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Anal *Ureaplasma* spp. positivity among HIV positive men who have sex with men may be associated with high-risk-type HPV infections

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ABSTRACT

Objective: HIV positive individuals, particularly men having sex with men (MSM), are at increased risk of sexually transmitted infections (STIs) at genital and extra-genital sites. Data on anorectal *Ureaplasma* infections are lacking. The aim of our study was to characterize anal *Ureaplasma* positivity among a cohort of HIV positive MSM and evaluate possible association with papillomavirus infection at the same site.
Methods: Anal swab samples, collected as part of routine screening for *Chlamydia trachomatis* and *Neisseria gonorrhea*, were additionally tested for HPV genotypes as well as for *Ureaplasma* and *Mycoplasma* using nucleic acid amplification method.
Results: Out of a total of 222 study participants, 195 (89%, 95% CI (84.9–93.2)) were positive for HPV, approximately three quarter being high-risk genotypes. Forty three individuals (19.4%, 95% CI (14.4–24.3)) harbored *Ureaplasma* spp. Infection with high-risk HPV types was significantly associated with co-presence of *Ureaplasma* with an odds ratio (95% confidence-interval) of 2.59 (1.03–6.54), P = 0.04.
Conclusion: Besides a high predominance of HPV infection, asymptomatic HIV positive MSM had a high prevalence of anal *Ureaplasma* positivity. Concomitant infections with high-risk HPV genotypes were common and statistically significant. The role of this co-existence as a potential risk factor for anal carcinogenesis needs further elucidation.

Introduction

Ureaplasma species (*Ureaplasma* spp.*)* are commonly considered to be part of the normal flora but are also capable of causing clinical illnesses. Among women they are responsible mainly for non-gonococcal urethritis and bacterial vaginosis. Serious complications may occur during pregnancy and after delivery with manifestations like chorioamnionitis, post-partum and post-abortal fever, congenital pneumonia, neonatal bacteremia and neonatal abscesses (Zhang et al., 2014; de Cordova et al., 2016).

Among men, *Ureaplasma* spp. has significantly been associated with non-gonoccocal urethritis and chronic prostatitis proving that it is more than just a harmless commensal (de Cordova et al., 2016; Povlsen et al., 2002; Wetmore et al., 2011). Here, HIV positive men who have sex with men (MSM) are affected by an increasing incidence of sexually transmitted infections (STIs) (Kirby, 2014) and infections at extra-genital sites have gained increasing significance in this population (Marcus et al., 2011; Rieg et al., 2008; Barbee et al., 2017; Patton et al., 2014). Since untreated STIs at any site may serve as facilitators of HIV transmission, screening both for genital and extra-genital sites is recommended as standard of care among HIV positive individuals (Templeton et al., 2014; Pathela et al., 2013; Rotchford et al., 2000; Ghys et al., 1997; Politch et al., 2012).

In addition to facilitating HIV transmission, STIs including *Ureaplasma* spp. may co-exist with human papillomavirus (HPV)

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and may contribute significantly to the pathogenesis of HPV-associated dysplastic lesions in the cervix (Bellaminutti et al., 2014; Camporiondo et al., 2016; Liu et al., 2016; Verteramo et al., 2009; Kim et al., 2016; Parthenis et al., 2018; Biernat-Sudolska et al., 2008; Biernat-Sudolska et al., 2011; Lukic et al., 2006). Since HIV positive MSM have a particularly high prevalence of anal HPV infection, understanding the extent of *Ureaplasma* spp. and HPV co-existence is of great relevance.

Despite existing evidence of pathological outcomes following *Ureaplasma* spp. infections, data on the extent and the role of anal *Ureaplasma* spp. infections among HIV positive MSM are scarce (Abbott et al., 2017). In this study we evaluated asymptomatic anal colonization with *Ureaplasma* spp. among HIV positive MSM.

