Title

Identifying Longitudinal Risk Factors for Anal High-risk HPV Infections and Strategies to Better Detect Anal High-grade Dysplasia for Anal Cancer Screening in Older Men who have Sex with Men

Author

Hsu, Hilary Kyle

Publication Date

2017

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Identifying Longitudinal Risk Factors for Anal High-risk HPV Infections and Strategies to Better Detect Anal High-grade Dysplasia for Anal Cancer Screening in Older Men who have Sex with Men

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Epidemiology

by

Hilary Kyle Hsu

2017

ABSTRACT OF THE DISSERTATION

Identifying Longitudinal Risk Factors for Anal High-risk HPV Infections and Strategies to Better Detect Anal High-grade Dysplasia for Anal Cancer Screening in Older Men who have Sex with Men

by

Hilary Kyle Hsu

Doctor of Philosophy in Epidemiology

University of California, Los Angeles 2017

Professor Marjan Javanbakht, Committee Co-Chair

Professor Dorothy J. Wiley, Committee Co-Chair

Human papillomaviruses (HPVs) are common sexually transmitted infections in the epithelium, some genotypes of which are oncogenic and capable of causing cervical and anal cancers. While HPV-associated cervical cancer rates have declined among U.S. women as a result of effective screening and treatment of cervical precancers, anal cancer is increasing in incidence nationwide and is quickly becoming a new threat to public health. While anal cancer is rare in the general U.S. population with an annual incidence rate of 1.8 cases per 100,000 men and women, gay, bisexual, and other men who have sex with men (MSM) have exponentially higher anal cancer incidence rates: 78-137 cases per 100,000 HIV-infected MSM and 5-35 cases per 100,000 HIV-uninfected MSM. The highest estimates for anal cancer in MSM exceed the incidence of the most common cancer in the world (prostate cancer: 120 cases per 100,000), emphasizing the disparate cancer risk in this population. Older MSM are at the highest risk since they have often gone their

whole lives without screening, allowing disease to progress unchecked. Risk factors and screening protocols for cervical cancer have long been established and informs anal cancer practices, though for anal cancer, more information is needed and a standard of care remains a work in progress. The purpose of this dissertation is to increase the knowledge and data surrounding anal HPV infections and resulting anal cancer precursors, to inform anal cancer screening and prevention protocols.

This dissertation uses data from the Multicenter AIDS Cohort Study (MACS), the longest running longitudinal study tracking the natural history of HIV/AIDS in MSM, and an independent clinical trial for anal cancer screening in MSM ("Improving Screening Tools for Anal cancer" (ISTA)). Chapter 2 analyzes longitudinal anal HPV data to characterize HPV infection in older MSM, and uses multivariable time-to-event proportional hazards models to identify predictors for incidence and clearance of anal HPV16 and 18, the high-risk HPVs (hrHPVs) accounting for up to 90% of anal cancers. Chapter 3 examines a sample of men with anal biopsy data, to compare anal cytology and hrHPV testing strategies to identify the most effective screening tool to predict histological high-grade squamous intraepithelial lesions (hHSILs): the precursors of anal cancer. Lastly, Chapter 4 assesses self-reported data on testosterone replacement therapy (TRT) and associations with anal cytology test performance and risk for hHSIL. Findings from this dissertation may provide invaluable information to develop standardized anal cancer screening and prevention protocols.

This dissertation of Hilary Kyle Hsu is approved.

Roger Detels

Pamina M. Gorbach

Marjan Javanbakht, Committee Co-Chair

Dorothy J. Wiley, Committee Co-Chair

University of California, Los Angeles

2017

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ACKNOWLEDGEMENTS

Thank you to my esteemed committee. Dr. Detels, it has been a great honor working with you and your research legacy over the past 7 years. Thank you for your continuous support and words of wisdom. Dr. Gorbach, thank you for being an encouraging masters advisor and for your valued contributions to the committee. Dr. Javanbakht, thank you for being an amazing doctoral advisor. I could not have made this great achievement without your support and mentoring. Dr. Wiley, thank you for taking a chance on me since I came to your lab in undergrad as a research assistant. Your mentorship, personally and professionally, and the numerous opportunities you have provided me have culminated in my successful completion of this dissertation. Thank you to my family and friends. Thank you to my girlfriend for your love and support and for helping me see this through. I would like to thank the Wiley lab for tuition support and to the UCLA Graduate Division for the generous additional funding.

Data used in Chapters 2 to 4 were collected by the Multicenter AIDS Cohort Study (MACS). MACS (Principal Investigators): Johns Hopkins University Bloomberg School of Public Health (Joseph Margolick, Todd Brown), U01-AI35042; Northwestern University (Steven Wolinsky), U01-AI35039; University of California, Los Angeles (Roger Detels, Oto Martinez-Maza, Otto Yang), U01-AI35040; University of Pittsburgh (Charles Rinaldo), U01-AI35041; the Center for Analysis and Management of MACS, Johns Hopkins University Bloomberg School of Public Health (Lisa Jacobson, Gypsyamber D'Souza), UM1-AI35043. The MACS is funded primarily by the National Institute of Allergy and Infectious Diseases (NIAID), with additional co-funding from the National Cancer Institute (NCI), the National Institute on Drug Abuse (NIDA), and the National Institute of Mental Health (NIMH). Targeted supplemental funding for specific projects was also provided by the National Heart, Lung, and Blood Institute (NHLBI), and the National Institute on Deafness and Communication Disorders (NIDCD). MACS data collection is also supported by UL1-TR001079 (JHU ICTR) from the National Center for Advancing Translational Sciences (NCATS) a component of the National Institutes of Health (NIH), and NIH Roadmap for Medical

Research. The contents of this publication are solely the responsibility of the authors and do not represent the official views of the National Institutes of Health (NIH), Johns Hopkins ICTR, or NCATS. The MACS website is located at http://aidscohortstudy.org/.

Additional data used in Chapters 3 and 4 were collected by the Wiley research team for the "Improving Screening Tools for Anal cancer" or "ISTA" study. Funding was provided by the National Cancer Institute (R01CA169508).

VITA

Education

June 2011	B.S., Biology University of California, Los Angeles Los Angeles, CA
June 2014	M.P.H., Epidemiology University of California, Los Angeles Los Angeles, CA

Employment

Oct. 2010 – June 2011	Research Assistant UCLA School of Nursing Los Angeles, CA
June 2011 – Sept. 2014	Research Project Coordinator UCLA School of Nursing Los Angeles, CA
Sept. 2014 – Current	Graduate Student Researcher UCLA School of Nursing Los Angeles, CA
Apr. 2015 – June 2015	Special Reader Behavioral Epidemiology University of California, Los Angeles Los Angeles, CA

Extracurriculars

Sept. 2009 – June 2014	Private Academic Tutor Los Angeles, CA
Aug. 2010 – June 2011	Vice President Community Medicine in Koreatown University of California, Los Angeles Los Angeles, CA
Aug. 2012 – June 2013	Graduate Student Association Representative UCLA Community Activities Committee University of California, Los Angeles Los Angeles, CA
June 2013 – June 2014	Activities Chair Epidemiology Student Association University of California, Los Angeles Los Angeles, CA

Awards

Sept. 2013 – June 2014	Fellowship in Epidemiology UCLA Graduate Division Los Angeles, CA
Sept. 2014 – June 2015	Fellowship in Epidemiology UCLA Graduate Division Los Angeles, CA
Sept. 2016 – June 2017	Fellowship in Epidemiology UCLA Graduate Division Los Angeles, CA
Apr. 2017	UCLA Grad Slam Competition Finalist Los Angeles, CA

PUBLICATIONS AND PRESENTATIONS

Manuscripts: **Hsu H**, Brown TT, Li X, Young S, Cranston RD, D'Souza G, Jacobson LP, Martinez-Maza O, Seaberg EC, Margolick JB, Jenkins FJ, Moran MG, Chua K, Bolan RK, Detels R, Wiley DJ. (2015) Association between Free Testosterone Levels and Anal Human Papillomavirus Types 16/18 Infections in a Cohort of Men Who Have Sex with Men. PLoS One. 2015 Mar 20;10(3):e0119447. doi: 10.1371/journal.pone.0119447. PMCID: PMC4368778

Wiley, D.J., Li, X., **Hsu, H.**, Seaberg, E.C., Cranston, R.D., Young, S., D'Souza, G., Martínez-Maza, O., DeAzambuja, K., Chua, K., Hussain, S.K., and Detels, R. Factors Affecting the Prevalence of Strongly and Weakly Carcinogenic and Lower-risk Human Papillomaviruses in Anal Specimens in a Cohort of Men who have Sex with Men (MSM). PLoS ONE. 2013 Nov; 8(11): e79492. doi: 10.1371/journal.pone.0079492.

Wiley, D., **Hsu, H.**, Bolan, R., Voskanian, A., Elashoff, D., Young, S., Dayrit, R., Barman, P., DeAzambuja, K., Masongsong, E., Martínez-Maza, O., and Detels, R. Comparison Of Two Anal Cytology Protocols To Predict High-Grade Anal Intraepithelial Neoplasia. J Low Genit Tract Dis. 2013 Oct; 17(4): 414-24. doi: 10.1097/LGT.0b013e318281d36e.

Abstracts: **Hsu, H.**, Brown, T., Li, X., Young, S., Cranston, R.D., D'Souza, G., Jacobson, L.P., Martinez-Maza, O., Seaberg, E.C., Detels, R., and Wiley, D.J. Serum free testosterone levels associated with anal human papillomavirus types 16/18 in a cohort of men who have sex with men. Sex Health. 2013 Nov; 10(6):578 doi: 10.1071/SHv10n6ab17.

Wiley, D., Li, X., **Hsu, H.**, Young, S., Cranston, R.D., D'Souza, G., Martinez, O., Seaberg, E., Dayrit, R., Hussain, S.K., Simard, E., Detels, R. Human papillomavirus (HPV) infection characteristics in a cohort of men who have sex with men (MSM) during long-term follow-up. Cancer Research. 2012 Apr 15; 72(8): Suppl. 1. doi: 10.1158/1538-7445.AM2012-551 (poster).

Wiley, D., Bolan, R., Voskanian, A., Young, S., Elashoff, D., **Hsu, H.**, Masongsong, E., Barman, P., and Detels, R. Nylon-flocked swab collection method better predicts high-grade AIN than does Dacron swab method. Infect Agent Cancer. 2012; 7(Suppl 1): 49. doi: 10.1186/1750-9378-7-S1-P49.

Oral and Poster Presentations: 17 Available Upon Request

Chapter 1. Introduction and Background

1.1 Introduction

Human papillomaviruses (HPVs) are the most common sexually transmitted infections in the United States [3]. HPVs are small, double-stranded DNA viruses that infect epithelium through microabrasions resulting from skin-to-skin contact, typically during sexual contact. It is estimated that all sexually active individuals will contract at least one HPV infection in their lifetime; however, up to 90% of infected individuals clear their infections in under 2 years [4-9]. Persistent HPV infections are of greater epidemiological concern than transient infections as long-term persistence of some HPV types are necessary to develop cancers after a latent period of ten or more years. There are over 200 genotypes of HPVs, twelve of which are classified by the International Agency for Research on Cancer as Group 1 high-risk (hr) strong carcinogens: HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 [10, 11]. Another thirteen hrHPV types are classified as Group 2 weak carcinogens: 26, 30, 34, 53, 66, 67, 68, 69, 70, 73, and 82, 85, and 97. Sixteen other types, which rarely cause cancer, are classified as low-risk (lr) HPV types 6, 11, 40, 42, 54, 55, 61, 62, 64, 71, 72, 81, 83, 84, Is39, and CP6108; types 6 and 11 are known to cause genital warts. HrHPV types 16 and 18 alone are estimated to cause 90% of anal cancers and 70% of cervical cancers [10-14]. While cervical cancer is the seventh most common cancer worldwide in incidence (4.4 to 42.7 cases per 100,000 women) and accounts for 90% of all HPV cancers, anal cancer is rare with an annual incidence rate of 1.8 cases per 100,000 men and women in the United States [15, 16]. However, in gay, bisexual, and other men who have sex with men (MSM), anal cancer incidence rates are exponentially higher: 5-35 cases per 100,000 in HIV-uninfected MSM and 78-137 cases per 100,000 in HIV-infected MSM [17-19]. Highest estimates for anal cancer in MSM exceed the incidence of the most common cancer affecting all U.S. men (prostate cancer: 120 cases per 100,000), emphasizing the disparate cancer risk in this population [20]. While HPV-associated cervical cancer rates have declined among U.S. women

as a result of effective screening and treatment of cervical precancers, anal cancer is increasing in incidence nationwide and is quickly becoming a new threat to public health [16, 21].

1.2 HPV Pathogenesis

The main target for HPV infection are basal cells on the basement membrane of epithelium [22]. Most infections and consequent disease occur at the squamocolumnar junction, specifically in the transformation zone (TZ), of the cervix and the anus where columnar and squamous epithelia meet [22]. This is likely due to lower immunity in these areas. There are decreased levels of Langerhans cells in the TZ which are responsible for immunosurveillance and stimulating innate and adaptive immune responses [9]. After HPV enters the skin through a microabrasion, it takes approximately 24 hours before it infects the basal cell layer and can become a productive infection [23](Figure 1 [1]). Mechanisms within HPVs allow them to evade innate immune responses of the host for weeks to years as evidenced by persistent infections [23-25]. Persistent infections are generally defined as consecutive visits testing positive for the same HPV type infections. In cervical infections, the minimum time between tests to define persistence can be as low as 4 months, all the way up to 7 years [7, 26, 27]. Persistent HPV infections can occur in the epithelium of various anatomical sites: most commonly the cervix, anal canal, and oropharynx, and in the cervix and anal canal. They can manifest as abnormal cell growths also known as lesions, dysplasias, or sometimes precancers [23]. These dysplasias can only develop from HPV infections and progress in severity from low- to high-grade squamous intraepithelial lesions, the latter of which are capable of further progressing to squamous cell cancers [4, 25, 28](Figure 1 [1]). Several behavioral and biological risk factors may influence persistence of HPV infections and lead to more severe disease.

1.3 Screening and Prevention

Screening and prevention for cervical cancer has been well-established since the 1960s, however, a standard for anal cancer remains to be established [18, 29-31]. Cervical cancer screening began with the Pap cytology test: a procedure implementing a brush or swab to collect cervical epithelial cells into preservative and tested to detect abnormal changes in cells resulting from HPV infections. Pap testing has been implemented for anal cancer screening though there is no current standard. Cell specimens are assessed similarly for both cervical and anal Paps typically classified using the Bethesda System standard reporting terms: negative for intraepithelial lesion (NIL), atypical squamous cells of unknown significance (ASC-US), atypical squamous cells – cannot exclude high-grade squamous intraepithelial lesion (ASC-H), low-grade squamous intraepithelial lesion (LSIL), and high-grade squamous intraepithelial lesion (HSIL) [32, 33]. A cytology specimen may be deemed unsatisfactory or uninterpretable if there is an insufficient amount of cells that can be evaluated, or it is obscured by contaminants (blood, lubricant, other discharge, or inflammation) [32, 34]. Overall, cytology specimens show low sensitivity and moderate specificity and require repeat testing or co-testing to improve detection.

Since the development of Pap testing, various DNA and RNA HPV tests have been developed and appended to primary cervical cancer screening protocols and implemented by some for anal cancer screening [35, 36]. There are genotyping tests that determine specific HPV type infections, while other HPV tests that group HPV types providing more general infection characteristics (eg. Group 1 hrHPV-positive vs. negative). HPV tests generally have higher sensitivity but lower specificity than cervical and anal cytology [37-39]. For cervical cancer screening, some experts advocate for screening solely using repeat HPV molecular testing to identify and track persistent hrHPV infections, while others support co-testing with cytology or just cytology alone [40]. Current cervical cancer screening guidelines recommend primary screening with cytology every 3 years for women ages 21-65 and testing with both cytology and HPV testing every 5 years for women ages 30-65, though no consensus is available for anal cancer screening

[37]. For cervical screening, subjects with abnormal cytology results (ASC-US or more severe) and/or positivity for hrHPVs are referred for a diagnostic examination to confirm disease called a colposcopy. During colposcopy exams, the cervix is examined, tissue stained with acetic acid, assessed, and biopsies are collected using forceps into preservative. High-resolution anoscopy has been recommended as the equivalent diagnostic examination of the anal canal to confirm disease and procedures are developed from the colposcopy model. However, the only current standard of care for anal cancer screening is a digital rectal examination where clinicians use their fingers to feel for any masses in the anal canal. Certified pathologists use a standard classification to evaluate both anal and cervical biopsy specimens as NIL, LSIL, HSIL, which has additional sub-classifications, [anal/cervical intraepithelial lesion (AIN/CIN) 2 (moderate dysplasia), AIN/CIN 2/3 (moderate-to-severe dysplasia), AIN/CIN 3 (severe dysplasia)], carcinoma in-situ (CIS), and invasive anal/cervical cancer (IAC/ICC). When precancers (LSIL or more severe) are observed during a colposcopy or after biopsy confirmation, they are often treated using cryotherapy, laser ablation, loop excision, or cold-knife conization [37]. With these effective strategies, cervical cancer is considered a wholly preventable disease. However, it has yet to be confirmed whether treatment of anal HSIL will reduce the likelihood of progression to anal cancer, though a large cohort study is underway to establish this link [18, 41-43] .

Despite expansive knowledge and established protocols for cervical cancer screening and treatment, there is no standard of care for anal cancer screening and prevention [44-46]. So far, practices from cervical cancer screening and prevention have been recommended for anal cancer, including repeat cytology and HPV testing, high-resolution anoscopy examinations with biopsy for diagnosis (equivalent to colposcopy), the use of identical cytological and histological classifications, and treatment approaches [33, 41, 42, 47-50]. However, distinctions between the two anatomical sites and broader susceptibility of anal disease to both men and women requires further research to independently establish a standard for anal cancer screening and prevention

[51, 52]. Focusing on MSM who are the highest risk for anal HPV infections and anal disease is key.

1.4 Measures of Anal HPV Infections in MSM

The prevalence of anal HPV infections in MSM have been reported in a number of studies with estimates of any HPV infection as high as 99% in HIV-infected MSM and 94% of HIV-uninfected MSM [53, 54]. Prevalent anal HPV16 and 18 infections range from 10-40% and 5-40%, respectively. A summary of anal HPV prevalence from a literature review of forty studies have been summarized in Supplementary Tables 1 and 2. Only eleven studies found reported the incidence rates of anal HPVs in MSM and are summarized in Supplementary Table 3 and 4. Lastly, only seven studies found report the clearance rates of anal HPVs in MSM and are summarized in Supplementary Tables 5 and 6. In large, most studies have small sample sizes and focus on HIV-infected MSM, with half of the HPV tests used testing only a portion of the 37 HPV types commonly reported. Estimating these infection characteristics in a large group of HIV-infected and -uninfected MSM would potentially add great value to better understand anal HPV infections and their risk factors over time.

1.5 Risk Factors for Anal HPV Infections

While inherent physiological differences exist between the cervix and anal canal, they share embryologic origins and similar HPV pathogenesis mechanisms are observed in each site suggesting shared risk factors for infection. A preliminary cross-sectional analysis performed in a large group of HIV-infected and -uninfected MSM showed HIV infection was associated with increased prevalence in all HPV groups (Figure 2 [2]). Multiple studies have verified the association with HIV infection, and have identified additional risk factors for incident and persistent HPV infection: the number of sex partners, race, age, smoking tobacco, receipt of a solid organ transplant; CD4+ count, HIV viral load, and adherence to highly active antiretroviral therapy (HAART) in HIV-infected individuals [2, 22, 54-64]. Receptive anal intercourse, specifically, is

associated with higher risk for anal HPV incidence, though experts agree non-intercourse anal behaviors, such as digital anal stimulation, play a role in transmission and infection risk [22]. Recent number of receptive anal intercourse partners was associated with increased prevalence of all HPV groups, whereas lifetime number of sex partners was only associated with increased prevalence of lrHPVs [2]. Factors associated with HPV clearance in MSM have not been well-studied, however one study in HIV-infected MSM found a strong association between young age (<25 years) and lower HIV RNA viral load with higher clearance of anal HPVs [64]. Other strong predictors of clearance of anal HPV infections may exist and remain to be identified [64].

1.6 Role of Sex Hormones on Disease

In women, persistent cervical HPV infections and cervical cancer risk are positively associated with higher parity, early sexual debut, and long-term oral contraceptive use which are associated with increased lifetime exposure to estrogen [65-72]. Similar HPV pathogenesis mechanism in the cervix suggest sex hormones may similarly influence anal HPV infection and cancer risk. Preliminary analyses show each half-$\log_{10}$ increase in serum free testosterone (FT) was associated with a 1.9-fold higher prevalence of anal HPV16/18 infections in a cohort of MSM [73]. These findings warrant a closer study of potential risks associated with supraphysiological testosterone levels in men, particularly in older HIV-infected MSM who comprise the highest risk group for anal cancer. For HIV-infected MSM, testosterone replacement therapy is often clinically indicated as their testosterone levels are lower compared to HIV-uninfected men and testosterone levels naturally decline with age in men [74]. Free steroid hormones are unbound and active, entering target cells primarily by passive diffusion through the cell membrane. Acting as ligands, free hormones bind to nuclear receptors and can cause epithelial cell proliferation [75]. Our group has tested a sample of biopsy specimens drawn from MSM with varying anal dysplasia and HPV16 infection, and all specimens tested positive for T receptors. This finding may link elevated

FT levels to HPV16/18 prevalence if FT binds to receptors in anal epithelium, allowing proliferation of infected cells, increasing viral production and persistent infection.

In addition to potentially increasing risk for anal HPV infection, supraphysiological testosterone levels, often induced by exogenous testosterone use, may affect the efficacy of anal cancer screening strategies in MSM. One limitation in screening comes from unsatisfactory or indeterminate Paps which indicate uninterpretable cytology results. This can be due to an insufficient amount of cells that can be evaluated, or obstruction by contaminants (blood, lubricant, other discharge, or inflammation) [32, 34]. One study showed women with unsatisfactory cervical cytology results had a 4-fold higher odds of having a follow-up abnormal Pap test, and a 5-fold higher odds of having an abnormal cervical biopsy compared to a negative control group with satisfactory cytology results [76]. Since anal cytology often yields a higher proportion of unsatisfactory (1-7.4%) results than cervical cytology (0.3-3.4%), unsatisfactory results may have a greater implication in anal cancer screening and determining the potential influence of testosterone is important [77-80]. In a study by Peitzmeier et al., researchers performed a large medical chart review to identify factors contributing to a high rate of unsatisfactory cervical Paps in a group of female-to-male transgender men (FTMTM) [34]. Many FTMTM retain their cervices and are still at risk for cervical disease, therefore, are recommended to follow cervical cancer screening guidelines for cisgender women. Peitzmeier et al. found that, compared to cisgender women, FTMTM had an 11-fold higher odds of receiving an unsatisfactory cervical Pap using liquid-based cytology, after adjusting for race, age, and BMI. For each additional year of testosterone use, odds of receiving an unsatisfactory Pap were 20% higher for FTMTM compared to cisgender women. Among FTMTM, the proportion of those with unsatisfactory Pap tests increased with duration of testosterone use. Researchers hypothesize one explanation may be histological changes in the cervix from testosterone therapy. Exogenous testosterone use may have a greater impact in FTMTM than in cisgender men due to a more drastic disruption of homeostatic testosterone levels, therefore, it is reasonable to surmise supraphysiological

testosterone levels may not similarly affect anal tissue in MSM. However, identifying the relationship between exogenous testosterone use in MSM and risk for anal disease is a novel exploration. Determining the causes of unsatisfactory cytology results is particularly important due to the potential to misclassify more severe dysplasia [34, 81-83].

1.7 Summary

A standard for anal cancer screening and prevention has not yet been established as further research is required. There is significant utility in HPV testing and cytology in screening for anal cancer and it is important to identify the optimal test. Few studies have focused on identifying risk factors influencing persistence and clearance of anal hrHPV infections necessary for anal cancer development. Identifying these risk groups is important for targeted screening approaches. Exogenous testosterone use may be important risk factors for anal dysplasia, and affect cytology screening efficacy. These associations have not been well-explored in MSM despite frequent exogenous testosterone use and high risk for anal cancer. Researching these topics may provide invaluable information to develop anal cancer screening and prevention strategies.

