

Microbiota for HPV Infection

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The microbiome is able to modulate immune responses, alter the physiology of the human organism, and increase the risk of viral infections and development of diseases such as cancer. Herein, we address changes in the cervical microbiota as potential biomarkers to identify the risk of cervical intraepithelial neoplasia (CIN) development and invasive cervical cancer in the context of human papillomavirus (HPV) infection.

Keywords: microbiota ; cervical displasia ; HPV ; cervical cancer

1. Introduction

Although HPV infection is a necessary cause, it is not determinant for cervical cancer development. HPV infects squamous epithelial basal cells, inducing lesions and even cervical cancer when it is not eliminated [1]. However, the majority of HPV infections are cleared and only a small fraction of infected women progress to premalignant lesions and cancer [1]. Pap smear has been used for cervical screening, which resulted in a decrease in deaths from cervical cancer. Nevertheless, the assay has low sensitivity (60–80%), high false-negative rates (30%), and significant false-positive rates, ranging from 15–50% [2][3]. On the other hand, introduction of HPV-DNA assays for screening has improved the results from equivocal cytology triage with Pap smear [4].

In many countries, Pap cytology is the primary screening test either alone or in conjunction with HPV DNA test (co-testing), although in some European countries a switch to primary HPV-DNA technique followed by cytology (Pap smear) has been recommended [5]. The American Cancer Society recommends the use of the HPV test as part of follow-up for an abnormal Pap result in women aged 21–29, while for women aged >31 co-testing screening is recommended every five years [6].

2. Microbiota as a Biomarker for HPV Infection and Cervical Dysplasia

The HPV-DNA test is a high-sensitive method, so the absence of high-risk HPV-DNA indicates low risk for cervical intraepithelial neoplasia grade 3 (CIN3) and cancer development, which may allow safe prolonging of cervicovaginal screening test intervals [5]. Additionally, even when HPV-DNA scores positive, the majority of HPV infections are eliminated and do not progress to cervical dysplasia. Nevertheless, since the risk of cancer development still exists, screening at short intervals is strongly recommended. Therefore, the characterization of novel biomarkers is important in order to decide precisely how each HPV-positive women will be treated (colposcopy) and whether HPV-DNA-negative women have high risk to acquire a new HPV infection and progress into CIN. They may function as secondary markers after HPV-DNA test and Pap smear to identify women under risk to HPV acquisition, persistent infection and cervical cancer, contributing to better follow-up/treatment strategies.

Acknowledging a biomarker potential, high diversity microbiota has been frequently shown to correlate with HPV status and different severities of cervical dysplasia, suggesting a potential in indicating vaginal health and disease (Figure 1). Therefore, we further discuss the cervicovaginal microbiome as a promising biomarker not only for HPV status but also for cytologic abnormalities.

