Pathology - Research and Practice 216 (2020) 153234

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Gardnerella vaginalis and *Trichomonas vaginalis* infections and the risk of persistence or progression of low-grade cervical intraepithelial neoplasia

Antonio Raffone [a], Antonio Travaglino [b,*], Antonio Angelino [a], Rosanna Esposito [a], Martina Pontillo [a], Antonio Mollo [c], Angela Santoro [d], Gian Franco Zannoni [d], Luigi Insabato [b], Matilde Sansone [a], Fulvio Zullo [a]

[a] Gynecology and Obstetrics Unit, Department of Neuroscience, Reproductive Sciences and Dentistry, School of Medicine, University of Naples Federico II, Naples, Italy
[b] Anatomic Pathology Unit, Department of Advanced Biomedical Sciences, School of Medicine, University of Naples Federico II, Naples, Italy
[c] Department of Medicine, Surgery and Dentistry, Schola Medica Salernitana, University of Salerno, Baronissi, Salerno, Italy
[d] Pathology Unit, Department of Woman and Child Health, Agostino Gemelli University Polyclinic, Catholic University of the Sacred Heart, Rome, Italy

ARTICLE INFO

Keywords:
Premalignant
Tailored
Prediction
Cervix
Cancer
Tumor
Response

ABSTRACT

Gardnerella vaginalis (GV) and *Trichomonas vaginalis* (TV) infections have been proposed as risk factors for persistence or progression of low-grade precancerous cervical lesions (CIN1/L-SIL). However, their role is still undefined. We aimed to assess if GV and TV infections affect the risk of persistence/progression of CIN1/L-SIL.

A retrospective cohort study was performed to assess the risk of CIN1/L-SIL persistence or progression, persistence alone and progression alone in patients with GV and/or TV infections (GV + and/or TV+), only GV (GV+), only TV (TV+), or GV and TV coinfections compared to patients without these infections. Relative risk (RR) with 95 % confidence intervals (CI) was adopted (significant p-value>0.05).

Two hundred and seventy patients were included. RR for CIN1/L-SIL persistence or progression was 1.63 in GV + and/or TV+ (p = 0.02), 1.99 in GV+ (p = 0.0008), 0.25 in TV+ (p = 0.32), 1.78 in coinfection (p = 0.26). RR for persistence was 1.55 in GV + and/or TV+ (p = 0.1), 2.179 in GV+ (p = 0.0013), 0.32 in TV+ (p = 0.41), 0.45 in coinfection (p = 0.55). RR for progression was 1.92 in GV + and/or TV+ (p = 0.22), 1.34 in GV+ (p = 0.68), 1.16 in TV+ (p = 0.91), 8.39 in coinfection (p = 0.0002).

In conclusion, GV infection may be a risk factor for CIN1/L-SIL persistence. TV infection alone does not significantly affect the risk of persistence or progression of such lesions, while it may greatly increase the risk of progression when associated with GV infection.

1. Introduction

Human papillomavirus (HPV) is the most frequent sexually transmitted infection in women, and the most important risk factor for cervical cancer [1,2]. Squamous cervical carcinoma arises from precancerous lesions termed “cervical intraepithelial neoplasia” (CIN) or “squamous intraepithelial lesion” (SIL). These lesions are classified into low-grade (CIN1/L-SIL) and high-grade (CIN2−3/ H-SIL), based on the grade of dysplasia [3–7]. While an intervention (*i.e.* cryotherapy, large loop excision of the transformation zone or cold knife conization) is necessary for high-grade lesions, a follow-up approach may be adopted for low-grade lesions, given their low risk of progression [8,9]. Several risk factors may act in the increase the risk of persistence or progression of these low-grade lesions [10–13]. Among these, *Gardnerella vaginalis* (GV) and *Trichomonas vaginalis* (TV) infections might play a role as risk factors for both HPV infection and progression of low-grade cervical precancerous lesions [14–16].

GV is an anaerobic bacterium which is involved in bacterial vaginosis [17]. It determines a predominance of anaerobic bacteria and a decrease in protective vaginal lactobacilli, favoring HPV and other sexually transmitted infections [18].

TV is a flagellate protozoan of the lower female genital tract that induce inflammation and damage in the cervico-vaginal epithelium, increasing the risk of HPV persistence and thus cervical cancer [19,20].