Figure 1.1 Progression of initial infection to persistent human papillomavirus (HPV) infection in the cells of the cervix. From left to right, there is a progression from initially normal cells. [1]

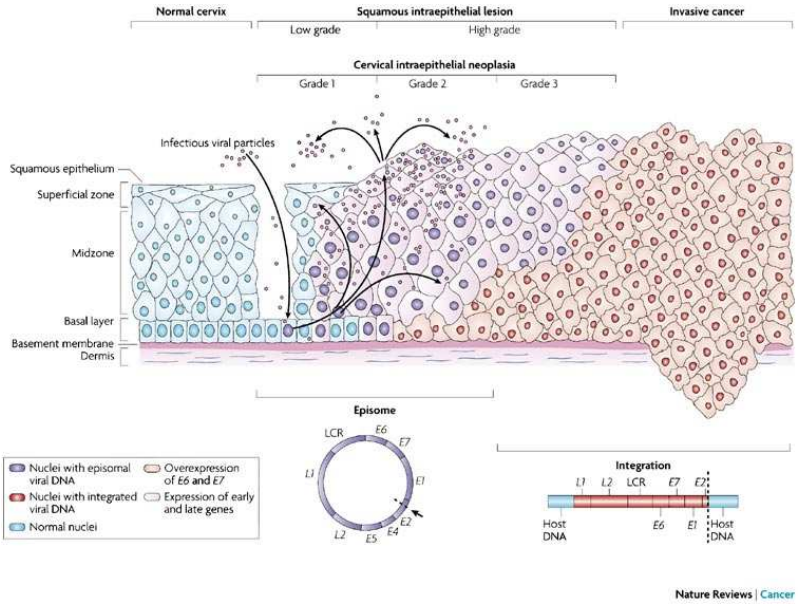
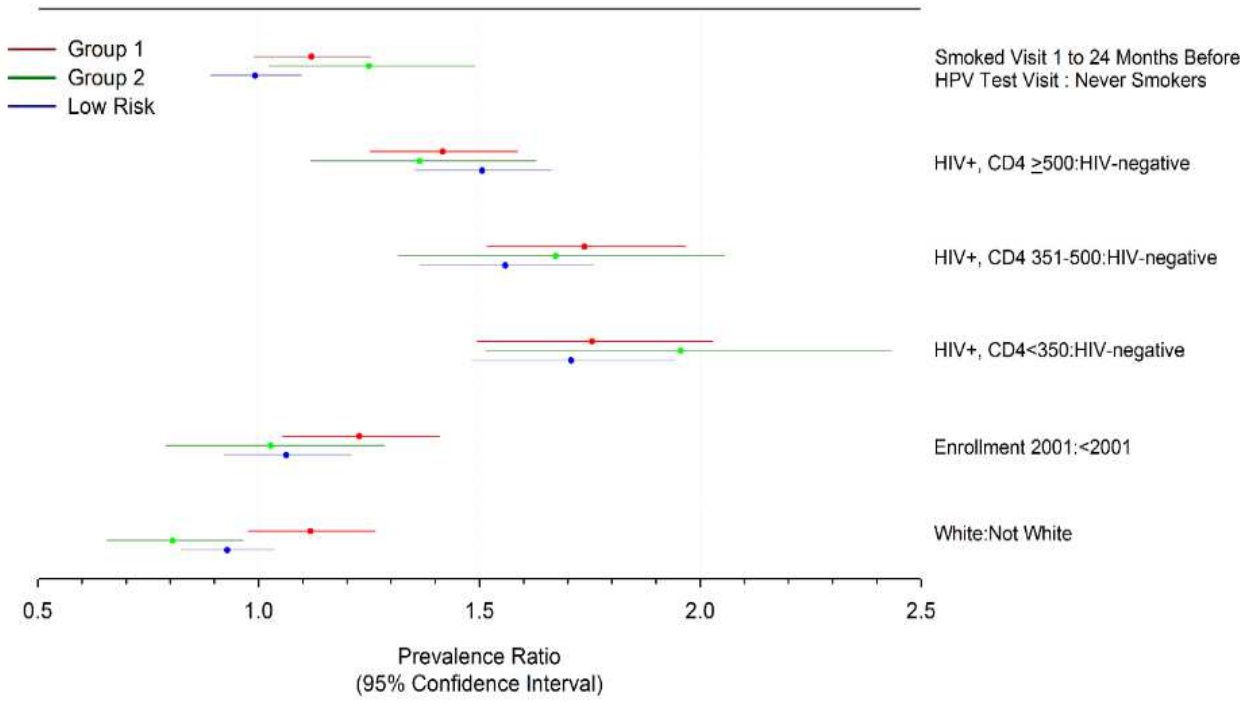


Figure 1.2 Comparison of Prevalence Ratios for Group 1 and 2 High-risk and Lower-risk HPVs for 1262 Men Enrolled in the Multicenter AIDS Cohort Study Anal Health Sub-study.[a] [2]



[a] Prevalence ratios are simultaneously adjusted for the effect of age, race, recruitment period (2001 vs <2001), study site; HIV infection (yes/no) and CD4 cell count (<350, 351-500, >500 cells/mm^3) among the infected, lifetime number of male sex partners at MACS visit 1 (<30, 30-99, 100-299, ≥300), number of sex partners reported between MACS visit 1 and 24 months before HPV testing (<30, 30-99, 100-199, ≥200), number of RAI partners during 24 months prior to HPV testing (0, 1-3, ≥4), tobacco smoking (yes/no) during two study periods: MACS visit 1 to the study visit 24 months before HPV testing, and the last 24 months of the study period.

1.8 References

1. Woodman CB, Collins SI, Young LS. The natural history of cervical HPV infection: unresolved issues. Nat Rev Cancer. 2007;7(1):11-22. doi: 10.1038/nrc2050. PubMed PMID: 17186016.

2. Wiley DJ, Li X, Hsu H, Seaberg EC, Cranston RD, Young S, et al. Factors affecting the prevalence of strongly and weakly carcinogenic and lower-risk human papillomaviruses in anal specimens in a cohort of men who have sex with men (MSM). PLoS One. 2013;8(11):e79492. doi: 10.1371/journal.pone.0079492. PubMed PMID: 24278140; PubMed Central PMCID: PMC3835810.

3. Satterwhite CL, Torrone E, Meites E, Dunne EF, Mahajan R, Ocfemia MC, et al. Sexually transmitted infections among US women and men: prevalence and incidence estimates, 2008. Sex Transm Dis. 2013;40(3):187-93. doi: 10.1097/OLQ.0b013e318286bb53. PubMed PMID: 23403598.

4. Ault KA. Epidemiology and natural history of human papillomavirus infections in the female genital tract. Infect Dis Obstet Gynecol. 2006;2006 Suppl:40470. doi: 10.1155/IDOG/2006/40470. PubMed PMID: 16967912; PubMed Central PMCID: PMC1581465.

5. (CDC) CfDCaP. HPV Vaccine Information For Young Women [updated 03/26/2015]. Available from: http://www.cdc.gov/std/hpv/stdfact-hpv-vaccine-young-women.htm.

6. Foundation THaAC. HPV: The Facts 2014 [updated 07/2014]. Available from: http://www.analcancerfoundation.org/wp-content/uploads/2014/12/HPV_9_print-1_G9.pdf.

7. Koshiol J, Lindsay L, Pimenta JM, Poole C, Jenkins D, Smith JS. Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis. Am J Epidemiol. 2008;168(2):123-37. doi: 10.1093/aje/kwn036. PubMed PMID: 18483125; PubMed Central PMCID: PMC2878094.

8. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. New England Journal of Medicine. 1998;338(7):423-8.
9. Deligeoroglou E, Giannouli A, Athanasopoulos N, Karountzos V, Vatopoulou A, Dimopoulos K, et al. HPV infection: immunological aspects and their utility in future therapy. Infect Dis Obstet Gynecol. 2013;2013:540850. doi: 10.1155/2013/540850. PubMed PMID: 24023507; PubMed Central PMCID: PMC3762170.

10. International Agency for Research on Cancer (IARC) Working Group on Evaluation of Carcinogenic Risks to Humans. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Volume 90 Human Papillomaviruses. Lyon, France: World Health Organization, International Agency for Research on Cancer; 2007. 689 p.

11. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. A review of human carcinogens--Part B: biological agents. Lancet Oncology. 2009;10(4):321-2. Epub 2009/04/08. PubMed PMID: 19350698.

12. Abramowitz L, Jacquard AC, Jaroud F, Haesebaert J, Siproudhis L, Pradat P, et al. Human papillomavirus genotype distribution in anal cancer in France: the EDiTH V study. International

Journal of Cancer. 2011;129(2):433-9. Epub 2010/09/15. doi: 10.1002/ijc.25671. PubMed PMID: 20839262.

13. Prevention CfDCa. HPV and Cancer 2017 [updated March 3, 2017; cited 2017 June 15]. Available from: https://www.cdc.gov/cancer/hpv/statistics/cases.htm.

14. Valmary-Degano S, Jacquin E, Prétet JL, Monnien F, Girardo B, Arbez-Gindre F, et al. Signature patterns of human papillomavirus type 16 in invasive anal carcinoma. Human Pathology. 2013;44(6):11. Epub 2012 Dec 23. doi: 10.1016/j.humpath.2012.08.019.

15. (IARC) IAfRoC. GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012 2012 [cited 2015 07/13]. Available from: http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx.

16. SEER S, Epidemiology and End Results Program,. SEER Cancer Statistics Factsheets: Anal Cancer Bethesda, MD: National Cancer Institute; [cited 2015 July 29]. Available from: http://seer.cancer.gov/statfacts/html/anus.html.

17. D'Souza G, Wiley DJ, Li X, Chmiel JS, Margolick JB, Cranston RD, et al. Incidence and epidemiology of anal cancer in the multicenter AIDS cohort study. J Acquir Immune Defic Syndr. 2008;48(4):491-9. doi: 10.1097/QAI.0b013e31817aebfe. PubMed PMID: 18614927; PubMed Central PMCID: PMC3991563.

18. Machalek DA, Poynten M, Jin F, Fairley CK, Farnsworth A, Garland SM, et al. Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: a systematic review and meta-analysis. Lancet Oncol. 2012;13(5):487-500. doi: 10.1016/S1470-2045(12)70080-3. PubMed PMID: 22445259.

19. Silverberg MJ, Lau B, Justice AC, Engels E, Gill MJ, Goedert JJ, et al. Risk of anal cancer in HIV-infected and HIV-uninfected individuals in North America. Clin Infect Dis. 2012;54(7):1026-34. doi: 10.1093/cid/cir1012. PubMed PMID: 22291097; PubMed Central PMCID: PMC3297645.

20. SEER S, Epidemiology and End Results Program. SEER Cancer Statistics Factsheets: Prostate Cancer Bethesda, MD: National Cancer Institute; [cited 2015 July 13]. Available from: https://seer.cancer.gov/statfacts/html/prost.html.

21. Benard VB, Thomas CC, King J, Massetti GM, Doria-Rose VP, Saraiya M, et al. Vital signs: cervical cancer incidence, mortality, and screening - United States, 2007-2012. MMWR Morb Mortal Wkly Rep. 2014;63(44):1004-9. PubMed PMID: 25375072.

22. Grulich AE, Poynten IM, Machalek DA, Jin F, Templeton DJ, Hillman RJ. The epidemiology of anal cancer. Sex Health. 2012;9(6):504-8. doi: 10.1071/SH12070. PubMed PMID: 22958581.

23. Stanley MA. Epithelial cell responses to infection with human papillomavirus. Clin Microbiol Rev. 2012;25(2):215-22. doi: 10.1128/CMR.05028-11. PubMed PMID: 22491770; PubMed Central PMCID: PMC3346303.

24. Longworth MS, Laimins LA. Pathogenesis of human papillomaviruses in differentiating epithelia. Microbiology and Molecular Biology Reviews. 2004;68(2):362-72. doi:

10.1128/MMBR.68.2.362-372.2004. PubMed PMID: 15187189; PubMed Central PMCID: PMC419925.

25. Moscicki AB, Schiffman M, Kjaer S, Villa LL. Chapter 5: Updating the natural history of HPV and anogenital cancer. Vaccine. 2006;24 Suppl 3:S3/42-51. doi: 10.1016/j.vaccine.2006.06.018. PubMed PMID: 16950017.

26. Schiffman M, Kjaer SK. Chapter 2: Natural history of anogenital human papillomavirus infection and neoplasia. J Natl Cancer Inst Monogr. 2003;(31):14-9. PubMed PMID: 12807940.

27. Castle PE, Schiffman M, Herrero R, Hildesheim A, Rodriguez AC, Bratti MC, et al. A prospective study of age trends in cervical human papillomavirus acquisition and persistence in Guanacaste, Costa Rica. J Infect Dis. 2005;191(11):1808-16. doi: 10.1086/428779. PubMed PMID: 15871112.

28. Ortoski RA, Kell CS. Anal cancer and screening guidelines for human papillomavirus in men. J Am Osteopath Assoc. 2011;111(3 Suppl 2):S35-43. PubMed PMID: 21415379.

29. Safaeian M, Solomon D, Castle PE. Cervical cancer prevention--cervical screening: science in evolution. Obstet Gynecol Clin North Am. 2007;34(4):739-60, ix. doi: 10.1016/j.ogc.2007.09.004. PubMed PMID: 18061867; PubMed Central PMCID: PMC2762353.

30. McNamara M, Batur P, Walsh JM, Johnson KM. HPV Update: Vaccination, Screening, and Associated Disease. J Gen Intern Med. 2016. doi: 10.1007/s11606-016-3725-z. PubMed PMID: 27184752.

31. Patel J, Salit IE, Berry MJ, de Pokomandy A, Nathan M, Fishman F, et al. Environmental scan of anal cancer screening practices: worldwide survey results. Cancer Med. 2014;3(4):1052-61. doi: 10.1002/cam4.250. PubMed PMID: 24740973; PubMed Central PMCID: PMC4303174.

32. Nayar R, Wilbur DC. The Pap Test and Bethesda 2014. "The reports of my demise have been greatly exaggerated." (after a quotation from Mark Twain). Acta Cytol. 2015;59(2):121-32. doi: 10.1159/000381842. PubMed PMID: 25997404.

33. Darragh TM, Colgan TJ, Cox JT, Heller DS, Henry MR, Luff RD, et al. The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. J Low Genit Tract Dis. 2012;16(3):205-42. doi: 10.1097/LGT.0b013e31825c31dd. PubMed PMID: 22820980.

34. Peitzmeier SM, Reisner SL, Harigopal P, Potter J. Female-to-male patients have high prevalence of unsatisfactory Paps compared to non-transgender females: implications for cervical cancer screening. J Gen Intern Med. 2014;29(5):778-84. doi: 10.1007/s11606-013-2753-1. PubMed PMID: 24424775; PubMed Central PMCID: PMC4000345.

35. FDA. FDA approves first human papillomavirus test for primary cervical cancer screening 2014 [updated 04/24/2014; cited 2015 10 24]. Available from: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm394773.htm.

36. Castle PE, Sadorra M, Garcia F, Holladay EB, Kornegay J. Pilot study of a commercialized human papillomavirus (HPV) genotyping assay: comparison of HPV risk group to cytology and

histology. J Clin Microbiol. 2006;44(11):3915-7. doi: 10.1128/JCM.01305-06. PubMed PMID: 16971652; PubMed Central PMCID: PMC1698309.

37. (USPSTF) USPSTF. Final Update Summary: Cervical Cancer: Screening 2015 [updated 07/2015]. Available from: http://www.uspreventiveservicestaskforce.org/Page/Document/UpdateSummaryFinal/cervical-cancer-screening.

38. Salit IE, Lytwyn A, Raboud J, Sano M, Chong S, Diong C, et al. The role of cytology (Pap tests) and human papillomavirus testing in anal cancer screening. AIDS. 2010;24(9):1307-13. doi: 10.1097/QAD.0b013e328339e592. PubMed PMID: 20442633.

39. Maia LB, Marinho LC, Barbosa TW, Velasco LF, Costa PG, Carneiro FP, et al. Value of human papillomavirus typing for detection of anal cytological abnormalities. Indian J Sex Transm Dis. 2013;34(2):102-6. doi: 10.4103/0253-7184.120540. PubMed PMID: 24339460; PubMed Central PMCID: PMC3841659.

40. Huh WK, Ault KA, Chelmow D, Davey DD, Goluart RA, Garcia FA, et al. Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance. Gynecol Oncol. 2015;136(2):5. Epub 1/8/2015. doi: doi:10.1016/j.ygyno.2014.12.022.

41. Moscicki AB, Darragh TM, Berry-Lawhorn JM, Roberts JM, Khan MJ, Boardman LA, et al. Screening for Anal Cancer in Women. J Low Genit Tract Dis. 2015;19(3 Suppl 1):S27-42. doi: 10.1097/LGT.0000000000000117. PubMed PMID: 26103446; PubMed Central PMCID: PMC4479419.

42. Palefsky J, Berry JM, Jay N. Anal cancer screening. Lancet Oncol. 2012;13(7):e279-80; author rreply e80. doi: 10.1016/S1470-2045(12)70215-2. PubMed PMID: 22748261.

43. Berry-Lawhorn JM, Chrobak D, Jay N, Palefsky JM. Who is ready to screen for anal squamous intraepithelial lesions and why should they perform high-resolution anoscopy? Sex Transm Dis. 2014;41(4):254-6. doi: 10.1097/OLQ.0000000000000119. PubMed PMID: 24622637.

44. Pils S, Joura EA. From the monovalent to the nine-valent HPV vaccine. Clin Microbiol Infect. 2015. doi: 10.1016/j.cmi.2015.05.001. PubMed PMID: 25980355.

45. Wells JS, Holstad MM, Thomas T, Bruner DW. An integrative review of guidelines for anal cancer screening in HIV-infected persons. AIDS Patient Care STDS. 2014;28(7):350-7. doi: 10.1089/apc.2013.0358. PubMed PMID: 24936878.

46. (USPSTF) USPSTF. Final Recommendation Statement Oral Cancer: Screening, November 2013 2013 [updated November 2013; cited 2015 10/16]. Available from: http://www.uspreventiveservicestaskforce.org/Page/Document/RecommendationStatementFinal/oral-cancer-screening1.

47. Goldstone SE, Moshier E. Detection of oncogenic human papillomavirus impacts anal screening guidelines in men who have sex with men. Dis Colon Rectum. 2010;53(8):1135-42. doi: 10.1007/DCR.0b013e3181e10842. PubMed PMID: 20628276.

48. Gimenez F, Costa-e-Silva IT, Daumas A, Araujo J, Medeiros SG, Ferreira L. The value of high-resolution anoscopy in the diagnosis of anal cancer precursor lesions in HIV-positive patients. Arq Gastroenterol. 2011;48(2):136-45. PubMed PMID: 21709956.

49. Health Quality O. Anal dysplasia screening: an evidence-based analysis. Ont Health Technol Assess Ser. 2007;7(4):1-43. PubMed PMID: 23074504; PubMed Central PMCID: PMC3377578.

50. Maniar KP, Nayar R. HPV-related squamous neoplasia of the lower anogenital tract: an update and review of recent guidelines. Adv Anat Pathol. 2014;21(5):341-58. doi: 10.1097/PAP.0000000000000035. PubMed PMID: 25105936.

51. Palefsky JM, Holly EA, Hogeboom CJ, Berry JM, Jay N, Darragh TM. Anal cytology as a screening tool for anal squamous intraepithelial lesions. J Acquir Immune Defic Syndr Hum Retrovirol. 1997;14(5):415-22. PubMed PMID: 9170415.

52. Darragh TM, Tokugawa D, Castle PE, Follansbee S, Borgonovo S, LaMere BJ, et al. Interrater agreement of anal cytology. Cancer Cytopathol. 2013;121(2):72-8. doi: 10.1002/cncy.21218. PubMed PMID: 22811048; PubMed Central PMCID: PMC3479324.

53. Li Z, Zhang H, Li X, Yang Y, Xin H, Li M, et al. Anal Human Papillomavirus Genotyping among HIV-Positive Men Who Have Sex with Men in Xi'an, China. PLoS One. 2015;10(4):e0125120. doi: 10.1371/journal.pone.0125120. PubMed PMID: 25923768; PubMed Central PMCID: PMC4414525.

54. Vajdic CM, van Leeuwen MT, Jin F, Prestage G, Medley G, Hillman RJ, et al. Anal human papillomavirus genotype diversity and co-infection in a community-based sample of homosexual men. Sex Transm Infect. 2009;85(5):330-5. Epub 2009/04/04. doi: sti.2008.034744 [pii] 10.1136/sti.2008.034744. PubMed PMID: 19342375.

55. Bertisch B, Franceschi S, Lise M, Vernazza P, Keiser O, Schoni-Affolter F, et al. Risk Factors for Anal Cancer in Persons Infected With HIV: A Nested Case-Control Study in the Swiss HIV Cohort Study. Am J Epidemiol. 2013. Epub 2013/08/01. doi: kwt153 [pii] 10.1093/aje/kwt153. PubMed PMID: 23900553.

56. Gage JC, Katki HA, Schiffman M, Fetterman B, Poitras NE, Lorey T, et al. Age-stratified 5-year risks of cervical precancer among women with enrollment and newly detected HPV infection. Int J Cancer. 2015;136(7):1665-71. doi: 10.1002/ijc.29143. PubMed PMID: 25136967; PubMed Central PMCID: PMC4314342.

57. Demers AA, Shearer B, Severini A, Lotocki R, Kliewer EV, Stopera S, et al. Distribution of human papillomavirus types, cervical cancer screening history, and risk factors for infection in Manitoba. Chronic Dis Inj Can. 2012;32(4):177-85. PubMed PMID: 23046799.

58. Madeleine MM, Finch JL, Lynch CF, Goodman MT, Engels EA. HPV-related cancers after solid organ transplantation in the United States. Am J Transplant. 2013;13(12):3202-9. doi: 10.1111/ajt.12472. PubMed PMID: 24119294; PubMed Central PMCID: PMC4049182.

59. Van Aar F, Mooij SH, Van Der Sande MA, Speksnijder AG, Stolte IG, Meijer CJ, et al. Anal and penile high-risk human papillomavirus prevalence in HIV-negative and HIV-infected MSM. AIDS. 2013. doi: 10.1097/01.aids.0000432541.67409.3c. PubMed PMID: 23921617.

60. Mooij SH, van Santen DK, Geskus RB, van der Sande MA, Coutinho RA, Stolte IG, et al. The effect of HIV infection on anal and penile human papillomavirus incidence and clearance: a cohort study among MSM. AIDS. 2016;30(1):121-32. doi: 10.1097/QAD.0000000000000909. PubMed PMID: 26474302.

61. Schwartz LM, Castle PE, Follansbee S, Borgonovo S, Fetterman B, Tokugawa D, et al. Risk Factors for Anal HPV Infection and Anal Precancer in HIV-Infected Men Who Have Sex With Men. J Infect Dis. 2013. Epub 2013 Sep 4. doi: 10.1093/infdis/jit374.

62. Couture MC, Page K, Stein ES, Sansothy N, Sichan K, Kaldor J, et al. Cervical human papillomavirus infection among young women engaged in sex work in Phnom Penh, Cambodia: prevalence, genotypes, risk factors and association with HIV infection. BMC Infect Dis. 2012;12:166. doi: 10.1186/1471-2334-12-166. PubMed PMID: 22839728; PubMed Central PMCID: PMC3436768.

63. Bahmanyar RE, Paavonen J, Naud P, Salmeron J, Chow SN, Apter D, et al. Prevalence and risk factors for cervical HPV infection and abnormalities in young adult women at enrolment in the multinational PATRICIA trial. Gynecol Oncol. 2012;127(3):11. doi: doi:10.1016/j.ygyno.2012.08.033.

64. Geskus RB, Gonzalez C, Torres M, Del Romero J, Viciana P, Masia M, et al. Incidence and clearance of anal high-risk human papillomavirus in HIV-positive men who have sex with men: estimates and risk factors. AIDS. 2016;30(1):37-44. doi: 10.1097/QAD.0000000000000874. PubMed PMID: 26355673; PubMed Central PMCID: PMC4674141.

65. Munoz N, Franceschi S, Bosetti C, Moreno V, Herrero R, Smith JS, et al. Role of parity and human papillomavirus in cervical cancer: the IARC multicentric case-control study. Lancet. 2002;359(9312):1093-101. PubMed PMID: 11943256.

66. Smith JS, Green J, Berrington de Gonzalez A, Appleby P, Peto J, Plummer M, et al. Cervical cancer and use of hormonal contraceptives: a systematic review. Lancet. 2003;361(9364):1159-67. PubMed PMID: 12686037.

67. Moreno V, Bosch FX, Munoz N, Meijer CJ, Shah KV, Walboomers JM, et al. Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case-control study. Lancet. 2002;359(9312):1085-92. doi: 10.1016/S0140-6736(02)08150-3. PubMed PMID: 11943255.

68. Singer A. The uterine cervix from adolescence to the menopause. Br J Obstet Gynaecol. 1975;82(2):81-99. PubMed PMID: 1125147.

69. Burk RD, Ho GY, Beardsley L, Lempa M, Peters M, Bierman R. Sexual behavior and partner characteristics are the predominant risk factors for genital human papillomavirus infection in young women. J Infect Dis. 1996;174(4):679-89. PubMed PMID: 8843203.

70. Ley C, Bauer HM, Reingold A, Schiffman MH, Chambers JC, Tashiro CJ, et al. Determinants of genital human papillomavirus infection in young women. J Natl Cancer Inst. 1991;83(14):997-1003. PubMed PMID: 1649312.

71. Chung SH, Franceschi S, Lambert PF. Estrogen and ERalpha: culprits in cervical cancer? Trends Endocrinol Metab. 2010;21(8):504-11. doi: 10.1016/j.tem.2010.03.005. PubMed PMID: 20456973; PubMed Central PMCID: PMC2914219.

72. Hormonal contraceptives, progestogens only. IARC Monogr Eval Carcinog Risks Hum. 1999;72:339-97. PubMed PMID: 10533178.

73. Hsu HK, Brown TT, Li X, Young S, Cranston RD, D'Souza G, et al. Association between Free Testosterone Levels and Anal Human Papillomavirus Types 16/18 Infections in a Cohort of Men Who Have Sex with Men. PLoS One. 2015;10(3):e0119447. doi: 10.1371/journal.pone.0119447. PubMed PMID: 25794147.

74. Slama L, Jacobson LP, Li X, Palella FJ, Jr., Margolick JB, Kingsley LA, et al. Longitudinal Changes Over 10 Years in Free Testosterone Among HIV-Infected and HIV-Uninfected Men. J Acquir Immune Defic Syndr. 2016;71(1):57-64. doi: 10.1097/QAI.0000000000000821. PubMed PMID: 26761271; PubMed Central PMCID: PMC4712718.

75. Chen HC, Farese RV. Steroid hormones: Interactions with membrane-bound receptors. Curr Biol. 1999;9(13):R478-81. PubMed PMID: 10395533.

76. Alsharif M, McKeon DM, Gulbahce HE, Savik K, Pambuccian SE. Unsatisfactory SurePath liquid-based Papanicolaou tests: causes and significance. Cancer. 2009;117(1):15-26. doi: 10.1002/cncy.20009. PubMed PMID: 19347825.

77. Zhao C, Domfeh AB, Austin RM. Histopathologic outcomes and clinical correlations for high-risk patients screened with anal cytology. Acta Cytol. 2012;56(1):62-7. doi: 10.1159/000331431. PubMed PMID: 22236747.

78. Conley L, Bush T, Darragh TM, Palefsky JM, Unger ER, Patel P, et al. Factors associated with prevalent abnormal anal cytology in a large cohort of HIV-infected adults in the United States. J Infect Dis. 2010;202(10):1567-76. doi: 10.1086/656775. PubMed PMID: 20925532.

79. Fontaine D, Narine N, Naugler C. Unsatisfactory rates vary between cervical cytology samples prepared using ThinPrep and SurePath platforms: a review and meta-analysis. BMJ Open. 2012;2(2):e000847. doi: 10.1136/bmjopen-2012-000847. PubMed PMID: 22505312; PubMed Central PMCID: PMC3332241.

80. Cranston RD, Hart SD, Gornbein JA, Hirschowitz SL, Cortina G, Moe AA. The prevalence, and predictive value, of abnormal anal cytology to diagnose anal dysplasia in a population of HIV-positive men who have sex with men. Int J STD AIDS. 2007;18(2):77-80. doi: 10.1258/095646207779949772. PubMed PMID: 17331275.

81. Bofin AM, Nygard JF, Skare GB, Dybdahl BM, Westerhagen U, Sauer T. Papanicolaou smear history in women with low-grade cytology before cervical cancer diagnosis. Cancer. 2007;111(4):210-6. doi: 10.1002/cncr.22865. PubMed PMID: 17567833.

82. Hock YL, Ramaiah S, Wall ES, Harris AM, Marston L, Marshall J, et al. Outcome of women with inadequate cervical smears followed up for five years. J Clin Pathol. 2003;56(8):592-5. PubMed PMID: 12890808; PubMed Central PMCID: PMC1770040.

