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Title page

Title

Antibody responses following incident anal and penile infection with human papillomavirus in teenage men who have sex with men

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Running title

HPV serological responses in MSM

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Abbreviations

HPV: human papillomavirus

MSM: men who have sex with men

AIN: Anal intraepithelial neoplasia

ART: anti-retroviral treatment

SEER: Surveillance, Epidemiology, and End Results Program

OR: odds ratios

CI: confidence intervals

IQR: interquartile range

Word count

Abstract: 248; text: 3319; 3 tables and 2 figures

Brief description

Anal infection with high risk HPV types can lead to anal cancer. In this paper we describe HPV antibody responses following incident anal infection within a cohort of teenage men who have sex with men in the very early stages of their sexual careers.

Clinical trial registration

Registry URL:

https://clinicaltrials.gov/ct2/show/NCT01422356?term=HPV&state1=PA%3AAU%3AVC&rank=8

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Abstract

Men who have sex with men (MSM) are at risk for human papillomavirus (HPV) related anal cancer. Few data exist on antibody responses following incident anogenital infection with HPV in teenage MSM. A cohort of 200 MSM aged 16-20 years from Melbourne, Australia were assessed at baseline, 3, 6, and 12 months. At each visit anal and penile swabs were collected for HPV DNA and serum for HPV antibodies for genotypes 6, 11, 16, and 18 (Merck's Multiplex Assays using Luminex). The main outcome, seroconversion, was defined as the detection of HPV antibodies following a negative antibody result for the same HPV type at baseline. The seroincidence rates for HPV types 6, 11, 16, and 18 were: 19 (95% CI 12-26), 7 (3-12), 4 (1-8), and 6 (3-11) per 100 person-years respectively. Men who experienced incident anal HPV infections from types 6/11 were significantly more likely to develop serum antibodies to the same HPV type(s) than those who experienced incident anal infections from types 16/18 [73% vs 18%, Odds ratio (OR) = 15, 95% CI: 2 -118]. The median time between incident anal HPV infection and seroconversion for HPV 6, 11, 16, and 18 was: 91, 38, 161 and 182 days respectively. Antibody responses against HPV types 6/11 were significantly more likely to occur following incident anal compared with incident penile infection with HPV types 6/11 (OR=6, 95% CI: 2-21). The likelihood of antibody responses following anogenital HPV infections depends on the HPV type and site of infection.

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Text

Introduction

Human papillomavirus (HPV) infection is common among men who have sex with men (MSM) and a cause of anogenital warts as well as anal cancer.[1] Anal cancers are overrepresented among HIV positive MSM.[2] Anogenital warts are most commonly caused by HPV types 6 and 11. The majority of anal cancers are caused by HPV types 16 and some anal cancers are caused by HPV types 18. In men the quadrivalent HPV vaccine is effective at preventing infection with HPV types 6, 11, 16, and 18 and related anogenital lesions.[3] With high coverage of the target population HPV vaccination should help to prevent anal cancer.[4]

Data suggest that in some populations of MSM the incidence of anal cancer has increased.[5] This includes increases in the incidence of anal cancer among HIV positive MSM despite the greater uptake of anti-retroviral treatment (ART) which has been associated with a fall in other HIV-associated cancers.[6, 7] Data from the US National Cancer Institute show that the 5-year survival for patients diagnosed with anal cancer to be 65.7% with poorer outcomes for more advanced cancers.[8]

Although only a proportion of anogenital HPV infections elicit a serum antibody response, antibodies following natural infection with HPV can provide data on cumulative exposure to HPV. In contrast, cross-sectional studies of genital HPV DNA provide information about current HPV infection.[9, 10] In women, high titres of

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naturally acquired antibodies to HPV 16 have been shown to be associated with a reduction in the risk of subsequent infection with HPV 16 suggesting that antibody mediated immune responses following natural infection may confer some degree of protection against repeat infection.[11, 12]

The probability and timing of type specific HPV antibody responses following incident HPV genital infection of the same type have been compared in prospective cohorts of women and heterosexual males.[13, 14] While a number of studies have examined HPV seroprevalence and seroincidence in MSM,[15-17] no previous studies have determined the rates of HPV antibody responses following incident anogenital HPV infection among teenage MSM. Most previous HPV serological surveys of MSM have been in older MSM who would on the whole have had greater exposure to HPV and therefore a higher likelihood of prior immune responses to HPV. These include HIV positive MSM who would be expected to have been at high sexual risk and among whom antibody responses may be altered because of immune suppression.

