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# Smoking in women with chronic vaginal discomfort is not associated with decreased abundance of *Lactobacillus* spp. but promotes *Mobiluncus* and *Gardnerella* spp. overgrowth: secondary analysis of trial data including microbiome analysis

## Kouření u žen s chronickým vaginálním diskomfortem není spojeno se sníženým výskytem *Lactobacillus* spp. ale podporuje nadměrný růst bakterií *Mobiluncus* a *Gardnerella* spp.: sekundární analýza dat z klinické studie zahrnující mikrobiální analýzu

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**Summary:** **Background:** Smoking is considered a risk factor for bacterial vaginosis. It is currently unknown which parameters of the vaginal environment are affected and how smoking triggers the disease. **Aim of the study:** The primary objective is to estimate the effect size of smoking on vaginal pH and the Nugent score in patients with chronic vulvovaginal discomfort prior to the development of episode of vaginosis. The secondary goal is to investigate the effect of smoking on individual microscopic parameters of the vaginal environment and on subjectively reported symptoms of vaginal discomfort. **Methods:** Smoking reported by patients was tested as a predictor, using multivariate logistic and ordinal logistic regression analysis on a dataset from the first visit of a randomized trial NCT04171947, which enrolled patients with intermediate vaginal environment. We tested the primary hypothesis (odds ratio (OR) for vaginal pH > 4.5 and Nugent score > 3 in smokers) at the significance level $\alpha = 5\%$. For exploratory analyses of the effect of smoking on the parameters of the vaginal environment, $\alpha$ was corrected as per Bonferoni. Results: In a cross-sectional sample of 250 women after adjusting for other risk factors, smoking had an impact on the Nugent score (OR = 3.3 (1.3–8.5), P = 0.011), while pH was not affected (OR = 1.2 (0.5–2.8), P = 0.698). Smoking was associated with the prevalence of clue cells (P < 0.000), *Gardnerella* spp. (P = 0.001) and *Mobiluncus* spp. (P = 0.001), while the prevalence of *Lactobacillus* remained unchanged (P = 0.049). **Conclusion:** Contrarily to common assumptions, vaginal *Lactobacillus* is not directly affected by smoking, which rather promotes the growth of bacteria of *Gardnerella* and *Mobiluncus* spp. Given that other parameters remained unaffected, it appears that smoking leads to vaginal dysbiosis by creating specific favourable conditions for these two opportunistic pathogens.

**Key words:** bacterial vaginosis – smoking – Nugent score – vaginal pH – vaginal flora – *Lactobacillus* – *Gardnerella* – *Mobiluncus*

**Souhrn:** **Východiska:** Kouření je považováno za rizikový faktor vzniku bakteriální vaginózy. V současnosti však není známo, které parametry vaginálního prostředí jsou ovlivněny a jak kouření spouští bakteriální vaginózu. **Cíl studie:** Primárním cílem je odhadnout velikost efektu kouření na vaginální pH a Nugentovo skóre u pacientek s chronickým vulvovaginálním diskomfortem před vznikem epizody manifestní vaginózy. Sekundárním cílem je prozkoumat vliv kouření na jednotlivé mikroskopické parametry vaginálního prostředí a na subjektivně reportované příznaky vaginálního diskomfortu. **Metodika:** Kouření reportované pacientkami bylo testováno jako prediktor pomocí multivariantní logistické a ordinální logistické regresní analýzy na souboru dat z první návštěvy randomizované studie NCT04171947, která zařazovala pacientky s přechodným vaginálním prostředím. Pro ověření primární hypotézy (poměr šancí OR na vaginální pH > 4,5 a Nugentovo skóre > 3 u kuřáků) byla uplatněna hladina významnosti $\alpha$ = 5 %. Pro exploratorní analýzu vlivu kouření na parametry vaginálního prostředí byla uplatněna $\alpha$ očištěna dle Bonferoniho. **Výsledek:** V průřezovém vzorku 250 žen po očištění o vliv dalších rizikových faktorů, že mělo kouření, má dopad na Nugentovo skóre (OR = 3,3 (1,3–8,5), p = 0,011), přičemž pH nebylo ovlivněno (OR = 1,2 (0,5–2,8), p = 0,698). Kouření bylo spojeno s prevalencí klíčových buněk (p < 0,000), bakterií *Gardnerella* spp. (p = 0,001) a *Mobiluncus* spp. (p = 0,001), zatímco zastoupení bakterií *Lactobacillus* zůstalo nedotčeno (p = 0,049). **Závěr:** Z pozorování lze odvodit, že na rozdíl od obvyklých předpokladů není vaginální *Lactobacillus* přímo ovlivňován kouřením, které spíše vytváří specifické příznivé podmínky a podporuje růst bakterií *Gardnerella* spp. a *Mobiluncus* spp. Vzhledem k tomu, že další parametry zůstaly nedotčeny, lze předpokládat, že kouření vede k vaginální dysbióze vytvořením příznivých podmínek speciálně pro tyto dva oportunní patogeny.