83. Owens CL, Buist DS, Peterson D, Kamineni A, Weinmann S, Ross T, et al. Follow-up and clinical significance of unsatisfactory liquid-based Papanicolaou tests. Cancer Cytopathol. 2015;123(1):59-65. doi: 10.1002/cncy.21490. PubMed PMID: 25346238; PubMed Central PMCID: PMC4356524.

Chapter 2. Longitudinal Analysis of Risk Factors for Incidence and Clearance of Anal HPV Types 16 and 18 Infections in the Multicenter AIDS Cohort Study

2.1 Abstract

Introduction: Human papillomaviruses (HPVs) commonly infect anal epithelium. Of 41 HPV types that can infect the anus and other genitals, twelve are classified as high-risk (hr) Group 1 HPVs which are strongly carcinogenic. HPV types 16 and 18 are two Group 1 viruses that cause 80-90% of anal cancers. Older men who have sex with men (MSM) are disproportionally affected by anal cancer, for which no standard procedure for screening or treatment exists. The objective of this study is to characterize incidence and clearance of anal HPV 16 and 18 infections in a cohort of older MSM.

Methods: 1,259 HIV-infected and –uninfected MSM from the Multicenter AIDS Cohort Study were followed between 2010 and 2014 in six-month intervals as part of the Anal Health Substudy, a nested longitudinal study, where a Dacron swab was collected from the anal canal and tested for 37 HPV types using the Linear Array HPV DNA PCR assay. HIV-stratified incidence and clearance rates and rate ratios (RRs) were calculated. Cox proportional hazards regression models estimated hazards ratios (HRs) for incidence and clearance of HPV16 and 18 infections by HIV infection status, controlling for age, race, smoking, CD4 count, receptive anal intercourse partnerships, and history of other sexually transmitted infections.

Results: Men were on average 55 ($\pm$9) years old, most were White, non-Hispanic (74%, 929/1259) and nearly half were HIV-infected (48%, 610/1259). Men contributed an average of 3.4 test visits over a 27-month period. At baseline, most men (79.6%) tested positive (+) for one or more anal HPVs. HPV16 and 18 infections were common, with 25.2% of men testing positive for one or both at baseline. Incidence and clearance of both types were common as well.

In multivariable models, age was inversely associated with incident HPV16 (HR: 0.97 (0.94, 0.99)). Among HIV-uninfected men, having four or more RAI partners in the last 2 years was associated with higher risk of incident HPV16 (HR: 2.5 (1.1, 5.3)), as were having a prevalent HPV18 infection, and history of anal warts and syphilis (HRs: 3.7 (1.5, 8.9); 6.1 (2.0, 19.0); 3.7 (1.1, 12.2)). Ever having symptomatic oro-facial herpes was associated with incident HPV18 in HIV-infected men (HR: 2.3 (1.1, 4.8)), while among HIV-uninfected men, being non-white was associated with a higher risk of incident HPV18 (HR: 3.6 (1.4, 9.1)). In HIV-infected men, age, never smoking, and never having anal herpes were associated with clearance of HPV16 (HRs 1.04 (1.00,1.07), 1.9 (1.1, 3.1), 1.8 (1.0, 3.2)). In HIV-uninfected men, not having a prevalent other Group 1 infection was associated with increased HPV16 clearance (HRs: 1.9 (1.0, 3.5)). Never smoking was associated with higher clearance of HPV18 in HIV-infected men (HR: 3.7 (1.4, 9.7)).

Discussion: Using PCR testing, anal HPV infections are common in this older group of MSM. Incidence and clearance of HPV 16 and 18 remains high, especially among HIV-infected men, suggesting risk for ongoing HPV exposure with age. Several risk factors were found to be associated with incidence and clearance, that may function by increasing exposure or influencing susceptibility. Overall, risk of anal HPV persistence was high, confirming the disproportionate risk MSM confer for HPV-related anal cancer.

2.2 Introduction

Human papillomaviruses (HPVs) are the most common sexually transmitted infections in the United States [1]. There are 41 HPV genotypes (types) that infect anogenital epithelium [2, 3]. Twelve types are classified by the International Agency for Research on Cancer as Group 1 high-risk (hr) strong carcinogens: HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 [2, 3]. Another thirteen hrHPV types are classified as Group 2 weak carcinogens: HPV26, 30, 34, 53, 66, 67, 68, 69, 70, 73, and 82, 85, and 97. Sixteen other types, which rarely cause cancer, are classified as low-risk (lr) HPVs: HPV6, 11, 40, 42, 54, 55, 61, 62, 64, 71, 72, 81, 83, 84, Is39, and CP6108; types 6 and 11 are known to cause genital warts. Persistent infection with hrHPV types 16 and 18 are estimated to cause 78 to 90% of invasive anal cancers (IACs) and 70% of cervical cancers [2-6]. Although invasive anal cancers (IACs) are rare in most US men with an estimated 1.5 cases per 100,000 person-years (py), HIV-infected and -uninfected men who have sex with men (MSM) have rates that are up to 75-fold higher: 5-35 and 78-137 cases/100,000 py, respectively [7-11]. Highest estimates for anal cancer in MSM exceed incidence of even the most common cancer in the US for men (prostate cancer: 97-120 cases per 100,000), emphasizing the serious cancer risk in this population [7, 12]. Anal cancer incidence is increasing nationwide and is quickly becoming a new threat to public health [13].

The prevalence of anal HPV infections in MSM has been reported in numerous studies with infection estimates as high as 99% in HIV-infected men and 94% in HIV-uninfected men [14, 15]. Prevalent anal HPV16 and 18 infections range from 10-40% and 3-40%, respectively [14, 16-19]. Most studies characterizing anal HPV incidence and clearance use small samples, mostly focusing on HIV-infected MSM, with some using HPV test assays that do not test for all anal HPV types commonly identified. Estimating infection characteristics in a large group of HIV-infected and -uninfected MSM would add great value in better understanding anal HPV infections and their risk factors.

A preliminary cross-sectional analysis performed in a large group of HIV-infected and -uninfected MSM showed HIV infection was associated with increased prevalence in all HPV groups (Figure 2.1 [20]). Multiple studies have verified the association between HPV infection acquisition and persistence, and anal cancer with HIV infection in men and women, and identified other risk factors including: number of sex partners, race, age, and smoking tobacco; among HIV-infected individuals, CD4+ count, HIV viral load and adherence to highly active antiretroviral therapy (HAART) [15, 20-31]. Receptive anal intercourse (RAI), specifically, is associated with higher risk for anal HPV incidence, though experts agree non-intercourse anal behaviors, such as digital anal stimulation, play a role in transmission and infection risk [27]. The recent number of RAI partners was associated with increased prevalence of all HPV groups, whereas lifetime number of sex partners was only associated with increased prevalence of lrHPVs [20]. Additionally, there is evidence to indicate higher hormone levels may also be associated with HPV infection. One analysis showed each half-$\log_{10}$ increase in serum free testosterone (FT) was associated with a 1.9-fold higher prevalence of anal HPV16/18 infections in a cohort of MSM, suggesting exogenous testosterone use may increase susceptibility to HPV infection [32]. Factors affecting anal HPV clearance have not been well studied in any group, however one study in HIV-infected MSM found a strong association between young age (<25 years) and lower HIV RNA viral load with higher clearance of anal HPVs [31]. Other strong predictors of clearance of anal HPV infections may exist and have yet to be identified [31].

Because anal HPV infections are more common in MSM than in the general population and factors associated with incidence and clearance of anal hrHPVs are not well understood, more research is needed to determine which MSM are the highest risk for persistent hrHPV infection. Better characterizing anal HPV16 and 18 infections among the group at highest risk for anal cancer may help to inform anal cancer screening guidelines and prevention strategies. Our goal for this study is to describe incidence and clearance of anal HPVs in a large cohort of MSM and identify risk factors for incidence and clearance of HPV types 16 and 18.

2.3 Methods

Participants

MSM enrolled in the Multicenter AIDS Cohort Study (MACS), the longest continuing U.S. longitudinal study tracking the natural history of HIV/AIDS in MSM) were recruited over three main enrollment periods (1984-87, 1987-91, and 2001-03) in four major U.S. cities: Baltimore, Chicago, Los Angeles, and Pittsburgh [33, 34]. In total, 6,972 HIV-infected and -uninfected MSM were enrolled and provided written consent for participation in the MACS. As of 2011, a remaining 2,216 participants were still active and enrolled. Men attended routine study visits every six months where laboratory specimens were collected, and sociodemographic, medical, and behavioral data were collected using self- and interview-guided questionnaires [34].

From 2010 to 2014, 68% (1,512/2,216) of the active MACS MSM provided written informed consent for periodic anal cytology and HPV testing as part of a nested cohort substudy: the Anal Health Substudy (AHS). The goal of the AHS was to assess the prevalence and risk factors for anal precancers. Enrollment was open to all MACS participants with no exclusion criteria. AHS visits were performed during regularly scheduled MACS visits. Men were followed from one to eight visits where an anal swab was collected and tested for cytology and HPV genotyping. To evaluate incidence and clearance of any HPV longitudinally, multiple visits over time were needed. Therefore, the sample was limited to 1,259 men who had two or more interpretable HPV samples from visits at least 4 months apart.

Procedures

Anal Pap testing was performed and a single swab was collected at every visit, and tested for cytology annually for HIV-infected men and every two years for HIV-uninfected men. Testing frequency differed due to the high risk for anal dysplasia in HIV-infected MSM. A Dacron swab was inserted ~2.5 inches beyond the anal verge, approximated to the wall, and rotated slowly (against the anal wall) while being extracted and immediately placed into ThinPrep Preservcyt® solution (Cytyc Corporation, Boxborough, MA). HPV genotyping was performed at every visit with

a swab specimen. Specimens were centrifuged (~10,000 rpm) for 15 minutes, pelleted, resuspended, centrifuged in phosphate-buffered saline, and dried. Cell pellets were resuspended again in 0.3mL of 20 mM Tris buffer (pH: 8.3). MasterPure Purification Kit (Epicentre, Madison, WI) was used to purify the DNA for testing. Anal HPV genotyping was performed from extracted DNA of residual cytology specimens (~75µg) using PCR and line-blot hybridization (Linear Array® HPV Genotyping Test, Roche Molecular Diagnostics, Pleasanton, CA) by Tricore Reference Laboratories (Albuquerque, NM). Linear Array uses the PGMY09-PGMY11 primer set, to amplify a 450 base-pair fragment of the L1 gene, to detect 37 HPV types: Group 1 hrHPVs: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59; Group 2 hrHPVs: 26, 53, 66, 67, 68, 69, 70, 73, and 82; and lrHPVs: 6, 11, 40, 42, 54, 55, 61, 62, 64, 71, 72, 81, 83, 84, Is39, and CP6108 [2, 3].

Sociodemographic and sexual-behavioral characteristics were collected using a standardized interviewer-guided questionnaire. Serum was collected and tested to evaluate Hepatitis B and HIV infection characteristics and are described elsewhere [34-38]. HIV RNA levels were measured using an ultrasensitive RNA PCR Assay (Hoffman-LaRoche, Nutley, NJ, USA) with a cutoff limit of 50 copies/mL; standard flow cytometry was used to quantify CD4 counts [39].

The exposures of interest collected from the baseline MACS visit to the last MACS visit coinciding with the last AHS visit were assessed for an association with anal HPV infection. Data for this analysis were requested and obtained from the Center for the Analysis of MACS Data (CAMACS, Johns Hopkins University, Baltimore, MD). Covariate data of interest were collected at baseline, and captured at every MACS visit (six months) to update changes since the last visit. These covariates include: age, race, HIV status and HIV characteristics (last seronegative and first seropositive visit date, CD4 count, RNA set point, AIDS diagnoses, and HAART use), study center, enrollment cohort, use of exogenous testosterone, number of receptive anal intercourse partners, body mass index (BMI), tobacco and alcohol use, and sexually transmitted infections (STIs): Hepatitis B and C virus (HBV/HCV), gonorrhea, syphilis, genital warts, anal warts, herpes,

and genital sores. The 35 other HPV types also tested for on the same swab were included as covariates grouped as other Group 1 hrHPVs, Group 2 hrHPVs, and lrHPVs.

The outcomes of interest were incidence and clearance of HPV types 16 and 18. Incident infections were defined as a positive test following a negative test. Cleared infections were defined by a negative test following a positive test.

Analysis

Descriptive analyses were used to estimate the baseline characteristics of study group and identify differences across variables by HIV status. Data for HIV-infected and –uninfected men were compared using Chi-squared (categorical variables) and Wilcoxon Rank Sum test statistics (continuous variables). HPV type-specific incidence and clearance rates were calculated for each of the 37 HPV types and for groups by pathogenicity: HPV16/18, Group 1 hrHPVs (with and without HPV16/18), nine Group 2 hrHPVs, and sixteen lrHPVs [2, 3]. Additionally, Kaplan Meier cumulative incidence and clearance curves for HPV16 and 18 were plotted and compared by HIV status.

Cox Proportional regression models were used to calculate hazards ratios (HRs) as an estimate of the relative risk of experiencing the outcome at any fixed time. Bivariate Cox regression models were performed to assess individual relationships between covariates and HPV16 and 18 incidence and clearance. Covariates were a combination of variables summarized from historical visits, and data collected at the baseline visit for HPV testing. Incidence models estimated time to incident HPV16 and 18 infections, separately, versus not getting each type. Time to incident infection was defined as the time from baseline or first negative visit to an incident positive visit. For men with prevalent or incident HPV16 and 18 infections, models estimated time to clearance of HPV16 and 18 infections, separately, versus not clearing each type. Time to clearance was defined as the time from baseline (for prevalent infection) or incident infection to the first negative visit.

Final models were stratified by HIV status to address violations of the proportional hazards assumption, resulting in a total of eight Cox regression models for incidence and clearance of HPV 16 and 18, individually. Since individuals vary in risk for incidence and clearance by HPV type, i.e., the participant needed to be negative to be at risk for incidence, and positive to be at risk for clearance, samples differed across models. The sample sizes were: 1,076 for HPV16 incidence (506 HIV-infected and 570 HIV-uninfected), 1,192 for HPV18 incidence (567 HIV-infected and 625 HIV-uninfected), 324 for HPV16 clearance (194 HIV-infected and 130 HIV-uninfected), and 161 for HPV18 clearance (106 HIV-infected and 55 HIV-uninfected). All models controlled for age, race, ever smoking tobacco, and, among the four HIV-infected only models, also recent CD4 count (>500 cells/mm^3 vs. $\leq$500 cells/mm^3). The incidence models additionally controlled for the number of receptive anal intercourse (RAI) partners in the last two years, while the clearance models controlled for the number of lifetime RAI partners. Individual models included some additional covariates including: ever using testosterone, ever having anal warts, anal herpes, syphilis, oro-facial herpes, or prevalent infections with other anal HPVs. Analyses were performed with SAS V9.4 (Cary, NC).

2.4 Results

At baseline, men were 55 ($\pm$9) years old, on average, and 74% (929/1259) were White, non-Hispanic, 18% (227/1259) were Black, non-Hispanic, and 8.2% (103/1259) reported another race/ethnicity. Nearly half of all participants were HIV-infected (610/1259). The participants contributed a total of 4,253 HPV test visits. On average, each man had 3.4 visits (range: 2-7); HIV-infected men had 3.8 visits and HIV-uninfected had 3 visits (p<0.05). The total follow-up time was 33,815 person-months, with each participant averaging 26.9 months on study (range: 4.1-45.5 months); 29.5 months for HIV-infected and 24.4 months for HIV-uninfected men (p<0.05).

Compared to HIV-uninfected men, HIV-infected men were younger, more likely to be non-White, and less likely to be overweight (p-values<0.001) (Table 2.1). HIV-infected men were more

likely to be current smokers than HIV-uninfected men, but drinking frequency did not differ (Table 2.1). Lastly, HIV-infected men more often reported five or more RAI partners than HIV-uninfected men in the two years prior to the baseline AHS visit (33.7% vs. 19.8%) (Table 2.1).

HPV Prevalence

The prevalence of both hrHPVs and lrHPVs was high in this group. Most men showed positivity for at least one HPV type at baseline, (79.6% (1002/1259)) (Table 2.2a). HPV16/18 infections were common with 25.2% (317/1259) having one or both types. While 55.1% (694/1259) of participants tested positive for any Group 1 HPV, 46.5% (586/1259) of men had positivity for a Group 1 type other than HPV16/18, 34.9% (439/1259) were positive for a Group 2 virus, and 60.2% (758/1259) were positive for low-risk viruses. Prevalence characteristics for the other 35 types are listed in Supplementary Table 2.1a.

HPV Incidence

During follow-up, there were 872 men with at least one new incident infection by any of the 37 HPV types detected at a rate of 35 men per 1,000 person-months (pm). There were 108 men who had incident HPV16 infections at a rate of 4/1,000 pm, with HIV-infected men having a higher incidence rate than HIV-uninfected men with an unadjusted rate ratio (RR) of 1.8 (95%CI: 1.1, 2.5) (Table 2.2a). Among those who had incident HPV16, the median time to infection was 12.2 months for HIV-infected men and 24.5 months for HIV-uninfected men. Sixty-eight men had incident HPV18 infections at a rate of 2.2/1,000 pm, with HIV-infected men similarly yielding a 2.1-fold higher incident rate than HIV-uninfected men (1.2, 3.4) (Table 2.2a). The median time to incident HPV18 infection was 22.9 for HIV-infected men and 12.6 months for HIV-uninfected men. Out of the 37 types, 27 yielded statistically significantly different crude RRs where rates of incident infection in HIV-infected men ranged from 1.7 to 5.6 higher than rates in HIV-uninfected men (Supplementary Table 2.1a).

HPV Clearance

Clearance of infections were frequent with 897 men clearing at least one of any HPV type at a rate of 53.4 men per 1,000 pm. A total of 129 men cleared HPV16 at a rate of 19.6 per 1,000 pm (Table 2.2b). The median time for clearing HPV16 infection was 12.2 for HIV-infected men and 19.0 months for HIV-uninfected men. There were 81 men who cleared HPV18 at a rate of 31.5 per 1,000 pm. The median survival time for clearing HPV18 infection was 12.2 for HIV-infected men and 6.5 months for HIV-uninfected men. Overall, HIV-infected men had a 27% higher rate of clearing any HPV type infections compared to HIV-uninfected men (RR: 1.3 (1.1, 1.5). When assessed by type, only clearance of HPV61 was statistically significantly different by HIV status with 88% higher rate of clearance in HIV-infected versus –uninfected men (RR: 1.9 (1.2, 2.9)). HPV16 and HPV18 showed higher clearance in HIV-uninfected than -infected men, though this did not reach statistical significance (Table 2.2b). The number of cleared infections and rates for each type and virus groupings are listed by HIV-infection status in Supplementary Table 2.1b.

Multivariable Analyses

Incident HPV16

In the multivariable survival Cox model for incident HPV16 in HIV-infected men, in addition to controlling for the effects of age, race, recent CD4 count, ever smoking, and the number of RAI partners in the last two years, we controlled for ever having anal warts and prevalent infection with other Group 1 viruses (Table 2.3). Only age was associated with incident HPV16 showing a 3.5% decrease in risk with each year of age (HR: 0.97; 95% CI (0.94, 0.99), p=0.03). Several risk factors for incident HPV16 were identified only among HIV-uninfected men. In addition to age, race, recent CD4 count, ever smoking, and the number of RAI partners in the last two years, we controlled for ever having anal warts or syphilis, and prevalent HPV18 infection (Table 2.4). Having a history of anal warts yielded a 6.1-fold higher risk of incident HPV16 compared to never having anal warts (p=0.002). Ever having syphilis infection conferred higher

3.7-fold higher risk (p=0.03), as did prevalent anal HPV18 at baseline (HR: 3.6; 95% CI (1.49, 8.94), p=0.005). Lastly, reporting four or more RAI partners in the last two years yielded a 2.5-fold higher risk of incident HPV16 infection (p=0.02).

Incident HPV18

The multivariable models for incident HPV18 also varied by HIV infection status. In the model for HIV-infected men, ever using testosterone and ever having symptomatic oro-facial herpes were additional covariates (Table 2.3). Prior symptomatic oro-facial herpes was associated with increased risk for incident HPV18 (HR: 2.3 p=0.03). Although not statistically significant, there was a 62% increase in risk for infection among men with CD4 count lower than 500 cells/mm^3 compared to those with more (p=0.1), and a 77% increase in incident HPV18 risk among ever testosterone users versus never users (p=0.07). In the multivariable model for incident HPV18 infection among HIV-uninfected men, prevalent HPV16 infection was uniquely included in the model (Table 2.4). Results showed being Black, non-Hispanic yielded a 3.6-fold higher risk compared to White, non-Hispanic men (p=0.01). Ever smoking had a 2.5-fold higher risk compared to non-smokers, and those reporting one to three RAI partners in the past two years had a 2.3-fold higher risk for incident HPV18 than those with none, but neither association reached statistical significance (p-values=0.1).

HPV16 Clearance

In the multivariable model for HPV16 clearance in HIV-infected men, the model additionally controlled for ever having anal herpes (Table 2.3). Each year of age was associated with a 4% increase in clearance for HPV16 infection (p=0.02), and never having smoked was associated with a 1.9-fold increase in clearance (p=0.02). However, ever having anal herpes showed a 45% decrease in clearance (p=0.04). When assessing HPV16 clearance in HIV-uninfected men, the model uniquely controlled for prevalent infection with another Group 1 virus which was associated with a 47% decrease in HPV16 clearance (p=0.04) (Table 2.4).

HPV18 Clearance

The results in multivariable models for HPV18 clearance differed slightly by HIV status. Among HIV-infected men, a recent CD4 count less than 500 cells/mm^3 was associated with a 38% decrease in clearance, but did not reach statistical significance (p=0.09) (Table 2.3). In the model for HIV-uninfected men, participants who never smoked had a 3.7-fold increase in HPV18 clearance compared to ever smokers (p<0.01) (Table 2.4).

2.5 Discussion

Anal HPV infections are very common in this cohort of mostly middle-aged MSM. Prevalent HPV infections were detected in 80% of all men at baseline. Every two out of three participants experienced at least one incident anal HPV infection, the slight majority being Group 1 hrHPVs, suggesting risk for new exposures was still high in older MSM. However, clearance was just as common, signifying transient infections were common. HIV-infected men demonstrated higher rates of incidence and clearance when compared to HIV-uninfected men, suggesting a difference in anal HPV infection risk profiles by HIV status.

Infection with HPV16 and 18 were common in this group affecting a quarter of all men. Incidence of HPV16 and HPV18 each occurred at a higher rate in HIV-infected men than in HIV-uninfected men. Stratified rates were within or below levels that others have reported [31, 40-42] [26]. The risk for incident new anal HPV16/18 infections was positively associated with the number of RAI partners as expected, however this was only observed in HIV-uninfected men, possibly due to more prevalent HPV16/18 infections in HIV-infected men. A history of anal warts and syphilis were associated with incident HPV16 infection in HIV-uninfected men, and ever having symptomatic oro-facial herpes was associated with incident HPV18 infection in HIV-infected men after controlling for sexual behaviors, suggesting coinfection with other STIs may increase risk for hrHPV infection. Other studies have found infection with syphilis and other STIs like chlamydia are associated with increased risk for incident hrHPV infection [28, 43]. Similarly, infection with HPVs can increase risk of acquisition of other HPV types, and as most anal warts are caused by

lrHPVs, it likely explains the association observed in this study [44]. In this cohort, exogenous testosterone use was not associated with risk for HPV16/18 incidence; however, this may be due to an inaccurate measure of lifetime use due to later editing of questions capturing this data in the MACS behavioral questionnaire. Though hypothesized, CD4 count did not affect incidence or clearance among HIV-infected men.

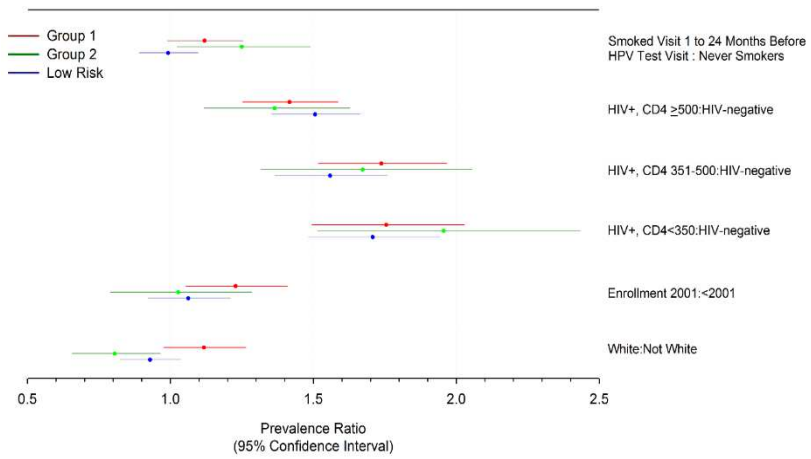
There were no differences in clearance of HPV16 or HPV18 by HIV-infection status. HAART adherence was not associated with anal HPV infection among HIV-infected men, as found by other investigators [20]. Smoking had an inverse association with clearance of infection suggesting higher clearance of anal hrHPVs among never smokers. However, smoking was not statistically significantly associated with incident infection suggesting it may only affect persistence and not acquisition of infection [42]. More research is needed to study the factors that predict clearance of anal hrHPVs.

These analyses are limited. Participants were followed in unequal intervals and have varying numbers of visits. This may lead to hidden bias if loss to follow-up was differential with respect to the exposures and outcome. However, this was unlikely as participants were not provided with HPV results until after the study was completed. With respect to defining infections, when an infection clears and reappears at a later visit, it is unclear whether this is a true incident infection or if the clinical specimen collection or testing assay missed a persistent infection. For any infections demonstrating clearance, it is possible they are not completely cleared but their viral loads remain at low undetectable levels that the Linear Array assay is not sensitive enough to detect. Censoring of data may also be an issue. Left censoring occurs from infections prevalent at baseline and may not be comparable to incident infections for assessing clearance, and the sample size did not provide adequate power to assess them separately. Additionally, right censoring occurs from loss to follow-up as we do not know infection characteristics beyond the end of study. As with many studies, self-reported data, such as for reporting sexual behaviors and some STIs, are inherent to biases related to recall or sensitivity. Lastly, these may not be

generalizable to other populations of MSM or other demographic groups in the general population. However, this study is one of the largest longitudinal analyses of anal HPV infection in HIV-infected and -uninfected MSM and these findings may inform other studies.

This study shows anal hrHPV infections are very common in older MSM suggesting risk for ongoing exposure with age. In the absence of a standard of care for screening for anal cancer, we hope these results will guide clinicians to identify the highest risk individuals among older MSM who would benefit most from regular monitoring for hrHPV infection.