However, the role of GV and TV infections as risk factor for persistence or progression of CIN1/L-SIL is still undefined [20–23,24]. We

* Corresponding author at: Anatomic Pathology Unit, Department of Advanced Biomedical Sciences, School of Medicine, University of Naples Federico II, Via Sergio Pansini, 5, Naples, 80131, Italy.
E-mail address: antonio.travaglino.ap@gmail.com (A. Travaglino).

https://doi.org/10.1016/j.prp.2020.153234
Received 7 August 2020; Received in revised form 26 September 2020; Accepted 29 September 2020
Available online 1 October 2020

aimed to assess how GV and TV infections affect the risk persistence or progression of CIN1/L-SIL.

2. Material and methods

2.1. Study protocol, selection criteria and ethical approval

The study protocol was *a priori* defined, and the study was reported according to the STROBE guidelines [25]. This study had a single-center observational retrospective cohort study design.

We reviewed clinical records and pathologic archives for consecutive patients diagnosed with CIN1/L-SIL from January 1999 to May 2019 at the Department of Neuroscience, Reproductive Sciences and Dentistry and the Department of Advanced Biomedical Sciences, School of Medicine, University of Naples Federico II, Naples, Italy.

We assessed the risk of progression or persistence of CIN1/L-SIL [26] in patients with GV and/or TV infections and patients without these infections.

We excluded: patients with a follow-up lower than 6 months; patients with CIN2−3/ H-SIL or invasive cervical carcinoma; patients with HIV infection; patients who did not provide a written consent for the use of their data and biospecimen for research purpose; patients whose pathological slides were not available for review.

Two blinded pathologists (AT, LI) reviewed all pathological slides in order to confirm the initial diagnosis. A review at a two-headed microscope was performed in the case of disagreements.

Before the beginning of the study, we obtained approval by the Institutional Review Board of the University Federico II of Naples, Italy (No.: 226/19). All data were anonymized, and the whole study was performed in accordance with the declaration of Helsinki.

2.2. Study endpoints

The primary endpoint was *a priori* established as the risk of persistence or progression of CIN1/L-SIL in patients with GV and/or TV infections (GV + and/or TV+) compared to patients without these infections.

Secondary endpoints were *a priori* established as follows:

- the risk of persistence of CIN1/L-SIL in GV + and/or TV + compared to patients without these infections;
- the risk of progression of CIN1/L-SIL in GV + and/or TV + compared to patients without these infections;
- the risk of persistence or progression of CIN1/L-SIL in patients with GV infection (GV+) compared to patients without GV or TV infections;
- the risk of persistence of CIN1/L-SIL in GV + compared to patients without GV or TV infections;
- the risk of progression of CIN1/L-SIL in GV + compared to patients without GV or TV infections;
- the risk of persistence or progression of CIN1/L-SIL in patients with TV infection (TV+) compared to patients without GV or TV infections;
- the risk of persistence of CIN1/L-SIL in TV + compared to patients without GV or TV infections;
- the risk of progression of CIN1/L-SIL in TV + compared to patients without GV or TV infections;
- the risk of persistence or progression of CIN1/L-SIL in patients with GV and TV coinfection compared to patients without GV or TV infections;
- the risk of persistence of CIN1/L-SIL in patients with GV and TV coinfection compared to patients without GV or TV infections;
- the risk of progression of CIN1/L-SIL in patients with GV and TV coinfection compared to patients without GV or TV infections.

Regression of CIN1/L-SIL was defined as the absence of the lesion at cytological or histological specimen after at least 6-month follow-up. Persistence of CIN1/L-SIL was defined as the persistence of CIN1/L-SIL at cytological or histological specimen after at least 6-month follow-up. Progression of CIN1/L-SIL was defined as worsening of lesion to CIN2−3/H-SIL or invasive carcinoma at cytological or histological specimen after at least 6-month follow-up.

2.3. Pathological and microbiological methods

Cytobrush (Pap-Test) was used to obtain cervical cytological samples, which were put on slides and fixed with a mix of wax and alcohol. Wax was then removed through a treatment with ethyl alcohol for 15 min. The Papanicolau staining was performed by using a Leica ST5020 automatized stainer. Nuclei were stained with hematoxylin, while cytoplasm was stained with OG6 and EA50.