**Klíčová slova:** bakteriální vaginóza – kouření – Nugentovo skóre – vaginální pH – vaginální flora – *Lactobacillus* – Gardenrella – *Mobiluncus* – lidský mikrobiom

## Introduction

Vaginal microflora dominated by *Lactobacillus* species represent the main pillar of mucosal homeostasis [1–4]. Along with *Lactobacillus*, less frequent elements coexist on the vaginal mucosa including bacteria, yeast, protozoa and blood cells [2,5,6]. In healthy women, lactic bacteria actively maintain low pH by anaerobic glycolysis of mucosal glycogen deposits which, along with the production of hydrogen peroxide, restricts the spread of other strains [2,5]. Opportunistic pathogens are thus present in latent numbers unless the conditions favour their overgrowth [6]. The stability of such an ecosystem is inversely proportional to the community diversity [3,5].

Contrarily to lay assumption, vaginal mucosa is a dynamic environment. Natural fluctuations in the composition relate to pregnancy and menstruation. In the course of life, vaginal mucosa is also repeatedly exposed to secretions, hormonal changes, and external influences; some of which have immediate negative effect on the ecosystem stability [7]. In healthy women, however, the microenvironment presents a tendency to spontaneously normalize towards *Lactobacillus*-dominated microflora [5] and short episodes of bacterial infection resolve spontaneously [7].

The specific conditions allow other strains – typically *Mobiluncus* and *Gardnerella* species, to compete with symbiotic *Lactobacillus*. Albeit often asymptomatic [8], roughly one third of patients with this intermediate flora progress to bacterial vaginosis [7,9] during which the bacteria exceed 100 to 1000-fold the numbers seen in healthy women [1].

Globally, bacterial vaginosis is the most common vaginal infection among women of reproductive age [10] having a significant impact on their quality of life [11]. Bacterial vaginosis is also responsible for problematic conception [12,13] adverse pregnancy outcomes [6,8], and increased risk of sexually transmitted infections [14–16], which imposes a large economic burden on society [10].

The treatment strategies for bacterial vaginosis are few [17] and they fail in over one half of patients [17,18]. Moreover, the repeated use of antibiotics represents a risk of the spread of resistant species. From the perspective of public health, the prevention of bacterial vaginosis is vital. Several risk factors have been identified and their avoidance is encouraged via health education [19]. In the common practice, smoking is considered one of them, although the epidemiological evidence is restricted to exploratory analyses burdened with multiple testing. Hellberg et al [20] showed roughly three-fold risk of bacterial vaginosis in smokers. The diagnosis was determined using Amsel criteria not allowing to uncover the impact of smoking on individual parameters. More detailed results were reported by Alnaif et al [21] describing the association between smoking and semiquantitative score developed by Nugent [22]. Again, smoking increased the risk about three-fold. Bradshaw et al [23] showed a dose-dependent relationship between the amount of cigarettes smoked and the risk of increased Nugent score in women and their female partners. Taken together, we know that smoking is associated with bacterial vaginosis in observational studies but we do not know what parameters of vaginal environment are affected and how is vaginosis triggered. We do not know if smoking favours pathogenic bacteria, if it restricts the symbiotic organisms and protective leukocytes, or if it acts directly on the mucosal pH.

