

Detection of *Gardnerella* spp. in patients with HSIL: preliminary results

BÁRBARA PONTAROLLO DAL SANTOS¹, VIVIAN HEIMBECKER¹, MARIA CLARA PICININ², KEITE DA SILVA NOGUEIRA^{1,3}, MARCIA GUIMARÃES DA SILVA⁴, PATRICIA SAVIO DE ARAUJO SOUZA⁵, MARIA ISABEL LAVORANTI⁶, DULCIMARY BITTENCOURT⁷, RITA MAIRA ZANINE^{6,7}, CAMILA MARCON^{1,7}

¹. FEDERAL UNIVERSITY OF PARANÁ, POST-GRADUATE PROGRAM OF MICROBIOLOGY, PARASITOLOGY AND PATHOLOGY, CURITIBA/PR, BRAZIL

². FEDERAL UNIVERSITY OF PARANA, DEPARTMENT OF BASIC PATHOLOGY, CURITIBA/PR, BRAZIL

³. FEDERAL UNIVERSITY OF PARANÁ, CLINICAL HOSPITAL, CURITIBA/PR, BRAZIL

⁴. PAULISTA STATE UNIVERSITY, BOTUCATU SCHOOL OF MEDICINE, DEPARTMENT OF PATHOLOGY, BOTUCATU/SP, BRAZIL

⁵. FEDERAL UNIVERSITY OF PARANÁ, DEPARTMENT OF GENETICS, CURITIBA/PR, BRAZIL

⁶. FEDERAL UNIVERSITY OF PARANA, DEPARTMENT OF GYNECOLOGY AND OBSTETRICS, CURITIBA/PR, BRAZIL

⁷. FEDERAL UNIVERSITY OF PARANA, PATHOLOGY OF THE LOWER GENITAL TRACT AND COLPOSCOPY, CURITIBA/PR, BRAZIL

Introduction: Infection with high-risk human papillomavirus (hrHPV) genotypes can lead to high-grade squamous intraepithelial lesions (HSIL), which may progress to cervical cancer. Persistence of hrHPV infection after HSIL treatment is frequent and may lead to lesions recurrence. Several factors are associated with hrHPV persistence, among which bacterial vaginosis (BV), which is the most prevalent vaginal dysbiosis. Virulent strains of *Gardnerella* spp. are involved in the pathogenesis of BV and their role in persistent hrHPV and HSIL recurrence remains to be addressed.

Objectives: The aim of this study is to identify vaginal microbiota covariates associated with the persistence of high-risk HPV (hrHPV) after surgical treatment, with particular interest in *Gardnerella* spp. isolates.

Methods: This cross-sectional study included 64 participants with HSIL who underwent surgical treatment for HSIL and 46 controls with normal cytological results and matched to HSIL participants by age and contraceptive method. Bacterial species were isolated utilizing two culture media: NYC III and the selective Columbia Colistin Nalidixic Acid (CNA) agar. Presumed *Gardnerella* spp. colonies were confirmed by MALDI-TOF-MS. Participants with HSIL were re-evaluated 6 months after surgery. The prevalence of VB and *Gardnerella* spp. colonization was compared between the groups using Fisher's exact test ($\alpha=0.05$).

Results: Prevalence of BV was 42.2% (n=27) among participants with HSIL and 23.9% (n=11) among controls. Among the 285 isolates in HSIL group, 23.8% (n=68) were *Gardnerella vaginalis* and 27.7% (n=79) were *Gardnerella swidsinskii*/*Gardnerella leopoldii*. In the control group, 146 isolates were recovered of which 14.4% (n=21) were *G. vaginalis* and 19.9% (n=29) were *G. swidsinskii*/*G. leopoldii*. There was no statistically significant difference in the detection of *Gardnerella* spp. between groups for *G. vaginalis* and for *G. swidsinskii*/*G. leopoldii* ($p=0.06$), nor between the groups with BV ($p=0.18$). Overall prevalence of the potentially virulent *G. vaginalis* was 26,6% (n=17) in HSIL and 19,6% (n=9) in control group. Data on hrHPV persistence after 6 months are available for 13 participants, of which one showed hrHPV positivity. In the upcoming weeks, HPV genotyping will be performed in 11 follow-up samples already available.

Discussion: It was observed that the prevalence of most virulent *Gardnerella* spp.(i. e. *G. vaginalis/piotti*) did not show a statistically significant difference between the groups ($p=0.66$), although previous studies have reported the opposite. This may be due to the fact that recruitment of the age-matched control group has not yet been completed. Further detection of virulence genes in *Gardnerella* spp. will be performed and will clarify whether there are differences in the distribution of *Gardnerella* spp. genotypes between groups. Also, further analysis on *Gardnerella* association with hrHPV-persistence after 6 months of surgery will be evaluated.

Conclusion: There was no statistically significant difference in the number of *Gardnerella* spp. isolates between groups, nor in the prevalence of BV. Nevertheless, this ongoing study will enable a molecular investigation of virulence factors associated with *Gardnerella* spp. isolates for a more detailed characterization, as well as their association with hrHPV persistence.

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