Figure 2.1 Comparison of Prevalence Ratios for Group 1 and 2 High-risk and Lower-risk HPVs for 1262 Men Enrolled in the Multicenter AIDS Cohort Study Anal Health Sub-study.[a][20]



[a] Prevalence ratios are simultaneously adjusted for the effect of age, race, recruitment period (2001 vs <2001), study site; HIV infection (yes/no) and CD4 cell count (<350, 351-500, >500 cells/mm^3) among the infected, lifetime number of male sex partners at MACS visit 1 (<30, 30-99, 100-299, ≥300), number of sex partners reported between MACS visit 1 and 24 months before HPV testing (<30, 30-99, 100-199, ≥200), number of RAI partners during 24 months prior to HPV testing (0, 1-3, ≥4), tobacco smoking (yes/no) during two study periods: MACS visit 1 to the study visit 24 months before HPV testing, and the last 24 months of the study period.

Figure 2.2a-d Kaplan-Meier Survival Curves by HIV-Infection Status for:
A) HPV16 Incidence, B) HPV18 Incidence, C) HPV16 Clearance, D) HPV18 Clearance

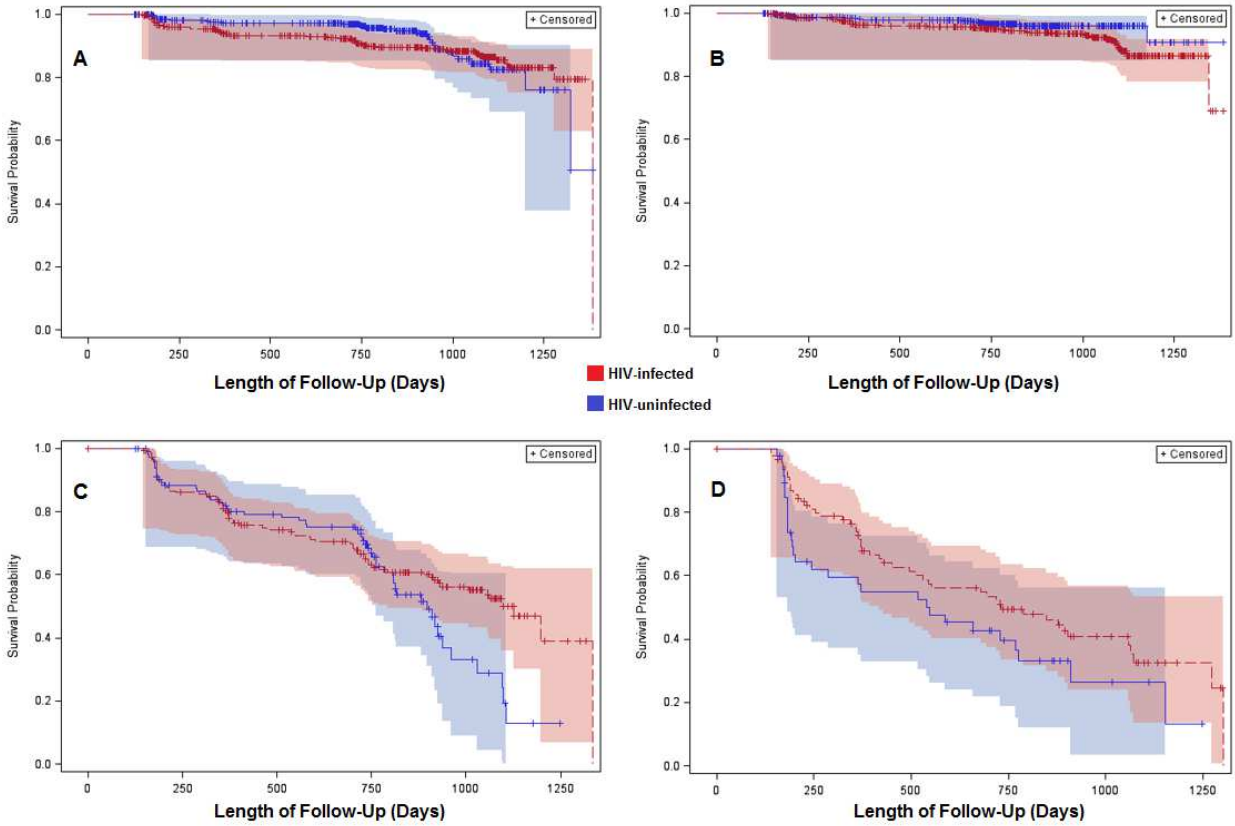


Table 2.1 Characteristics of AHS Study Group at Baseline by HIV Infection Status

	Total (n=1,259)	**HIV Seropositive (n=610)**	**HIV Seronegative (n=649)**	***p*-value**
	n (%)	n (%)	n (%)	
Characteristics				
Study Center				
Baltimore	328 (26.1)	148 (24.1)	181 (27.9)	*p<0.0001*
Chicago	266 (21.1)	183 (30.0)	83 (12.8)	
Pittsburgh	311 (24.7)	132 (21.6)	179 (27.6)	
Los Angeles	354 (28.1)	148 (24.3)	206 (31.7)	
Race				
White, non-Hispanic	929 (73.8)	395 (64.8)	534 (82.3)	*p<0.0001*
Black, non-Hispanic	227 (18.0)	154 (25.3)	73 (11.3)	
Other	103 (8.2)	61 (10.0)	42 (6.5)	
Age				
≤35 years old	43 (3.4)	26 (4.1)	18 (2.8)	*p<0.0001*
36-45 years old	126 (10.0)	78 (12.8)	48 (7.4)	
46-55 years old	488 (38.8)	272 (44.6)	216 (33.3)	
56-65 years old	467 (37.1)	205 (33.6)	262 (40.4)	
66-75 years old	124 (9.9)	30 (4.9)	94 (14.5)	
76+ years old	11 (0.9)	0 (0.0)	11 (1.7)	
Highest Level of Education Completed				
High school or Less	138 (12.0)	87 (15.5)	51 (8.6)	*p<0.0001*
Some college	246 (21.3)	149 (26.6)	97 (16.4)	
4-year college	280 (24.3)	133 (23.8)	147 (24.8)	
Some graduate school	129 (11.2)	59 (10.4)	71 (12.0)	
Post-graduate school	360 (31.2)	133 (23.8)	227 (38.3)	
BMI				
Normal Weight (BMI <25)	526 (42.7)	283 (47.9)	243 (38.0)	*p=0.0004*
Overweight (BMI ≥25)	705 (57.3)	308 (52.1)	397 (62.0)	
CD4 Count				
≤250 CD4 cells/mm3	50 (4.1)	50 (8.5)	0 (0)	*p<0.0001*
251-500 CD4 cells/mm3	218 (17.8)	180 (30.6)	38 (6.0)	
501+ CD4 cells/mm3	957 (78.1)	359 (61.0)	598 (94.0)	
Drinking Frequency Since Last Visit				
No drinks	217 (18.2)	120 (20.9)	97 (15.6)	*p=0.1058*
1-3 drinks/ week	728 (60.8)	339 (59.1)	387 (62.3)	
4-13 drinks/ week	174 (14.6)	77 (13.4)	97 (15.6)	
13+ drinks/ week	78 (6.5)	38 (6.6)	40 (6.4)	
Smoking Status				
Never smoked	345 (28.6)	157 (27.0)	188 (30.0)	*p<0.0001*
Former smoker	629 (52.1)	274 (47.2)	355 (56.6)	
Current smoker	234 (19.4)	150 (25.8)	84 (13.4)	
Ever Used Testosterone				
Yes	188 (15.1)	186 (31.0)	2 (0.3)	*p<0.0001*
No	1061 (85.0)	414 (69.0)	647 (99.7)	
Number of Receptive Anal Intercourse Partnerships in Last 2 Years				
No partners	633 (51.2)	268 (45.0)	367 (56.9)	*p<0.0001*
1-4 partners	277 (22.3)	127 (21.3)	150 (23.3)	
More than 4 partners	329 (26.5)	201 (33.7)	128 (19.8)	

Table 2.2a-b Anal HPV Prevalence at Beginning and at End of Follow-up in 1,259 MSM in the MACS Anal Health Substudy, Incidence and Clearance Rates for Anal HPV Groups and HPV16/18 Infections for All Men and Stratified by HIV Infection Status with Rate Ratios

Table 2.2a: Prevalence at Baseline and End of Follow-up and Incidence Rates with Incidence Rate Ratios (IRRs)

	All Men					HIV-Infected Men			HIV-Uninfected Men			Ratio
HPV TYPE	**Baseline Prevalence (%)**	**End Positivity (%)**	**Incidence**	**Person months**	**Incidence Rate**	**Incidence**	**Person months**	**Incidence Rate**	**Incidence**	**Person months**	**Incidence Rate**	**IRR (95%CI)**
Any Type	1002 (79.6)	991 (78.7)	872	24919.4	34.99	511	12047.1	42.42	361	12872.2	28.04	1.51 (1.32, 1.73)
Group 1	694 (55.1)	680 (54.0)	573	27627.3	20.74	381	13306.6	28.63	192	14320.7	13.41	2.14 (1.80, 2.54)
HPV 16	240 (19.1)	220 (17.5)	108	27204.0	3.97	70	13771.3	5.08	38	13432.7	2.83	1.80 (1.13, 2.49)
HPV 18	107 (8.5)	93 (7.4)	68	31132.1	2.18	47	16102.6	2.92	21	15029.5	1.40	2.09 (1.20, 3.36)
Other Group 1	586 (46.5)	572 (45.4)	514	28093.5	18.30	349	13597.0	25.67	165	14496.4	11.38	2.26 (1.87, 2.71)
Group 2	439 (34.9)	412 (32.7)	402	29200.0	13.77	263	14439.7	18.21	139	14760.3	9.42	1.93 (1.57, 2.38)
Low Risk	758 (60.2)	745 (59.2)	628	26998.6	23.26	399	13242.8	30.13	229	13755.8	16.65	1.81 (1.54, 2.13)

Table 2.2b**:** Clearance Rates with Clearance Rate Ratios (CRRs)

	All Men			HIV-Infected Men			HIV-Uninfected Men			Ratio
HPV TYPE	**Clearance**	**Person months**	**Clearance Rate**	**Clearance**	**Person months**	**Clearance Rate**	**Clearance**	**Person months**	**Clearance Rate**	**CRR (95%CI)**
Any Type	897	16801.5	53.39	523	8793.3	59.48	374	8008.2	46.70	1.27 (1.12, 1.45)
Group 1	603	13221.4	45.61	381	7449.9	51.14	222	5771.5	38.46	1.33 (1.13, 1.57)
HPV 16	129	6594.9	19.56	76	4136.1	18.38	53	2458.9	21.55	0.85 (0.60, 1.21)
HPV 18	81	2570.1	31.52	51	1807.0	28.22	30	763.1	39.31	0.72 (0.46, 1.13)
Other Group 1	516	7906.3	65.26	340	5080.9	66.92	176	2825.4	62.29	1.07 (0.90, 1.29)
Group 2	404	8941.2	45.18	265	5634.0	47.04	139	3307.2	42.03	1.12 (0.91, 1.37)

Low Risk	636	14112.0	45.07	405	7963.5	50.86	231	6148.5	37.57	1.35 (1.15, 1.59)

Table 2.3 Hazards Ratios for Multivariable Models for HIV-infected MSM for Incidence and Clearance of Anal HPV 16 and 18 Infections

Model	Age (per year)	Race (White vs. Non-white)	CD4 Count (≥500 vs <500 cells/mm3)	Smoking (Ever vs Never)	RAI Partners in the Last 2 years (4+ vs 0)	RAI Partners in the Last 2 years (1-3 vs 0)	Lifetime RAI Partners (11+ vs 0	Lifetime RAI Partners (1-10 vs 0)	Anal Warts (Ever vs Never)	Prevalent Other Group 1 (Yes vs No)	Use of Testosterone (Ever vs Never)	Oro-Facial Herpes (Ever vs Never)	Anal Herpes (Ever vs Never)
Incident 16	***0.97 (0.94, 0.99)***	1.02 (0.60, 1.71)	1.12 (0.67, 1.87)	0.94 (0.55, 1.59)	1.27 (0.71, 2.28)	1.28 (0.68, 2.43)	-	-	1.55 (0.83, 2.89)	1.59 (0.93, 2.70)	-	-	-
Incident 18	0.98 (0.94, 1.02)	1.67 (0.80, 3.50)	0.62 (0.34, 1.12)	1.28 (0.65, 2.52)	1.61 (0.83, 3.12)	0.98 (0.41, 2.34)	-	-	-	-	1.77 (0.95, 3.27)	***2.26 (1.07, 4.77)***	-
Clearance 16	***1.04 (1.00, 1.07)***	0.79 (0.45, 1.39)	1.02 (0.65, 1.62)	***0.54 (0.32, 0.90)***	-	-	0.69 (0.31, 1.57)	0.86 (0.35, 2.13)	-	-	-	-	***0.55 (0.31, 0.96)***
Clearance 18	0.99 (0.95, 1.03)	1.17 (0.54, 2.56)	1.08 (0.57, 2.02)	1.04 (0.53, 2.01)	-	-	0.56 (0.18, 1.74)	0.77 (0.24, 2.46)	-	-	-	-	-

Table 2.4: Hazards Ratios for Multivariable Models for HIV-uninfected MSM for Incidence and Clearance of Anal HPV 16 and 18 Infections

Model	Age (per year)	Race (White vs. Non-white)	Smoking (Ever vs Never)	RAI Partners in the Last 2 years (4+ vs 0)	RAI Partners in the Last 2 years (1-3 vs 0)	Lifetime RAI Partners (11+ vs 0)	Lifetime RAI Partners (1-10, vs 0)	Anal Warts (Ever vs Never)	Prevalent HPV16 (Yes vs No)	Prevalent HPV18 (Yes vs No)	Prevalent Other Group 1 (Yes vs No)	Syphilis (Ever vs Never)
Incident 16	1.02 (0.98, 1.06)	1.10 (0.41, 2.97)	0.67 (0.34, 1.33)	***2.45 (1.13, 5.32)***	1.08 (0.39, 3.00)	-	-	***6.14 (1.99, 19.01)***	-	***3.65 (1.49, 8.94)***	-	***3.70 (1.12, 12.19)***
Incident 18	1.02 (0.97, 1.08)	***0.28 (0.11, 0.74)***	2.48 (0.73, 8.50)	1.63 (0.47, 5.65)	2.28 (0.83, 6.29)	-	-	-	1.79 (0.64, 4.97)	-	-	-
Clearance 16	1.01 (0.97, 1.05)	0.65 (0.28, 1.52)	1.16 (0.62, 2.17)	-	-	1.91 (0.42, 8.64)	0.96 (0.20, 4.58)	-	-	-	***0.53 (0.29, 0.98)***	-
Clearance 18	1.01 (0.96, 1.05)	0.68 (0.25, 1.82)	***0.27 (0.10, 0.70)***	-	-	0.75 (0.23, 2.39)	0.39 (0.11, 1.35)	-	-	-	-	-

Supplemental Tables 2.1a-b Anal HPV Positivity at Beginning and at End of Follow-up in 1,259 MSM in the MACS Anal Health Substudy and Incidence and Clearance Rates for 35 Other Anal HPV Type Infections for All Men and Stratified by HIV Infection Status with Rate Ratios

Supplemental Table 2.1a Prevalence at Baseline and End of Follow-up and Incidence Rates with Incidence Rate Ratios (IRRs)

	All Men					**HIV-Infected Men**			**HIV-Uninfected Men**			**Ratio**
HPV TYPE	**Baseline Prevalence (%)**	**End Positivity (%)**	**Incidence**	**Person months**	**Incidence Rate**	**Incidence**	**Person months**	**Incidence Rate**	**Incidence**	**Person months**	**Incidence Rate**	**IRR (95%CI)**
Other Group 1												
HPV 31	101 (8.0)	101 (8.0)	88	30677.1	2.87	64	15903.0	4.02	24	14774.1	1.62	2.48 (1.53, 3.91)
HPV 33	72 (5.8)	76 (6.0)	75	31770.6	2.36	60	16467.8	3.64	15	15302.9	0.98	3.72 (2.08, 6.45)
HPV 35	80 (6.4)	75 (6.0)	74	31300.8	2.36	63	15851.1	3.97	11	15449.7	0.71	5.58 (2.79, 10.06)
HPV 39	89 (7.1)	85 (6.8)	77	31481.6	2.45	54	16478.0	3.28	23	15003.6	1.53	2.14 (1.30, 3.45)
HPV 45	128 (10.2)	98 (7.8)	71	30400.2	2.34	58	15389.6	3.77	13	15010.5	0.87	4.35 (2.24, 7.46)

HPV 51	112 (8.9)	118 (9.4)	102	30747.6	3.32	66	16050.5	4.11	36	14697.2	2.45	1.68 (1.09, 2.47)
HPV 52	294 (23.2)	276 (21.9)	211	26078.3	8.09	144	12409.7	11.60	67	13668.6	4.90	2.37 (1.53, 2.73)
HPV 56	66 (5.2)	64 (5.1)	80	31649.8	2.53	58	16471.6	3.52	22	15178.3	1.45	2.43 (1.48, 3.96)
HPV 58	114 (9.1)	100 (7.9)	80	30806.7	2.60	61	15716.4	3.88	19	15090.3	1.26	3.08 (1.76, 4.94)
HPV 59	100 (7.9)	96 (7.6)	74	30834.5	2.40	61	15677.9	3.89	13	15156.6	0.86	4.54 (2.38, 7.87)
Group 2												
HPV 26	11 (0.9)	7 (0.6)	13	33531.3	0.39	10	17744.8	0.56	3	15786.5	0.19	2.97 (0.81, 10.72)
HPV 53	163 (13.0)	152 (12.1)	131	29257.8	4.48	94	14984.9	6.27	37	14272.9	2.59	2.42 (1.61, 3.44)
HPV 66	87 (6.9)	76 (6.0)	76	31443.8	2.42	53	16159.7	3.28	23	15284.1	1.50	2.18 (1.28, 3.41)
HPV 67	25 (2.0)	26 (2.1)	36	33005.8	1.09	29	17344.3	1.67	7	15661.5	0.45	3.74 (1.62, 8.47)
HPV 68	82 (6.5)	74 (5.9)	67	31341.4	2.14	48	16227.0	2.96	19	15114.5	1.26	2.35 (1.35, 3.92)
HPV 69	22 (1.8)	20 (1.6)	25	33155.5	0.75	18	17503.9	1.03	7	15651.5	0.45	2.30 (0.96, 5.47)
HPV 70	113 (9.0)	113 (9.0)	95	30715.0	3.09	65	15656.7	4.15	30	15058.2	1.99	2.08 (1.29, 3.06)
HPV 73	59 (4.7)	67 (5.3)	69	32226.9	2.14	39	16958.8	2.30	30	15268.1	1.96	1.17 (0.74, 1.93)
HPV 82	28 (2.2)	24 (1.9)	37	33114.6	1.12	24	17508.9	1.37	13	15605.7	0.83	1.65 (0.84, 3.23)
Low Risk												
HPV 6	192 (15.3)	166 (13.1)	82	29178.8	2.81	51	14630.1	3.49	31	14548.7	2.13	1.64 (0.95, 2.32)
HPV 11	61 (4.9)	48 (3.8)	30	32294.5	0.93	20	16989.7	1.18	10	15304.8	0.65	1.80 (0.83, 3.78)
HPV 40	18 (1.4)	14 (1.1)	23	33337.7	0.69	18	17596.1	1.02	5	15741.6	0.32	3.22 (1.19, 8.64)
HPV 42	95 (7.6)	98 (7.8)	91	31187.1	2.92	67	16074.7	4.17	24	15112.4	1.59	2.62 (1.59, 4.04)
HPV 54	107 (8.5)	117 (9.3)	110	30588.8	3.60	81	15596.4	5.19	29	14992.4	1.93	2.68 (1.68, 3.92)
HPV 55	148 (11.8)	144 (11.4)	97	29703.4	3.27	70	15065.8	4.65	27	14637.6	1.84	2.52 (1.51, 3.66)
HPV 61	163 (13.0)	149 (11.8)	98	29646.7	3.31	66	15336.3	4.30	32	14310.4	2.24	1.92 (1.22, 2.85)
HPV 62	167 (13.3)	165 (13.1)	118	29330.5	4.02	85	14929.3	5.69	33	14401.2	2.29	2.48 (1.58, 3.54)
HPV 64	5 (0.4)	4 (0.3)	4	33751.0	0.12	3	17938.1	0.17	1	15812.9	0.06	2.64 (0.27, 25.41)

HPV 71	9 (0.7)	9 (0.7)	9	33524.7	0.27	7	17767.3	0.39	2	15757.3	0.13	3.10 (0.64, 14.89)
HPV 72	85 (6.8)	87 (6.9)	69	31204.1	2.21	41	16133.9	2.54	28	15070.2	1.86	1.37 (0.81, 2.12)
HPV 81	73 (5.8)	72 (5.7)	49	32005.8	1.53	38	16494.7	2.30	11	15511.1	0.71	3.25 (1.59, 6.08)
HPV 83	60 (4.8)	63 (5.0)	52	32296.9	1.61	37	16837.4	2.20	15	15459.4	0.97	2.26 (1.22, 4.04)
HPV 84	129 (10.3)	141 (11.2)	136	29805.2	4.56	94	15210.2	6.18	42	14595.0	2.88	2.15 (1.44, 2.97)
IS39	16 (1.3)	16 (1.3)	17	33434.9	0.51	13	17645.0	0.74	4	15789.9	0.25	2.91 (0.94, 8.86)
CP6108	134 (10.6)	141 (11.2)	128	32409.4	3.95	86	15301.3	5.62	42	14638.2	2.87	1.96 (1.29, 2.70)
Alpha-7	343 (27.2)	302 (24.0)	245	30971.8	7.91	181	15658.5	11.56	64	15313.2	4.18	2.77 (2.08, 3.68)
Alpha-9	503 (40.0)	479 (38.1)	393	29304.4	13.41	267	14448.4	18.48	126	14856.0	8.48	2.18 (1.76, 2.69)

Supplemental Table 2.1b Clearance Rates with Clearance Rate Ratios (CRRs)

	All Men			**HIV-Infected Men**			**HIV-Uninfected Men**			**Ratio**
HPV TYPE	**Clearance**	**Person months**	**Clearance Rate**	**Clearance**	**Person months**	**Clearance Rate**	**Clearance**	**Person months**	**Clearance Rate**	**CRR (95%CI)**
Other Group 1										
HPV 31	89	2853.6	31.19	66	1834.1	35.98	23	1019.4	22.56	1.59 (0.99, 2.56)
HPV 33	73	1971.4	37.03	58	1424.7	40.71	15	546.6	27.44	1.48 (0.84, 2.62)
HPV 35	79	2160.0	36.57	66	1791.4	36.84	13	368.7	35.26	1.04 (0.58, 1.89)
HPV 39	80	2148.2	37.24	53	1323.6	40.04	27	824.6	32.74	1.22 (0.77, 1.94)
HPV 45	98	3206.6	30.56	73	2412.4	30.26	25	794.2	31.48	0.96 (0.61, 1.51)
HPV 51	95	2972.3	31.96	68	1912.5	35.56	27	1059.8	25.48	1.40 (0.89, 2.18)
HPV 52	229	7579.3	30.21	167	5376.0	31.06	62	2203.3	28.14	1.10 (0.82, 1.48)
HPV 56	78	1909.6	40.85	55	1236.9	44.47	23	672.7	34.19	1.30 (0.80, 2.12)
HPV 58	97	2863.6	33.87	77	2073.4	37.14	20	790.1	25.31	1.47 (0.90, 2.40)
HPV 59	86	2790.2	30.82	66	2111.6	31.26	20	678.6	29.47	1.06 (0.64, 1.75)
Group 2										
HPV 26	17	289.1	58.81	12	238.0	50.41	5	51.1	97.93	0.51 (0.18, 1.46)
HPV 53	143	4287.5	33.35	98	2743.6	35.72	45	1543.9	29.15	1.23 (0.86, 1.74)

HPV 66	88	2151.9	40.89	62	1622.5	38.21	26	529.4	49.11	0.78 (0.49, 1.23)
HPV 67	35	751.9	46.55	30	575.9	52.09	5	176.0	28.41	1.83 (0.71, 4.73)
HPV 68	79	2215.9	35.65	55	1547.3	35.54	24	668.5	35.90	0.99 (0.61, 1.60)
HPV 69	26	600.7	43.29	17	443.1	38.37	9	157.5	57.13	0.67 (0.30, 1.51)
HPV 70	97	2791.8	34.75	73	1980.2	36.87	24	811.6	29.57	1.25 (0.79, 1.98)
HPV 73	59	1417.0	41.64	42	875.0	48.00	17	541.9	31.37	1.53 (0.87, 2.69)
HPV 82	38	566.2	67.12	26	356.8	72.86	12	209.3	57.33	1.27 (0.64, 2.52)
Low Risk										
HPV 6	106	4595.1	23.07	70	3233.9	21.65	36	1361.2	26.45	0.82 (0.55, 1.22)
HPV 11	42	1463.8	28.69	31	938.4	33.03	11	525.4	20.94	1.58 (0.79, 3.14)
HPV 40	26	414.5	62.72	18	312.4	57.61	8	102.1	78.34	0.74 (0.32, 1.69)
HPV 42	92	2384.1	38.59	62	1696.4	36.55	30	687.7	43.62	0.84 (0.54, 1.30)
HPV 54	104	3121.1	33.32	78	2261.4	34.49	26	859.8	30.24	1.14 (0.73, 1.78)
HPV 55	106	3894.2	27.22	76	2779.7	27.34	30	1114.5	26.92	1.02 (0.67, 1.55)
HPV 61	110	3909.9	28.13	83	2425.0	34.23	27	1484.9	18.18	1.88 (1.22, 2.91)
HPV 62	116	4394.8	26.39	83	2953.4	28.10	33	1441.3	22.90	1.23 (0.82, 1.84)
HPV 64	5	63.8	78.35	4	39.2	102.07	1	24.6	40.61	2.51 (0.28, 22.49)
HPV 71	9	254.9	35.31	6	174.7	34.34	3	80.2	37.41	0.92 (0.23, 3.67)
HPV 72	71	2530.1	28.06	47	1767.7	26.59	24	762.4	31.48	0.84 (0.52, 1.38)
HPV 81	51	1754.1	29.07	39	1427.7	27.32	12	326.5	36.76	0.74 (0.39, 1.42)
HPV 83	51	1398.8	36.46	36	1052.2	34.21	15	346.6	43.28	0.79 (0.43, 1.44)
HPV 84	121	3703.2	32.67	82	2479.0	33.08	39	1224.2	31.86	1.04 (0.71, 1.52)
IS39	16	328.3	48.73	13	286.7	45.35	3	41.6	72.08	0.63 (0.18, 2.21)
CP6108	124	3662.3	33.86	84	2476.0	33.93	40	1186.3	33.72	1.01 (0.69, 1.47)
Alpha-7	291	233827	7687.5	192	150943	4962.5	99	82884	2725.0	1.06 (0.84, 1.36)
Alpha-9	292	280524	9222.7	199	170297	5598.8	93	110227	3623.9	1.39 (1.08, 1.77)

2.6 References

1. Satterwhite CL, Torrone E, Meites E, Dunne EF, Mahajan R, Ocfemia MC, et al. Sexually transmitted infections among US women and men: prevalence and incidence estimates, 2008. Sex Transm Dis. 2013;40(3):187-93. doi: 10.1097/OLQ.0b013e318286bb53. PubMed PMID: 23403598.