## Material and methods

Our primary hypothesis is that, among patients visiting outpatient gynaecology

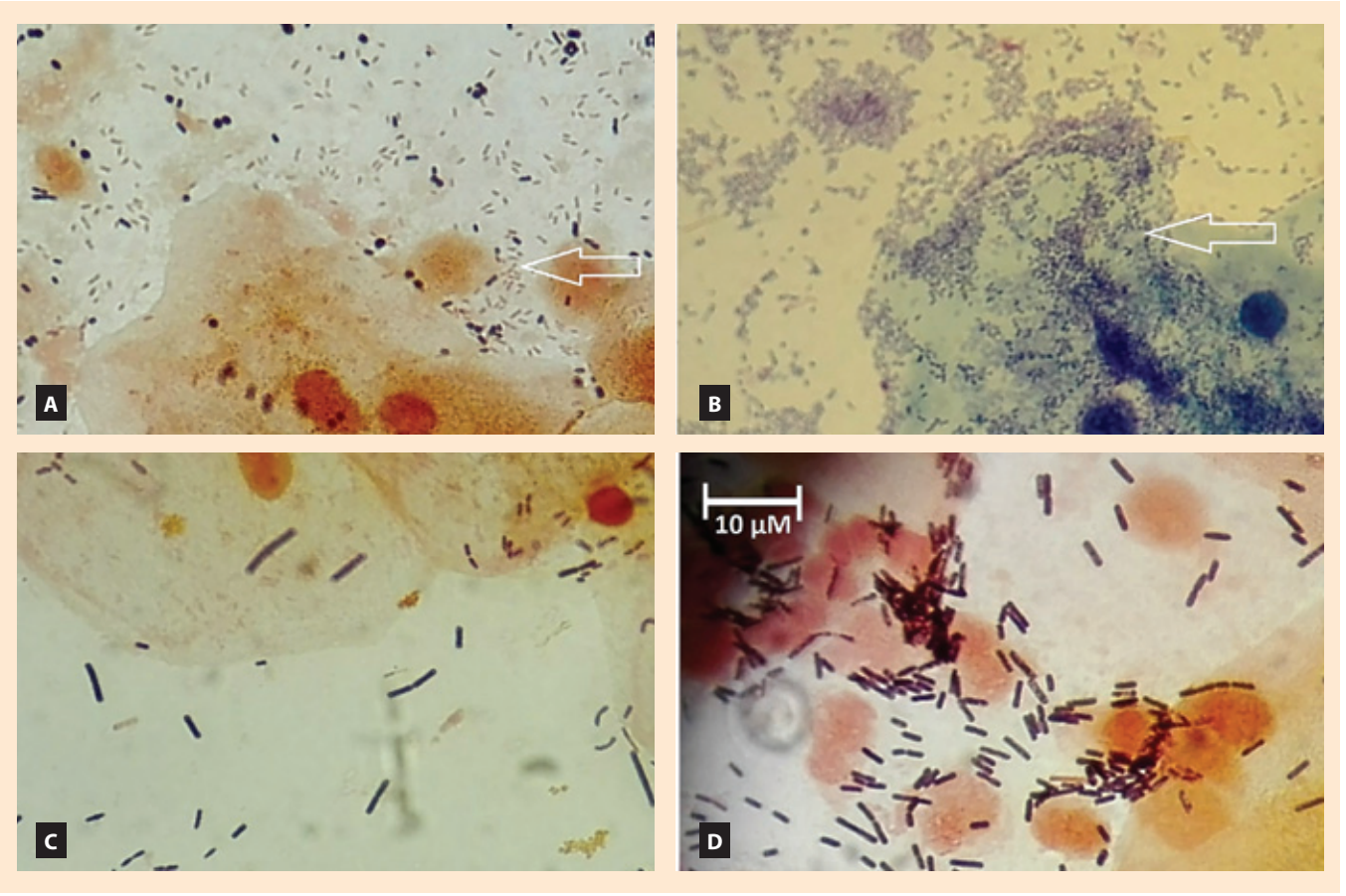


**Fig. 1. Representative micrograms of the vaginal environment.**
For each patient, one slide was stained by Giemsa-Romanowsky and the other by Gram stain. The slides were assessed using an oil immersion lens, providing total magnification of 1,000X. Nugent scores (NS) were determined according to Nugent et al [46]. Panels A and B represent a microscopic image of bacterial vaginosis. Panels C and D show a normal environment. Panel A: NS = 10 (2+, 4+, 3+); Panel B: NS = 8 (0, 4+, 0) is a typical *Gardnerella* infection; Panel C: NS = 1 (3+, 0, 0); Panel D: NS = 0 (4+, 0, 0) with frequent rod-like *Lactobacillus*. The arrows indicate high diversity mucosal flora typical for higher NS.