Histological specimens were obtained by colposcopic biopsy or conization. Biopsy specimens were fixed in buffered formalin for 6 h, while conization specimens were first sectioned *in toto* at gross handling and then fixed in buffered formalin for 24 h. Formalin-fixed samples were dehydrated through an automatized procedure, and they were embedded in paraffin the day after. Four μm-thick sections were obtained from paraffin-embedded tissue blocks. Routine hematoxylin-eosin staining was performed through an automatized procedure.

The presence of GV and TV was assessed by culture of cervico-vaginal swab: GV was assessed by culture in Gardnerella selective agar with 5% human blood, while TV was assessed by in-pouch culture test.

2.4. Statistical analyses

The risk of persistence or progression of CIN1/L-SIL was assessed by relative risk (RR) with 95 % confidence intervals (CI) and significant p-value <0.05. MedCalc (MedCalc Software bv, Ostend, Belgium) was used for statistical analysis.

3. Results

3.1. Study population

Two hundred and seventy women met the selection criteria and were included in the study. Of total, 9% were GV+, 2% TV+, 1% coinfections, and 87 % patients without GV or TV infections (Table1).

One hundred and eighty-eight patients showed CIN1/L-SIL regression, 64 persistence, and 18 progression. Regression was observed in 44 % of GV+, 50 % of coinfections, 54.3 % of GV + and/or TV+, 55.6 % of patients without GV or TV infection. Persistence was observed in 48 % of GV+, 0 coinfections, 41.5 % of GV + and/or TV+, 22.1 % of patients without GV or TV infection. Progression was observed in 8% of GV+, 50 % of coinfections, 11.4 % of GV and/or TV+, 6% of patients without GV or TV infection. No TV + showed persistence or progression (Table 2).

Table 1
Patient characteristics.

	GV+	TV+	Coinfection	GV + and/or TV+	No infection	TOTAL
CIN1 / L-SIL, n (%)	25 (9)	6 (2)	4 (1)	35	235 (87)	270
Age, mean years ± SD	39.4 ± 11.5	45.2 ± 14.5	40.5 ± 16.5	40.2 ± 12.7	42.9 ± 13.9	42.5 ± 13.8
Smoker, n	2	1	2	6	51	56

GV+: patients with *Gardnerella vaginalis* infection.
TV+: patients with *Trichomonas vaginalis* infection.
GV + and/or TV+: patients with *Gardnerella* and/or *Trichomonas vaginalis* infection.

Table 2
Cervical lesions outcomes.

	GV+	TV+	Coinfection	GV + and/or TV+	No infection	TOTAL
Regression, n (%)	11 (44)	6 (100)	2 (50)	19 (54.3)	169 (71.9)	**188**
Persistence, n (%)	12 (48)	0 (0)	0 (0)	12 (34.3)	52 (22.1)	**64**
Progression, n (%)	2 (8)	0 (0)	2 (50)	4 (11.4)	14 (6)	**18**
Persistence or progression, n (%)	14 (56)	0 (0)	2 (50)	16 (45.7)	66 (28.1)	**82**
TOTAL	**25**	**6**	**4**	**35**	**235**	**270**

GV+: women with *Gardnerella vaginalis* infection.
TV+: women with *Trichomonas vaginalis* infection.
GV + and/or TV+: women with *Gardnerella* and/or *Trichomonas vaginalis* infection.

3.2. Study endpoints

3.2.1. Patients with GV and/or TV infection

RR for CIN1/L-SIL persistence or progression in GV + and/or TV + was 1.63 (95 %CI, 1.07–2.46) compared to patients without these infections (p = 0.02).

RR for CIN1/L-SIL persistence in GV + and/or TV + was 1.55 (95 % CI, 0.92–2.6) compared to patients without these infections (p = 0.1).

RR for CIN1/L-SIL progression in GV + and/or TV + was 1.92 (95 % CI, 0.67–5.5) compared to patients without these infections (p = 0.22).

3.2.2. Patients with GV infection

RR for CIN1/L-SIL persistence or progression in GV + was 1.99 (95 % CI, 1.33–2.98) compared to patients without GV or TV infection (p = 0.0008).

RR for CIN1/L-SIL persistence in GV + was 2.17 (95 %CI, 1.35–3.48) compared to patients without GV or TV infection (p = 0.0013).