2. International Agency for Research on Cancer (IARC) Working Group on Evaluation of Carcinogenic Risks to Humans. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Volume 90 Human Papillomaviruses. Lyon, France: World Health Organization, International Agency for Research on Cancer; 2007. 689 p.

3. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. A review of human carcinogens--Part B: biological agents. Lancet Oncology. 2009;10(4):321-2. Epub 2009/04/08. PubMed PMID: 19350698.

4. Abramowitz L, Jacquard AC, Jaroud F, Haesebaert J, Siproudhis L, Pradat P, et al. Human papillomavirus genotype distribution in anal cancer in France: the EDiTH V study. International Journal of Cancer. 2011;129(2):433-9. Epub 2010/09/15. doi: 10.1002/ijc.25671. PubMed PMID: 20839262.

5. Prevention CfDCa. HPV and Cancer 2017 [updated March 3, 2017; cited 2017 June 15]. Available from: https://www.cdc.gov/cancer/hpv/statistics/cases.htm.

6. Valmary-Degano S, Jacquin E, Prétet JL, Monnien F, Girardo B, Arbez-Gindre F, et al. Signature patterns of human papillomavirus type 16 in invasive anal carcinoma. Human Pathology. 2013;44(6):11. Epub 2012 Dec 23. doi: 10.1016/j.humpath.2012.08.019.

7. (IARC) IAfRoC. GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012 2012 [cited 2015 07/13]. Available from: http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx.

8. SEER S, Epidemiology and End Results Program,. SEER Cancer Statistics Factsheets: Anal Cancer Bethesda, MD: National Cancer Institute; [cited 2015 July 29]. Available from: http://seer.cancer.gov/statfacts/html/anus.html.

9. Daling JR, Weiss NS, Hislop TG, Maden C, Coates RJ, Sherman KJ, et al. Sexual practices, sexually transmitted diseases, and the incidence of anal cancer. New England Journal of Medicine. 1987;317(16):973-7.

10. D'Souza G, Wiley DJ, Li X, Chmiel JS, Margolick JB, Cranston RD, et al. Incidence and epidemiology of anal cancer in the multicenter AIDS cohort study. J Acquir Immune Defic Syndr. 2008;48(4):491-9. doi: 10.1097/QAI.0b013e31817aebfe. PubMed PMID: 18614927; PubMed Central PMCID: PMC3991563.

11. Machalek DA, Poynten M, Jin F, Fairley CK, Farnsworth A, Garland SM, et al. Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: a systematic review and meta-analysis. Lancet Oncol. 2012;13(5):487-500. doi: 10.1016/S1470-2045(12)70080-3. PubMed PMID: 22445259.

12. SEER S, Epidemiology and End Results Program. SEER Cancer Statistics Factsheets: Prostate Cancer Bethesda, MD: National Cancer Institute; [cited 2015 July 13]. Available from: https://seer.cancer.gov/statfacts/html/prost.html.

13. Benard VB, Thomas CC, King J, Massetti GM, Doria-Rose VP, Saraiya M, et al. Vital signs: cervical cancer incidence, mortality, and screening - United States, 2007-2012. MMWR Morb Mortal Wkly Rep. 2014;63(44):1004-9. PubMed PMID: 25375072.

14. Li Z, Zhang H, Li X, Yang Y, Xin H, Li M, et al. Anal Human Papillomavirus Genotyping among HIV-Positive Men Who Have Sex with Men in Xi'an, China. PLoS One. 2015;10(4):e0125120. doi: 10.1371/journal.pone.0125120. PubMed PMID: 25923768; PubMed Central PMCID: PMC4414525.

15. Vajdic CM, van Leeuwen MT, Jin F, Prestage G, Medley G, Hillman RJ, et al. Anal human papillomavirus genotype diversity and co-infection in a community-based sample of homosexual men. Sex Transm Infect. 2009;85(5):330-5. Epub 2009/04/04. doi: sti.2008.034744 [pii] 10.1136/sti.2008.034744. PubMed PMID: 19342375.

16. Nyitray AG, Carvalho da Silva RJ, Chang M, Baggio ML, Ingles DJ, Abrahamsen M, et al. Incidence, Duration, Persistence, and Factors Associated With High-risk Anal Human Papillomavirus Persistence Among HIV-negative Men Who Have Sex With Men: A Multinational Study. Clin Infect Dis. 2016;62(11):1367-74. doi: 10.1093/cid/ciw140. PubMed PMID: 26962079; PubMed Central PMCID: PMC4872291.

17. Supindham T, Chariyalertsak S, Utaipat U, Miura T, Ruanpeng D, Chotirosniramit N, et al. High Prevalence and Genotype Diversity of Anal HPV Infection among MSM in Northern Thailand. PLoS One. 2015;10(5):e0124499. doi: 10.1371/journal.pone.0124499. PubMed PMID: 25932915; PubMed Central PMCID: PMC4416722.

18. Palefsky JM, Holly EA, Ralston ML, Jay N. Prevalence and risk factors for human papillomavirus infection of the anal canal in human immunodeficiency virus (HIV)-positive and HIV-negative homosexual men. Journal of Infectious Diseases. 1998;177(2):361-7.

19. Blas MM, Brown B, Menacho L, Alva IE, Silva-Santisteban A, Carcamo C. HPV Prevalence in Multiple Anatomical Sites among Men Who Have Sex with Men in Peru. PLoS One. 2015;10(10):e0139524. doi: 10.1371/journal.pone.0139524. PubMed PMID: 26437318; PubMed Central PMCID: PMC4593601.

20. Wiley DJ, Li X, Hsu H, Seaberg EC, Cranston RD, Young S, et al. Factors affecting the prevalence of strongly and weakly carcinogenic and lower-risk human papillomaviruses in anal specimens in a cohort of men who have sex with men (MSM). PLoS One. 2013;8(11):e79492. doi: 10.1371/journal.pone.0079492. PubMed PMID: 24278140; PubMed Central PMCID: PMC3835810.

21. Bertisch B, Franceschi S, Lise M, Vernazza P, Keiser O, Schoni-Affolter F, et al. Risk Factors for Anal Cancer in Persons Infected With HIV: A Nested Case-Control Study in the Swiss HIV Cohort Study. Am J Epidemiol. 2013. Epub 2013/08/01. doi: kwt153 [pii] 10.1093/aje/kwt153. PubMed PMID: 23900553.

22. Gage JC, Katki HA, Schiffman M, Fetterman B, Poitras NE, Lorey T, et al. Age-stratified 5-year risks of cervical precancer among women with enrollment and newly detected HPV

infection. Int J Cancer. 2015;136(7):1665-71. doi: 10.1002/ijc.29143. PubMed PMID: 25136967; PubMed Central PMCID: PMC4314342.

23. Demers AA, Shearer B, Severini A, Lotocki R, Kliewer EV, Stopera S, et al. Distribution of human papillomavirus types, cervical cancer screening history, and risk factors for infection in Manitoba. Chronic Dis Inj Can. 2012;32(4):177-85. PubMed PMID: 23046799.

24. Madeleine MM, Finch JL, Lynch CF, Goodman MT, Engels EA. HPV-related cancers after solid organ transplantation in the United States. Am J Transplant. 2013;13(12):3202-9. doi: 10.1111/ajt.12472. PubMed PMID: 24119294; PubMed Central PMCID: PMC4049182.

25. Van Aar F, Mooij SH, Van Der Sande MA, Speksnijder AG, Stolte IG, Meijer CJ, et al. Anal and penile high-risk human papillomavirus prevalence in HIV-negative and HIV-infected MSM. AIDS. 2013. doi: 10.1097/01.aids.0000432541.67409.3c. PubMed PMID: 23921617.

26. Mooij SH, van Santen DK, Geskus RB, van der Sande MA, Coutinho RA, Stolte IG, et al. The effect of HIV infection on anal and penile human papillomavirus incidence and clearance: a cohort study among MSM. AIDS. 2016;30(1):121-32. doi: 10.1097/QAD.0000000000000909. PubMed PMID: 26474302.

27. Grulich AE, Poynten IM, Machalek DA, Jin F, Templeton DJ, Hillman RJ. The epidemiology of anal cancer. Sex Health. 2012;9(6):504-8. doi: 10.1071/SH12070. PubMed PMID: 22958581.

28. Schwartz LM, Castle PE, Follansbee S, Borgonovo S, Fetterman B, Tokugawa D, et al. Risk Factors for Anal HPV Infection and Anal Precancer in HIV-Infected Men Who Have Sex With Men. J Infect Dis. 2013. Epub 2013 Sep 4. doi: 10.1093/infdis/jit374.

29. Couture MC, Page K, Stein ES, Sansothy N, Sichan K, Kaldor J, et al. Cervical human papillomavirus infection among young women engaged in sex work in Phnom Penh, Cambodia: prevalence, genotypes, risk factors and association with HIV infection. BMC Infect Dis. 2012;12:166. doi: 10.1186/1471-2334-12-166. PubMed PMID: 22839728; PubMed Central PMCID: PMC3436768.

30. Bahmanyar RE, Paavonen J, Naud P, Salmeron J, Chow SN, Apter D, et al. Prevalence and risk factors for cervical HPV infection and abnormalities in young adult women at enrolment in the multinational PATRICIA trial. Gynecol Oncol. 2012;127(3):11. doi: doi:10.1016/j.ygyno.2012.08.033.

31. Geskus RB, Gonzalez C, Torres M, Del Romero J, Viciana P, Masia M, et al. Incidence and clearance of anal high-risk human papillomavirus in HIV-positive men who have sex with men: estimates and risk factors. AIDS. 2016;30(1):37-44. doi: 10.1097/QAD.0000000000000874. PubMed PMID: 26355673; PubMed Central PMCID: PMC4674141.

32. Hsu HK, Brown TT, Li X, Young S, Cranston RD, D'Souza G, et al. Association between Free Testosterone Levels and Anal Human Papillomavirus Types 16/18 Infections in a Cohort of Men Who Have Sex with Men. PLoS One. 2015;10(3):e0119447. doi: 10.1371/journal.pone.0119447. PubMed PMID: 25794147.

33. Kaslow RA, Ostrow DG, Detels R, Phair JP, Polk BF, Rinaldo CR, Jr. The Multicenter AIDS Cohort Study: rationale, organization, and selected characteristics of the participants. American Journal of Epidemiology. 1987;126(2):310-8.

34. Detels R, Jacobson L, Margolick J, Martinez-Maza O, Munoz A, Phair J, et al. The Multicenter AIDS Cohort Study, 1983 to ...,. Public Health. 2012;126(3):196-8. doi: 10.1016/j.puhe.2011.11.013. PubMed PMID: 22206985; PubMed Central PMCID: PMC3324261.

35. Wada NI, Jacobson LP, Margolick JB, Breen EC, Macatangay B, Penugonda S, et al. The effect of HAART-induced HIV suppression on circulating markers of inflammation and immune activation. AIDS. 2015;29(4):463-71. doi: 10.1097/QAD.0000000000000545. PubMed PMID: 25630041; PubMed Central PMCID: PMC4311407.

36. Garzino-Demo A, Moss RB, Margolick JB, Cleghorn F, Sill A, Blattner WA, et al. Spontaneous and antigen-induced production of HIV-inhibitory beta-chemokines are associated with AIDS-free status. Proc Natl Acad Sci U S A. 1999;96(21):11986-91. PubMed PMID: 10518563; PubMed Central PMCID: PMC18399.

37. Falade-Nwulia O, Seaberg EC, Rinaldo CR, Badri S, Witt M, Thio CL. Comparative risk of liver-related mortality from chronic hepatitis B versus chronic hepatitis C virus infection. Clin Infect Dis. 2012;55(4):507-13. doi: 10.1093/cid/cis432. PubMed PMID: 22523269; PubMed Central PMCID: PMC3520384.

38. Seaberg EC, Witt MD, Jacobson LP, Detels R, Rinaldo CR, Young S, et al. Differences in hepatitis C virus prevalence and clearance by mode of acquisition among men who have sex with men. J Viral Hepat. 2014;21(10):696-705. doi: 10.1111/jvh.12198. PubMed PMID: 25280229; PubMed Central PMCID: PMC4187219.

39. Viswanathan S, Detels R, Mehta SH, Macatangay BJ, Kirk GD, Jacobson LP. Level of adherence and HIV RNA suppression in the current era of highly active antiretroviral therapy (HAART). AIDS Behav. 2015;19(4):601-11. doi: 10.1007/s10461-014-0927-4. PubMed PMID: 25342151; PubMed Central PMCID: PMC4393774.

40. Darwich L, Canadas MP, Videla S, Coll J, Molina-Lopez RA, Sirera G, et al. Prevalence, clearance, and incidence of human papillomavirus type-specific infection at the anal and penile site of HIV-infected men. Sex Transm Dis. 2013;40(8):611-8. doi: 10.1097/01.OLQ.0000430798.61475.08. PubMed PMID: 23859907.

41. Nyitray AG, Carvalho da Silva RJ, Baggio ML, Lu B, Smith D, Abrahamsen M, et al. Age-specific prevalence of and risk factors for anal human papillomavirus (HPV) among men who have sex with women and men who have sex with men: the HPV in men (HIM) study. J Infect Dis. 2011;203(1):49-57. Epub 2010/12/15. doi: 10.1093/infdis/jiq021. PubMed PMID: 21148496; PubMed Central PMCID: PMC3086435.

42. Phanuphak N, Teeratakulpisarn N, Pankam T, Kerr SJ, Barisri J, Deesua A, et al. Anal human papillomavirus infection among Thai men who have sex with men with and without HIV infection: prevalence, incidence, and persistence. J Acquir Immune Defic Syndr. 2013;63(4):472-9. doi: 10.1097/QAI.0b013e3182918a5a. PubMed PMID: 23514956; PubMed Central PMCID: PMC3700660.

43. Zhang DY, Yin YP, Feng TJ, Hong FC, Jiang N, Wang BX, et al. HPV infections among MSM in Shenzhen, China. PLoS One. 2014;9(5):e96364. doi: 10.1371/journal.pone.0096364. PubMed PMID: 24801331; PubMed Central PMCID: PMC4011743.

44. Goodman MT, Shvetsov YB, McDuffie K, Wilkens LR, Zhu X, Ning L, et al. Acquisition of anal human papillomavirus (HPV) infection in women: the Hawaii HPV Cohort study. J Infect Dis. 2008;197(7):957-66. PubMed PMID: 18429348.

Chapter 3. Comparison of Anal Cytology and PCR Anal HPV Testing Strategies to Predict High-grade Anal Precancers among Older MSM with Persistent Anal HPV Infections

3.1 Abstract

Background: Men who have sex with men (MSM) compose the group at highest risk for anal cancer. High-grade squamous intraepithelial lesions, identified by anal biopsy and histology (hHSILs), are precursors for anal cancer caused by persistent infection with high-risk human papillomaviruses (hrHPVs). There are 12 strongly carcinogenic hrHPVs (Group 1); these include HPV16 and 18, which account for up to 90% of anal cancers. There is no current standard protocol for anal cancer screening, although experts recommend repeated screening using anal cytology. Cytology shows poor sensitivity to detect hHSIL, and HPV testing may be a viable screening alternative. The objective of this study is to compare the efficacy of anal cytology with hrHPV testing to predict hHSIL in older MSM.

Methods: Participants are 183 U.S. MSM from the Anal Health Substudy (AHS), a nested cohort substudy of the Multicenter AIDS Cohort Study, who were followed for multiple visits. A single Dacron swab was collected from the anal canal, and tested for cytology and 37 HPV types (Linear Array HPV DNA PCR assay). Following the AHS, men were assessed using the gold-standard: high-resolution anoscopy (HRA) with anal biopsy. Biopsies were classified as hHSIL or <hHSIL. Multivariable logistic regression models estimated odds ratios (ORs) and areas under the receiver-operating curve (AUCs) were calculated for five screening test strategies to predict hHSIL. Strategies compared were: having any abnormal cytology (abCyt) versus normal, persistent positivity (positive at two or more consecutive visits) for HPV types 16/18 (pHPV16/18+), and persistent positivity for Group 1 hrHPVs (pGroup 1+); two combined serial strategies were further assessed: abCyt & pHPV16/18+, and abCyt & pGroup 1+. Each model controlled for age, center, race, HIV, number of male receptive anal intercourse partners in the last two years, and smoking.

Results: Men were, on average, 59 (±8) years old; 58% (107/183) were HIV-infected, and 87% (159/183) were White, non-Hispanic. 73% (134/193) had abCyt and 54% (98/183) had hHSIL. Persistently detectable HPVs were common, with 44% (80/183) testing pHPV16/18+ and 87% (159/183) testing pGroup 1+. On average, HIV-infected men were younger, more likely to have a lower CD4 count (<500 CD4 cells/mm^3), have abCyt, and test pHPV16/18+ (*p*-values<0.05). In multivariable models, abCyt alone had 2.2-fold higher odds of hHSIL (95% CI 1.1, 4.6), pHPV16/18+ alone had 3.6-fold higher odds of hHSIL (1.8, 7.2), and pGroup 1+ alone was not associated with hHSIL (OR: 1.5 (0.6, 3.7)). When abCyt and pHPV16/18+ were combined in a model, positivity on both tests was associated with 3.6-fold higher odds of hHSIL (1.7, 7.3). When abCyt and pGroup 1+ were combined, positivity on both tests was associated with 2.3-fold higher odds of hHSIL (1.2, 4.6). Diagnostic accuracy of individual test strategies: abCyt (AUC: 0.65), pHPV16/18+ (0.70), and pGroup 1+ (0.63), and combined strategies (AUCs: 0.68 & 0.66) were compared; no single strategy was significantly more accurate than another (*p*-values>0.05).

Discussion: Findings suggest testing for persistent HPV16/18-positivity, even using a low-threshold PCR assay, may be an effective strategy for detecting hHSIL in older HIV-infected and -uninfected MSM. The high hHSIL prevalence in this sample (54%) emphasizes an urgent need for developing a standard approach to screening.

3.2 Introduction

Human papillomaviruses (HPVs) are very common sexually transmitted infections that infect epithelium through microabrasions [1]. Most sexually active individuals will contract at least one HPV infection in their lifetime; however, as many as 90% of infections can clear within two years [2-7]. Forty-one HPV genotypes can infect male and female genitalia: twelve types are classified as Group 1 high-risk (hr) strong carcinogens (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59), thirteen hrHPV types as Group 2 weak carcinogens (26, 30, 34, 53, 66, 67, 68, 69, 70, 73, and 82, 85, and 97), and sixteen as low-risk (lr) HPV types, which are not carcinogenic (6, 11, 40, 42, 54, 55, 61, 62, 64, 71, 72, 81, 83, 84, Is39, and CP6108) [8, 9]. Persistent, long-term infections with the Group 1 hrHPVs are estimated to cause 31,500 new HPV-related cancers annually in the United States [10]. HPV types 16 and 18 alone are estimated to cause 78 to 90% of anal cancer cases [10-12]. Anal cancer incidence rates in the general population are low at 1.8 cases per 100,000 persons, however, among gay, bisexual, and other men who have sex with men (MSM), rates are significantly higher, especially among those with HIV infection where estimates may reach as high as 137 cases/100,000 [13-17]. Despite these disproportionately high rates, there is no standard of care for anal cancer screening [18].

Screening as a secondary prevention for cervical cancer has been well-established since the 1960s, beginning with the Pap cytology test where a brush or swab is used to collect cervical epithelial cells into preservative and tested to detect abnormal cellular changes or lesions resulting from persistent HPV infections [16, 19-21]. Lesions vary in severity, the most severe being high-grade squamous intraepithelial lesions (HSILs) which are considered precancers that can further progress into cancer [22]. Pap testing is often recommended for primary anal cancer screening for at-risk populations where clinical facilities and experts are available to provide services and follow-up [23]. However, for either organ, cytology can have low sensitivity and moderate specificity requiring repeat testing or co-testing for HPVs to detect disease [24, 25].

HPV tests were developed more recently and some are clinically approved for use in some primary cervical cancer screening protocols [26, 27]. As an alternative screening tool to cytology, some experts advocate for screening using HPV molecular testing alone to identify persistent hrHPV infections, while current guidelines offer HPV co-testing with cytology at wider time intervals [28, 29]. HPV tests have higher sensitivity but lower specificity than cytology due to high HPV prevalence implying applications for anal cancer screening need development [29-32]. Generally, abnormal anal cytology results and/or positivity for hrHPVs are recommended to be followed up with a diagnostic examination called high-resolution anoscopy (HRA) to confirm disease. Anal tissue is examined and biopsies of possible lesions are collected to confirm disease suggested by screening. Certified pathologists evaluate anal biopsy specimens similar to cytology, but with increased granularity for HSIL results. Some recommend utilizing HRA as the primary form of screening since cytology often misses disease and HPV testing over diagnoses disease, however, the cost of HRA and lack of trained clinicians limits feasibility [33]. Anal histological-confirmed HSILs (hHSILs) are treatable with ablative and topical methods and treatment is thought to prevent progression to anal cancer as has been confirmed for cervical hHSIL and cervical cancer [29]. The goal of this study is to compare anal cytology versus anal HPV testing to predict anal hHSILs to inform screening strategies for high-risk individuals.

3.3 Methods

Participants

Participants were recruited from the Multicenter AIDS Cohort Study (MACS), the longest running U.S. longitudinal study tracking the natural history of HIV/AIDS in MSM [34, 35]. Since 1984, enrollment has continued over three periods (1984-87, 1987-91, and 2001-03) in four major U.S. cities disproportionately affected by the HIV epidemic: Baltimore, Chicago, Los Angeles, and Pittsburgh, with renewed enrollment ongoing since 2010. Over the duration of the MACS, 7,352

HIV-infected and -uninfected MSM have been enrolled and provided written consent for participation, with 2,216 participants active in 2010-2011.

From 2010-2014, 1,512 MACS men were seen in a substudy called the Anal Health Substudy (AHS), where an anal swab was collected at regular 6-month MACS visits and tested for cytology and HPVs. The study is described in more detail elsewhere and additional details are provided below [36]. These analyses are limited to 183 men followed in the AHS for at least two visits (≥4 months apart) in whom persistent hrHPV infection was detected and at least one interpretable cytology result was available. These men also had an HRA examination with biopsy subsequent to AHS visits.

Procedures

AHS subjects contributed up to eight study visits at which an anal Pap test was performed. Briefly, the protocol planned for cytology and HPV specimen collection annually for HIV-infected subjects, and every two years for HIV-uninfected subjects. For cytology specimens, a single Dacron swab was inserted ~2.5 inches beyond the anal verge, approximated to the wall, and rotated slowly against the anal wall while being extracted and immediately placed into ThinPrep Preservcyt® solution (Cytyc Corporation, Boxborough, MA). Specimens were centrifuged (~10,000 rpm) for 15 minutes, pelleted, resuspended, centrifuged in phosphate-buffered saline, dried and evaluated by trained cytopathologists at a single laboratory (Tricore Reference Laboratories, Albuquerque, NM). Anal specimens were classified as negative for intraepithelial lesion (NIL), atypical squamous cells of unknown significance (ASC-US), atypical squamous cells – cannot exclude high-grade squamous intraepithelial lesion (ASC-H), low-grade squamous intraepithelial lesion (LSIL), and high-grade squamous intraepithelial lesion (HSIL) [37, 38]. For these analyses, specimens were categorized as abnormal (ASC-US or worse) versus normal (NIL). Residual cytology specimens (~75µg) were used to extract DNA for anal HPV genotyping. Cell pellets were resuspended in 0.3mL of 20 mM Tris buffer (pH: 8.3) and a MasterPure Purification Kit (Epicentre, Madison, WI) was used to purify the DNA to perform anal HPV

genotyping with PCR and line-blot hybridization (Linear Array® HPV Genotyping Test, Roche Molecular Diagnostics, Pleasanton, CA). Linear Array uses the PGMY09-PGMY11 primer set, to amplify a 450 base-pair fragment of the L1 gene, to detect 37 HPV types: Group 1 hrHPVs -16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59; Group 2 hrHPVs - 26, 53, 66, 67, 68, 69, 70, 73, and 82; and lrHPVs - 6, 11, 40, 42, 54, 55, 61, 62, 64, 71, 72, 81, 83, 84, Is39, and CP6108 [8, 9]. Persistent HPV infections were defined as two or more consecutive positive visits. These specimens form three main exposures of interest: 1) abnormal cytology (abCyt), 2) persistent HPV16 and/or 18 infection (pHPV16/18+), and 3) Group 1 hrHPV infection (pGroup 1+).

Sociodemographic, sexual-behavioral covariate data were collected using a standardized interviewer-guided questionnaire from the subject's baseline MACS visit, and in six-month intervals thereafter at scheduled MACS visits [35]. Covariates collected and assessed in this analysis include age, race, HIV infection status, number of male receptive anal intercourse partners in the last two years, and ever smoked tobacco. Covariates were each summarized longitudinally from baseline data up until the visit where outcome was measured.

The primary outcome of interest was hHSIL identified from anal biopsies collected during HRA. HRA was performed using bright light and magnification to examine anal tissue around the transformation zone for dysplasia. Tissue was stained with acetic acid and Lugol's iodine to contrast possibly affected areas suspicious for lesion. Biopsies were collected using endoscopic forceps from acetowhite lesions or Lugol's negative lesions which showed punctation, friability, or high vascularity. Biopsy specimens were collected individually into 20mL screw-top cups containing 10% neutral buffered formalin solution. Board certified pathologists (Tricore Reference Laboratories, Albuquerque, NM) processed and evaluated biopsy specimens using LAST terminology: NIL, LSIL, and HSIL which was further specified as anal intraepithelial neoplasia (AIN) 2 (moderate dysplasia), AIN 2/3 (moderate-to-severe dysplasia), AIN 3 (severe dysplasia)], CIS, and invasive anal cancer (IAC). For these analyses, any HSIL grade is classified as hHSIL.

Analysis

Men were censored at the outcome visit (HRA visit) to exclude subsequent cytology and HPV test visits. In the event a subject had multiple HRAs while enrolled in the AHS, data from a later HRA proximal to the AHS visit was used where sufficient time accrued to define hrHPV persistence. Subjects with at least one abCyt (≥ASCUS) were classified as abCyt. Persistent hrHPVs were grouped as pHPV16/18+ versus not and pGroup 1+ versus not. Indeterminate HPV results and uninterpretable (unsatisfactory) cytology were excluded.