Obr. 1. Reprezentativní mikroskopické snímky vaginálního prostředí.
U každé pacientky byly provedeny dva stěry, jeden byl obarven dle Giemsy-Romanowského a druhý dle Grama. Roztěr na sklíčku byl odečten imerzním objektivem pod 1 000násobným celkovým zvětšením. Nugentovo skóre (NS) bylo spočteno dle Nugenta et al [46]. Panely A a B představují pacientky s mikroskopickým obrazem bakteriální vaginózy. Panely C a D ukazují normální prostředí. Panel A: NS = 10 (2+, 4+, 3+); Panel B: NS = 8 (0, 4+, 0) je typickou infekcí bakterie *Gardnerella*; Panel C: NS = 1 (3+, 0, 0); Panel D: NS = 0 (4+, 0, 0) s častými tyčinkami rodu *Lactobacillus*. Šipky ukazují smíšenou slizniční flóru typickou pro zvýšené NS.

clinics for vulvovaginal discomfort, smokers have higher odds of Nugent score of > 3 and vaginal pH > 4.5. We further test the relationship between self-reported current smoking, individual subjective symptoms and individual microscopic parameters of the vaginal microenvironment.

This is a secondary analysis of the data from the first visit of the clinical trial MAT072017 [24] (ClinicalTrials.gov Identifier: NCT04171947) which took place in 14 outpatient gynaecology clinics in the Czech Republic in 2018. We included premenopausal patients aged 18 to 55 attending outpatient gynaecology for vulvovaginal discomfort. The subjects had negative pregnancy test, were not breastfeeding, with no antibiotics during the previous month, no bleeding of unknown aetiology, no severe infection requiring antibiotic treatment, and without diabetes mellitus or any oncological condition. For more details, refer to the ClinicalTrials registry.

At the first visit, the patients signed informed consent and consent to the processing of personal information. The patients then filled in questionnaires, rated their symptoms on the scale 0 to 4, and underwent initial general examination, their medical history was assessed, pH measurements were taken, and wet mounts were prepared. In all patients, pH was determined using uniform gynaecology test strips (MColorpHast, Merck, Germany) according to a predefined procedure. Wet mounts were prepared from the posterior vaginal wall using a cotton swab. Fixed and blinded

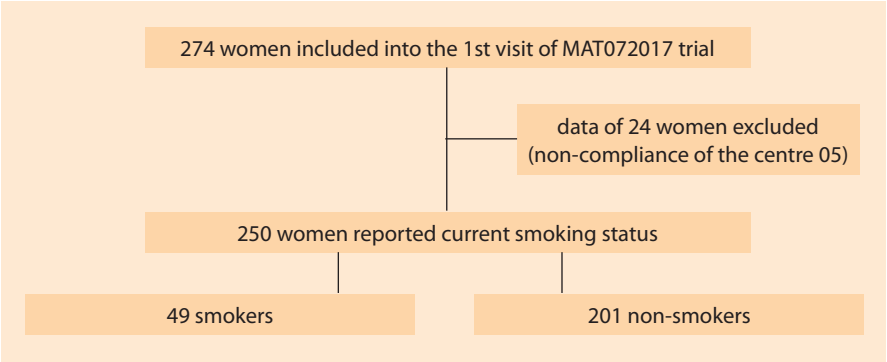


**Fig. 2. Patient flow.**
The data of 24 women (entire centre) were excluded from the analysis for repeated non-compliance of this centre uncovered during several on-site monitoring visits.