Methods

Study population

The study population consisted of HIV positive MSM — a sub-cohort of HIV positive patients in the Austrian HIV Cohort Study (AHIVCOS) (35th Report of the Austrian HIV Cohort Study, 2018) — undertaking follow up at the Medical University of Innsbruck, Department of Dermatology and Venereology and at the Medical University of Salzburg, Department of Internal Medicine. The study has been approved by the ethic committees of the Medical University of Innsbruck and Salzburg Federal Government. After obtaining written informed consent, data on sociodemographic variables (age, smoking status, body-mass index (BMI), educational and marital status and reproductive/sexual behavior (number of lifetime sexual partners, age at first sexual contact) were obtained using questionnaires. Clinical data on HIV progression markers were also linked to the questionnaire and to the respective samples based on a unique patient identifying code.

Study samples

Anal-swab samples were collected between May 2015 and October 2016. Samples were self-collected as part of a routine screening program for *Chlamydia trachomatis* (*C. trachomatis*) and *Neisseria gonorrhoea* (N. *gonorrhoea*) infections using the Multi-Collect Specimen Collection Kit (Abbott Molecular).

HPV DNA detection and genotyping

After real-time amplification of the HPV-L1 genome using 5 μl of purified nucleic acid, genotyping followed using allele-specific reverse line-blot hybridization of the PCR products permitting the differentiation of 40 high-risk (hrHPV) and low-risk HPV genotypes (Ampliquality Type Express, AB ANALITICA, Padua, Italy). Beta-globin was used as an internal control for the confirmation of the validity of the test.

STI diagnosis

Ten μl of the remaining DNA was used for the amplification of *Ureaplasma* spp. DNA using a validated multiplex nucleic acid amplification kit (AmpliSens® multiprime-FRT, Moscow, Russia) which also detected *Mycoplasma genitalium*, *Mycoplasma hominis* and *C. trachomatis* in addition to Ureaplasma spp. In a further step *Ureaplasma* spp. positive samples were sub-classified into *Ureaplasma urealyticum* and *Ureaplasma parvum* (AmpliSens® Florocenosis/Mycoplasma-FRT, Moscow, Russia).

In this study we refer to *M. genitalium*, *M. hominis and Ureaplasma* spp. as Mollicutes. All test kits used in this study are well validated (CE marked for IVD) and are all equipped with amplification as well as positive and negative controls for each run.

Statistical analysis

The distribution HPV and other STIs were presented across sociodemographic and behavioural characteristics and HIV progression markers of the study population. Univariate and multivariate adjusted logistic regression models were applied to characterize the association between anal HPV infection pattern and prevalence of *Ureaplasma spp*. at the same site. P-values <0.05 were considered significant.

Results

A total of 222 individuals were evaluated. The mean age (standard deviation) of the study participants was 45.1 (12.5). Table 1 presents sociodemographic and behavioural characteristics of the study population across *Ureaplasma* spp. positivity. *Ureaplasma* spp. positive individuals reported to have a significantly higher number of lifetime sexual partners. Age at baseline, age at first sexual contact, smoking, BMI, marital or educational factors were not significantly associated with *Ureaplasma* spp. positivity. The proportion of individuals with abnormal HIV progression markers was low with only 36(16.2%) of the study participants having detectable HIV viral load of ≥40 Copies/mL and 47(21.2%) with CD4 count less than 500 cells/mm3. As presented in

Table 1
Sociodemographic, behavioural characteristics and HIV progression markers of study participants by *Ureaplasma* spp. positivity status (N = 222).