The primary aim of this study was to examine type-specific HPV serum antibody responses following incident infections of the anus and penis with HPV types 6, 11, 16, and 18 in a cohort of teenage MSM in the HPV in the Young People Epidemiological Research (HYPER) Study.[18, 19] The HYPER Study included MSM with limited sexual experiences and therefore for many their likely first exposure and immune response to HPV occurred during the study.

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Material and Methods

Men were recruited to the HYPER Study between October 2010 and September 2012 via a number of avenues in Melbourne, Australia including the gay community, clubs, universities, and sexual health clinics. Eligible participants were men aged 16 and 20 years reporting sex with men of any age. Details of the study methods are described elsewhere.[18] Briefly, men provided informed consent and attended study visits at baseline, then at 3, 6, and 12 months when specimens were obtained for HPV DNA testing using separate swabs from each of the following sites: anal canal, perianus, penis, and an oral rinse. In addition, blood was obtained for HPV serology at each visit.[18] Men were screened for HIV at baseline. Men also completed questionnaires at each visit which included details on sexual behaviours and whether the participant had been diagnosed with anal or genital warts.

Laboratory methods

HPV DNA detection and genotyping

Samples were tested at the Regional HPV Labnet Reference Laboratory, Molecular Microbiology Department, Royal Women's Hospital, Melbourne. Details of the HPV DNA testing methods have been published elsewhere.[18] Briefly, extracted DNA was amplified using Ll consensus primers PGMY09/11. Amplification products were hybridised with a biotin-labelled HPV Ll generic probe and captured on streptavidin-coated plates (Roche Biochemicals). The bound hybrid was detected by

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an anti-digoxigenin peroxidase conjugate by use of the colourimetic substrate ABTS. Samples that were positive by HPV L1 consensus were further genotyped by HPV Linear Array genotyping assay (Roche Molecular Systems, Pleasanton, California) for detection of 37 HPV genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 82v, and 89).

HPV serology

Serum samples were tested at PPD Vaccines and Biologics Lab, Wayne, Pennsylvania. Antibodies against HPV types 6, 11, 16, and 18 were measured using Merck's multiplexed, type specific competitive Luminex immunoassay as previously described.[20] Seropositivity was defined using the following cut offs previously established based on milli Merck units per mL (mMU/mL): HPV 6 (≥20 mMU/mL); HPV 11 (≥16 mMU/mL); HPV 16 (≥20 mMU/mL); and HPV 18 (≥24 mMU/mL).[21]

This study was approved by the Alfred Hospital Research Ethics Committee (174/10) and the University of Melbourne Ethics Committee (1034462). HYPER was registered on the Australia New Zealand Clinical Trials Registry (ACTRN12611000857909) and with the National Institutes of Health (NCT01422356).

Statistical analyses

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The sample size (n=200) was chosen to provide acceptable upper and lower 95% CIs around the expected proportion of men with any HPV DNA at each timepoint: between 2% and 7%. Confidence intervals for proportions were calculated using exact methods.

Incident HPV infection was defined as the detection of HPV DNA on at least one follow-up visit following a negative HPV DNA result at baseline from the same site and the same HPV type. Based on positive results for any HPV DNA at baseline in which anal (30.8%, 61/198) and perianal (30.6%, 60/196) HPV DNA detection was almost identical (60 of the anal HPV types were the same as 60 of the perianal types), we tested anal and perianal DNA as a combined sample and report the results here combined as anal DNA.[18, 19] Only one case of oral HPV infection from among the quadrivalent vaccine types was detected at baseline (HPV16) with no incident oral infections from quadrivalent types during follow-up.[18, 19] As such, we excluded oral HPV DNA from further analyses.

Seroprevalence was defined as seropositivity for a particular HPV type at baseline. Seroprevalence ratios comparing seroprevalence in men with and without DNA of the same HPV type as found on serology detected at the anus or penis during the study, and their 95% CIs, were calculated.

Seroconversion, the main outcome of interest, was defined as the detection of HPV

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antibodies following incident HPV infection of the same type. Seroincidence was calculated for each HPV type based on seroconversion expressed in person-years. Rates of HPV seroconversion following incident HPV infection were calculated for each specific HPV type, for example, seroconversion with HPV16 specific antibodies following incident HPV16 DNA detection.