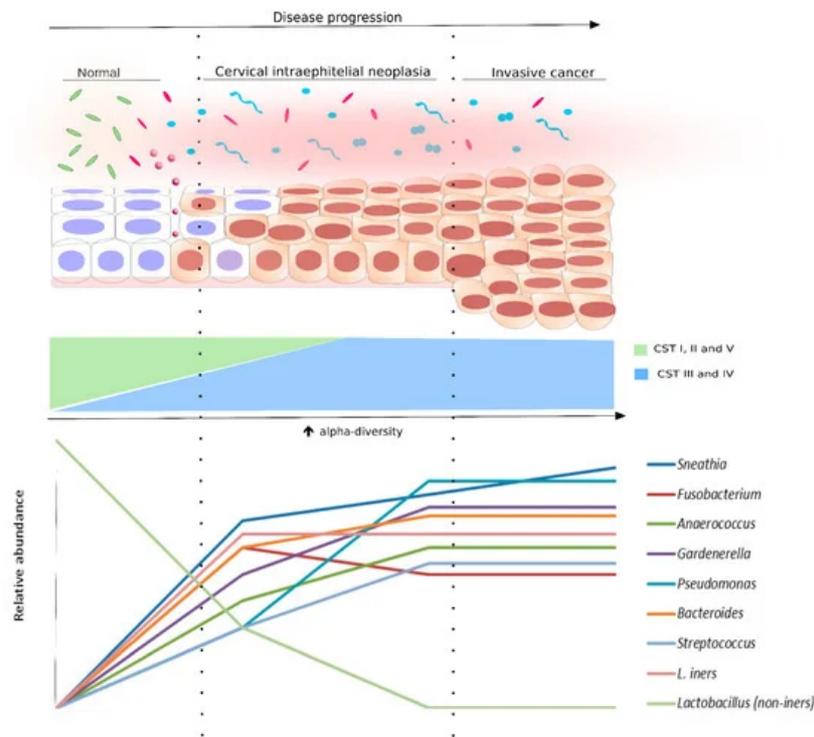


Figure 1. Bacterial diversity distribution in intraepithelial neoplasia progression. The scheme displays the progression of the cervical epithelium from normal to invasive cervical cancer, as well as the bacterial diversity (alpha-diversity) and the species abundance in the cervical microenvironment at each cytological stage. The normal cytology is commonly associated with community state types (CSTs) I, II, or V, which are *Lactobacillus* species (non-*iners*)-dominant (light green rods). However, following the cervical disease progression, the relative abundance of *Lactobacillus* non-*iners* species start to decrease. Concomitant to that, alpha-diversity increases and the microbiota is changed to CST III (pink rods and circles) or IV (light blue and pink shapes). Some bacterial species were found, in different studies, associated with cervical disease progression. They are also displayed in this figure in a representative graph of relative abundance (lower panel).

Different reports consistently demonstrated the relationship between HPV infection and/or persistence and microbiome composition. In fact, a study reported that HPV+ women exhibited a more complex microbiome diversity than HPV- counterparts [7]. Similarly, a work with Korean twins reported that HPV+ patients had a high-diversity vaginal microbiome with reduced proportion of *Lactobacillus* spp. when compared to their HPV- matches. Moreover, bacteria of the *Sneathia* genus was remarkably associated with HPV positivity and suggested as a biomarker for viral status [8]. Taken together, those reports strongly suggest a relationship between microbiome increased diversity and HPV infection. On the other hand, they do not demonstrate a causality link between a shift in microbiota composition and HPV acquisition, since those studies were not longitudinal with a patient's follow-up through time.

In this scenario, a study following women for 16 weeks showed that a vaginal microbiota dominated by *L. gasseri* was related to a faster HPV clearance, while microbiomes with lower *Lactobacillus* levels and higher abundance of *Atopobium*, *Gardnerella*, and *Prevotella* were associated with a slower infection resolution [9]. Further, *L. crispatus* was the most prevalent *Lactobacillus* spp. in Italian women that cleared HPV infection or were consistently HPV-, while a microbiome composition characterized for lower *Lactobacillus* spp. counts and higher abundance of *Gardnerella*, *Prevotella*, *Atopobium*, and *Sneathia* (a combination commonly associated with bacterial vaginosis) was the most frequent among women with persistent hr-HPV infection during one year. Also, among patients with persistent HPV infection, *L. iners* was the most abundant *Lactobacillus* species [9]. Accordingly, a longitudinal observation of African/Caribbean women living in Canada showed that a microbiome with lower abundance of *Lactobacillus* spp. and greater representation of anaerobic bacteria was more frequent in HPV+ than in HPV- subjects [10]. Altogether, those studies are important to elucidate the cause-effect relationship between vaginal microbiota and HPV status. Since they report a temporal dynamics in which infection resolution and persistence are associated with different bacterial compositions, microbiome alteration is suggested as a factor that could occur before HPV acquisition, modulating, and facilitating viral maintenance. Moreover, a possible protective role for *Lactobacillus* spp. dominated microbiota is noteworthy, while its paucity and increased amounts of other bacterial genera is related with higher HPV risk. Nevertheless, a study has recently provided some insights into possible interactions between viruses and the vaginal microbiome. The authors demonstrated that cervicovaginal samples with CSTs I or IV that were positive for oncogenic viruses (HPV and/or polyomaviruses) showed increased abundance of *L. crispatus* as well as *P. timonensis* and *S. sanguinegens*, respectively, when compared to its counterparts without any virus detected [11]. That suggested that the presence of viruses may also exert influence in the

cervicovaginal microbiome composition. Ethnicity is another important factor that must be considered, since *L. gasseri* (together with *G. vaginalis*) was more frequent in HPV+ subjects compared to HPV- in a Chinese cohort [11] as well as *L. mucosae* and *Enterococcus faecalis* were the most dominant species among women from Northeast India [12], which opposes previous findings.

It is also important to highlight that microbiome composition has also been related to other viral infections. For example, African women with *L. crispatus*-dominated microbiota registered significantly less frequent HIV, HSV-2, and HPV infections and bacterial STIs when compared to other compositions [13]. A report studying Caucasian Italian women demonstrated higher rates of HPV infection among samples belonging to CSTs III or IV, as well as a higher frequency of polyomaviruses in women with CSTs III or I. Although the *L. crispatus*-dominated environment has been associated with the presence of polyomaviruses, a longitudinal analysis of those patients revealed that CST I was associated with increased rates of viral clearance, while women harboring CSTs III or IV commonly progressed to persistent infections [14].