Variables across Ureaplasma infection		P value[a]
Age, years (mean (SD))		
Overall	**45.1 (12.2)**	
Negative for *Ureaplasma* (n = 175)	45.7 (12.5)	
Positive for *Ureaplasma* (n = 41)	44.6(12.5)	0.55
Educational status beyond high school, n (%)		
Overall	122 (55)	
Negative for *Ureaplasma*	99 (58.6)	
Positive for *Ureaplasma*	23 (57.5)	0.90
Married/living in partnership, n (%)		
Overall	**78 (35.1)**	
Negative for *Ureaplasma*	60 (37.7)	
Positive for *Ureaplasma*	18 (48.6)	0.26
Current smokers, n (%)		
Overall	**102 (45.9)**	
Negative for *Ureaplasma*	81(48.2)	
Positive for *Ureaplasma*	21 (52.5)	0.72
BMI, kg/m2, (mean (SD))		
Overall	**24.6 (3.9)**	
Negative for *Ureaplasma* (n = 159)	24.8 (4.1)	
Positive for *Ureaplasma* (n = 37)	24.1 (2.8)	0.33
Age at first sexual contact, years(mean (SD))		
Overall	**16.3 (3.4)**	
Negative for *Ureaplasma* (n = 108)	15.9(3.0)	
Positive for *Ureaplasma* (n = 24)	16.3 (2.9)	0.58
Life time sexual Partners ≥ 10, n (%)		
Overall	**88 (39.6)**	
Negative for *Ureaplasma*	67 (60.9)	
Positive for *Ureaplasma*	20 (83.3)	**0.037**[b]
Current CD4 count (mean (SD))		
Overall	**717 (320)**	
Negative for *Ureaplasma* (n = 167)	725 (333)	
Positive for *Ureaplasma* (n = 43)	691 (265)	0.55
Nadir CD4 count (mean (SD))		
Overall	**289 (191)**	
Negative for *Ureaplasma*(n = 170)	284 (198)	
Positive for *Ureaplasma* (n = 43)	308 (162)	0.48
HIV detected (≥ 40 Copies/mL), n (%)		
Overall	**36 (16.2)**	
Negative for *Ureaplasma*	31 (28.7)	
Positive for *Ureaplasma*	5 (17.2)	0.22

SD = standard deviation, BMI = body mass index (kg/m^2).
[a] P-Value significance <0.05, significance for *Ureaplasma* positive individuals as compared to *Ureaplasma* negative individuals.
[b] Statistically significant.

Table 2
Prevalence (95% CI) of various sexually transmitted pathogens detected in the anal mucosa of HIV positive MSM (N = 222).

	n	Prevalence% (95% CI)
HPV	195	89 (84.9–93.2)
HrHPV	154	73 (66.8–79.1)
Multiple HPV	159	75.4 (69.2–81)
Mollicutes[a]	56[b]	25.2 (19.8–31.1)
Ureaplasma spp.[c]	43	19.4 (14.4–24.3)
Ureaplasma urealyticum	22	10.9 (6.4–15.3)
Ureaplasma parvum	1	0.5 (0–1.5)
Mycoplasma genitalium	11	5.0 (2.3–8.1)
Mycoplasma hominis	19	8.6 (5.0–12.2)
Chlamydia trachomatis	12	5.4 (2.7–10.9)
Neisseria gonorroea	3	2.0 (0.0–4.8)

[a] Mollicutes refer to *Ureaplasma* spp., *Mycoplasma genitalium*, *Mycoplasma hominis*.
[b] Not equal to the sum of the rest due to co-infections.
[c] For 18 of the *Ureaplsma* spp. positive study participants we lacked adequate anal swab samples for genotyping and one sample tested negative for both genotypes.

Table 1 none of the HIV progression markers showed significant difference across *Ureaplasma* spp. positivity status.