Throughout the paper, "HPV 6/11" refers to HPV 6 and/or 11; "HPV 16/18" refers to HPV 16 and/or 18. Univariate Cox regression was used to calculate the hazard ratio (HR) of seroconversion based on the number of recent receptive anal sex partners. Cox regression clustered on individual participants was used to calculate the hazard ratio for seroconversion comparing scenarios with incident anal HPV and those without. Kaplan Meier plots with log-rank tests were used to compare the cumulative proportion of seroconversions from baseline between men with and without incident anal HPV infection of the same type. This analysis was separated by low (6/11) and high (16/18) risk HPV types, excluding men with prevalent or incident penile HPV DNA and those with prevalent anal HPV DNA of the same type. These exclusions were made to ensure calculation of the association between seroconversion and incident HPV infection. Logistic regression accounting for repeat observations from individual participants was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for site-specific HPV seroconversion rates for HPV 6/11 versus HPV16/18. Logistic regression accounting for repeat observations from individual participants was also used to calculate OR and 95% CI for HPV seroconversion rates

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between men with incident anal infection and men with incident penile infection for HPV 6/11, and then 16/18. The repeat observations were accounted for as each participant was counted multiple times to categorise them based on site (anus vs penis) and incident HPV infection (yes vs no). Statistical analyses were conducted using STATA 13.

Results

Characteristics of study population

The median age of the 200 men was 19 years (IQR 18-20). Median age at first insertive or receptive anal sex was 17 years. Men had a median of 3 insertive and 3 receptive anal sex partners in their lifetime. From baseline to 12 months, men reported a median of 2 insertive and 3 receptive anal sex partners with 54% reporting inconsistent condom use for either insertive or receptive anal sex. At baseline all men tested negative for HIV. More detailed demographic characteristics and sexual behaviours are reported elsewhere.[18, 22] No men received HPV vaccination before or during the study period.[23]

Seroprevalence of HPV at baseline

The type specific seroprevalence rates for HPV 6, 11, 16, and 18 among the 200 men at baseline are shown in Table 1. Prevalent HPV seropositivity ranged from 3.0% for HPV 18 to 12.5% for HPV 6. The proportion of men who were seropositive for HPV types 6/11 was significantly higher than for HPV types 16/18 (16.5% versus 5.5% p

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<0.001). Baseline type specific seropositivity was significantly higher among those who had DNA of the same HPV type detected at any site at any study visit for HPV types 6 (p<0.001), 11 (p<0.001), and 16 (p=0.001) but not for HPV 18 (p=0.114).

Patterns of HPV seropositivity

Among the 200 men enrolled in the study, 168 (84.0%) completed the study to the 12 month visit. Patterns of HPV seropositivity to types 6, 11, 16, and 18 observed over the 12 month period of follow up are illustrated in Figure 1. Most men who were seropositive to one of the HPV types at baseline remained seropositive to that type through to the 12 month visit: 76.2% (16/21) for HPV 6; 87.5% (7/8) for HPV 11; 100.0% (6/6) for HPV 16; and 83.3% (5/6) for HPV 18.

Seroincidence of HPV

A quarter of men seroconverted to at least one of the four HPV types during 12 months of follow up (Table 2). The seroincidence rates for HPV types 6, 11, 16, and 18 were: 18.8 (95% CI: 12.5-26.5), 6.7 (95% CI: 3.2-11.9), 3.9 (95% CI: 1.4-8.2), and 5.8 (95% CI: 2.7-10.7) per 100 person-years respectively. Most men developed seropostivity to only one of these four types. In addition, six men developed antibodies to both HPV 6 and 11; two men to both HPV 16 and 18; and one man to all four HPV types. No association was found between the likelihood of seroconversion and the number of receptive anal sex partners in the 3 months prior to seroconversion (≤1 vs >1, HR=2.33, 95%CI: 0.90-6.03).

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The proportion of incident HPV DNA infections associated with seroconversion to the same HPV type is shown in Table 3. Men who experienced incident anal HPV infections from types 6/11 were significantly more likely to develop serum antibodies to the same HPV type than those who experienced incident anal infections from types 16 and 18 (72.7% vs 18.2%, OR = 12.0, 95% CI: 2.5-58.1). However, the likelihood of seroconverting did not differ between men who experienced incident penile HPV infections from types 6/11 and those who experienced incident penile infections from types 16/18 (42.9% vs 20.0%, OR=3.0, 95%CI: 0.2-53.2). The median time between incident anal infection and seroconversion for HPV 6, 11, 16 and 18 was: 91 days (range 0-281), 46 days (range 0-106), 165 days, and 183 days (range 85-259), respectively. The number of seroconversions following incident penile infections was too small to allow an estimation of the median time between penile infection and seroconversion.

Serum antibody responses against HPV types 6/11 were significantly more likely to occur following incident anal compared with incident penile infection with HPV types 6/11 (OR=5.9, 95% CI: 1.7-21.0) (Table 3). Serum antibody responses against HPV types 16/18 following incident anal and incident penile infections with HPV types 16/18 were similar (OR=0.99, 95% CI: 0.06-15.92).