Descriptive analyses were performed to characterize the study sample. For each characteristic, differences between HIV-infected and –uninfected men were evaluated. Fisher's exact test, chi-square, and Wilcoxon Rank Sum tests were used to evaluate differences for categorical and continuous variables, respectively. Each of the three testing strategies were plotted on Receiver Operating Curves (ROCs), and Areas under the curve (AUCs) were estimated. The AUCs were assessed to determine the overall diagnostic ability of each test and were compared to one another using a non-parametric approach; pHPV16/18+ vs. ab (PROC LOGISTIC; ROCCONTRAST). Additionally, test performance characteristics (sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV)) of each test strategy were calculated to provide more detail.

Three final multivariable logistic regression models assessed each screening strategy individually for their predictive ability for the outcome, hHSIL: pHPV16/18+, pGroup 1+, and abCyt. To assess whether using two tests together had a significant improvement over any single test, a second analysis was performed. Two multivariable logistic regression models assessed serial positivity (positive on both) of pHPV16/18+ and abCyt combined, and pGroup 1+ and abCyt combined, with hHSIL as the outcome. To build the models, unadjusted logistic regression models were used to assess independent associations between main exposures, covariates, and the outcome. Potential confounding relationships and effect measure modification within covariates were assessed and inferred from published literature. Pearson correlation coefficients were

calculated to quantify correlations between continuous variables and pseudo R^2 for categorical variables to exclude highly correlated variables in the model. Final multivariable models included age, center, race, HIV infection status, number of male receptive anal intercourse partners in the last two years, and ever tobacco smoking. Analyses were all performed using SAS V9.4 (Cary, NC).

3.4 Results

At the time of the HRA visit, men were 59 years old on average; 87% (159/183) were White, non-Hispanic and 58% (107/183) were HIV-infected. Ninety-eight men had hHSIL (54%) demonstrating a high burden of anal disease in this group. Men on average had 2.4 ($\pm$0.9) cytology results, 3.2 ($\pm$1) HPV test results and 1.9 ($\pm$1.4) HRAs. The swabs showed 73% had abCyt (134/183), 44% were pHPV16/18+ (80/183), and 87% were pGroup 1+ (159/183). HIV-infected men were younger on average, more likely to have a lower CD4 count (<500 CD4 cells/mm^3), have abCyt, and to test pHPV16/18+ (*p*-values<0.05, Table 3.1).

Diagnostic Ability and Test Performance Characteristics

Of the five single and combined test strategies, only pHPV16/18+ yielded moderate estimates for all four test characteristics, in both unadjusted and adjusted models (Table 3.2a&b). In unadjusted models, comparing AUCs suggested pHPV16/18+ had greater diagnostic ability (AUC=0.66) than pGroup 1+ (0.53, $p<0.05$), but not when compared to having abCyt (0.59, $p=0.1$) (Table 3.2a). In fully adjusted models, the AUC for pHPV16/18+ (0.70) remained statistically significantly higher than pGroup 1+ (0.63, $p<0.05$) (Figure 3.1). The AUC was still not higher than abCyt (0.65, $p=0.2$) alone (Fig. 3.1), or either of the two combined strategies (0.68, $p=0.3$ & 0.66, $p=0.3$, Table 3.2b). Overall, these results did not definitively suggest that any screening strategy was the most effective strategy for diagnosing hHSIL in this small group of older MSM.

Multivariable Regression Results

In the multivariable models, four of the five testing strategies yielded an association with hHSIL. abCyt was associated with 2.2-fold higher odds of hHSIL (95% CI: 1.1, 4.6) when compared to men with no abCyt findings (Table 3.2b). pHPV16/18+ was associated with 3.6-fold higher odds of hHSIL (1.8, 7.2), compared to men who did not have pHPV16/18+. pGroup 1+ was not associated with odds of hHSIL (1.5 (0.6, 3.7)) compared to men who did not have pGroup 1+. When abCyt and pHPV16/18+ were combined in the same model, positivity for both was associated with 3.6-fold higher odds of hHSIL (1.7, 7.3) when compared to men who were positive on only one or neither test. When abCyt and pGroup 1+ were combined in the same model, positivity for both was associated with 2.3-fold higher odds of hHSIL (1.2, 4.6) when compared to men positive on one or neither test.

3.5 Discussion

Anal hHSIL was common, affecting 54% of this sample of middle-aged MSM. The proportion affected was high regardless of HIV-infection status, highlighting the high proportion of anal disease in older MSM especially when compared to a 2% estimate observed in HIV-uninfected women [39]. Currently, there is no standard procedure for screening for anal hHSIL, and screening is not widely available. As a result, many older MSM are never screened for anal cancer, despite being at much higher risk for developing it compared to the general population [13-17]. Therefore, identifying an effective screening strategy for detecting anal hHSIL, by testing for abCyt, pHPV16/18+, and pGroup 1+, is critical.

Testing for abCyt, the recommended screening strategy for anal cancer, was predictive for hHSIL as expected. However, testing for pHPV16/18+ yielded even higher odds of hHSIL. These results are not surprising considering pHPV16/18+ is found in up to 87% of anal cancer cases and has been well-established as a strong risk factor for hHSIL [10, 12]. pHPV16/18+ also yielded the largest AUC estimate, suggesting better diagnostic ability than pGroup1 1+, but was

inconclusive for other strategies. Interestingly, pGroup 1+ was not predictive for hHSIL which could be explained by the high prevalence of these infections. Lastly, serial testing for combined positivity for pHPV16/18+ and abCyt showed no difference from testing for pHPV16/18+ alone. This suggests screening for pHPV16/18+ alone could maintain standard clinical performance, while minimizing resources over paired testing. Overall, this study provides evidence to suggest testing for pHPV16/18+ alone may be an effective screening strategy for hHSIL in older MSM.

Utilizing hrHPV testing for anal precancer screening is not a novel concept. Current guidelines for cervical cancer screening employ hrHPV and cytology co-testing which allows for less frequent testing [29, 42]. Researchers have shown hrHPV testing alone to be effective for primary cervical screening, and performed better than cytology in multiple comparison trials [28, 41, 43]. Furthermore, testing for only HPV16/18 has been assessed, and it performed well in several study samples [44-47]. Another advantage hrHPV testing has over cytology is the lower probability of producing an uninterpretable specimen, which increases reliability and efficiency by avoiding unnecessary clinical visits [40, 41]. Clinically, the value of high sensitivity that HPV testing provides for hHSIL over cytology is important for older MSM to accurately detect hHSIL and potentially avoid cancer progression by treating for hHSIL. Persistent anal HPV16/18 testing could provide optimal clinical performance while reducing burden on medical institutions and patients alike.

These analyses may be limited. The sample size may not be sufficiently powered to assess these screening strategies. Self-report data are subject to misclassification bias, however if present, it is likely biased towards the null as it would be non-differential with respect to the exposure and outcome. At the time of covariate data collection, participants were unaware of the test strategies used for the exposure and HRA results for the outcome were not yet available. Though necessary, limiting the sample to hrHPV-persistent individuals may have underpowered the analysis, in addition to attenuate the effects of HPV testing when excluding all non-hrHPV persistent infections from the control groups. The research PCR-based HPV assay used is very

sensitive and detects viruses at low levels, while also yielding high agreement with clinically-approved high-threshold HPV tests [48]. However, this would suggest estimates are biased towards the null as high-threshold HPV tests would use a higher cut-off and yield a stronger association between pHPV16/18+ and hHSIL. The results from this study may not be generalizable to other MSM. This group was followed for anal disease for a few years as part of the Multicenter AIDS Cohort Study, most of the men had a clinical indication for HRA with biopsy from abCyt or positive hrHPV results. However, though this group was better screened than most, the proportion of hHSIL is not likely a dramatic overrepresentation of disease in other older MSM as other studies have reported similar prevalence.

In the absence of a standard of care for screening, older MSM are at significant risk for developing anal cancer [13-17]. Several options for screening exist that are informed by cervical cancer screening [16, 19-21]. The findings from this study suggest repeat testing for persistent HPV16/18+, even with a low-threshold research assay, may be more effective to screen for anal hHSIL than cytology, the most commonly recommended test for screening. Screening for the highest risk HPVs may be considered as the primary screening strategy for older MSM where anal hHSIL is common, though more research is needed to confirm these findings.

Figure 3.1 Comparisons of AUCs for High Grade Anal Dysplasia Screening Strategies: Persistent HPV16/18+ vs. Abnormal Cytology and Persistent HPV16/18+ vs Persistent Group 1+ for 183 Men

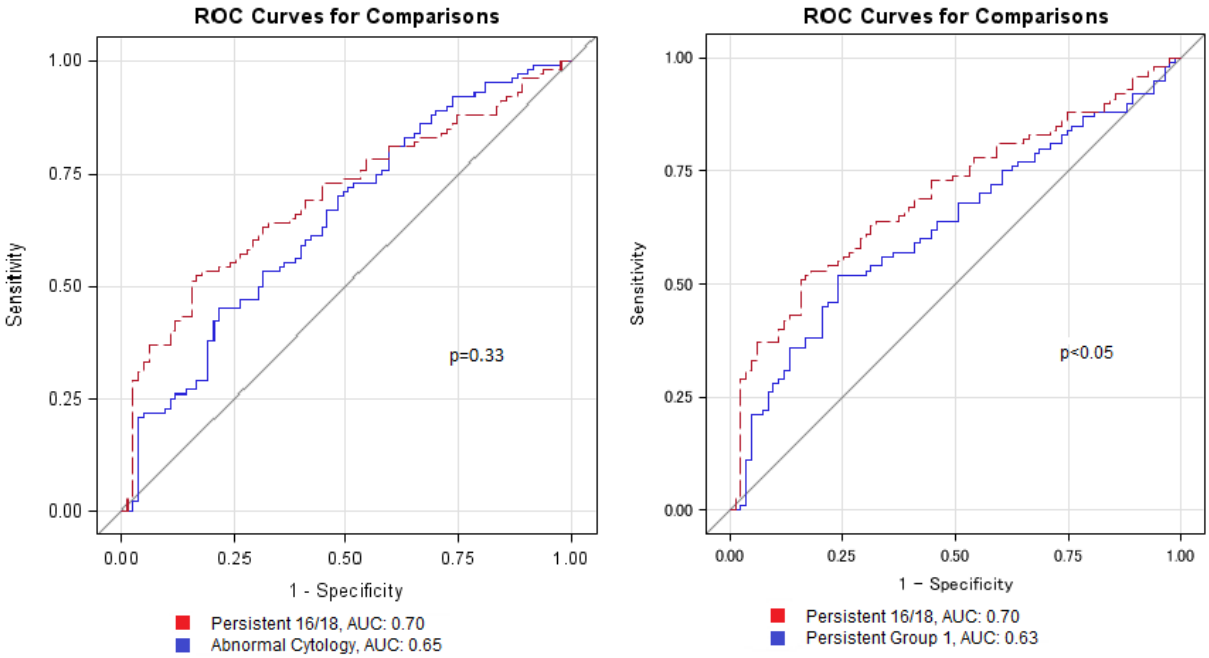


Table 3.1 Characteristics of 183 MSM Screened with Anal Swab and HRA by HIV-Infection Status				
	Total (n=183)	**HIV-Infected (n=107)**	**HIV-Uninfected (n=76)**	***p***
Characteristic	n (col %)	n (col %)	n (col %)	
Age	59.1 (+-8.4)	57 (+-8.3)	62.2 (+-7.6)	***<0.01***
Race				
White, non-Hispanic	159 (86.9)	89 (83.2)	70 (92.1)	0.11
Other	24 (13.1)	18 (16.8)	6 (7.9)	
BMI				
Normal Weight (<25)	93 (50.8)	60 (56.1)	33 (43.4)	0.1
Overweight (≥25)	90 (49.2)	47 (43.9)	43 (56.6)	
CD4 Count				
≤500 CD4 cells/mm^3	38 (20.8)	33 (30.8)	5 (6.6)	***<0.01***
501+ CD4 cells/mm^3	145 (79.2)	74 (69.2)	71 (93.4)	
Smoking Status				
Ever Smoker	131 (71.6)	74 (69.2)	57 (75.0)	0.41
Never Smoker	52 (28.4)	33 (30.8)	19 (25.0)	
Number of Receptive Anal Intercourse Partnerships in the Last 2 Years				
No partners	96 (52.5)	51 (47.7)	45 (59.2)	0.06
1-3 partners	36 (19.7)	19 (17.8)	17 (22.4)	
4+ partners	51 (27.9)	37 (34.6)	14 (18.4)	
Anal Cytology Result				
Abnormal	1347 (73.2)	88 (82.2)	46 (60.5)	***<0.01***
Normal	49 (26.8)	19 (17.8)	30 (39.5)	
Anal HPV16/18 Persistent Infection				
Positive	80 (43.7)	55 (51.4)	25 (32.9)	***0.02***
Negative	103 (56.3)	52 (48.6)	51 (67.1)	
Anal Group 1 HPV Persistent Infection				
Positive	159 (86.9)	97 (90.7)	62 (81.6)	0.08
Negative	24 (13.1)	10 (9.4)	14 (18.4)	
HRA Anal Biopsy Result				
HSIL	98 (53.6)	58 (54.2)	40 (52.6)	0.88
<HSIL	85 (46.5)	49 (45.8)	36 (47.4)	

Table 3.2a Test Characteristics of Cytology and HPV Testing to Predict Histological High-Grade Anal Dysplasia in in 183 MSM Screened with HRA – Unadjusted Model

Test Strategy Model	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	OR (95% CI)	AUC	95% CI
Abnormal Cytology	81.0	36.1	60.4	61.2	2.41 (1.23, 4.72)	0.59	(0.52, 0.65)
Persistent HPV16/18+	578.0	73.5	72.5	59.2	3.83 (2.04, 7.18)	0.66	(0.59, 0.73)
Persistent Group 1+	90.0	16.9	56.6	58.3	1.83 (0.77, 4.36)	0.53	(0.48, 0.58)
Abnormal Cytology and Persistent HPV16/18+	50.0	79.5	74.6	56.9	3.88 (2.00, 7.52)	0.65	(0.58, 0.71)
Abnormal Cytology and Persistent Group 1+	74.0	48.2	63.2	60.6	2.65 (1.42, 4.92)	0.61	(0.54, 0.68)

Table 3.2b Test Characteristics of Cytology and HPV Testing to Predict Histological High-Grade Anal Dysplasia in 183 MSM Screened with HRA – Adjusted[1] Model

Test Strategy Model	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Adjusted OR (95% CI)	AUC	95% CI
Abnormal Cytology	61.0	37.3	54.0	44.3	2.24 (1.08, 4.61)	0.65	(0.57, 0.73)
Persistent HPV16/18+	59.0	60.2	64.1	54.9	3.62 (1.84, 7.16)	0.70	(0.62, 0.77)
Persistent Group 1+	62.0	33.7	53.0	42.4	1.48 (0.59, 3.67)	0.63	(0.55, 0.71)
Abnormal Cytology and Persistent HPV16/18+	51.0	56.6	58.6	49.0	3.55 (1.72, 7.33)	0.68	(0.60, 0.76)
Abnormal Cytology and Persistent Group 1+	68.0	41.0	58.1	51.5	2.34 (1.20, 4.59)	0.66	(0.58, 0.74)

[1]Models adjusted for age, center, race, HIV-infection, number of receptive anal intercourse partners in the last two years, and ever tobacco smoking

3.6 References

1. Satterwhite CL, Torrone E, Meites E, Dunne EF, Mahajan R, Ocfemia MC, et al. Sexually transmitted infections among US women and men: prevalence and incidence estimates, 2008. Sex Transm Dis. 2013;40(3):187-93. doi: 10.1097/OLQ.0b013e318286bb53. PubMed PMID: 23403598.

2. Ault KA. Epidemiology and natural history of human papillomavirus infections in the female genital tract. Infect Dis Obstet Gynecol. 2006;2006 Suppl:40470. doi: 10.1155/IDOG/2006/40470. PubMed PMID: 16967912; PubMed Central PMCID: PMC1581465.

3. (CDC) CfDCaP. HPV Vaccine Information For Young Women [updated 03/26/2015]. Available from: http://www.cdc.gov/std/hpv/stdfact-hpv-vaccine-young-women.htm.

4. Foundation THaAC. HPV: The Facts 2014 [updated 07/2014]. Available from: http://www.analcancerfoundation.org/wp-content/uploads/2014/12/HPV_9_print-1_G9.pdf.

5. Koshiol J, Lindsay L, Pimenta JM, Poole C, Jenkins D, Smith JS. Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis. Am J Epidemiol. 2008;168(2):123-37. doi: 10.1093/aje/kwn036. PubMed PMID: 18483125; PubMed Central PMCID: PMC2878094.

6. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. New England Journal of Medicine. 1998;338(7):423-8.

7. Deligeoroglou E, Giannouli A, Athanasopoulos N, Karountzos V, Vatopoulou A, Dimopoulos K, et al. HPV infection: immunological aspects and their utility in future therapy. Infect Dis Obstet Gynecol. 2013;2013:540850. doi: 10.1155/2013/540850. PubMed PMID: 24023507; PubMed Central PMCID: PMC3762170.

8. International Agency for Research on Cancer (IARC) Working Group on Evaluation of Carcinogenic Risks to Humans. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Volume 90 Human Papillomaviruses. Lyon, France: World Health Organization, International Agency for Research on Cancer; 2007. 689 p.

9. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. A review of human carcinogens--Part B: biological agents. Lancet Oncology. 2009;10(4):321-2. Epub 2009/04/08. PubMed PMID: 19350698.

10. Prevention CfDCa. HPV and Cancer 2017 [updated March 3, 2017; cited 2017 June 15]. Available from: https://www.cdc.gov/cancer/hpv/statistics/cases.htm.

11. Abramowitz L, Jacquard AC, Jaroud F, Haesebaert J, Siproudhis L, Pradat P, et al. Human papillomavirus genotype distribution in anal cancer in France: the EDiTH V study. International Journal of Cancer. 2011;129(2):433-9. Epub 2010/09/15. doi: 10.1002/ijc.25671. PubMed PMID: 20839262.

12. Valmary-Degano S, Jacquin E, Prétet JL, Monnien F, Girardo B, Arbez-Gindre F, et al. Signature patterns of human papillomavirus type 16 in invasive anal carcinoma. Human Pathology. 2013;44(6):11. Epub 2012 Dec 23. doi: 10.1016/j.humpath.2012.08.019.

13. (IARC) IAfRoC. GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012 2012 [cited 2015 07/13]. Available from: http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx.

14. SEER S, Epidemiology and End Results Program,. SEER Cancer Statistics Factsheets: Anal Cancer Bethesda, MD: National Cancer Institute; [cited 2015 July 29]. Available from: http://seer.cancer.gov/statfacts/html/anus.html.

15. D'Souza G, Wiley DJ, Li X, Chmiel JS, Margolick JB, Cranston RD, et al. Incidence and epidemiology of anal cancer in the multicenter AIDS cohort study. J Acquir Immune Defic Syndr. 2008;48(4):491-9. doi: 10.1097/QAI.0b013e31817aebfe. PubMed PMID: 18614927; PubMed Central PMCID: PMC3991563.

16. Machalek DA, Poynten M, Jin F, Fairley CK, Farnsworth A, Garland SM, et al. Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: a systematic review and meta-analysis. Lancet Oncol. 2012;13(5):487-500. doi: 10.1016/S1470-2045(12)70080-3. PubMed PMID: 22445259.

17. Silverberg MJ, Lau B, Justice AC, Engels E, Gill MJ, Goedert JJ, et al. Risk of anal cancer in HIV-infected and HIV-uninfected individuals in North America. Clin Infect Dis. 2012;54(7):1026-34. doi: 10.1093/cid/cir1012. PubMed PMID: 22291097; PubMed Central PMCID: PMC3297645.

18. Benard VB, Thomas CC, King J, Massetti GM, Doria-Rose VP, Saraiya M, et al. Vital signs: cervical cancer incidence, mortality, and screening - United States, 2007-2012. MMWR Morb Mortal Wkly Rep. 2014;63(44):1004-9. PubMed PMID: 25375072.

19. Safaeian M, Solomon D, Castle PE. Cervical cancer prevention--cervical screening: science in evolution. Obstet Gynecol Clin North Am. 2007;34(4):739-60, ix. doi: 10.1016/j.ogc.2007.09.004. PubMed PMID: 18061867; PubMed Central PMCID: PMC2762353.

20. McNamara M, Batur P, Walsh JM, Johnson KM. HPV Update: Vaccination, Screening, and Associated Disease. J Gen Intern Med. 2016. doi: 10.1007/s11606-016-3725-z. PubMed PMID: 27184752.

21. Patel J, Salit IE, Berry MJ, de Pokomandy A, Nathan M, Fishman F, et al. Environmental scan of anal cancer screening practices: worldwide survey results. Cancer Med. 2014;3(4):1052-61. doi: 10.1002/cam4.250. PubMed PMID: 24740973; PubMed Central PMCID: PMC4303174.

22. Berry JM, Jay N, Cranston RD, Darragh TM, Holly EA, Welton ML, et al. Progression of anal high-grade squamous intraepithelial lesions to invasive anal cancer among HIV-infected men who have sex with men. Int J Cancer. 2014;134(5):1147-55. doi: 10.1002/ijc.28431. PubMed PMID: 23934991.

23. Palefsky J, Cranston RD. Anal squamous intraepithelial lesions: Diagnosis, screening, prevention, and treatment: Wolters Kluwer; 2017 [updated 1/26/2017; cited 2017 August 30]. Available from: https://www.uptodate.com/contents/anal-squamous-intraepithelial-lesions-diagnosis-screening-prevention-and-treatment?source=search_result&search=anal%20cytology&selectedTitle=1~12.

24. Panther LA, Wagner K, Proper J, Fugelso DK, Chatis PA, Weeden W, et al. High resolution anoscopy findings for men who have sex with men: inaccuracy of anal cytology as a predictor of

histologic high-grade anal intraepithelial neoplasia and the impact of HIV serostatus. Clin Infect Dis. 2004;38(10):1490-2. PubMed PMID: 15156490.

25. Palefsky JM, Holly EA, Hogeboom CJ, Berry JM, Jay N, Darragh TM. Anal cytology as a screening tool for anal squamous intraepithelial lesions. Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology. 1997;14(5):415-22.

26. FDA. FDA approves first human papillomavirus test for primary cervical cancer screening 2014 [updated 04/24/2014; cited 2015 10 24]. Available from: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm394773.htm.

27. Castle PE, Sadorra M, Garcia F, Holladay EB, Kornegay J. Pilot study of a commercialized human papillomavirus (HPV) genotyping assay: comparison of HPV risk group to cytology and histology. J Clin Microbiol. 2006;44(11):3915-7. doi: 10.1128/JCM.01305-06. PubMed PMID: 16971652; PubMed Central PMCID: PMC1698309.

28. Huh WK, Ault KA, Chelmow D, Davey DD, Goluart RA, Garcia FA, et al. Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance. Gynecol Oncol. 2015;136(2):5. Epub 1/8/2015. doi: doi:10.1016/j.ygyno.2014.12.022.

29. (USPSTF) USPSTF. Final Update Summary: Cervical Cancer: Screening 2015 [updated 07/2015]. Available from: http://www.uspreventiveservicestaskforce.org/Page/Document/UpdateSummaryFinal/cervical-cancer-screening.

30. Salit IE, Lytwyn A, Raboud J, Sano M, Chong S, Diong C, et al. The role of cytology (Pap tests) and human papillomavirus testing in anal cancer screening. AIDS. 2010;24(9):1307-13. doi: 10.1097/QAD.0b013e328339e592. PubMed PMID: 20442633.

31. Maia LB, Marinho LC, Barbosa TW, Velasco LF, Costa PG, Carneiro FP, et al. Value of human papillomavirus typing for detection of anal cytological abnormalities. Indian J Sex Transm Dis. 2013;34(2):102-6. doi: 10.4103/0253-7184.120540. PubMed PMID: 24339460; PubMed Central PMCID: PMC3841659.

32. Burd EM. Human Papillomavirus Laboratory Testing: the Changing Paradigm. Clin Microbiol Rev. 2016;29(2):291-319. doi: 10.1128/CMR.00013-15. PubMed PMID: 26912568; PubMed Central PMCID: PMC4786885.

33. Schofield AM, Sadler L, Nelson L, Gittins M, Desai M, Sargent A, et al. A prospective study of anal cancer screening in HIV-positive and negative MSM. AIDS. 2016;30(9):1375-83. doi: 10.1097/QAD.0000000000001045. PubMed PMID: 26836788.

34. Kaslow RA, Ostrow DG, Detels R, Phair JP, Polk BF, Rinaldo CR, Jr. The Multicenter AIDS Cohort Study: rationale, organization, and selected characteristics of the participants. American Journal of Epidemiology. 1987;126(2):310-8.

35. Detels R, Jacobson L, Margolick J, Martinez-Maza O, Munoz A, Phair J, et al. The Multicenter AIDS Cohort Study, 1983 to ...,. Public Health. 2012;126(3):196-8. doi: 10.1016/j.puhe.2011.11.013. PubMed PMID: 22206985; PubMed Central PMCID: PMC3324261.

36. Wiley DJ, Li X, Hsu H, Seaberg EC, Cranston RD, Young S, et al. Factors Affecting the Prevalence of Strongly and Weakly Carcinogenic and Lower-Risk Human Papillomaviruses in Anal Specimens in a Cohort of Men Who Have Sex with Men (MSM). PLoS One. 2013;8(11):e79492. doi: 10.1371/journal.pone.0079492. PubMed PMID: 24278140; PubMed Central PMCID: PMC3835810.

37. Nayar R, Wilbur DC. The Pap Test and Bethesda 2014. "The reports of my demise have been greatly exaggerated." (after a quotation from Mark Twain). Acta Cytol. 2015;59(2):121-32. doi: 10.1159/000381842. PubMed PMID: 25997404.

38. Darragh TM, Colgan TJ, Cox JT, Heller DS, Henry MR, Luff RD, et al. The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. J Low Genit Tract Dis. 2012;16(3):205-42. doi: 10.1097/LGT.0b013e31825c31dd. PubMed PMID: 22820980.

39. Holly EA, Ralston ML, Darragh TM, Greenblatt RM, Jay N, Palefsky JM. Prevalence and risk factors for anal squamous intraepithelial lesions in women. J Natl Cancer Inst. 2001;93(11):843-9.

40. Barut MU, Kale A, Kuyumcuoglu U, Bozkurt M, Agacayak E, Ozekinci S, et al. Analysis of Sensitivity, Specificity, and Positive and Negative Predictive Values of Smear and Colposcopy in Diagnosis of Premalignant and Malignant Cervical Lesions. Med Sci Monit. 2015;21:3860-7. PubMed PMID: 26655816; PubMed Central PMCID: PMC4678924.