RR for CIN1/L-SIL progression in GV + was 1.34 (95 %CI, 0.32–5.57) compared to patients without GV or TV infection (p = 0.68).

3.2.3. Patients with TV infection

RR for CIN1/L-SIL persistence or progression in TV + was 0.25 (95 % CI, 0.02–3.69) compared to patients without GV or TV infection (p = 0.32).

RR for CIN1/L-SIL persistence in TV + was 0.32 (95 %CI, 0.02–4.69) compared to patients without GV or TV infection (p = 0.41).

RR for CIN1/L-SIL progression in TV + was 1.16 (95 %CI, 0.07–17.6) compared to patients without GV or TV infection (p = 0.91).

3.2.4. Patients with GV and TV coinfection

RR for CIN1/L-SIL persistence or progression in GV + and TV + was 1.78 (95 %CI, 0.65–4.84) compared to patients without GV or TV infection (p = 0.26).

RR for CIN1/L-SIL persistence in GV + and TV + was 0.45 (95 %CI, 0.03–6.3) compared to patients without GV or TV infection (p = 0.55).

RR for CIN1/L-SIL progression in GV + and TV + was 8.39 (95 %CI, 2.78–25.31) compared to patients without GV or TV infection (p = 0.0002).

3.2.5. Synthesis of results

Results about each study endpoint in each study sub-population are summarized in Table 3.

4. Discussion

This study showed that GV infection is a significant risk factor for CIN1/L-SIL persistence, while GV and TV coinfection is a significant risk factor for CIN1/L-SIL progression. In fact, patients with GV infection may have a 2-fold increased risk of persistence of low-grade precancerous cervical lesion, while patients with GV and TV coinfection may have an 8-fold increased risk of progression of such lesions. On the other hand, TV infection alone does not appear to significantly affect the risk of persistence or progression of precancerous cervical lesions compared to women without this infection.

Table 3
Relative risk for CIN1/L-SIL persistence/progression in each study sub-population.

STUDY ENDPOINT	STUDY SUB-POPULATION			
	GV+	TV+	Coinfection	GV + and/or TV+
Persistence	**2.17**	0.32*	0.45*	1.55*
Progression	1.34*	1.16*	**8.39**	1.92*
Persistence or progression	**1.99**	0.25*	1.78*	**1.63**

* Not significant p value.

Several factors have been investigated as risk factors for persistence and/or progression of precancerous cervical lesions [27]. In this regard, on a hand, some cervico-vaginal infections (*e.g. Chlamydia trachomatis* and *Ureaplasma urealyticum*) seem to increase the risk of persistence of HPV infection; on the other hand, cervical mucosa inflammation related to chronic infections may itself favor carcinogenesis [28]. In fact, non-specific antimicrobial oxidants as products of inflammation may generate an oxidative impairment to host DNA [29].

We found GV as risk factor for persistence of cervical precancerous lesions. Several mechanisms may support this finding. Firstly, GV infection increase the vaginal pH and reduces protective vaginal lactobacilli, which have shown to be cytotoxic on cervical tumor cells *in vitro* [30–32]. Secondly, GV infection reduces levels of secretory leukocyte protease inhibitor, which is a defensive mechanism against viral infections [30,33]. Thirdly, GV infection may alter the protective cervical mucosa barrier by increasing the level of mucin-degrading enzymes [34,35]. These effects may lead to a delayed HPV infection's clearance and thus to a persistence of precancerous cervical lesions [36,37].

On the other hand, also TV infection has shown several mechanisms possibly related to bad outcomes of precancerous cervical lesions. In particular, it has shown to be able to upregulate macrophage proinflammatory responses and cell proliferation *in vitro*, with also changes in vaginal epithelium and cervical mucus [38–41]. Moreover, cytotoxic substances released during infection, such as cell-detaching factor and N-nitrosamines, also may promote epithelial atypia and dysplasia [42]. Despite these findings, we found a not-significant effect of TV infection on cervical precancerous lesions outcomes.

Finally, patients with GV and TV coinfection showed the highest RR for progression, suggesting a synergic effect of GV and TV in affecting CIN1/L-SIL progression. These results seem to indicate that TV infection may not be a stand-alone risk factor for persistence or progression of cervical precancerous lesions, but that it may greatly enhance the effect of other factors, such as GV infection.

