Exploring the Microbiota for the Diagnosis of Anal Precancerous Lesions in MSM

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ABSTRACT #276

INTRODUCTION

- Anal cancer is a leading neoplasia in HIV-infected MSM. The current screening strategy is based on the detection of high-degree squamous intraepithelial lesions (HSIL), using anal cytology. While this approach shows good sensitivity, the specificity is poor.
- We aimed to identify in MSM a set of anal-associated bacterial biomarkers for the diagnosis of biopsy-proven HSIL (bHSIL).

METHODS

- Cross-sectional prospective study performed in HIV+ and HIV- MSM referred to a high-resolution anoscopy clinic.
- The primary outcome was the presence of bHSIL at the inclusion or during the previous year.
- We analyzed fecal and mucosal microbiota to search for biomarkers predictive of the presence of bHSIL.
- We collected fecal samples in specific containers and obtained anal mucosa specimens with an anal cytobrush. The V3-V4 region of the 16S rRNA gene was sequenced using the Illumina platform.
- We selected the biomarkers based on their LDA scores, AUC-ROC in logistic regression models and concomitant predictive value in feces.

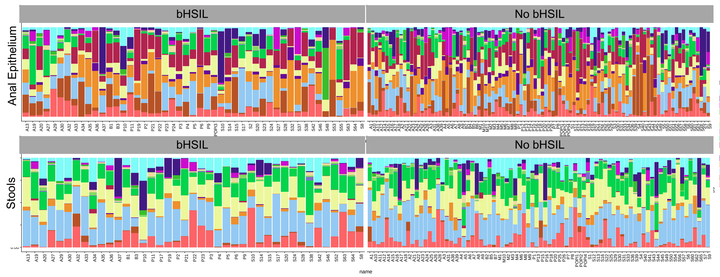
RESULTS

General characteristics of the study population

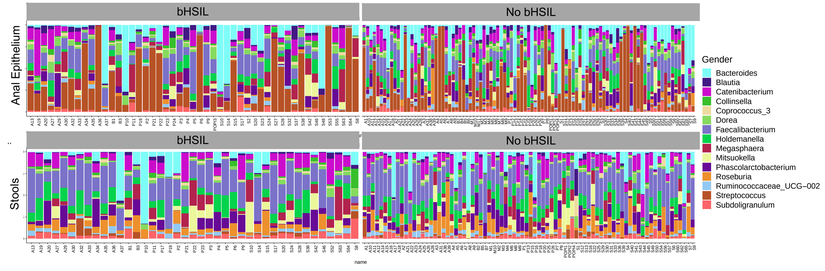
Variable	bHSIL	No HSIL	P value
	n=51	n=99	
Age, mean (SD), years	46.9 (10.9)	45.5 (12.5)	0.403
Male, n (%)	51 (100)	99 (100)	1
Body mass index, mean (SD), kg/m2	24.6 (3.2)	25.8 (11.5)	0.791
Time with undetectable plasma HIV RNA, median (P25-P75), months	32 (20-64)	2.6 (1.2-6.2)	
HIV diagnosis, n (%)	44 (86.3)	73 (73.7)	0.079
Undetectable plasma HIV RNA if HIV-infected, n (%)	41 (98%)	64 (88%)	0.068
Nadir CD4+ T cell counts, median (P25-P75), cells/uL	389 (210-588)	386 (268-487)	0.641
CD4+ T cell counts, median (P25-P75), cells/uL	800 (588-1008)	751 (592-953)	0.660
CD4/CD8 ratio, median (P25-P75), cells/uL	0.94 (0.57-1.12)	0.86 (0.59-1.05)	0.805
Sexual behaviour			
Active	7 (13.7)	10 (10.2)	0.363
Passive	14 (27.4)	19 (19.4)	
Both	30 (58.8)	69 (70.4)	
Use of antibiotic in past 3 months, n (%)	16 (33.3)	30 (32.6)	0.931