41. Rijkaart DC, Berkhof J, van Kemenade FJ, Coupe VM, Rozendaal L, Heideman DA, et al. HPV DNA testing in population-based cervical screening (VUSA-Screen study): results and implications. Br J Cancer. 2012;106(5):975-81. doi: 10.1038/bjc.2011.581. PubMed PMID: 22251922; PubMed Central PMCID: PMC3305964.

42. Meijer CJ, Berkhof J, Castle PE, Hesselink AT, Franco EL, Ronco G, et al. Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. Int J Cancer. 2009;124(3):516-20. doi: 10.1002/ijc.24010. PubMed PMID: 18973271; PubMed Central PMCID: PMC2789446.

43. Cuzick J, Clavel C, Petry KU, Meijer CJ, Hoyer H, Ratnam S, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. Int J Cancer. 2006;119(5):1095-101. doi: 10.1002/ijc.21955. PubMed PMID: 16586444.

44. Lloveras B, Gomez S, Alameda F, Bellosillo B, Mojal S, Muset M, et al. HPV testing by cobas HPV test in a population from Catalonia. PLoS One. 2013;8(3):e58153. doi: 10.1371/journal.pone.0058153. PubMed PMID: 23483984; PubMed Central PMCID: PMC3590124.

45. Ge Y, Christensen P, Luna E, Armylagos D, Schwartz MR, Mody DR. Performance of Aptima and Cobas HPV testing platforms in detecting high-grade cervical dysplasia and cancer. Cancer. 2017;125(8):652-7. doi: 10.1002/cncy.21875. PubMed PMID: 28574670.

46. Castle PE, Stoler MH, Wright TC, Jr., Sharma A, Wright TL, Behrens CM. Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical

cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. Lancet Oncol. 2011;12(9):880-90. doi: 10.1016/S1470-2045(11)70188-7. PubMed PMID: 21865084.

47. Zhang Q, Zhao M, Cao D, Wei X, Wang L, Li Y, et al. Assessment of the Effectiveness of HPV16/18 Infection Referred for Colposcopy in Cervical Cancer Screening in Northwest of China. J Med Virol. 2017. doi: 10.1002/jmv.24902. PubMed PMID: 28710863.

48. Gage JC, Partridge EE, Rausa A, Gravitt PE, Wacholder S, Schiffman M, et al. Comparative performance of human papillomavirus DNA testing using novel sample collection methods. J Clin Microbiol. 2011;49(12):4185-9. Epub 2011/10/15. doi: JCM.01254-11 [pii] 10.1128/JCM.01254-11. PubMed PMID: 21998422; PubMed Central PMCID: PMC3232937.

Chapter 4. Role of Testosterone Replacement Therapy with Unsatisfactory Anal Cytology and Histological High-grade Anal Lesions in Older Men Who Have Sex with Men

4.1 Abstract

Background: Testosterone replacement therapy (TRT) is prescribed often for men symptomatic for hypogonadism, who tend to be older or have a strong risk factor such as HIV infection. Older men who have sex with men (MSM) disproportionately form this risk group, and also are at highest risk for anal cancer. Risks of TRT are currently debated with recent studies reporting risk for prostate cancer and cardiovascular disease among TRT users. One study identified a link between increased serum free testosterone levels and anal Human papillomavirus (HPV) 16/18 infection, the leading cause for 90% of anal cancers. Persistent HPV16/18 infections can cause high-grade squamous intraepithelial lesions (HSILs) which then can progress to anal cancer. Screening with a swab for anal cytology can detect HSIL for follow-up diagnoses and care. A study in female-to-male transgender men suggested TRT may cause unsatisfactory swab results for cervical cancer screening, which informs anal screening practices. The goals of this study were to identify whether TRT affected anal cytology performance and risk for anal HSIL.

Methods: Two hundred and ninety-six HIV-infected and -uninfected MSM were screened with anal cytology and histology in a randomized clinical trial. One Dacron and one nylon-flocked swab were collected from the anal canal and tested for cytology; results were combined to form the cytology result, unsatisfactory vs. satisfactory. Men were assessed using the gold-standard: high-resolution anoscopy (HRA) with anal biopsy to determine histological HSIL (hHSIL). Men were interviewed with two surveys, one for sociodemographic and behavioral data, with another specifically for self-report of lifetime TRT use. Two multivariable logistic regression models estimated odds ratios (ORs) and 95% confidence intervals between TRT and unsat, and TRT and hHSIL. Each model controlled for age, race, HIV infection, and number of male receptive anal

intercourse (RAI) partners in the last two years, with the unsat model additionally controlling for swab collection order.

Results: On average, participants were 55 (±11) years old, White, non-Hispanic (75%, 221/296), and HIV-infected (56%, 167/296). Nearly half of all men (46%, 135/296) reported ever using TRT, most were HIV-infected (107/135); 85 men were using testosterone at their HRA visit. hHSIL was found 43% of men with histology (121/281). In the multivariable model for unsatisfactory cytology, there was no statistically significant association between current use of TRT and risk for unsatisfactory cytology (OR: 0.8 (95% CI: 0.4, 1.5)). However, swab order was associated with unsatisfactory cytology with 2.9-fold higher odds when the nylon-flocked swab was drawn first (OR: 2.9 (1.7, 4.6)). Multivariable results for hHSIL also suggested no association between self-reported current use of TRT and risk for hHSIL (OR: 1.1 (0.6, 2.1)). Men who reported two or more RAI partners within the previous 2 years had 89% higher odds of hHSIL than men who reported none (OR: 1.9, (1.0, 3.5)).

Discussion: TRT use is high in this group of older MSM, with four times higher use in HIV-infected men than -uninfected men. This analysis found no association between TRT and unsatisfactory cytology, however, findings may inform swab collection practices that implement multiple swabs or nylon-flocked swabs. No relationship was found between TRT and hHSIL, however hHSIL prevalence was common, stressing the need for effective screening practices. Due to limitations of self-report, this study should be repeated using biological measurements for serum testosterone since physiological uptake of TRT varies by individual and therapy.

4.2 Introduction

Exogenous testosterone use has significantly increased in the US with an estimated ten-fold increase in prescriptions between 2000-2011 [1]. Testosterone replacement therapy (TRT) may be recommended for men with hypogonadism, low serum testosterone levels, which can occur naturally with increasing age or from health conditions such as HIV [2-5]. However, decreased testosterone level alone is often not sufficient to warrant TRT as it is possible to have lower levels without any measurable impact on daily living. Measuring free testosterone (FT) in serum, the biologically active form of testosterone, is the minimally recommended practice for a clinical indication to prescribe TRT [6]. For precision, expert endocrinologists recommend that multiple measurements should be performed over time using the same assay, due to assay-to-assay variations, and only using blood specimens drawn in the morning, due diurnal changes in hormones [6]. Since individuals vary biologically, low readings should also be paired with clinical symptoms like decreased muscle mass, sexual function, and bone mass, and the absence of contraindications [6]. Data from a large cohort of men has shown that 95% of TRT prescriptions were against recommended guidelines, suggesting a large contributor to the recent growth of TRT may be due to inappropriate prescription practices [7, 8]. In the wake of ubiquitous TRT, there is also insufficient data to prove the proposed benefits of TRT outweigh its potential risks, which is particularly important for immunodeficient individuals where additional disease can have a serious impact [5, 9, 10].

Some data suggest exogenous sex hormones increase cancer risk, and additional data show higher risk for HPV-infections and –associated neoplasm related to exogenous female sex hormones. Historically, hormone replacement therapy (HRT) using estrogen, with and without progestins, was commonly prescribed for symptomatic menopausal women [11, 12]. However, in 1999, Weiderpass et al., identified a strong association between five-year estrogen HRT use and a 6-fold increase in risk for endometrial cancer [13]. Shortly after in 2002, data from a cohort of 16,608 women in the Women's Health Initiative (WHI) study demonstrated a strong association

between randomized combined estrogen and progestin HRT and increased incidence of invasive breast cancer, stroke, and pulmonary embolism [14]. These findings have since informed prescribing practices and fewer menopausal women use HRT in the absence of a strong clinical indication. Further highlighting the risk of exogenous estrogen, independent of HRT, increased lifetime exposure from higher parity, early sexual debut, and long-term oral contraceptive use has been found to increase risk for persistent high-risk (hr) human papillomavirus (HPV) cervical infections and subsequent cervical disease [15-22]. Long-term cervical infection with hrHPVs can cause severe dysplasia, high-grade squamous intraepithelial lesions (HSILs), which can progress into cervical cancer.

A recent study has identified a positive association between serum free testosterone and risk for hrHPV types 16 or 18 infections in a cohort of older men who have sex with men (MSM), the two most carcinogenic hrHPVs that account for 78-90% of anal cancer cases [23-27]. While not directly studied, testosterone therapy may play role in anal hrHPV risk since successful testosterone therapy should increase FT levels. For HIV-infected MSM where anal cancer rates are at least 40-fold higher than the general population, better understanding the relationship between testosterone therapy and anal HPV-related disease is critical since HIV infection is also associated with lower FT [28-31]. Unlike the WHI study, no large cohort has assessed randomized testosterone therapy. However, smaller studies have reported mixed results between testosterone therapy and risk for prostate cancer, and stroke and pulmonary embolism: forms of cardiovascular disease (CVD) [32-36]. In 2015, the FDA released an update for TRT manufacturers to add warning labels of potential increased CVD risk and recommended clinicians to follow stricter guidelines for prescribing TRT, supporting the concerns regarding a lack of knowledge surrounding TRT risks [37, 38]. As the strong associations between exogenous estrogen and several diseases have been established in women, identifying risks of exogenous testosterone is critical, especially in the context of HPV-associated anal disease in MSM where prevalence of the exposure and outcome are high [39, 40].

There is no standardized practice for anal cancer screening, though the current recommendation for screening MSM is a repeated anal cytology test, modeled after the cervical Pap test, where a swab is used to collect squamous epithelial cells from the anal canal and tested for cellular abnormalities that may indicate anal lesions or precancers [41]. However, cytology often has low sensitivity and moderate specificity [42], often relying on repeat testing to detect disease [43]. Abnormal cytology results are followed by a diagnostic exam called a high-resolution anoscopy (HRA) where anal tissue is stained, examined, and biopsied to diagnose disease. Screening relies on a sufficient number of squamous cells collected by the swab to provide a readable or satisfactory specimen. Insufficiently cellular specimens, defined as having fewer than 2000 nucleated squamous cells (1-6 cells per high-powered field), or specimens obscured by blood, lubricant, mucous or inflammatory discharge contaminants are evaluated as *unsatisfactory* for cytology [44-46]. Anal cytology often yields a higher proportion of unsatisfactory (1-7.4%) results than cervical cytology (0.3-3.4%), therefore reducing unsatisfactory results in anal specimens is critical for improving screening to detect precancers and cancers early for treatment [47-50].

TRT may potentially be associated with unsatisfactory cytology. For example, a medical chart review conducted by Peitzmeier et al., identified factors contributing to a high rate of unsatisfactory cervical Paps in a large sample of cisgender women and female-to-male transgender men (FTMTM) with intact cervices [45]. Nearly 91% of the FTMTM reported ever being prescribed TRT [45]. After adjusting for race, age, and BMI, FTMTM showed an 11-fold higher odds of having an unsatisfactory cytology specimen compared to cisgender women [45]. For each additional year of testosterone use, the odds of an unsatisfactory Pap were 20% higher for FTMTM than cisgender women, and the proportion of unsatisfactory Pap tests increased with the duration of TRT [45]. The researchers hypothesize exogenous testosterone may be directly affecting cervical tissue and epithelial cell shedding, although provider or patient discomfort in the specimen collection may be a contributing factor. The relationship between TRT and

unsatisfactory anal cytology in MSM has not been studied and may be important to anal cancer screening practices [45, 51-53]. Thus, we studied 296 older MSM to explore the associations between TRT and the risk for anal histological-confirmed HSIL (hHSIL) and unsatisfactory anal cytology.

4.3 Methods

Participants

The 296 MSM who compose this study sample were from a randomized clinical trial evaluating cytology for anal cancer screening called the ISTA study (Improving Screening Tools for Anal cancer). Participants were screened at a single visit using anal swab specimens and high-resolution anoscopy (HRA) with biopsy specimens. The men were from Los Angeles, Palm Springs, and San Francisco, California, with approximately half (53%, 157/296) of whom were also participants of the Multicenter AIDS Cohort Study (MACS). The MACS is characterized as the longest running longitudinal study tracking the natural history of HIV/AIDS in MSM and is described in detail elsewhere [54, 55]. The remaining men who were non-MACS were recruited through community clinics. Enrollment was open to all HIV-infected and-uninfected adult MSM who were not experiencing anal bleeding or fissures at the time of the visit.

Procedures

MACS participants are seen every six months and sociodemographic and behavioral data are collected through interviewer- and self-guided questionnaires. For non-MACS men, similar data were collected in an interviewer-guided questionnaire at the ISTA study visit. All participants completed an additional interviewer-guided survey focused on exogenous testosterone use. The survey included questions on the form of testosterone used (gel, injectable, oral/buccal, patch, or implant), brand name if known, dosage (high/low), frequency, and duration (start/stop dates). Given the temporal effects of testosterone, current use of any form of exogenous testosterone at the time of their HRA visit was assessed as the exposure of interest, compared to past but not current use, and never use.

Anal cytology was collected by a trained examiner using two swabs, a nylon-flocked swab (Copan Italia International, Brescia, Italy) collected into SurePath™ (BD Diagnostics TriPath, Burlington, NC) preservative and the standard swab, a Dacron swab collected into Preservcyt® (Hologic, Inc., Marlborough, MA) preservative. Each swab was inserted approximately 5 centimeters, and rotated against the anal wall with pressure for 10 to 20 seconds before being withdrawn. Swab collection order was randomized by swab type and preservative. Due to the larger swab size, the nylon-flocked swab was collected through a lubricated clear plastic anoscope to facilitate swab insertion. Where Dacron swab was randomized for collection after the nylon-flocked swab, the Dacron swab was collected through the anoscope to reduce lubricant contamination. Both swabs were processed identically and blinded specimens were interpreted by cytopathologists at Tricore Reference Laboratories (Albuquerque, New Mexico) using standard cytologic classification for interpretable specimens. The ISTA protocol collected additional swabs, however only data from these two swabs are used in this analysis. Ultimately, cytology results from both swabs were combined and dichotomized as satisfactory when both swabs were interpretable, and unsatisfactory when one or both were uninterpretable.

Following swab collection, HRA was performed using standard recommended protocols [56]. Briefly, anal epithelium was stained with acetic acid and Lugol's iodine to differentiate tissue potentially containing abnormal cellular changes. A high-resolution scope with bright light and magnification was used by the anoscopist to examine anal tissue and biopsies were collected where lesions were suspected. Biopsies were collected into formalin and sent to pathologists at Tricore Laboratories for processing and assessment to diagnose anal disease. Lesions were classified using standard terminology, negative for lesion (NIL), low-grade squamous intraepithelial lesion (LSIL), and high-grade squamous intraepithelial lesion (HSIL) with additional granular classifications. For this analysis, a participant with HSIL on any biopsy was classified as having histologically-confirmed HSIL (hHSIL).

Analysis

Descriptive analyses were performed to characterize the study sample. The differences for each characteristic were evaluated by HIV-infection status using Chi-square and Wilcoxon Rank for categorical and continuous variables, respectively. Unadjusted bivariate and adjusted multivariable logistic regression models were used to assess the association between self-reported testosterone use with unsatisfactory cytology and hHSIL, individually. Potential confounders and effect modifiers were assessed and inferred from published literature. For MACS men, covariates were a combination of summary variables from historical cohort data and data collected at the MACS visit most proximal to the ISTA examination visit. For non-MACS men, covariate data reported on ISTA questionnaires were used. Pearson correlation coefficients assessed associations between continuous variables and pseudo R^2 for categorical variables to determine multicollinearity. Covariates included in the multivariable models were selected from bivariate models and from published literature. The final model for unsatisfactory cytology included current testosterone use, age, race, swab order, the number of anal receptive intercourse partners in the last two years, and HIV-infection (HIV+, $\geq$500 CD4 cells/mm^3, HIV+, <500 CD4 cells/mm^3, vs. HIV-uninfected). The final model for hHSIL included the same covariates except for swab order.

4.4 Results

Descriptive Statistics

On average, participants were 55 years old, with the majority being White, non-Hispanic, and 56% (167/296) were HIV-infected (Table 4.1). 57% (170/296) of men reported no receptive anal intercourse (RAI) partners in the past two years, 18% (55/296) reported one, and 24% (71/296) reported two or more. HIV-infected men were more likely to have one RAI partner than HIV-uninfected men who were more likely to have none (p=0.02).

Nearly half of all men (46%, 135/296) reported ever using any form of TRT in their lifetime. Most TRT users were HIV-infected with 64% (107/167) lifetime TRT use compared to 22%

(28/129) of HIV-uninfected men (Supplementary Table 4.1). Sixty-one percent (82/135) of TRT users reported one form, while 39% (53/135) reported having used two to six different formulations. The most common brand-name testosterone used was AndroGel™ (AbbVie Inc., North Chicago, IL) (82/135). Nearly a third of all men (29%; 85/296) were using testosterone at their ISTA visit, most of whom were HIV-infected (n=75). Current and historical TRT users showed similar use patterns (Figure 4.1). Compared to never users, current users were more likely to be White, non-Hispanic, and HIV-infected (Supplementary Table 4.2).

Cytology Model

Of 296 men sampled, three were excluded from analyses due to missing cytology data from both anal swabs (n=293). Five additional participants showed missing data for one swab but were included. Overall, 93% (272/293) of men showed one or more interpretable cytology results from the two swabs, with 7% (21/293) showing unsatisfactory results for both. However, 41% (119/293) of men showed at least one unsatisfactory cytology.

Bivariate analyses showed no association between current or ever testosterone use and unsatisfactory cytology using the combined data (Table 4.2). When swabs were assessed individually, current testosterone use showed no association with cytology for the nylon-flocked swab, however, current testosterone use appeared to be protective for unsatisfactory cytology on the Dacron swab, i.e., OR: 0.49 (0.26, 0.92). HIV infection was not associated with unsatisfactory cytology, though drawing the nylon-flocked swab first appeared to be significant (OR: 2.86 (1.75, 4.55)).

In the multivariable model for unsatisfactory cytology, there was no statistically significant association between current use of exogenous testosterone and risk for unsatisfactory cytology, i.e., OR: 0.75 (0.39, 1.45) (Table 4.2). However, swab order was associated with unsatisfactory cytology. When the nylon-flocked swab was first drawn, there was 2.9-fold higher odds of either swab being unsatisfactory (95% CI: 1.72, 4.55). These findings suggest first-drawn nylon-flocked swab penalizes the Dacron swab. When this adjusted model was limited to HIV-infected MSM

alone, high recent CD4 count (>500 cells/mm^3) was protective for unsatisfactory cytology: for men with $\geq$500 cells/mm^3 vs. <500 cells/mm^3, i.e., OR=0.44, (0.22, 0.89).

Histology Model

Forty-three percent (121/281) of men showed hHSIL. HIV infection and CD4 count were not associated with hHSIL (p>0.05). While unsatisfactory cytology and hHSIL were not associated with one another (p>0.05), abnormal cytology was strongly associated with hHSIL on either swab (nylon-flocked, OR: 4.96 (2.56, 9.62); Dacron, OR: 2.88 (1.61, 5.15)). Assessed alone, current testosterone use was not statistically significantly associated with hHSIL, i.e., OR: 1.24, (0.72, 2.12) (Table 4.3).

The multivariable model for hHSIL, controlling for the effects of other covariates, suggested no association between self-reported current use of exogenous testosterone and risk for hHSIL, i.e., OR: 1.09 (0.58, 2.06) (Table 4.3). However, men that reported two or more receptive anal intercourse partners in the 2 years prior to the examination had 89% higher odds of hHSIL than men who reported none (OR=1.89, 95% CI: 1.04, 3.45).

4.5 Discussion

Exogenous testosterone use was commonly reported in this group of HIV-infected and –uninfected MSM supporting previous studies suggesting high usage due to HIV infection and age. The proportion of having one of two anal swabs collected being unsatisfactory for anal cytology was very high (41%), however being unsatisfactory on both swabs drawn was much lower at 7%, falling within the range of published studies on anal cytology [47-49]. Prevalence of hHSIL was also high in this group, highlighting the disproportionate burden of anal disease affecting MSM. No relationship was identified between current TRT and unsatisfactory anal cytology or hHSIL in this group of older MSM. These results showed the Dacron swab, commonly recommended for anal cytology, was penalized when drawn after other swabs, while the nylon-flocked swab was not. This may be informative for clinicians who utilize protocols where multiple swabs or nylon-

flocked swabs are collected. Because the effects of current TRT in the context of anal cancer are inconclusive, future study is warranted.

These analyses do not assess differences in anal and cervical tissues, nor in sex hormone effects across organs or gender groups. To better understand the role testosterone plays in anal epithelium, anal tissue studies assessing local responses to exogenous testosterone may be helpful. Interestingly, one genotyping study of cervical-cancer affected women with a history of negative cytology findings suggested e-cadherin polymorphisms were associated with false negative screening tests [57]. E-cadherin is a cytoskeletal glycoprotein that binds epithelial cells together, and factors that influence adhesion would likely affect exfoliation of HPV-affected cells (leading to false-negative cervical cytology). These findings suggest more research is needed to better understand the role exogenous testosterone might be playing in anal epithelium that may affect screening, or directly affect risk for anal dysplasia.

This study is limited by sample size and we may not have the power to detect small effects. Self-report of the exposure and the covariates are subject to imperfect recall and social desirability that could yield misclassification bias. Longitudinal biological measures of serum FT may be a more informative metric for the exposure over self-report alone, since systemic absorption (of testosterone) may vary across men. Covariate data gathered from community participants may be less reliable than contemporaneously collected self-report data gathered by the MACS cohort. Lastly, high TRT use patterns suggest study results may not be generalizable to all MSM.

Though the link between TRT and anal dysplasia is not confirmed, clinicians are best advised to adhere to expert guidelines, and to cautiously approach TRT therapy to balance risks and benefits for individual patients for other diseases. To further explore the role of TRT in anal screening and disease, other methods may be appropriate including serum measurements and longitudinal study of a larger study sample. The high prevalence of testosterone use and anal hHSIL in this group of older MSM should give pause to men and providers. Further efforts are

needed to research long-term effects of TRT, particularly among HIV-infected men where therapy is frequently prescribed and risk for other disease is high.

Figure 4.1 Frequency of Ever and Current Use of Exogenous Testosterone by Form Among 296 Older MSM Screened for Anal Dysplasia

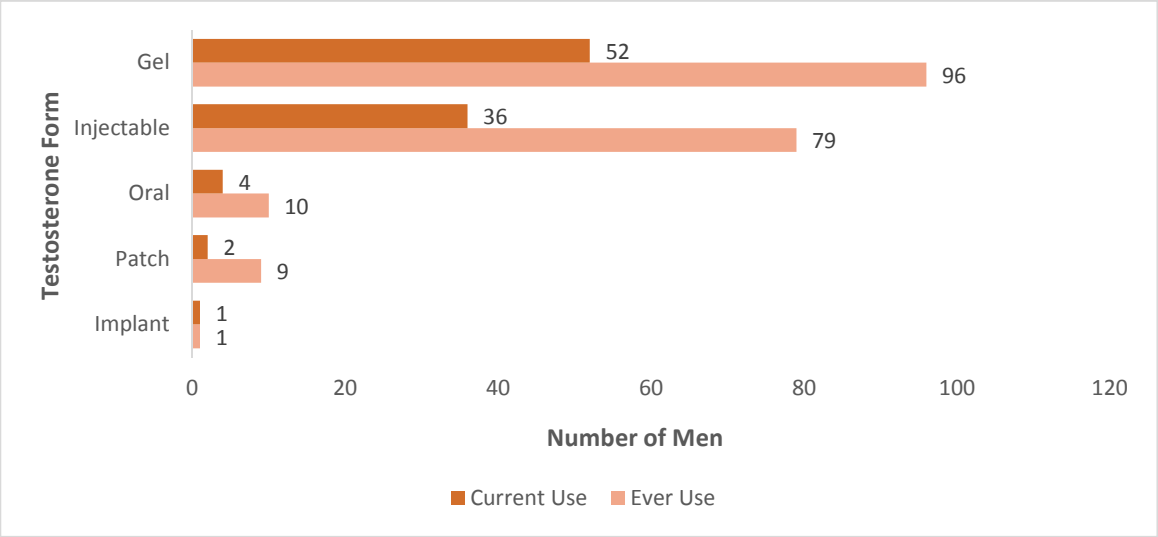


Table 4.1 Characteristics of 296 Older HIV-Infected and -Uninfected MSM Screened for Anal Dysplasia	
Characteristic	
Mean Age (SD)	55.5 (11.2)
	Frequency (Col %)
Race	
White, non-Hispanic	221 (74.7)
Other	75 (25.3)
HIV Status, CD4 Count	
HIV-infected, <500 CD4 cells/mm^3	58 (19.6)
HIV-infected, 500+ CD4 cells/mm^3	113 (38.2)
HIV-uninfected	125 (42.2)
Smoking Status	
Ever Smoker	220 (74.3)
Never Smoker	76 (25.7)
Number of Receptive Anal Intercourse Partnerships in the Last 2 Years	
No partners	170 (57.4)
1 partners	55 (18.6)
2+ partners	71 (24.0)
Use of Testosterone	
Current Use	85 (28.7)
Ever Use (Not Current)	50 (16.9)
Never Use	161 (54.4)
Number of Testosterone Forms Used Among Current Users	
0	211 (71.3)
1	77 (26.0)
2	6 (2.0)
3	2 (0.7)
Anal Cytology Quality	
Unsatisfactory (at least one swab)	119 (40.6)
Satisfactory	173 (59.4)
Anal Biopsy Result	
HSIL	121 (42.9)
<HSIL	161 (57.1)