Patients with CIN1/L-SIL and GV infection, and even more with GV and TV coinfection, may require a closer follow-up because of the significantly higher risk of persistence or progression of cervical precancerous lesions. Furthermore, patients with GV and TV coinfection might require treatment due to the 8-fold higher risk of progression; in these patients, the possible benefits of treatment need to be evaluated.

A strength of our study is the exclusion of HIV + patients. In fact, these patients might have a higher baseline risk of both vaginal infections and CIN1/L-SIL persistence/progression, constituting a serious confounding factor. Furthermore, we assessed progression and persistence both together and separately, defining the effect of GV and TV infection on CIN1/L-SIL more clearly. In fact, we found that GV alone

increased the risk of persistence but not of progression, while the coexistence of TV with GV greatly increased the risk of progression but not of persistence.

However, some limitations affect our results, such as the retrospective design and the low number of patients with TV infections in our cohort.

Further studies are necessary in this field to investigate GV and TV infection in affecting precancerous cervical lesions outcomes, as well as other risk factors to be integrated in a predictive algorithm of response in the future.

5. Conclusion

GV infection may be a risk factor for CIN1/L-SIL persistence. TV infection alone does not appear to significantly affect the risk of persistence or progression of such lesions, while when associated with GV infection, it may greatly increase the risk of progression. Patients with GV infection, and even more with GV and TV coinfection, may require a closer follow-up of CIN1/L-SIL. Future studies are necessary to further investigate this field.

Ethical approval

Before the beginning of the study, we obtained approval by the Institutional Review Board of the University Federico II of Naples, Italy (No.: 226/19). data were anonymized. All patients included in this study provided their written consent.

Informed Consent

All included patients provided a written consent for the use of their data and biospecimen for research purpose.

Author Contribution

AR: study conception, data analysis, results interpretation, manuscript preparation; **AT**: study conception, data analysis, results interpretation, manuscript preparation; **AA**: study conception, data collection, manuscript preparation; **RE**: data collection, data analysis, manuscript preparation; **MP**: data collection, data analysis, manuscript preparation; **AM**: data analysis, manuscript revision, supervision; **AS:** data analysis, results interpretation, manuscript revision; **GFZ:** data collection, results interpretation, manuscript revision, supervision; **LI**: data collection, results interpretation, manuscript revision, supervision; **MS**: study conception, data collection, manuscript revision, supervision; **FZ**: study conception, results interpretation, manuscript revision, supervision.

Declaration of Competing Interest

The authors report no declarations of interest.

References

[1] E.F. Dunne, E.R. Unger, M. Sternberg, et al., Prevalence of HPV infection among females in the United States, J. Am. Med. Assoc. 297 (8) (2007) 813–819.

[2] IARC monographs on the evaluation of carcinogenic risks to humans, in: Human Papillomaviruses, Volume 90, International Agency for Research on Cancer, Lyon, France, 2007.

[3] R. Kurman, M. Carcangiu, C. Herrington, R. Young, WHO Classification of Tumours of Female Reproductive Organs, 4th edn., International Agency for Research on Cancer, Lyons, 2014.

[4] A.G. Waxman, D. Chelmow, T.M. Darragh, et al., Revised terminology for cervical histopathology and its implications for management of high-grade squamous intraepithelial lesions of the cervix, Obstet. Gynecol. 120 (1465) (2012).

[5] T.M. Darragh, T.J. Colgan, J. Thomas Cox, et al., The lower anogenital squamous terminology standardization project for HPV-associ- ated lesions: background and consensus recommendations from the college of american pathologists and the american society for colposcopy and cervical pathology, Int. J. Gynecol. Pathol. 32 (76) (2013).

[6] R. Nayar, D.C. Wilbur, The Pap Test and Bethesda 2014, Acta Cytol. (59) (2015) 121–132.

[7] A. Raffone, A. Travaglino, A. D'Antonio, M. De Marco, M. Caccese, M. Mascolo, L. Insabato, P. Zeppa, A. Rosati, A. Mollo, F. Zullo, M. Guida, BAG3 expression correlates with the grade of dysplasia in squamous intraepithelial lesions of the uterine cervix, Acta Obstet. Gynecol. Scand. 99 (January (1)) (2020) 99–104.