Cumulative HPV seroincidence

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The cumulative proportion of HPV seroconversions following incident anal HPV infection are shown in Figure 2. Figure 2A shows the cumulative proportion of seroconversions to HPV 6/11 stratified by the presence or absence of incident anal HPV 6/11 DNA infection. Men with prevalent or incident penile HPV 6/11 DNA and those with prevalent anal HPV 6/11 DNA were excluded from this analysis. Figure 2B shows the cumulative proportion of seroconversions to HPV 16/18 stratified by the presence or absence of incident anal HPV 16/18 DNA infection. Men with prevalent or incident penile HPV 16/18 DNA and those with prevalent anal HPV 16/18 DNA were excluded from this analysis. For the above comparisons in seroconversion, we applied sensitivity analyses by including person-time from baseline to incident penile HPV infection for HPV 6/11 (log-rank test $p<0.001$) and HPV 16/18 (log-rank test $p<0.001$).

Men with incident anal HPV DNA types 6/11 were more likely to develop antibodies to HPV types 6/11 than men without incident anal HPV DNA 6/11 (hazard ratio (HR) = 22.1, 95% CI: 9.7-50.1). Similarly, but to a lesser degree, men with incident anal HPV DNA types 16/18 were more likely to develop antibodies to types 16/18 than men without incident anal HPV DNA 16/18 (HR = 8.9, 95% CI: 2.2-35.1).

During follow up, 4 men reported the development of new anal warts, all 4 of whom seroconverted to HPV 6, with 2 also seroconverting to HPV11. One man reported the development of new penile warts but did not seroconvert to either HPV 6/11.

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Discussion

To our knowledge this is the first study to examine serum antibody responses to HPV following incident anal infection with HPV in teenage MSM. The results indicate that the likelihood of antibody responses following anogenital HPV infections among young MSM depended on the HPV type and site of infection. Seroconversion after anal HPV infection was more likely following infection with HPV types 6/11 compared with 16/18. Furthermore, antibody responses to HPV 6/11 were more likely following anal compared with penile infection. Seroconversion occurred at a variable number of months after anal HPV infection depending on HPV type.

In a study of women aged 18-20 by Carter *et al*. serum antibody responses to HPV types 6, 16, and 18 occurred in 68.8%, 59.5% and 54.1% of women following incident genital DNA infection with the same HPV type.[13] In contrast, a much lower rate of seroconversion was observed among heterosexual males in a study by Edelstein *et al.*, where only 7.1% of men developed serum antibody responses to HPV type 16 after incident genital HPV 16 infection.[14] In that study anal HPV was not included. In our study, MSM who encountered incident anal HPV infection from low risk HPV types (6/11) were significantly more likely to develop type specific antibody responses than those who experienced incident anal infections from high risk HPV types (16/18) with respective seroconversion rates of 72.7% compared with 18.2%.

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In all three studies, serum antibodies following infection at three different anatomical sites (female genital, male genital, and anal) took several months to develop. Among women, the median time between incident genital HPV infection and type specific seroconversion was approximately 12 months with no significant difference between HPV types 6, 16, and 18. Among heterosexual males, the median time between incident genital infection α9 HPV (HPV 16, 31, 33, 35, 52, 58 and 67) infection and type specific seroconversion was 3.9 months. In our study the median time between incident anal infection and seroconversion was variable: for HPV 6, 11, 16, and 18 it was 91 days, 46 days, 165 days, and 183 days respectively.

Animal studies suggest that microtrauma to the epithelium with exposure of the basement membrane and basal cells may be important in the establishment of HPV infection.[24] While the determinants of antibody responses to HPV infection are yet to be fully elucidated, it has been postulated that the higher rate of seroconversion seen in women may be attributable to infection of the mucosal epithelium of the female genital tract, in contrast to the keratinized stratified squamous epithelium lining the penis, with greater likelihood of microtrauma in the former. In contrast to the studies by Carter and Edelstein, HPV infections among MSM in this study were predominantly anal. In theory, the relatively high rate of seroconversion seen with HPV types 6 and 11 in this study could reflect microtrauma to the anorectal epithelium via receptive anal sex. Transmission of HPV from the penis to the anus appears to be easier than transmission from the anus to the penis which is consistent

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with this theory.[17] Our finding that seroconversion to HPV types 6/11 is more likely following anal compared with penile infection is also consistent with this. Why lower seroconversion rates were seen following anal infection with HPV types 16 and 18 warrants further research.