As discussed above, the vaginal microbiota composition has been extensively associated with HPV positivity and suggested as a promising biomarker for HPV risk. Accordingly, HPV infection is strongly recognized as a necessary, but not sufficient, cause for cervical carcinogenesis. Therefore, since specific bacterial compositions are linked with increased viral infections, the microbial community could also be associated with cervical dysplasia development and inform about cytological abnormalities. Indeed, different studies have already described a cervicovaginal microbiome shift with increased proportions of bacteria such as *Gardnerella*, *Prevotella*, *Atopobium*, and decreased abundance of *Lactobacillus* spp. occurring together with CIN and cancer. However, again, the cause-effect relationship between microbiome and cervical dysplasia has not been elucidated throughly [14].

Differences in microbiota composition were found between normal cytology, cervical lesions and cancer. That is, while *L. crispatus* and *L. iners* were respectively the predominant species for HPV- and HPV+ women without cytologic alterations, *Sneathia* spp. and *Fusobacterium* spp. were predominant in squamous intraepithelial lesions and cervical cancer, respectively [15]. Similarly, CST IV (high diversity microbiome lacking *Lactobacillus* spp.) frequency was shown to be directly proportional to cervical abnormalities severity: the cluster was gradually more abundant in low-grade squamous intraepithelial lesions (LSIL), high-grade SIL (HSIL) and cervical cancer. Moreover, HSIL samples had greater abundance of *Sneathia sanguinegens*, *Anaerococcus tetradius*, and *Peptostreptococcus anaerobius* than LSIL, suggesting shifts in microbiome composition according to disease severity [16]. Likewise, *Lactobacillus* dominance decreased together with cervical dysplasia severity while *Sneathia* spp. were increased in low/ high grade precancerous lesions and invasive cervical carcinoma [17]. In this context, different analyses suggest a paucity of *Lactobacillus* and increased relative abundance of other species as a factor associated with cervical abnormality development. Those reports also indicate that each stage of cervical dysplasia is associated with a corresponding microbial pattern. For example, while *Lactobacillus* spp. are more associated with normal cytology and absence of HPV, a higher abundance of *Sneathia* spp. was observed throughout cancer development and, therefore, could be used as indicative of vaginal disease together with other species mentioned above.

Additional evidence corroborates the findings described above. First, the presence of *A. vaginae*, *G. vaginalis*, and *L. iners* together with *L. crispatus* in low levels was suggested as the most hazardous combination for CIN development, with an odds ratio of 34.1 for CIN in the presence of hr-HPV [18]. Moreover, a report indicated that *B. fragilis*, *L. delbrueckii*, and *S. agalactiae* had an indirect effect on cervical cancer mediated by HPV infection, while *A. vaginae* and *P. stutzeri* also exhibited a direct effect on cervical carcinogenesis independently of HPV status [19]. Of note, a study evaluated the impact of loop electrosurgical excision procedure (LEEP), a method to treat CIN 2/3 to avoid cancer development, in the microbiome composition. The authors noticed that a microbiota containing *Prevotella* and lacking a consistent dominant species shifted significantly to an *L. iners* dominated community after three months of LEEP intervention [20]. However, in sharp contrast to those findings, it has been reported that a microbiome dominated by unclassified *Lactobacillus* spp. and *L. iners* was significantly associated with CIN 2 and CIN 3 in women with hr-HPV [21]. Although *L. iners* was already described to be related with CIN [18] or HPV positivity [15], the dominance by other *Lactobacillus* species was previously reported as a protective factor, but this cohort showed a different observation for unclassified *Lactobacillus* spp. In this scenario, Hispanic ethnicity by itself was associated with a decrease in *Lactobacillus* dominance and *Sneathia* spp. enrichment when compared to non-Hispanic women living in the U.S [17].

HIV-positive women show increased risk of HPV acquisition and CIN development [22][23][24]. A study that analyzed the cervicovaginal microbiome in the postpartum period of HIV-positive women showed a high frequency of *L. iners*, *Moryella*, *Schlegelella*, and *Gardnerella* associated with CIN with significant odds ratios of 40 for *Moryella* and of 3.5 for *Schlegelella* [25]. In a longitudinal analysis, when comparing the bacterial microbiome of women that showed CIN regression to normal cytology, *Gardnerella* appeared with a higher frequency in CIN when compared to normal status [25]. Similarly, a report from HIV+ pregnant women in Zambia showed that they had higher microbiome diversity, greater

abundance of *G. vaginalis* and *A. vaginae* and lack of *L. crispatus* when compared to HIV- pregnant participants. Also, *L. iners* enrichment was observed in HIV- individuals and in HIV+ subjects with preconceptional ART exposure [26]. These findings corroborate the association of specific bacteria with cervical premalignant lesions. Despite such association, it is important to highlight that little is known about the longitudinal nature of changes in microbiome in the development of CIN and cervical cancer in HPV+ women. However, the association seen in cross-sectional and in some longitudinal studies indicates that the microbiome could be used as a sensor for cervical alteration and risk for CIN progression.

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