[8] L.S. Massad, M.H. Einstein, W.K. Huh, et al., ASCCP Consensus Guidelines Conference. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors, J. Low. Genit. Tract Dis. 17 (5 Suppl 1) (2012) S1–S27, 2013.

[9] S. Andersson, M. Mints, E. Wilander, Results of cytology and high-risk human papillomavirus testing in females with cervical adenocarcinoma in situ, Oncol. Lett. 6 (1) (2013) 215–219.

[10] L.A. Brinton, W.C. Reeves, M.M. Brenes, Parity as a risk factor for cervical cancer, Am. J. Epidemiol. 130 (3) (1989) 486–496.

[11] F.X. Bosch, N. Muñoz, S. de Sanjosé, et al., Risk factors for cervical cancer in Colombia and Spain, Int. J. Cancer 52 (1992) 750–758.

[12] E. Roura, X. Castellsagué, M. Pawlita, et al., Smoking as a major risk factor for cervical cancer and pre-cancer: results from the EPIC cohort, Int. J. Cancer 135 (2) (2014) 453–466, https://doi.org/10.1002/ijc.28666.

[13] S. Wardak, Human Papillomavirus (HPV) and cervical cancer, Med. Dosw. Mikrobiol. 68 (1) (2016) 73–84.

[14] A. Audirac-Chalifour, K. Torres-Poveda, M. Bahena-Román, et al., Cervical microbiome and cytokine profile at various stages of cervical Cancer: a pilot study, PLoS One 11 (4) (2016) e0153274, https://doi.org/10.1371/journal.pone.0153274. Published 2016 Apr 26.

[15] E. Gillet, J.F. Meys, H. Verstraelen, et al., Association between bacterial vaginosis and cervical intraepithelial neoplasia: systematic review and meta-analysis, PLoS One 7 (10) (2012) e45201, https://doi.org/10.1371/journal.pone.0045201.

[16] Y. Liang, M. Chen, L. Qin, B. Wan, H. Wang, A meta-analysis of the relationship between vaginal microecology, human papillomavirus infection and cervical intraepithelial neoplasia [published correction appears in Infect Agent Cancer. 2019 Dec 9;14:47], Infect Agent Cancer. 14 (2019) 29, https://doi.org/10.1186/s13027-019-0243-8. Published 2019 Oct 26.

[17] J.R. Schwebke, C.A. Muzny, W.E. Josey, Role of Gardnerella vaginalis in the pathogenesis of bacterial vaginosis: a conceptual model, J. Infect. Dis. 210 (3) (2014) 338–343.

[18] A. Swidsinski, W. Mendling, V. Loening-Baucke, et al., Adherent biofilms in bacterial vaginosis, Obstet. Gynecol. 106 (5 Pt 1) (2005) 1013–1023.

[19] M. Sutton, M. Sternberg, E.H. Koumans, et al., The prevalence of Trichomonas vaginalis infection among reproductive-age women in the United States, 2001–2004, Clin. Infect. Dis. 45 (10) (2007) 1319–1326.

[20] J.R. Schwebke, D. Burgess, Trichomoniasis, Clin. Microbiol. Rev. 17 (4) (2004) 794–803.

[21] M. Usyk, C.P. Zolnik, P.E. Castle, et al., Cervicovaginal microbiome and natural history of HPV in a longitudinal study, PLoS Pathog. 16 (3) (2020) e1008376, https://doi.org/10.1371/journal.ppat.1008376. Published 2020 Mar 26.

[22] A. Mitra, D.A. MacIntyre, G. Ntritsos, et al., The vaginal microbiota associates with the regression of untreated cervical intraepithelial neoplasia 2 lesions, Nat. Commun. 11 (1) (2020) 1999, https://doi.org/10.1038/s41467-020-15856-y. Published 2020 Apr 24.

[23] R.M. Feng, M. Wang, J.S. Smith, et al., Risk of high-risk human papillomavirus infection and cervical precancerous lesions with past or current trichomonas infection: a pooled analysis of 25,054 women in rural China, J. Clin. Virol. 99–100 (2018) 84–90.

[24] S. Yang, W. Zhao, H. Wang, Y. Wang, J. Li, X. Wu, Trichomonas vaginalis infection-associated risk of cervical cancer: a meta-analysis, Eur. J. Obstet. Gynecol. Reprod. Biol. 228 (2018) 166–173.