RESULTS

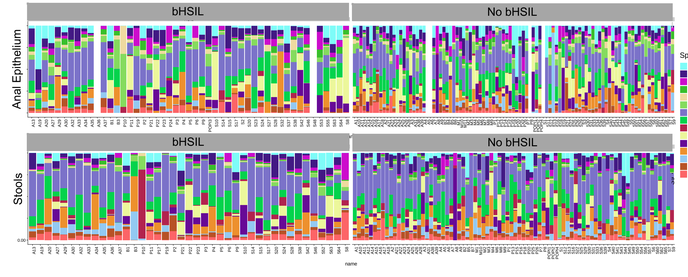
Relative proportion of taxa at the family level (top 15 families)



Relative proportion of taxa at the family level (top 15 genus)



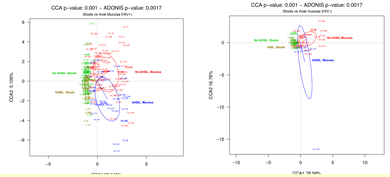
Relative proportion of taxa at the family level (top 15 species)



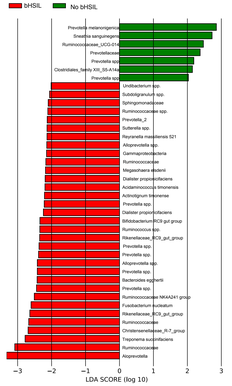
Alpha diversity was higher in anal epithelium compared to feces but did not differ between patients with or without bHSIL.

	Stools			Anal Mucosa		
	bHSIL	No bHSIL	p value	bHSIL	No bHSIL	p value
No. OTUs	323 (288-372)	297 (260-359)	0.181	354 (222-397)	314 (245-373)	0.359
Shannon	3.6 (4.4-4.8)	4.5 (4.3-4.8)	0.345	4.4 (3.9-4.8)	4.4 (3.8-4.7)	0.937
Chao1	326 (288-374)	299 (260-365)	0.166	358 (223-398)	315 (246-373)	0.381
ACE	324 (288-372)	298 (260-359)	0.172	356 (223-398)	315 (245-374)	0.369

The differences in beta diversity between sampling locations were significant, although no clustering was observed in the analysis by study group or HIV status.



Biomarkers of precancerous anal lesions in anal epithelium using LEfSe analysis



After exploring the predictive value of the 15 taxa with greater LDA scores in logistic regression models, we selected four taxa in anal samples based on their individual predictive performance and their concomitant predictive value in feces.

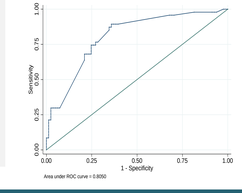
Logistic regression (robust)			
bHSIL	**Coef.**	**Std. Err.**	**P value**
Ruminococcaceae_NK4A214_group abundance	0.77	0.32	0.016
Alloprevotella spp. abundance	0.36	0.18	0.041
Prevotella melanonigenica presence	-1.67	0.80	0.039
Ruminococcaceae_UCG-014	0.97	0.54	0.072

Continuous variables were log10 transformed to meet the regression assumptions.

- Each 25% increase in the abundance of the Ruminococcaceae NK4A214 group and Alloprevotella genus were associated with a 17% (p=0.041) and 8% (p=0.016) increased risk of bHSIL, respectively.
- The absence of Prevotella melanonigenica and Ruminococcaceae UCG-014 was predictive of bHSIL (OR 6.1, P=0.018 and OR 3.2, P=0.026, respectively).
- From 35 (94%) of false positive cytologic results, the combination of these four biomarkers reclassified to true negative 33 (94%), significantly improving the predictive performance of anal cytology alone to AUC 0.805.

Diagnostic test	Sensitivity (%)	Specificity (%)	AUC ROC	P value
Anal citology[1]	72 (59-85)	57 (46-68)	-	0.002
Bacterial biomarkers[2]	37 (23-51)	90 (84-96)	0.737	0.002
Bacterial biomarkers[2] + anal citology	65 (51-79)	83 (74-90)	0.805	0.0006

[1]High or low intraepithelial squamous lesions = positive
[2]Abundance of Ruminococcaceae NK4A214 and Alloprevotella, absence of Prevotella melanonigenica and Ruminococcaceae UCG-014



CONCLUSIONS

- We found anal-associated bacteria indicative of higher risk of precancerous anal lesions, which combination was highly specific.
- The microbiota could be exploited as a complementary diagnostic tool for anal cytology to overcome the low specificity and high rate of false positive results of the current screening strategy for anal cancer screening.

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