Obr. 2. Výběr subjektů.
Data 24 pacientek (zařazených v jediném centru) byla vyřazena z analýzy pro opakované porušení protokolu zkoušejícími v tomto centru.

slides (4-digit codes) from all study centers were transported to a central accredited laboratory (private accredited laboratory AeskuLab k.s, Prague). One slide was stained by Giemsa-Romanowsky and the other by Gram stain. The blinded slides were assessed by a trained microbiologist using an oil immersion lens, providing total magnification of 1000X. The following elements were quantified by a microbiologist: squamous epithelial cells; clue cells; mixed flora; yeast as pseudomycelia; Gram neg. diplococci; fibrous *Lactobacillus*; *Gardnerella*; spirochetes; parabasal epithelial cells; leukocytes; yeast as blastospores; Gram pos. cocci in chains; *Lactobacillus*; *Mobiluncus*; Leptotrichia, and Trichomonas vaginalis. The Nugent score was determined, according to Nugent et al [22] (Fig. 1). Pointwise semiquantitative rating was performed according to the laboratory standardized procedure. Briefly, the number of elements per field were categorized into 5 points. For *Mobiluncus* and *Gardnerella*, 0 = none; 1~tens; 2~hundreds, 3~thousands; and 4 means abnormal infection. For *Lactobacillus*, 0 = none; 1~10 to 15; 2~15 to 50, 3~50 to 100; and 4 means over 100 bacteria per field. All ratings were done by the same microscopists. Unclear findings were resolved by another independent expert.

Current smoking was reported with the patient by the gynaecologist via an electronic case-report form based on the interview with the patient. The smoking status recorded in the electronic case report forms was verified during monitoring visits against the patient's medical record; any inconsistency was reconciled with the gynaecologist.

The hypothesis was tested in the cross-sectional sample using multivariate logistic regression (binary outcomes) and ordinal logistic regression (pointwise outcomes) at $\alpha$ level 0.05. The same was used to test the relationship between smoking and individual symptoms. The association between smoking and individual microscopic parameters was tested at $\alpha$ level 0.005 (corrected as per Bonferoni). The estimates were adjusted to the prevalence of patient-reported psychological distress, BMI, age, recent menstruation, recent coitus, multiple male sexual partners, soap with normal pH, regular usage of tampons and presence of a female sexual partner, using multivariate regression analysis. These factors were considered potential confounders based on a) the baseline differences between smokers and non-smokers and b) risk factors previously published in the literature. The data was complete thanks to thorough monitoring of the trial MAT072017. No imputation was done.

We report in line with the STROBE statement [25].

## Results

Of 274 patients enrolled in 14 out-patient gynaecology clinics, the data of 24 were excluded from the analysis due to repeated non-compliance of one centre. Of 250 patients attending out-patient gynaecology for vulvovaginal discomfort fulfilling the abovementioned criteria, 136 had vaginal pH > 4.5 (107/201 non-smokers vs. 29/49 smokers). Seventy-two subjects had Nugent score > 3 (51/201 non-smokers vs. 21/49 smokers) (Fig. 2). Smokers reported psychological distress (depression, anxiety, bereavement, divorce, job difficulties) roughly twice more often. The remaining characteristics did not differ significantly between smokers and non-smokers (Tab. 1).

Comparing the differences in outcomes between smokers and non-smokers, we identified higher mean Nugent score and more severe discharge in smokers. The vaginal microenvironment in smokers was characterized by higher abundance of clue cells, *Gardnerella* species and *Mobiluncus* species (Tab. 2). The multivariate adjustment showed that smoking was associated with a 3.3-fold (1.3–8.5) higher odds of abnormal Nugent score (adjusted to psychological distress, BMI, age, recent menstruation, recent coitus, multiple male sexual partners, soap with normal pH, usage of tampons and presence of a female sexual partner) (Tab. 3). The estimate remains significant even after further adjustment to previous pregnancy, vaginal douching, allergy to pessary or condoms, use of hormonal contraception, antibiotics, antimycotics or corticosteroids (p = 0.04).

Increased vaginal pH was not associated with subjectively reported smoking (p = 0.577). None of the subjective symptoms are associated with smoking. Among 15 microscopic parameters, only the presence of clue cells, *Gardnerella*

**Tab. 1. Characteristics of smokers and non-smoker attending out-patient gynaecology for vulvovaginal discomfort.**
The differences were tested via Chi2 or t-tests. The smokers suffered more frequently from self-reported psychologic difficulties. The remaining overall characteristics are balanced.

