to maintain *Candida* attachment to the vaginal surface and not different from those produced by any

FIGURE 3
Uneven infiltration of the mucosa



Candida hyphae on the surface of the vaginal biopsy in a patient with VVC (DAPI stain ×100, *blue fluorescence* **[A** and **B]**, Caal Cy3 ×1000, *Candida*, *yellow fluorescence* **[B]**). Unlike in healthy women, bacteria in patients with VVC are not restricted to vaginal secretions but follow the *Candida* infiltration, growing inside the epithelial layer. Lactobacilli remain predominant and include lactobacilli other than *Lactobacillus iners*. The yeast infiltration of the vaginal surface is uneven, with some of the regions being completely intact, as shown in the marked areas a1 to a3. **C**, Lab Cy3 (lactobacilli, *yellow fluorescence*). **D**, Same microscopic field as panel C hybridized with Lcrisp Cy5 (*Lactobacillus crispatus*, *dark red fluorescence*) as a part of the lactobacilli population presented in the figure. The hybridizations with *Gardnerella* and *Atopobium* FISH probes were negative in this case (not shown).

DAPI, 4′,6′-diamino-2-phenylindole; *FISH*, fluorescent in situ hybridization; *VVC*, vulvovaginal candidiasis.

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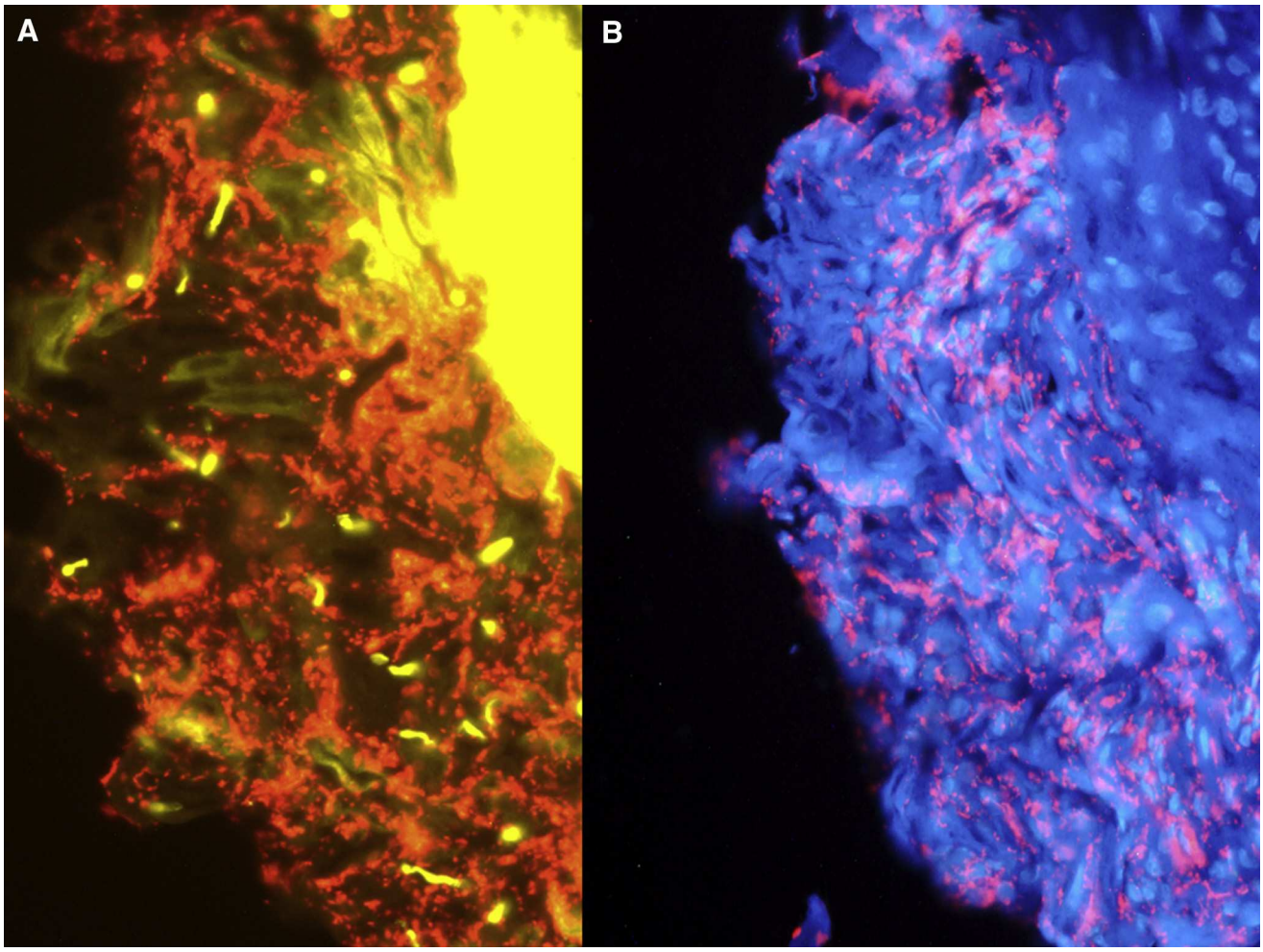
other microorganisms growing in a colony on a culture plate.