Table 4.2 Prevalence and Factors Associated with Unsatisfactory Anal Cytology in 296 MSM Screened for Anal Dysplasia			
Characteristic	Unsatisfactory Cytology (% of row)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Age (years)			
64-78	32 (44.4)	1.60 (0.82, 3.12)	1.51 (0.68, 3.37)
58-63	32 (44.4)	1.60 (0.82, 3.12)	1.69 (0.78, 3.71)
51-57	30 (40.5)	1.36 (0.70, 2.66)	1.40 (0.68, 2.91)
21-50	25 (33.3)	Reference	Reference
Race			
White, non-Hispanic	89 (40.5)	0.97 (0.57, 1.67)	0.81 (0.43, 1.54)
Other	30 (41.1)	Reference	Reference
HIV Status, CD4 Count			
HIV-infected, <500 CD4	28 (48.3)	1.13 (0.61, 2.12)	1.36 (0.67, 2.77)
HIV-infected, 500+ CD4	35 (31.5)	0.56 (0.33, 0.95)	0.64 (0.35, 1.18)
HIV-uninfected	56 (45.2)	Reference	Reference
Number of Receptive Anal Intercourse Partnerships in the Last 2 Years			
2+ partners	29 (40.9)	0.94 (0.53, 1.66)	1.00 (0.54, 1.85)
1 partner	19 (35.2)	0.74 (0.39, 1.40)	0.70 (0.35, 1.43)
0 partners	71 (42.3)	Reference	Reference
Swab Randomization			
Nylon-Flocked First	78 (52.7)	**2.86 (1.75, 4.55)**	**2.86 (1.72, 4.55)**
Dacron First	41 (28.3)	Reference	Reference
Use of Testosterone			
Current Use	28 (34.2)	0.67 (0.38, 1.16)	0.75 (0.39, 1.45)
Ever Use (Not Current)	21 (41.2)	0.90 (0.48, 1.71)	0.84 (0.42, 1.68)
Never Use	70 (43.8)	Reference	Reference

Table 4.3 Prevalence and Factors Associated with Histological Anal High-Grade Lesions in 281 MSM Screened for Anal Dysplasia			
Characteristic	hHSIL (row %)	Bivariate OR (95% CI)	Multivariable OR (95% CI)
Age (years)			
64-78	27 (38.6)	0.67 (0.34, 1.30)	0.74 (0.34, 1.62)
58-63	29 (40.3)	0.71 (0.37, 1.39)	0.73 (0.34, 1.55)
51-57	31 (44.3)	0.84 (0.43, 1.64)	0.84 (0.41, 1.70
21-50	34 (28.1)	Reference	Reference
Race			
White, non-Hispanic	92 (43.4)	1.08 (0.63, 1.87)	1.20 (0.64, 2.27)
Other	29 (41.4)	Reference	Reference
HIV Status, CD4 Count			
HIV-infected, <500 CD4	28 (50.0)	1.51 (0.80, 2.87)	1.32 (0.65, 2.70)
HIV-infected, 500+ CD4	46 (42.6)	1.12 (0.66, 1.91)	1.00 (0.54, 1.87)
HIV-uninfected	47 (39.8)	Reference	Reference
Number of Receptive Anal Intercourse Partnerships in the Last 2 Years			
2+ partners	36 (53.7)	**2.05 (1.15, 3.64)**	**1.89 (1.04, 3.45)**
1 partner	26 (50.0)	1.76 (0.94, 3.31)	1.51 (0.77, 2.95)
0 partners	59 (36.2)	Reference	Reference
Use of Testosterone			
Current Use	39 (48.8)	1.24 (0.72, 2.12)	1.09 (0.58, 2.06)
Ever Use (Not Current)	15 (31.3)	0.59 (0.30, 1.18)	0.58 (0.28, 1.18)
Never Use	67 (43.5)	Reference	Reference

Supplementary Table 4.1 Characteristics of 296 Older HIV-Infected and -Uninfected MSM Screened for Anal Dysplasia by Ever Testosterone Use			
Characteristic	**Ever Testosterone User**	**Never User**	***p*-values**
Mean Age (SD)	56.1 (10.3)	55.0 (11.8)	0.39
	Frequency (Row %)	***Frequency (Row %)***	
Race			
White, non-Hispanic	105 (47.5)	116 (52.5)	0.26
Other	30 (40.0)	45 (60.0)	
HIV Status, CD4 Count			
HIV-infected, <500 CD4	35 (60.3)	23 (39.7)	<0.0001
HIV-infected, 500+ CD4	72 (63.7)	41 (36.3)	
HIV-uninfected	28 (22.4)	97 (77.6)	
Smoking Status			
Ever Smoker	97 (44.1)	123 (55.9)	0.37
Never Smoker	38 (50.0)	38 (50.0)	
Number of Receptive Anal Intercourse Partnerships			
No partners	74 (43.5)	96 (56.5)	0.70
1 partners	27 (49.1)	28 (50.9)	
2+ partners	34 (47.9)	37 (52.1)	
Anal Cytology Quality			
Unsatisfactory (at least	49 (41.2)	70 (58.8)	0.23
Satisfactory	84 (48.3)	90 (51.7)	
Anal Biopsy Result			
HSIL	54 (42.2)	74 (57.8)	0.82
<HSIL	67 (43.5)	87 (56.5)	

Supplementary Table 4.2 Characteristics of 296 Older HIV-Infected and -Uninfected MSM Screened for Anal Dysplasia by Current Testosterone Use			
Characteristic	**Current Testosterone User**	**Not Current User**	***p*-values**
Mean Age (SD)	57.0 (9.3)	54.8 (11.8)	0.13
	Frequency (Row %)	***Frequency (Row %)***	
Race			
White, non-Hispanic	72 (32.6)	149 (67.4)	0.01
Other	13 (17.3)	62 (82.7)	
HIV Status, CD4 Count			
HIV-infected, <500 CD4	23 (39.7)	35 (60.3)	<0.001
HIV-infected, 500+ CD4	52 (46.0)	61 (54.0)	
HIV-uninfected	10 (8.0)	115 (92.0)	
Smoking Status			
Ever Smoker	59 (26.8)	161 (73.2)	0.22
Never Smoker	26 (34.2)	50 (65.8)	
Number of Receptive Anal Intercourse Partnerships			
No partners	41 (24.1)	129 (75.9)	0.07
1 partners	22 (40.0)	33 (60.0)	
2+ partners	22 (31.0)	49 (69.0)	
Anal Cytology Quality			
Unsatisfactory (at least	29 (24.4)	90 (75.6)	0.21
Satisfactory	54 (31.0)	120 (69.0)	
Anal Biopsy Result			
HSIL	40 (33.1)	81 (66.9)	0.16
<HSIL	41 (25.5)	120 (74.5)	

4.6 References

1. Handelsman DJ. Global trends in testosterone prescribing, 2000-2011: expanding the spectrum of prescription drug misuse. Med J Aust. 2013;199(8):548-51.

2. Lunenfeld B, Mskhalaya G, Zitzmann M, Arver S, Kalinchenko S, Tishova Y, et al. Recommendations on the diagnosis, treatment and monitoring of hypogonadism in men. Aging Male. 2015;18(1):5-15. doi: 10.3109/13685538.2015.1004049. PubMed PMID: 25657080; PubMed Central PMCID: PMC4648196.

3. Corona G, Rastrelli G, Forti G, Maggi M. Update in testosterone therapy for men. J Sex Med. 2011;8(3):639-54; quiz 55. doi: 10.1111/j.1743-6109.2010.02200.x. PubMed PMID: 21711483.

4. Basaria S. Male hypogonadism. Lancet. 2014;383(9924):1250-63. doi: 10.1016/S0140-6736(13)61126-5. PubMed PMID: 24119423.

5. Huo S, Scialli AR, McGarvey S, Hill E, Tugertimur B, Hogenmiller A, et al. Treatment of Men for "Low Testosterone": A Systematic Review. PLoS One. 2016;11(9):e0162480. doi: 10.1371/journal.pone.0162480. PubMed PMID: 27655114; PubMed Central PMCID: PMC5031462

6. Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, et al. Testosterone therapy in adult men with androgen deficiency syndromes: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2006;91(6):1995-2010. doi: 10.1210/jc.2005-2847. PubMed PMID: 16720669.

7. Morgan DJ, Dhruva SS, Wright SM, Korenstein D. 2016 Update on Medical Overuse: A Systematic Review. JAMA Intern Med. 2016;176(11):1687-92. doi: 10.1001/jamainternmed.2016.5381. PubMed PMID: 27654002.

8. Jasuja GK, Bhasin S, Reisman JI, Berlowitz DR, Rose AJ. Ascertainment of Testosterone Prescribing Practices in the VA. Med Care. 2015;53(9):746-52. doi: 10.1097/MLR.0000000000000398. PubMed PMID: 26196850.

9. Gomes AR, Souteiro P, Silva CG, Sousa-Pinto B, Almeida F, Sarmento A, et al. Prevalence of testosterone deficiency in HIV-infected men under antiretroviral therapy. BMC Infect Dis. 2016;16(1):628. doi: 10.1186/s12879-016-1892-5. PubMed PMID: 27809804; PubMed Central PMCID: PMC5096002.

10. Corona G, Rastrelli G, Maggi M. Diagnosis and treatment of late-onset hypogonadism: systematic review and meta-analysis of TRT outcomes. Best Pract Res Clin Endocrinol Metab. 2013;27(4):557-79. doi: 10.1016/j.beem.2013.05.002. PubMed PMID: 24054931.

11. Kaplan B, Aschkenazi-Steinberg S, Yogev Y, Nahum R, Sulkes J, Phisher M. Gynecologists' trends and attitudes toward prescribing hormone replacement therapy during menopause. Menopause. 2002;9(5):354-9. PubMed PMID: 12218724.

12. Chang C, Lin CH. Hormone replacement therapy and menopause: a review of randomized, double-blind, placebo-controlled trials. Kaohsiung J Med Sci. 2003;19(6):257-70. doi: 10.1016/S1607-551X(09)70472-3. PubMed PMID: 12873034.

13. Weiderpass E, Adami HO, Baron JA, Magnusson C, Bergstrom R, Lindgren A, et al. Risk of endometrial cancer following estrogen replacement with and without progestins. J Natl Cancer Inst. 1999;91(13):1131-7. PubMed PMID: 10393721.

14. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA. 2002;288(3):321-33. PubMed PMID: 12117397.

15. Munoz N, Franceschi S, Bosetti C, Moreno V, Herrero R, Smith JS, et al. Role of parity and human papillomavirus in cervical cancer: the IARC multicentric case-control study. Lancet. 2002;359(9312):1093-101. PubMed PMID: 11943256.

16. Smith JS, Green J, Berrington de Gonzalez A, Appleby P, Peto J, Plummer M, et al. Cervical cancer and use of hormonal contraceptives: a systematic review. Lancet. 2003;361(9364):1159-67. PubMed PMID: 12686037.

17. Moreno V, Bosch FX, Munoz N, Meijer CJ, Shah KV, Walboomers JM, et al. Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case-control study. Lancet. 2002;359(9312):1085-92. doi: 10.1016/S0140-6736(02)08150-3. PubMed PMID: 11943255.

18. Singer A. The uterine cervix from adolescence to the menopause. Br J Obstet Gynaecol. 1975;82(2):81-99. PubMed PMID: 1125147.

19. Burk RD, Ho GY, Beardsley L, Lempa M, Peters M, Bierman R. Sexual behavior and partner characteristics are the predominant risk factors for genital human papillomavirus infection in young women. J Infect Dis. 1996;174(4):679-89. PubMed PMID: 8843203.

20. Ley C, Bauer HM, Reingold A, Schiffman MH, Chambers JC, Tashiro CJ, et al. Determinants of genital human papillomavirus infection in young women. J Natl Cancer Inst. 1991;83(14):997-1003. PubMed PMID: 1649312.

21. Chung SH, Franceschi S, Lambert PF. Estrogen and ERalpha: culprits in cervical cancer? Trends Endocrinol Metab. 2010;21(8):504-11. doi: 10.1016/j.tem.2010.03.005. PubMed PMID: 20456973; PubMed Central PMCID: PMC2914219.

22. Hormonal contraceptives, progestogens only. IARC Monogr Eval Carcinog Risks Hum. 1999;72:339-97. PubMed PMID: 10533178.

23. Prevention CfDCa. HPV and Cancer 2017 [updated March 3, 2017; cited 2017 June 15]. Available from: https://www.cdc.gov/cancer/hpv/statistics/cases.htm.

24. Abramowitz L, Jacquard AC, Jaroud F, Haesebaert J, Siproudhis L, Pradat P, et al. Human papillomavirus genotype distribution in anal cancer in France: the EDiTH V study. International

Journal of Cancer. 2011;129(2):433-9. Epub 2010/09/15. doi: 10.1002/ijc.25671. PubMed PMID: 20839262.

25. Valmary-Degano S, Jacquin E, Prétet JL, Monnien F, Girardo B, Arbez-Gindre F, et al. Signature patterns of human papillomavirus type 16 in invasive anal carcinoma. Human Pathology. 2013;44(6):11. Epub 2012 Dec 23. doi: 10.1016/j.humpath.2012.08.019.

26. International Agency for Research on Cancer (IARC) Working Group on Evaluation of Carcinogenic Risks to Humans. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Volume 90 Human Papillomaviruses. Lyon, France: World Health Organization, International Agency for Research on Cancer; 2007. 689 p.

27. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. A review of human carcinogens--Part B: biological agents. Lancet Oncology. 2009;10(4):321-2. Epub 2009/04/08. PubMed PMID: 19350698.

28. SEER S, Epidemiology and End Results Program,. SEER Cancer Statistics Factsheets: Anal Cancer Bethesda, MD: National Cancer Institute; [cited 2015 July 29]. Available from: http://seer.cancer.gov/statfacts/html/anus.html.

29. Machalek DA, Poynten M, Jin F, Fairley CK, Farnsworth A, Garland SM, et al. Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: a systematic review and meta-analysis. Lancet Oncol. 2012;13(5):487-500. doi: 10.1016/S1470-2045(12)70080-3. PubMed PMID: 22445259.

30. Silverberg MJ, Lau B, Justice AC, Engels E, Gill MJ, Goedert JJ, et al. Risk of anal cancer in HIV-infected and HIV-uninfected individuals in North America. Clin Infect Dis. 2012;54(7):1026-34. doi: 10.1093/cid/cir1012. PubMed PMID: 22291097; PubMed Central PMCID: PMC3297645.

31. Monroe AK, Dobs AS, Palella FJ, Kingsley LA, Witt MD, Brown TT. Morning free and total testosterone in HIV-infected men: implications for the assessment of hypogonadism. AIDS Res Ther. 2014;11(1):6. doi: 10.1186/1742-6405-11-6. PubMed PMID: 24450960; PubMed Central PMCID: PMC3900935.

32. Busnelli A, Somigliana E, Vercellini P. 'Forever Young'-Testosterone replacement therapy: a blockbuster drug despite flabby evidence and broken promises. Hum Reprod. 2017;32(4):719-24. doi: 10.1093/humrep/dex032. PubMed PMID: 28333214.

33. Tyagi V, Scordo M, Yoon RS, Liporace FA, Greene LW. Revisiting the role of testosterone: Are we missing something? Rev Urol. 2017;19(1):16-24. PubMed PMID: 28522926; PubMed Central PMCID: PMC5434832.

34. Corona G, Rastrelli G, Maseroli E, Sforza A, Maggi M. Corrigendum: Testosterone Replacement Therapy and Cardiovascular Risk: A Review. World J Mens Health. 2016;34(2):154. doi: 10.5534/wjmh.2016.34.2.154. PubMed PMID: 27574600; PubMed Central PMCID: PMC4999491.

35. Golla V, Kaplan AL. Testosterone Therapy on Active Surveillance and Following Definitive Treatment for Prostate Cancer. Curr Urol Rep. 2017;18(7):49. doi: 10.1007/s11934-017-0695-6. PubMed PMID: 28589395.

36. Hackett GI. Testosterone Replacement Therapy and Mortality in Older Men. Drug Saf. 2016;39(2):117-30. doi: 10.1007/s40264-015-0348-y. PubMed PMID: 26482385.

37. Desroches B, Kohn TP, Welliver C, Pastuszak AW. Testosterone therapy in the new era of Food and Drug Administration oversight. Transl Androl Urol. 2016;5(2):207-12. doi: 10.21037/tau.2016.03.13. PubMed PMID: 27141448; PubMed Central PMCID: PMC4837303.

38. Seftel AD. Re: Testosterone Products: Drug Safety Communication - FDA Cautions about Using Testosterone Products for Low Testosterone due to Aging; Requires Labeling Change to Inform of Possible Increased Risk of Heart Attack and Stroke. J Urol. 2015;194(3):759-60. doi: 10.1016/j.juro.2015.06.058. PubMed PMID: 26292880.

39. Bhatia R, Murphy AB, Raper JL, Chamie G, Kitahata MM, Drozd DR, et al. Testosterone replacement therapy among HIV-infected men in the CFAR Network of Integrated Clinical Systems. AIDS. 2015;29(1):77-81. doi: 10.1097/QAD.0000000000000521. PubMed PMID: 25387318; PubMed Central PMCID: PMC4379711.

40. Goldstone SE, Winkler B, Ufford LJ, Alt E, Palefsky JM. High prevalence of anal squamous intraepithelial lesions and squamous-cell carcinoma in men who have sex with men as seen in a surgical practice. Dis Colon Rectum. 2001;44(5):690-8.

41. Palefsky JM, Holly EA, Hogeboom CJ, Berry JM, Jay N, Darragh TM. Anal cytology as a screening tool for anal squamous intraepithelial lesions. Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology. 1997;14(5):415-22.

42. Barut MU, Kale A, Kuyumcuoglu U, Bozkurt M, Agacayak E, Ozekinci S, et al. Analysis of Sensitivity, Specificity, and Positive and Negative Predictive Values of Smear and Colposcopy in Diagnosis of Premalignant and Malignant Cervical Lesions. Med Sci Monit. 2015;21:3860-7. PubMed PMID: 26655816; PubMed Central PMCID: PMC4678924.

43. Rijkaart DC, Berkhof J, van Kemenade FJ, Coupe VM, Rozendaal L, Heideman DA, et al. HPV DNA testing in population-based cervical screening (VUSA-Screen study): results and implications. Br J Cancer. 2012;106(5):975-81. doi: 10.1038/bjc.2011.581. PubMed PMID: 22251922; PubMed Central PMCID: PMC3305964.

44. Darragh TM, Winkler B. Anal cancer and cervical cancer screening: key differences. Cancer Cytopathol. 2011;119(1):5-19. Epub 2011/02/15. doi: 10.1002/cncy.20126. PubMed PMID: 21319310.

45. Peitzmeier SM, Reisner SL, Harigopal P, Potter J. Female-to-male patients have high prevalence of unsatisfactory Paps compared to non-transgender females: implications for cervical cancer screening. J Gen Intern Med. 2014;29(5):778-84. doi: 10.1007/s11606-013-2753-1. PubMed PMID: 24424775; PubMed Central PMCID: PMC4000345.

46. Nayar R, Wilbur DC. The Pap Test and Bethesda 2014. "The reports of my demise have been greatly exaggerated." (after a quotation from Mark Twain). Acta Cytol. 2015;59(2):121-32. doi: 10.1159/000381842. PubMed PMID: 25997404.

47. Zhao C, Domfeh AB, Austin RM. Histopathologic outcomes and clinical correlations for high-risk patients screened with anal cytology. Acta Cytol. 2012;56(1):62-7. doi: 10.1159/000331431. PubMed PMID: 22236747.

48. Conley L, Bush T, Darragh TM, Palefsky JM, Unger ER, Patel P, et al. Factors associated with prevalent abnormal anal cytology in a large cohort of HIV-infected adults in the United States. J Infect Dis. 2010;202(10):1567-76. doi: 10.1086/656775. PubMed PMID: 20925532.

49. Cranston RD, Hart SD, Gornbein JA, Hirschowitz SL, Cortina G, Moe AA. The prevalence, and predictive value, of abnormal anal cytology to diagnose anal dysplasia in a population of HIV-positive men who have sex with men. Int J STD AIDS. 2007;18(2):77-80. doi: 10.1258/095646207779949772. PubMed PMID: 17331275.

50. Fontaine D, Narine N, Naugler C. Unsatisfactory rates vary between cervical cytology samples prepared using ThinPrep and SurePath platforms: a review and meta-analysis. BMJ Open. 2012;2(2):e000847. doi: 10.1136/bmjopen-2012-000847. PubMed PMID: 22505312; PubMed Central PMCID: PMC3332241.

51. Bofin AM, Nygard JF, Skare GB, Dybdahl BM, Westerhagen U, Sauer T. Papanicolaou smear history in women with low-grade cytology before cervical cancer diagnosis. Cancer. 2007;111(4):210-6. doi: 10.1002/cncr.22865. PubMed PMID: 17567833.

52. Hock YL, Ramaiah S, Wall ES, Harris AM, Marston L, Marshall J, et al. Outcome of women with inadequate cervical smears followed up for five years. J Clin Pathol. 2003;56(8):592-5. PubMed PMID: 12890808; PubMed Central PMCID: PMC1770040.

53. Owens CL, Buist DS, Peterson D, Kamineni A, Weinmann S, Ross T, et al. Follow-up and clinical significance of unsatisfactory liquid-based Papanicolaou tests. Cancer Cytopathol. 2015;123(1):59-65. doi: 10.1002/cncy.21490. PubMed PMID: 25346238; PubMed Central PMCID: PMC4356524.

54. Detels R, Jacobson L, Margolick J, Martinez-Maza O, Munoz A, Phair J, et al. The Multicenter AIDS Cohort Study, 1983 to ...,. Public Health. 2012;126(3):196-8. doi: 10.1016/j.puhe.2011.11.013. PubMed PMID: 22206985; PubMed Central PMCID: PMC3324261.

55. Kaslow RA, Ostrow DG, Detels R, Phair JP, Polk BF, Rinaldo CR, Jr. The Multicenter AIDS Cohort Study: rationale, organization, and selected characteristics of the participants. American Journal of Epidemiology. 1987;126(2):310-8.

56. Hillman RJ, Cuming T, Darragh T, Nathan M, Berry-Lawthorn M, Goldstone S, et al. 2016 IANS International Guidelines for Practice Standards in the Detection of Anal Cancer Precursors. J Low Genit Tract Dis. 2016;20(4):283-91. doi: 10.1097/LGT.0000000000000256. PubMed PMID: 27561134.

57. Felix JC, Lonky NM, Tamura K, Yu KJ, Naidu Y, Lai CR, et al. Aberrant expression of E-cadherin in cervical intraepithelial neoplasia correlates with a false-negative Papanicolaou smear. Am J Obstet Gynecol. 2002;186(6):1308-14. PubMed PMID: 12066114.

Chapter 5. Concluding Remarks

Anal cancer incidence is increasing in the U.S. for all men and women, remains disparately higher in older HIV-infected and -uninfected MSM [1-3]. Currently, there is no standard procedure for screening for anal high-grade squamous intraepithelial lesions (hHSILs) due to insufficient data, leaving screening underdeveloped and not widely available. As a result, many older MSM are never screened for anal cancer, despite being at higher risk than the general population [1-5]. Successful practices for cervical cancer screening may inform anal screening, though are inadequate as there remain unique differences between the two organs. The main goal of this dissertation is to provide more data on anal HPV infection and hHSIL in older MSM, in hopes of guiding the development of effective screening practices to ultimately reduce and prevent anal cancer.

Chapter 2 sought to characterize anal HPV infections in older MSM, and identify risk factors for incidence and clearance of infections. The results showed that, using PCR testing, anal HPV infections are common in this older group of MSM. Incidence and clearance of HPV 16 and 18 remains high, especially among HIV-infected men, suggesting ongoing HPV exposure with age. Infection with HPV16 and 18 was found in a quarter of the sample at baseline, and several risk factors were found to be associated with incidence and clearance, that may function by increasing exposure or influence susceptibility. Overall, risk of anal HPV persistence was high, confirming the disproportionate risk MSM confer for HPV-related anal cancer.

Chapter 3 aimed to determine whether anal high-risk HPV testing could be used as a primary screening tool when compared to anal cytology to detect hHSIL in older HIV-infected and-uninfected MSM. The findings suggest testing for persistent HPV16/18-positivity, even using a low-threshold PCR assay, may be an effective alternative to cytology. Estimates were higher than anal cytology, and even when testing was paired with cytology, there was no improvement from testing for persistent HPV16/18 alone. This suggests screening for persistent HPV16/18 alone could maintain standard clinical performance, while minimizing resources over paired testing. The

high hHSIL prevalence in this sample (54%), especially when compared to a 2% estimate observed in HIV-uninfected women [6], emphasizes the urgent need for developing a standard approach to screening.

Chapter 4 aimed to perform a novel exploration of the potential risk of testosterone replacement therapy (TRT) on anal cytology screening and hHSIL in older MSM. Results showed history of TRT was commonly reported in this group of older HIV-infected and –uninfected MSM, found in almost half of all men. No association was found between TRT and unsatisfactory cytology, however, the swab type and order was significant which may inform swab collection practices that implement multiple swabs or nylon-flocked swabs. No relationship was found between TRT and hHSIL, however hHSIL prevalence was common, stressing the need for effective screening practices. Due to limitations of self-report, this study should be repeated using biological measurements for serum testosterone since physiological uptake of TRT varies by the individual and therapy used.

The findings from this dissertation further expand the limited knowledge of anal HPV infection and hHSIL in older MSM. While limitations remain, sound research methodology was employed to conduct these analyses. This dissertation uniquely contributes longitudinal data from a large cohort of older MSM where similar longitudinal studies are very sparse. It also supports the use of hrHPV testing as a primary screening tool, which has greater potential to be more reliable and efficient than recommended anal cytology. Lastly, this dissertation employed a novel exploration on TRT and anal disease, which warrants more research on its long-term effects as TRT is frequently prescribed in HIV-infected MSM for whom risk for other disease is high. This dissertation contributes data to the study of anal cancer and may help to guide the development of anal cancer screening and the direction of future related research.

5.1 References

1. SEER S, Epidemiology and End Results Program,. SEER Cancer Statistics Factsheets: Anal Cancer Bethesda, MD: National Cancer Institute; [cited 2015 July 29]. Available from: http://seer.cancer.gov/statfacts/html/anus.html.

2. D'Souza G, Wiley DJ, Li X, Chmiel JS, Margolick JB, Cranston RD, et al. Incidence and epidemiology of anal cancer in the multicenter AIDS cohort study. J Acquir Immune Defic Syndr. 2008;48(4):491-9. PubMed PMID: 18614927.

3. Silverberg MJ, Lau B, Justice AC, Engels E, Gill MJ, Goedert JJ, et al. Risk of anal cancer in HIV-infected and HIV-uninfected individuals in North America. Clin Infect Dis. 2012;54(7):1026-34. doi: 10.1093/cid/cir1012. PubMed PMID: 22291097; PubMed Central PMCID: PMC3297645.

4. (IARC) IAfRoC. GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012 2012 [cited 2015 07/13]. Available from: http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx.

5. Machalek DA, Poynten M, Jin F, Fairley CK, Farnsworth A, Garland SM, et al. Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: a systematic review and meta-analysis. Lancet Oncol. 2012;13(5):487-500. doi: 10.1016/S1470-2045(12)70080-3. PubMed PMID: 22445259.

6. Holly EA, Ralston ML, Darragh TM, Greenblatt RM, Jay N, Palefsky JM. Prevalence and risk factors for anal squamous intraepithelial lesions in women. J Natl Cancer Inst. 2001;93(11):843-9.