Tab. 1. Charakteristiky pacientek kuřaček a nekuřaček navštěvujících ambulantní gynekologické ordinace z důvodu vulvovaginálního diskomfortu.
Rozdíly byly testovány pomocí Chi kvadrát testu. Kuřačky reportovaly častěji psychologické obtíže, zbývající charakteristiky byly vyvážené.

| | Smoking (49) | Not smoking (201) | P-value |
|---|---|---|---|
| Mean Age | 32.4 ± 6.9 | 34.1 ± 8.2 | 0.19 |
| Mean BMI | 23.7 ± 5.5 | 23.2 ± 4.7 | 0.50 |
| Menstruation at sampling | 5 (10%) | 18 (9%) | 0.56 |
| Psychologic troubles | 19 (39%) | 37 (18%) | 0.00 |
| Having more than 3 sexual male partners in the previous 12 years | 9 (18%) | 23 (11%) | 0.20 |
| Having female sexual partner in the previous 12 months | 2 (4%) | 2 (1%) | 0.13 |
| Having sexual activity prior to 18 y/o | 39 (80%) | 144 (72%) | 0.27 |
| Previous pregnancy | 24 (49%) | 104 (52%) | 0.61 |
| Vaginal douches in the previous 3 months | 8 (16%) | 19 (9%) | 0.21 |
| Cosmetics with normal/high pH | 20 (41%) | 65 (32%) | 0.17 |
| Regularly using tampons | 31 (63%) | 121 (60%) | 0.94 |
| Decreased immunity | 9 (18%) | 34 (17%) | 0.73 |
| Allergy to condoms or vaginal cosmetics | 5 (10%) | 15 (7%) | 0.56 |
| Previous successful treatment of colpitis | 8 (16%) | 52 (26%) | 0.17 |
| Hormonal contraception | 17 (35%) | 58 (29%) | 0.52 |
| Other hormonal treatment | 3 (6%) | 10 (5%) | 0.78 |
| Antibiotic/antimycotics in the previous 3 months | 8 (16%) | 49 (24%) | 0.20 |
| Probiotics or other OTC products in the previous 3 months | 13 (27%) | 45 (22%) | 0.61 |
| Per oral probiotics for this episode | 2 (4%) | 4 (2%) | 0.39 |
| Per vaginam probiotics for this episode | 3 (6%) | 10 (5%) | 0.75 |
| Personal hygiene products for this episode | 3 (6%) | 3 (1%) | 0.06 |
| Corticosteroids in the previous 3 months | 0 (0%) | 6 (3%) | 0.22 |
| This is the first episode of bacterial vaginosis | 15 (31%) | 64 (32%) | 0.87 |
| Patient suffers regularly from these symptoms | 16 (33%) | 54 (27%) | 0.42 |
| Previous (anamnestic) treatment of bacterial vaginosis | 29 (59%) | 121 (60%) | 0.90 |

spp. and *Mobilluncus* spp. are associated with smoking. The reduction in *Lactobacillus* content is not associated with smoking.

## Discussion

Smoking is known to largely affect human microbiome, notably with respect to oral, airway and gut health [26]. We show that, in addition to bacterial vaginosis [20], smoking is also associated with abnormal microflora during chronic recurrent vulvovaginal discomfort.

To date, a specific role of smoking in the aetiology of bacterial vaginosis has not been described. A pilot project using 16S rRNA gene sequencing in vaginal swabs of 20 smokers [27] aimed to identify the species that are most influenced by smoking. The study suggests that smoking correlates with decreased mucosal colonization by symbiotic *Lactobacillus* spp. and smoking cessation may help restore normal microflora in women already suffering from bacterial vaginosis. Contrarily to this finding, smoking was not related with vaginal pH, nor with the decrease in *Lactobacillus* content in our cohort of women suffering from recurrent vaginal discomfort. Our results indicate that smoking generates favourable conditions for pathogenic bacteria rather than unfavourable conditions for symbiotic *Lactobacillus*. Adjusted regression analysis based on the semi-quantitative microscopy shows that *Gardnerella* and *Mobiluncus* content increases in smokers along with the frequency of clue cells. Our result complements previous findings and shows that smoking promotes *Gardnerella* and *Mobiluncus* growth prior to the development of the clinical episode of bacterial vaginosis.