A strength of this study is that males were teenagers relatively early in their sexual experiences meaning that for many these were their first sexual exposures and immune responses to HPV. Another strength is that samples were collected reasonably frequently over 12 months, at baseline, 3, 6 and 12 months.[18] In contrast, previous HPV serological surveys of MSM have included older, more sexually experienced men where the great majority of men are found to have prevalent anal HPV infection.[15-17, 25, 26] Mooij et al., reported data on HPV seroconversion following incident HPV DNA infection in MSM [27]. That study included much older MSM (median age: 38 years for HIV-negative MSM, 47 for HIV-positive MSM) where 38% were HIV-infected. In that study serum samples were collected at baseline and 12 months only.

There are limitations to this study. First, anogenital infection with HPV was defined on the basis of DNA detection at a single time point. It is possible some of these led to detection of inconsequential surface DNA rather than established productive viral infections.[28] However, the high rate of seroconversion with low risk HPV types would suggest most were true infections. There were too few HPV infections defined by

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repeat DNA positivity at two or more time points to compare seroconversion rates between infections defined by persistent HPV DNA of the same type versus cases which might ostensibly represent transient DNA detection. Second, serological follow up was limited to 9 months from incident anogenital infection. It is uncertain whether further seroconversions would have been detected had follow up had been longer. If this was the case, the median time to seroconversion would be longer than our estimation.

In this group of teenage MSM, overall seroincidence rates to HPV types 6, 11, 16 and 18 was high with a quarter of males developing antibodies to at least one of these HPV types over 12 months of follow up. Overall, this was a sexually active group of men with a high incidence of anogenital HPV infection as detailed elsewhere.[18, 19, 22] Seroincidence could differ in other populations of young MSM depending on the extent of anal sex and partner change.

Our study adds to existing literature on HPV antibody responses following exposure to genital HPV by providing data on HPV serological response rates following acquisition of anal HPV, the major site of HPV infection among MSM and the cause of anal cancer. Future research should be directed at determining whether serum antibodies that develop following natural HPV infection of the anus protect against reinfection with the same HPV type in MSM.[29] If antibodies following anal HPV infection are not protective against repeat anal HPV infections then this would make

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the case for HPV vaccination of already sexually active MSM to prevent anal cancer more compelling.

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Author Contributions

This study was conceived and designed by MYC and CKF in consultation with the other authors. HZ designed the questionnaire and was responsible for data collection and analysis. SNT and AMC supervised HPV testing. All authors have contributed to interpretation of data and study findings. HZ, MC and CKF drafted the manuscript with all authors critically reviewing the paper.

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Conflict of Interest Disclosure

This investigator initiated study was funded by Merck. Merck had no input into the design, analysis or reporting of the study. HZ has received travel sponsorship from CSL. CKF has received honoraria from CSL Biotherapies and Merck and research funding from CSL Biotherapies. CKF owns shares in CSL Biotherapies the manufacturer for Gardasil. JSH has received an honorarium from CSL Biotherapies and is an investigator on an Australian Research Council funded project (LP0883831) that includes CSL Biotherapies as a research partner. AEG has received honoraria and untied research funding from CSL biotherapies, and has received honoraria from Merck. SMG has received advisory board fees and grant support from CSL and

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References

1. Schiffman M, Clifford G, Buonaguro FM. Classification of weakly carcinogenic human papillomavirus types: addressing the limits of epidemiology at the borderline. *Infect Agent Cancer* 2009;**4**: 8.

2. Machalek DA, Poynten M, Jin F, Fairley CK, Farnsworth A, Garland SM, Hillman RJ, Petoumenos K, Roberts J, Tabrizi SN, Templeton DJ, Grulich AE. 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**: 487-500.

3. Giuliano AR, Palefsky JM, Goldstone S, Moreira ED, Jr., Penny ME, Aranda C, Vardas E, Moi H, Jessen H, Hillman R, Chang YH, Ferris D, et al. Efficacy of quadrivalent HPV vaccine against HPV Infection and disease in males. *N Engl J Med* 2011;**364**: 401-11.

4. Palefsky JM, Giuliano AR, Goldstone S, Moreira ED, Jr., Aranda C, Jessen H, Hillman R, Ferris D, Coutlee F, Stoler MH, Marshall JB, Radley D, et al. HPV vaccine against anal HPV infection and anal intraepithelial neoplasia. *N Engl J Med* 2011;**365**: 1576-85.

5. Jin F, Stein AN, Conway EL, Regan DG, Law M, Brotherton JM, Hocking J, Grulich AE. Trends in anal cancer in Australia, 1982-2005. *Vaccine* 2011;**29**: 2322-7.