The fixation or adherence of *Candida* microorganisms to the vaginal surface is possible or likely through hyphal mucosal invasion. Visually apparent white membranes, which cover the vaginal epithelium in vulvovaginal candidiasis and appear to be biofilms, are actually deep inflammatory infiltrates.

In the past, few studies have included vaginal biopsies in women with VVC. In the absence of such critical tissue status information, vaginal candidiasis has been considered as entirely superficial mucosal infection and the lack of even superficial invasion emphasized. In fact, some clinicians refer to *Candida* vaginitis as infection of vaginal secretions only. Moreover, the histological appearance of nonvaginal *Candida* lesions presented in some publications are all definitively invasive (Gow and Hube, [17] page 407, Figure 1, A, B, and C, and Figure 2; Jabra-Rizk et al, [18] page 2729, Figure 9, B and C; Gao et al, [6] page 737, Figure 9; Allison et al, [7] page 8, Figure 6) and similar to our findings.

A most prominent feature in our study is a deep infiltration of vaginal tissue. The infiltration is not homogeneous with some regions affected more

FIGURE 4
***Candida-Gardnerella* co-invasion**



Biopsy from a woman with VVC, multicolor hybridization with a mix of probes Caal Cy3 (*Candida, yellow fluorescence* **[A]**) and Gard Cy5 (*Gardnerella*, *dark red* **[A and B]**) ×1000 and counterstained with DAPI (*blue fluorescence* showing all rich on DNA stuctures **[B]**) on the right side. Similar to the observations in bacterial vaginosis, *Gardnerella* can be demonstrated in VVC in high numbers in association with *Candida* **(panel A)** and a high density of *Gardnerella* proliferation resembling a biofilm formation similar to that observed in BV, shown in Figure 1B. However, the DAPI counterstain demonstrates clearly that in VVC *Gardenrellla* is no longer only adherently attached but invasive and located below the epithelal surface **(panel B)**.

BV, bacterial vaginosis; *DAPI*, 4′,6′-diamino-2-phenylindole; *VVC*, vulvovaginal candidiasis.

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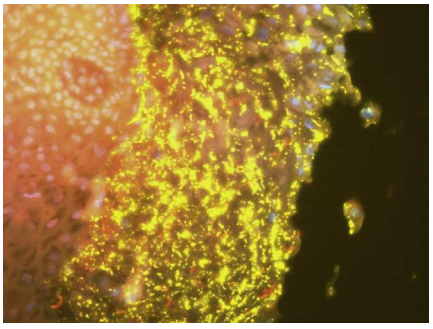
heavily than others and some remaining completely free. The removal of such lesions is impossible without stripping the integrity of the epithelial layer. In spite of the depth of infiltration, it is not full thickness, and disseminated infection is not reported clinically.

Candida colonization and infection occur in polymicrobial environments. Our findings suggest possible bacteria-yeast interactions in tissue invasion. With regard to VVC, *Gardnerella* spp. was claimed to promote and lactobacilli to supress *Candida* biofilms.[7,19] While our data support a contributory promoting role for *Gardnerella*, little was forthcoming to support lactobacilli being beneficial or protective.

On the contrary, lactobacilli were visualized accompanying *Candida* infection even more often than *Gardnerella*. The concentrations or density of lactobacilli within the vaginal epithelium was high, both for *L. iners* and other *Lactobacillus* species including *L. crispatus*. Because the lactobacilli were homogeneously distributed within the lesion and in some cases even enriched at the forefront of *Candida* infiltration, a pathogenic fungal-bacterial symbiosis seems more likely than a secondary saprophytic relationship.