HPV is the most commonly detected sexually transmitted pathogen with a prevalence (95% confidence interval (CI)) of 89% (84.9–93.2) and is characteristically predominated by high-risk and multiple genotypes. Three quarter of these men harboured one or more of HPV genotypes classified as high-risk and about a quarter were co-infected with one or more of the Mollicutes. *Ureaplasma* spp. were detected in 19.4%, 95% CI (14.4–24.3) of the anal mucosa samples tested. We were able to genotype 24 out of 43 *Ureaplasma* spp. positive samples. For 19 of the Ureaplsma spp. positive study participants we lacked adequate anal swab samples for genotyping. The majority of the *Ureaplasma* spp. positive study participants harboured *U. urealyticum* and only one was positive for *U. parvum* (Table 2). About 37% of *Ureaplasma* spp. positive patients, 95% CI (23.3–51.2) were co-infected with one or more of the other Mollicutes or *C. trachomatis* (Supplementary Table S1). The co-existence *Ureaplasma* spp. and *M. hominis* was statistically significant (P = 0.001).

As shown in Table 3 *Ureaplasma* spp. positive men are statistically significantly more likely to harbour hrHPV and multiple type HPV infections. The statistical significance persisted even after adjusting for the reported number of lifetime sexual partners. Several vaccine-type HPV genotypes, but not HPV 16 or 18, were significantly more detectable among patients positive for *Ureaplasma* spp. The magnitude of the association but not the statistical significance persisted when we limited our analysis to those individuals positive for *U. urealyticum* (Supplementary Table S2).

The association between anal HPV positivity and colonization with STI pathogens other than *Ureaplasma* spp. is presented in Supplementary Table S3. Some vaccine type hrHPV genotypes were statistically significantly co-detected with *M. hominis*. However, this association faded when excluding participants co-

Table 3
Characterizing HPV infection pattern by Ureaplasma positivity status among HIV positive MSM (N = 222).

	n (%)	Univaritae OR (95% CI)	Bivariate[c] OR (95% CI)	P value[d]
Hr-HPV				
Negative for *Ureaplasma*	118 (69.8)	1	1	
Positive for *Ureaplasma*	36 (85.7)	**2.59 (1.03–6.54)**[b]	**5.16 (1.13–23.6)**[b]	0.03
Multiple HPV types				
Negative for *Ureaplasma*	123 (69.9)	1	1	
Positive for *Ureaplasma*	36 (85.7)	**2.58 (1.03–6.50)**[b]	**4.85 (1.07–22.0)**[b]	0.04
Vaccine-type HPV[a]				
Negative for *Ureaplasma*	101 (60.5)	1	1	
Positive for *Ureaplasma*	28 (67.0)	1.41 (0.68–2.91)	1.70 (0.61–4.74)	0.31
HPV 16				
Negative for *Ureaplasma*	33 (19.5)	1	1	
Positive for *Ureaplasma*	13 (31)	1.85 (0.87–3.94)	1.82 (0.65–5.10)	0.26
HPV 18				
Negative for *Ureaplasma*	17 (10.1)	1	1	
Positive for *Ureaplasma*	3 (7.1)	0.69 (0.19–2.47)	0.45 (0.05–3.87)	0.45
HPV 6				
Negative for *Ureaplasma*	34 (20.1)	1	1	
Positive for *Ureaplasma*	11 (26.2)	1.41 (0.64–3.09)	1.53 (0.55–4.24)	0.41
HPV 11				
Negative for *Ureaplasma*	18 (10.7)	1	**1**	
Positive for *Ureaplasma*	7 (16.7)	1.67 (0.65–4.30)	**3.14 (1.05–9.43)**[b]	0.04
HPV 31				
Negative for *Ureaplasma*	23 (13.6)	1	1	
Positive for *Ureaplasma*	13 (31.0)	**2.85 (1.29–6.26)**[b]	**2.78 (1.00–7.77)**[b]	0.05
HPV 33				
Negative for *Ureaplasma*	9 (5.3)	1	1	
Positive for *Ureaplasma*	5 (11.9)	2.40 (0.76–7.59)	**4.90 (1.11–21.6)**[b]	0.03
HPV 45				
Negative for *Ureaplasma*	21 (8.9)	1	1	
Positive for *Ureaplasma*	7 (19.0)	1.41 (0.55–3.58)	1.00 (0.26–4.00)	0.9
HPV 52				
Negative for *Ureaplasma*	15(19.4)	1	1	
Positive for *Ureaplasma*	8 (40.0)	**2.40 (0.94–6.11)**[b]	3.15 (0.90–11.0)	0.07
HPV 58				
Negative for *Ureaplasma*	9 (5.4)	1	1	
Positive for *Ureaplasma*	1 (2.4)	0.43 (0.05–2.45)	0.80 (0.05–1.00)	0.43