6. Dandapani SV, Eaton M, Thomas CR, Jr., Pagnini PG. HIV- positive anal cancer: an update for the clinician. *J Gastrointest Oncol* 2010;**1**: 34-44.

7. Grulich AE, Poynten IM, Machalek DA, Jin F, Templeton DJ, Hillman RJ. The epidemiology of anal cancer. *Sex Health* 2012;**9**: 504-8.

8. National Cancer Institute 1975-2012. SEER Stat Fact Sheets: Anal Cancer. Available at: http://seer.cancer.gov/statfacts/html/anus.html. Verified on: August 27, 2015.

9. Markowitz LE, Sternberg M, Dunne EF, McQuillan G, Unger ER. Seroprevalence of human papillomavirus types 6, 11, 16, and 18 in the United States: National Health and Nutrition Examination Survey 2003-2004. *J Infect Dis* 2009;**200**: 1059-67.

10. Newall AT, Brotherton JM, Quinn HE, McIntyre PB, Backhouse J, Gilbert L, Esser MT, Erick J, Bryan J, Formica N, MacIntyre CR. Population seroprevalence of human papillomavirus types 6, 11, 16, and 18 in men, women, and children in Australia. *Clin Infect Dis* 2008;**46**: 1647-55.

11. Castellsague X, Naud P, Chow SN, Wheeler CM, Germar MJ, Lehtinen M, Paavonen J, Jaisamrarn U, Garland SM, Salmeron J, Apter D, Kitchener H, et al. Risk of newly detected infections and cervical abnormalities in women seropositive for naturally acquired human papillomavirus type 16/18 antibodies: analysis of the control arm of PATRICIA. *J Infect Dis* 2014;**210**: 517-34.

12. Wentzensen N, Rodriguez AC, Viscidi R, Herrero R, Hildesheim A, Ghosh A, Morales J, Wacholder S, Guillen D, Alfaro M, Safaeian M, Burk RD, et al. A competitive serological assay shows naturally acquired immunity to human papillomavirus infections in the Guanacaste Natural History Study. *J Infect Dis* 2011;**204**: 94-102.

13. Carter JJ, Koutsky LA, Hughes JP, Lee SK, Kuypers J, Kiviat N, Galloway DA. Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. *J Infect Dis* 2000;**181**: 1911-9.

14. Edelstein ZR, Carter JJ, Garg R, Winer RL, Feng Q, Galloway DA, Koutsky LA. Serum antibody response following genital {alpha}9 human papillomavirus infection in young men. *J Infect Dis* 2011;**204**: 209-16.

15. Lu B, Viscidi RP, Wu Y, Nyitray AG, Villa LL, Lazcano-Ponce E, Carvalho da Silva RJ, Baggio ML,

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Quiterio M, Salmeron J, Smith DC, Abrahamsen M, et al. Seroprevalence of human papillomavirus (HPV) type 6 and 16 vary by anatomic site of HPV infection in men. *Cancer Epidemiol Biomarkers Prev* 2012;**21**: 1542-6.

16. van Rijn VM, Mooij SH, Mollers M, Snijders PJ, Speksnijder AG, King AJ, de Vries HJ, van Eeden A, van der Klis FR, de Melker HE, van der Sande MA, van der Loeff MF. Anal, Penile, and Oral High-Risk HPV Infections and HPV Seropositivity in HIV-Positive and HIV-Negative Men Who Have Sex with Men. *PLoS One* 2014;**9**: e92208.

17. Poynten IM, Jin F, Templeton DJ, Prestage GP, Donovan B, Pawlita M, Fairley CK, Garland S, Grulich AE, Waterboer T. Prevalence, incidence, and risk factors for human papillomavirus 16 seropositivity in Australian homosexual men. *Sex Transm Dis* 2012;**39**: 726-32.

18. Zou H, Tabrizi SN, Grulich AE, Garland SM, Hocking JS, Bradshaw CS, Morrow A, Prestage G, Cornall AM, Fairley CK, Chen MY. Early acquisition of anogenital human papillomavirus among teenage men who have sex with men. *J Infect Dis* 2014;**209**: 642-51.

19. Zou H, Tabrizi SN, Grulich AE, Hocking JS, Bradshaw CS, Cornall AM, Morrow A, Prestage G, Law MG, Garland SM, Chen MY, Fairley CK. Site-specific human papillomavirus infection in adolescent men who have sex with men (HYPER): an observational cohort study. *Lancet Infect Dis* 2014.