Obviously pathogenic consortia include a variety of different types of bacteria as demonstrated in polymicrobial BV biofilms. The interaction between fungi and bacteria may contribute to the switch from saprophytic to invasive forms of fungal

FIGURE 5
Homogeneous *Candida* lactobacilli co-invasion



Biopsy from a woman with VVC, multicolor FISH using Liners Cy3 (*Lactobacillus iners*, *yellow fluorecence*) and Caal Cy5 probes (*Candida*, *dark red*), and counterstained with DAPI (*blue fluorescence*) ×1000. In contrast to patients with BV and healthy women, *Lactobacillus iners* were identified in dense association with *Candida* infiltration simulating cohesion. Lactobacilli are evenly distributed all over the region of yeast invasion. No *Gardnerella*- or *Atopobium*-positive signals were observed (not shown).

BV, bacterial vaginosis; *DAPI*, 4′,6′-diamino-2-phenylindole; *FISH*, fluorescent in situ hybridization; *VVC*, vulvovaginal candidiasis.

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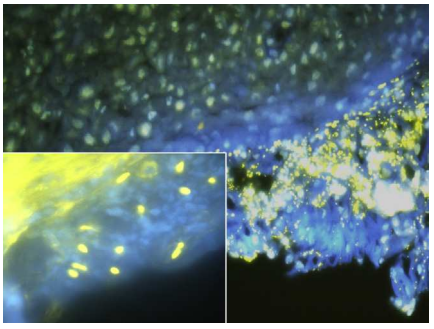
FIGURE 6
Lactobacilli on the forefront of the *Candida* invasion



Lactobacillus iners co-invasion of the epithelial layer is evident in a patient with VVC. Liners Cy3 probe (*Lactobacillus iners*, *yellow fluorecence*) and PF Cy3 probes (*Candida*, *yellow fluorescence* on the insertion left) were counterstained with DAPI (*blue fluorescence*) ×1000. Lactobacilli are concentrated on the forefront of the *Candida* invasion, indicating primarily co-invasion rather than secondary diffusion into the damaged epithelium.

DAPI, 4′,6′-diamino-2-phenylindole; *VVC*, vulvovaginal candidiasis.

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growth. Quorum-sensing research investigates stimuli that synchronize a correlated response of the microorganism to population density. Presently it is mainly focused on cross talking within single groups of microorganisms,[5,9,10] this but should be widened to polymicrobial populations.

In our series, 26% of the lesions did not hybridize with the *Candida albicans*-specific Caal FISH probe but were positive with the universal yeast probe. At present, we cannot designate the origin of these fungi. Either the Caal FISH probe hybridizes less stringently to other representatives of *Candida*, or the infiltration is indeed caused by non-*Candida* yeasts in some cases, which were clinically diagnosed as VVC. However, the extent of the infiltration by *Candida albicans* and other yeasts was identical, even in the absence of hyphae (Figure 6), indicating similar pathogenic potential.

Our data point to vaginal epithelial surface or mucosal invasion as an unrecognized feature of VVC. Although vaginal biopsies were not obtained in asymptomatic women without VVC but colonized with *Candida*, it is likely that such common saprophytic colonization occurs in the absence of any tissue invasion. Most importantly, no evidence of in situ, in vivo *Candida* biofilm existence emerged.

The clinical relevance of these findings is largely unknown, but the absence of finding a biofilm in VVC implies that antibiofilm therapy is not indicated because it may be required in BV. The finding of tissue invasion in VVC may have relevance to recolonization of vagina with candida organisms following antifungal therapy (ie, the vaginal mucosa may serve as organism reservoir to explain recolonization following treatment[20]). ■

Acknowledgments

The work has not been published previously and is not under consideration for publication elsewhere. The publication is approved by all authors, and tacitly or explicitly by the responsible authorities in which the work was carried out, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright holder. Each author named in the byline participated actively and sufficiently in the study reported. The author contributions included the following: Drs Swidsinski, Guschin, XY, and Tertychnyy designed the study; Drs Dörffel, Tang, Tertychnyy, Luo, and Jiang conducted the study; Drs Sobel and Verstraelen critically revised the manuscript; Drs Swidsinski, Guschin, and Dörffel performed the fluorescent in situ hybridization; and Drs Tertychnyy, Tang, Luo, and Jiang analyzed the data. All authors contributed to the conception of the work, revising of the data, shaping of the manuscript, and approved the final draft submitted.