Our cohort was selected with the aim to represent a typical patient consult-

**Tab. 2. Differences in symptoms and elements of vaginal environment between smokers and non-smokers are represented as mean values ± SD.**
The smokers had significantly higher mean Nugent score and more severe discharge. Vaginal microenvironemnt in smokers was characterized by higher abundance of clue cells, *Gardnerella* species and *Mobiluncus* species.

Tab. 2. Rozdíly mezi kuřačkami a nekuřačkami v příznacích a zastoupení mikroskopických prvků vaginálního prostředí jsou vyjádřeny jako průměr ± směrodatná odchylka.
Pacientky kuřačky měly v průměru významně vyšší Nugentovo skóre a závažnější výtok. Vaginální mikroskopické prostředí u kuřaček je charakterizováno vyšším zastoupením klíčových buněk, *Gardnerella* spp. a *Mobiluncus* spp.

| Objective symptoms | Smoking (49) | Not smoking (201) | P-value |
|---|---|---|---|
| Nugent score | 3.8 ± 3.9 | 2.1 ± 2.8 | 0.00 |
| pH | 5.0 ± 0.5 | 5.0 ± 0.6 | 0.91 |
| **Subjective symptoms (0 to 4)** | | | |
| itching | 0.80 ± 0.96 | 0.82 ± 0.90 | 0.86 |
| burning | 0.69 ± 0.92 | 0.78 ± 0.93 | 0.58 |
| redness | 0.51 ± 0.74 | 0.46 ± 0.75 | 0.69 |
| discharge | 1.56 ± 0.77 | 1.27 ± 0.89 | 0.04 |
| odour | 0.65 ± 0.78 | 0.49 ± 0.76 | 0.18 |
| **Elements of the vaginal microenvironment (0 to 4)** | | | |
| Squamous epithelial cells | 2.22 ± 0.62 | 2.24 ± 0.59 | 0.84 |
| Clue cells | 0.41 ± 0.61 | 0.12 ± 0.39 | 0.00 |
| Mixed flora | 1.10 ± 1.03 | 1.21 ± 1.00 | 0.49 |
| yeast as pseudomycelia | 0.08 ± 0.34 | 0.06 ± 0.28 | 0.64 |
| Gram neg. diplococci | none | none | – |
| Fibrous *Lactobacillus* | 0.02 ± 0.14 | 0.02 ± 0.14 | 0.98 |
| *Gardnerella* | 1.06 ± 1.38 | 0.45 ± 1.0 | 0.00 |
| Spirochetes | none | none | – |
| Parabasal epithelial cells | 0.02 ± 0.14 | 0.09 ± 0.31 | 0.10 |
| Leukocytes | 1.18 ± 1.07 | 1.27 ± 1.04 | 0.59 |
| Yeast as blastospores | 0.06 ± 0.24 | 0.12 ± 0.45 | 0.34 |
| Gram pos. cocci in chains | 0.04 ± 0.20 | 0.04 ± 0.25 | 0.91 |
| *Lactobacillus* | 1.43 ± 1.24 | 1.65 ± 1.13 | 0.22 |
| *Mobiluncus* | 0.27 ± 0.67 | 0.04 ± 0.26 | 0.00 |
| *Leptotrichia* | none | none | – |
| *Trichomonas vaginalis* | none | none | – |

ing the outpatient gynaecology clinic. Although roughly 70% women in our cohort had suffered with previous episodes of bacterial vaginosis, the swabs were taken prior to the development of the clinical episode or between the episodes. Thus, the conclusions drawn should not be extrapolated to bacterial vaginosis patients. Another limitation is that our sample consisted exclusively of Caucasian women which may somehow limit generalisation in other ethnic groups in which the microbiome dynamic follows different rules [4,28]. Third, inherent to observational research, our conclusion relies on the assumption of no unmeasured confounding which cannot be proved. A randomized study of smoking, however, is and will not be achievable with respect to the ethical considerations.