20. Opalka D, Lachman CE, MacMullen SA, Jansen KU, Smith JF, Chirmule N, Esser MT. Simultaneous quantitation of antibodies to neutralizing epitopes on virus-like particles for human papillomavirus types 6, 11, 16, and 18 by a multiplexed luminex assay. *Clin Diagn Lab Immunol* 2003;**10**: 108-15.

21. Dias D, Van Doren J, Schlottmann S, Kelly S, Puchalski D, Ruiz W, Boerckel P, Kessler J, Antonello JM, Green T, Brown M, Smith J, et al. Optimization and validation of a multiplexed luminex assay to quantify antibodies to neutralizing epitopes on human papillomaviruses 6, 11, 16, and 18. *Clin Diagn Lab Immunol* 2005;**12**: 959-69.

22. Zou H, Prestage G, Fairley CK, Grulich AE, Garland SM, Hocking JS, Bradshaw CS, Cornall AM, Tabrizi SN, Morrow A, Chen MY. Sexual behaviors and risk for sexually transmitted infections among teenage men who have sex with men. *J Adolesc Health* 2014;**55**: 247-53.

23. Zou H, Grulich AE, Cornall AM, Tabrizi SN, Garland SM, Prestage G, Bradshaw CS, Hocking JS, Morrow A, Fairley CK, Chen MY. How very young men who have sex with men view vaccination against human papillomavirus. *Vaccine* 2014;**32**: 3936-41.

24. Roberts JN, Buck CB, Thompson CD, Kines R, Bernardo M, Choyke PL, Lowy DR, Schiller JT. Genital transmission of HPV in a mouse model is potentiated by nonoxynol-9 and inhibited by carrageenan. *Nat Med* 2007;**13**: 857-61.

25. Mooij SH, van der Klis FR, van der Sande MA, Schepp RM, Speksnijder AG, Bogaards JA, de Melker HE, de Vries HJ, Snijders PJ, van der Loeff MF. Seroepidemiology of high-risk HPV in HIV-negative and HIV-infected MSM: the H2M study. *Cancer Epidemiol Biomarkers Prev* 2013;**22**: 1698-708.

26. Heiligenberg M, Alberts CJ, Waterboer T, Speksnijder AG, De Vries HJ, Pawlita M, Schim van der Loeff MF. Route of sexual exposure is independently associated with seropositivity to HPV-16 and HPV-18 among clients of an STI clinic in the Netherlands. *J Infect Dis* 2013;**208**: 1081-5.

27. Mooij SH, Landen O, van der Klis FR, van der Sande MA, de Melker HE, Xiridou M, van Eeden A, Heijman T, Speksnijder AG, Snijders PJ, Schim van der Loeff MF. HPV seroconversion following anal and penile HPV infection in HIV-negative and HIV-infected MSM. *Cancer Epidemiol Biomarkers Prev* 2014;**23**: 2455-61.

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28. Widdice L, Ma Y, Jonte J, Farhat S, Breland D, Shiboski S, Moscicki AB. Concordance and transmission of human papillomavirus within heterosexual couples observed over short intervals. *J Infect Dis* 2013;**207**: 1286-94.

29. Mooij SH, Landen O, van der Klis FR, van der Sande MA, de Melker HE, Coutinho RA, van Eeden A, van Rooijen MS, Meijer CJ, Schim van der Loeff MF. No evidence for a protective effect of naturally induced HPV antibodies on subsequent anogenital HPV infection in HIV-negative and HIV-infected MSM. *J Infect* 2014;**69**: 375-86.

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Tables

Table 1. Seroprevalence of HPV quadrivalent vaccine types among teenage men who have sex with men at baseline

HPV type	Overall seroprevalence % (n/N, 95% CI)	Seroprevalence ratio: anal DNA† (95% CI)	Seroprevalence ratio: penile DNA‡ (95% CI)	Seroprevalence ratio: anal or penile DNA§ (95% CI)
6	12.5 (25/200, 8.3-17.9)	8.27 (3.44-19.86)	2.01 (0.55-7.40)	6.33 (2.77-14.48)
11	7.0 (14/200, 3.9-11.5)	13.10 (3.62-47.43)	0	11.59 (3.18-42.19)
16	3.5 (7/200, 1.4-7.1)	13.18 (2.56-67.76)	3.44 (0.45-26.47)	10.21 (1.96-53.10)
18	3.0 (6/200, 1.1-6.4)	3.87 (0.76-19.72)	0	3.52 (0.69-18.07)
6/11	16.5 (33/200, 11.6-22.4)	5.90 (2.66-13.07)	0.51 (0.07-3.51)	4.63 (2.18-9.85)
16/18	5.5 (11/200, 2.8-9.6)	3.08 (1.01-9.51)	2.55 (0.63-10.34)	2.46 (0.79-7.67)
6/11/16/18	19.5 (39/200, 14.2-25.7)	2.90 (1.52-5.55)	0.69 (0.23-2.09)	2.41 (1.29-4.53)

Notes:

† Seroprevalence ratio comparing seroprevalence between men with and without DNA of the same HPV type as found on serology detected at the anus at least once during the study.