[25] E. Von Elm, D.G. Altman, M. Egger, et al., The strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies, Int. J. Surg. 12 (2014) 1495–1499.

[26] Gestione della paziente con pap test anormale - Linee Guida Edizione, La Colposcopia in Italia Anno XXI - N.1-dicembre 2006, 2006.

[27] A. Hildesheim, R. Herrero, P.E. Castle, et al., HPV co-factors related to the development of cervical cancer: results from a population-based study in Costa rica, Br. J. Cancer 84 (9) (2001) 1219–1226.

[28] P.E. Castle, S.L. Hillier, L.K. Rabe, et al., An association of cervical inflammation with high-grade cervical neoplasia in women infected with oncogenic human papillomavirus (HPV), Cancer Epidemiol. Biomarkers Prev. 10 (October (10)) (2001) 1021–1027.

[29] B.N. Ames, L.S. Gold, W.C. Willett, The causes and prevention of cancer, Proc Natl Acad Sci U S A. 92 (12) (1995) 5258–5265.

[30] S.M. Wahl, T.B. McNeely, E.N. Janoff, et al., Secretory leukocyte pro- tease inhibitor (SLPI) in mucosal fluids inhibits HIV-I, Oral Dis. 3 (1997) S64–S69.

[31] M. Morris, A. Nicoll, I. Simms, J. Wilson, M. Catchpole, Shift in flora suggestive of/compatible with bacterial vaginosis: a public health review, BJOG. 108 (2001) 439–450.

[32] E. Motevaseli, M. Shirzad, S.M. Akrami, A.S. Mousavi, A. Mirsalehian, M. Modarressi-Hossein, Normal and tumor cervical cells respond differently to vaginal lactobacilli, independent of pH and lactate, J. Med. Microbiol. 62 (2013) 1065–1072.

[33] A.M. Briselden, B.J. Moncla, C.E. Stevens, S.L. Hillier, Sialidases (neuraminidases) in shift in flora suggestive of/compatible with bacterial vaginosis and shift in flora

suggestive of/compatible with bacterial vaginosis-associated microflora, J. Clin. Microbiol. 30 (1992) 663–666.
[34] C.C. King, D.J. Jamieson, J. Wiener, et al., Bacterial vaginosis and the natural history of human papillomavirus, Infect. Dis. Obstet. Gynecol. 2011 (2011) 319460.
[35] R.M. Brotman, M.D. Shardell, P. Gajer, et al., Interplay between the temporal dynamics of the vaginal microbiota and human papilloma- virus detection, J. Infect. Dis. 210 (2014) 1723–1733.
[36] M.A. Clarke, A.C. Rodriguez, J.C. Gage, et al., A large, population- based study of age-related associations between vaginal pH and human papillomavirus infection, BMC Infect. Dis. 12 (33) (2012).
[37] Y.L. Guo, K. You, J. Qiao, Y.M. Zhao, L. Geng, Bacterial vaginosis is conducive to the persistence of HPV infection, Int. J. STD AIDS 23 (2012) 581–584.
[38] P.L. Fiori, N. Diaz, A.R. Cocco, P. Rappelli, D. Dessi, Association of trichomonas vaginalis with its symbiont mycoplasma hominis synergistically upregulates the in vitro proinflammatory response of human monocytes, Sex. Transm. Infect. 89 (2013) 449–454.
[39] N. Phukan, T. Parsamand, A.E. Brooks, T.N. Nguyen, A. Simoes-Barbosa, The adherence of trichomonas vaginalis to host ectocervical cells is influenced by Lactobacilli, Sex Transmission Infect. 89 (2013) 455–459.
[40] O. Twu, D. Dessi, A. Vu, F. Mercer, G.C. Stevens, N. de Miguel, et al., Trichomonas vaginalis homolog of macrophage migration inhibitory factor induces prostate cell growth, invasiveness, and inflammatory responses, Proc Natl Acad Sci U S A. 111 (2014) 8179–8184.
[41] M.E. Boon, P.A. Holloway, H. Breijer, Bontekoe T.R. Gardnerella, Trichomonas and Candida in cervical smears of 58,904 immigrants participating in the dutch national cervical screening program, Acta Cytol. 56 (2012) 242–246.
[42] S. Sood, A. Kapil, An update on Trichomonas vaginalis, Indian J. Sex. Transm. Dis. 29 (2008) 7–14.