References

1. Denning DW, Kneale M, Sobel JD, Rautemaa-Richardson R. Global burden of recurrent vulvovaginal candidiasis: a systematic review. Lancet Infect Dis 2018;18:PE339-E347.
2. Muzny CA, Schwebke JR. Biofilms: an underappreciated mechanism of treatment failure and recurrence in vaginal infections. Clin Infect Dis 2015;61:601–6.
3. Nobile CJ, Johnson AD. Candida albicans biofilms and human disease. Annu Rev Microbiol 2015;69:71–92.
4. Harriott MM, Noverr MC. Importance of Candida-bacterial polymicrobial biofilms in disease. Trends Microbiol 2011;19:557–63.
5. Höfs S, Mogavero S, Hube B. Interaction of Candida albicans with host cells: virulence factors, host defense, escapestrategies, and the microbiota. J Microbiol 2016;54:149–69.
6. Gao M, Wang H, Zhu L. Quercetin assists fluconazole to inhibit biofilm formations of fluconazole-resistant candida albicans in in vitro and in vivo antifungal managements of vulvovaginal candidiasis. Cell Physiol Biochem 2016;40:727–42.
7. Allison DL, Willems HM, Jayatilake JA, Bruno VM, Peters BM, Shirtliff ME. Candida-bacteria interactions: their impact on human disease. Microbiol Spectr 2016;4:1–26.
8. Hirota K, Yumoto H, Sapaar B, Matsuo T, Ichikawa T, Miyake Y. Pathogenic factors in Candida biofilm-related infectious diseases. J Appl Microbiol 2017;122:321–30.
9. Lohse MB, Gulati M, Johnson AD, Nobile CJ. Development and regulation of single- and multi-species Candida albicans biofilms. Nat Rev Microbiol 2018;16:19–31.
10. Blankenship JR, Mitchell AP. How to build a biofilm: a fungal perspective. Curr Opin Microbiol 2006;9:588–94.
11. Ganguly S, Mitchell AP. Mucosal biofilms of Candida albicans. Curr Opin Microbiol 2011;14:380–5.
12. Soll DR, Daniels KJ. Plasticity of Candida albicans biofilms. Microbiol Mol Biol Rev 2016;80:565–95.

13. Harriott MM, Lilly EA, Rodriguez TE, Fidel PL, Noverr MC. Candida albicans forms biofilms on the vaginal mucosa. Microbiology 2010;156:3635–44.

14. Swidsinski A, Mendling W, Loening-Baucke V, et al. Adherent biofilms in bacterial vaginosis. Obstet Gynecol 2005;106:1013–23.

15. Swidsinski A, Loening-Baucke V, Mendling W, et al. Infection through structured polymicrobial Gardnerella biofilms (StPM-GB). Histol Histopathol 2014;29:567–87.

16. Alexander Swidsinski, Loening-Baucke V. Evaluation of polymicrobial involvement using fluorescence in situ hybridization (FISH) in clinical practice—application guide. Liehr T (ed). Berlin, Germany: Springer-Verlag; 2017. p. 531-43.

17. Gow NA, Hube B. Importance of the Candida albicans cell wall during commensalism and infection. Curr Opin Microbiol 2012;15:406–12.

18. Jabra-Rizk MA, Kong EF, Tsui C, et al. Candida albicans pathogenesis: fitting within the host-microbe damage response framework. Infect Immun 2016;84:2724–39.

19. Matsubara VH, Bandara HM, Mayer MP, Samaranayake LP. Probiotics vas antifungals in mucosal candidiasis. Clin Infect Dis 2016;62:1143–53.

20. Sobel JD. Recurrent vulvovaginal candidiasis. Am J Obstet Gynecol 2016;214:15–21.

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Received July 3, 2018; revised Sept. 28, 2018; accepted Oct. 17, 2018.

The study was supported by a Charité University research promotion grant (2016) and The German Federation of Industrial Research Associations ZIM Project ZF4143701AJ5. Both funding sources were not involved in the study design, collection, analysis and interpretation of data, in writing a report, or the decision to submit the article for publication.

The authors report no conflict of interest.

The data of the publication were not presented previously.

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