OR = odds ratio, 95% CI = 95% confidence interval, MSM = men having sex with men.
[a] HPV 16, 18, 6, 11, 31, 33, 45, 52, 58.
[b] Statistically significant.
[c] Adjusted for number of life time sexual partners(n = 13)7.
[d] P value for bivariate adjusted regression.

infected with *Ureaplasma* spp. (63% of the *M. hominis* positive samples) which may have hampered the statistical power due to lower sample size.

Discussion

In this study we showed a high prevalence of anal *Ureaplasma* spp. positivity among apparently asymptomatic HIV positive MSM. Previous studies reported similar *Ureaplasma* spp. colonization patterns in the genitourinary tract in HIV positive individuals, (Martinelli et al., 1999; Wang et al., 2012; de Cordova et al., 2016). This is, at least to our knowledge, the first study to evaluate the extent of anal *Ureaplasma* spp. positivity among HIV positive men. Our study showed that *U. urealyticum* was the dominant type in the anal mucosa of HIV positive MSM accounting for 91% of the genotyped samples. Previous data showed that *Ureaplasma* spp. colonize genitourinary tracts of both healthy and symptomatic individuals. Whereas some studies suggested *U. parvum* to be more common in the urine samples of symptomatic men (Cox et al., 2016; Strauss et al., 2018; Deguchi et al., 2015) others found that *U. urealyticum* but not *U. parvum* to be associated with symptoms of urethritis (Zhang et al., 2014; Moi et al., 2015; Frølund et al., 2016). The role of *Ureaplasma* colonization in the anal mucosa has never been presented before. Since the samples in our study were collected as part of a routine STI screening program, the clinical significance of this colonization pattern cannot be fully explained at this stage. Further studies among patients presenting with symptoms of proctitis may shed light on the role of anal colonization with *Ureaplasma* spp. in general and *U. urealyticum* in particular, among susceptible patients.

Sexually transmitted infections play a significant role in facilitating HIV transmission (Ward and Rönn, 2010; Quilter et al. 2017; Sexton et al., 2005). Previous data have revealed that genitourinary STIs including *Ureaplasma* spp. may be associated with discrepantly higher HIV viral load in genital secretions as compared to peripheral blood samples probably due to higher concentration of HIV-harbouring CD4+ cells at the infection sites (Pathela et al., 2013; Rotchford et al., 2000; Ghys et al., 1997; Politch et al., 2012; Ward and Rönn, 2010; Fox and Fidler, 2010). HIV negative individuals with STIs, on the other hand, may be at increased risk of HIV acquisition due to infiltration of the infection site with susceptible inflammatory cells including CD4+ cells. Persistent HIV RNA shedding in the anorectal mucosa despite undetectable systemic viral load has been reported in association with co-existence of other STIs, possibly increasing the risk of infecting a discordant partner (García-Payá et al., 2018, Jansen et al., 2011). Despite the evidence that genitourinary *Ureaplasma* spp. infections are more prevalent in HIV positive as compared to HIV negative individuals, its explicit role as a risk factor for HIV acquisition is neither clearly demonstrated nor indisputably excluded. Whether or not anal *Ureaplasma* spp. infections contribute to an increased anorectal HIV shedding has never been shown before. Our finding of high prevalence of anal *Ureaplasma* spp. among HIV positive MSM warrants future investigations assessing particularly the risk of HIV seroconversion among *Ureaplasma* spp. positive HIV negative MSM.