## Conclusion

To conclude, we hope to have presented a piece of evidence on the role of smoking in the development of vaginal dysbiosis. A recent metabolomic study [29] shows increased concentration of nicotine metabolites and other amine compounds on the vaginal mucosa of smokers. Biogenic amine compounds have the ability to increase the virulence of latent opportunistic strains that are present even in healthy women. Further research should focus on the molecular mechanism and triggers responsible for *Gardnerella* and *Mobiluncus* overgrowth, notably with respect to smoking-cessation strategies such as nicotine-replacement therapy.

### Declaration of interests

J.T. and B.F. are employees of Value Outcomes, a consultancy and research organization that designed, monitored and conducted the primary randomized trial on behalf of the sponsor Nature Laboratories Ltd., T.D. is the director of Value Outcomes. J.M. is the director of the laboratory that performed blinded analysis of the vaginal swabs, J.K. is a co-owner of the Nature Laboratories Ltd. that provided data from the randomized trial MAT072017. There are no competing interests relevant to the analysis, conclusion or interpretation of the data.

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**Tab. 3. Unadjusted and adjusted odds ratios for increase in individual parameters of the vaginal environment between 201 non-smokers and 49 smokers.**
The effect of smoking (yes/no) on individual parameters was investigated using logistic regression and ordinal logistic regression. The alpha was corrected for multiple testing for all analyses except for the primary hypothesis testing of the pH (binary) and Nugent score (binary).

Tab. 3. Očištěné a neočištěné poměry šancí pro jednotlivé parametry vaginálního prostředí u 201 nekuřaček a 49 kuřaček.
Vliv kouření (ano/ne) na jednotlivé parametry byl testován pomocí logistické a ordinálně logistické regrese. Hladina alfa byla očištěna o mnohočetné testování u všech analýz kromě testu primární hypotézy u pH (binární) a Nugentova skóre (binární).

| Parameter yes/no | Crude odds ratio | Adjusted odds ratio* | P-value adjusted* |
|---|---|---|---|
| Nugent score (1 to 10) above 3 | 2.2 (1.2 to 4.2) | 3.3 (1.3 to 8.5) | 0.011 |
| pH (4 to 7) above 4.5 | 1.3 (0.7 to 2.4) | 1.2 (0.5 to 2.8) | 0.698 |
| Itching (0 to 4) above 1 | 1.2 (0.6 to 2.5) | 1.5 (0.6 to 4.1) | 0.415 |
| Burning (0 to 4) above 1 | 0.7 (0.3 to 1.6) | 1.0 (0.3 to 2.8) | 0.927 |
| Rubor (0 to 4) above 1 | 1.0 (0.3 to 2.7) | 0.8 (0.2 to 4.1) | 0.825 |
| Discharge (0 to 4) above 1 | 1.9 (1.0 to 3.6) | 1.5 (0.6 to 3.6) | 0.338 |
| Malodour (0 to 4) above 1 | 1.2 (0.5 to 2.9) | 0.6 (0.1 to 2.9) | 0.504 |
| **Pointwise parameter** | | | |
| Nugent continuous (0 to 10) | 2.35 (1.30 to 4.23) | 3.65 (1.62 to 8.25) | 0.002 |
| pH continuous (4 to 7) | 1.21 (0.70 to 2.08) | 1.18 (0.50 to 2.84) | 0.698 |
| Gardnerella (0 to 4) | 3.02 (1.55 to 5.90) | 5.74 (1.84 to 12.21) | 0.001 |
| Clue cells (0 to 4) | 4.46 (2.14 to 9.31) | 7.22 (2.50 to 20.87) | 0.000 |
| Mobiluncus (0 to 4) | 6.50 (2.14 to 19.72) | 11.84 (2.59 to 54.19) | 0.001 |
| Fibrous lactobacillus (0 to 4) | 1.03 (0.11 to 9.39) | 5.88 (0.4 to 87.37) | 0.198 |
| Lactobacillus (0 to 4) | 0.67 (0.38 to 1.23) | 0.46 (0.21 to 1.00) | 0.049 |

*adjusted to: psychological distress, BMI, age, recent menstruation, recent coitus, multiple male sexual partners, soap with normal pH, usage of tampons, female sexual partner.

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