Another aspect of clinical relevance is the interaction between HPV and other non-HPV STIs and their role in the pathogenesis of HPV associated dysplastic lesions. Previous studies have demonstrated that STIs, particularly, *C. trachomatis*, may co-exist with HPV and may contribute to the progression of cervical cancer through induction of profound inflammatory state (Anttila et al. 2001; Castle and Giuliano, 2003; Rasmussen et al. 1997; Kulkarni et al., 2001). The role of co-existence of *Ureaplasma spp*. with hrHPV as a promoting factor for HPV-associated dysplastic lesions was not studied adequately (Biernat-Sudolska et al., 2011). Our finding of a significant association between *Ureaplasma* spp. and hrHPV is in line with a recent work among a population of healthy women undergoing screening for cervical cancer which showed that cervical hrHPV infection is significantly associated with co-detection of *Ureaplasma* spp. at the same site with an odds ratio of 2.3, $p = 0.02$ (Parthenis et al., 2018). Our study is the first to evaluate co-existence of HPV and *Ureaplasma* spp. in the anal mucosa of HIV positive individuals practicing anal intercourse. The significant association supports a tempting speculation that the anal *Ureaplasma* spp. infection may be contributing to persistence of the HPV infection at this site. Since *Ureaplasma* spp. infections can induce inflammatory state (Castle and Giuliano, 2003; Novy et al., 2009; Lobão et al 2017), their potential role as co-factors for HPV induced anal carcinogenesis should not be ignored until proven otherwise in convincingly large prospective studies assessing anal high grade intra-epithelial neoplasia (AIN) or anal cancer as outcome variables. The association between anal STI pathogens other than *Ureaplasma* spp. and HPV co-infection needs to be assessed in a larger study as the sample size in our study for these pathogens does not allow an analysis with adequate statistical power.

We assume that factors other than a mere liberal sexual behaviour among our study population may be responsible for the observed significant HPV-*Ureaplasma* spp. co-existence since the statistical significance persisted even after adjusting for the reported number of lifetime sexual partners. A residual confounding due to this self-reported variable and hence shared risk factors between these two pathogens can, however, not be fully excluded and may be a potential limitation of the study.

A further limitation is the cross-sectional nature of our study making it impossible to infer causality in any direction regarding the co-existence HPV and *Ureaplasma* spp. If a further study proves *Ureaplasma* spp. colonization of the anal mucosa to be a predisposing factor to hrHPV infection and/or persistence, screening and control of otherwise asymptomatic non-HPV STIs including *Ureaplasma* spp. may help reduce the risk associated with hrHPV infection. Quality data on cytological and /or histological findings, which we lack in our study, would have provided a better picture of the role of *Ureaplasma* spp. on HPV-associated carcinogenesis. The fact that participants were not explicitly asked for the presence of clinical symptoms consistent with proctitis at the time of the study may be another limitation. However, since the samples were taken as part of a regular biannual STI screening program we can safely assume that the vast majority had no clinical peculiarities at the time of the study.

In conclusion, our study shows that anal *Ureaplsasm* spp. is frequently detected in a population of HIV positive MSM without symptoms of an STI. In the era of sensitive diagnostic methods and effective treatment for *Ureaplasma* spp., the high predominance of this pathogen, in the anal mucosa of HIV positive MSM warrants serious attention, particularly considering the potential risk of HIV transmission. Taking the fact that the great majority of our study population had anal infection with hrHPV and considering previous work, which proved STIs other than HPV to facilitate HPV-associated dysplasia, our finding of a significant association between hrHPV and *Ureaplasma* spp. co-infection warrants further elucidation, particularly with regards to anal carcinogenesis. The added value and cost-effectiveness of using a multiplex STI screening approach which detects *Ureaplasma* spp. in addition to the routinely tested *C. trachomatis* and *N. gonorrhoea* needs to be evaluated.

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Conflict of interest statement

Authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2019.04.025.

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