‡ Seroprevalence ratio comparing seroprevalence between men with and without DNA of the same HPV type as found on serology detected at the penis at least once during the study.

§ Seroprevalence ratio comparing seroprevalence between men with and without DNA of the same HPV type as found on serology detected at either the anus or the penis at least once during the study.

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Table 2. Seroconversion to HPV among teenage men who have sex with men

HPV type	No. men who seroconverted	Person-years at risk	Seroincidence (95% CI) per 100 PY†
6	25	133	18.8 (12.5-26.5)
11	10	150	6.7 (3.2-11.9)
16	6	155	3.9 (1.4-8.2)
18	9	155	5.8 (2.7-10.7)
6/11*	29	148	19.6 (13.5-26.9)
16/18*	13	157	8.3 (4.5-13.7)
6/11/16/18*	37	150	24.7 (18.0-32.4)

Notes:

6 men seroconverted to both HPV 6 and 11; 2 men seroconverted to both HPV 16 and 18; and 1 man seroconverted to all four types. No. = number.

* One would stop contributing person time once detected with antibodies to one of these types of HPV.

All men were not vaccinated against HPV before the study and during the study period.

† PY: person-years

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Table 3. Proportion of incident anal and penile HPV DNA infections associated with HPV seroconversion to the same HPV type by 12 months

	Anal DNA positive cases			
	No. incident DNA infections	**No. seroconversion cases**	**% (95% CI) seroconverted**	**Odds ratio (95% CI)**
HPV6	15	11	73.3 (44.9-92.2)	
HPV11	7	5	71.4 (29.0-96.3)	
HPV16	8	1	12.5 (0.3-52.7)	
HPV18	14	3	21.4 (4.7-50.8)	
6/11	22	16	72.7 (50.0-89.3)	12.0 (2.5-58.1)*
16/18	22	4	18.2 (5.2-40.3)	
	Penile DNA positive cases			
	No. Incident DNA infections	**No. seroconversion cases**	**% seroconverted**	
HPV6	4	2	50.0 (6.8-93.2)	
HPV11	3	1	33.3 (0.8-90.6)	
HPV16	4	1	25.0 (0.6-80.6)	
HPV18	1	0	0.0 (0.0-97.5)	
6/11	7	3	42.9(9.9-81.6)	3.0 (0.2-53.2)*
16/18	5	1	20.0 (0.5-71.6)	

Notes:

*We used logistic regression accounting for repeated measures from individual participants to compare the odds ratio of HPV seroconversion between HPV 6/11 and HPV16/18 (reference category).

A number of men with incident anal HPV infection also had incident penile HPV: 3/15 for type 6; 1/7 for type 11; 1/8 for type 16; and 1/14 for type 18.

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Figure legends

Figure 1. Patterns of HPV seropositivity among teenage men who have sex with men observed over 12 months

Ab: antibody. No Ab: no antibody detected at any visit. Black brackets refer to positive type-specific HPV serology. White brackets refer to negative type-specific HPV serology. Men who were seropositive but who had missing serum during follow up were excluded from the table: 4 seroprevalent and 1 seroincident for HPV6 Ab; 6 seroprevalent and 1 seroincident for HPV11 Ab; 1 seroprevalent and 1 seroincident for HPV16 Ab; and 1 seroincident for HPV18 Ab.

Figure 2. The cumulative proportion of HPV seroconversions stratified by the presence or absence of incident anal HPV DNA of the same type

Figure 2A: The cumulative proportion of seroconversions to HPV 6/11 among men who experienced or did not experience incident anal HPV 6/11 DNA infection. Men with prevalent or incident penile HPV 6/11 DNA and those with prevalent anal HPV 6/11 were excluded. Follow up period was from incident infection to seroconversion for either HPV 6/11. "HPV6/11-" refers to men with neither incident anal HPV6 nor 11. "HPV6/11+" refers to men with either incident anal HPV6 or 11.

Figure 2B: The cumulative proportion of seroconversions to HPV 16/18 among men who experienced or did not experience incident anal HPV 16/18 DNA infection. Men with prevalent or incident penile HPV 16/18 DNA and those with prevalent anal HPV 16/18 were excluded. Follow up period was from

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incident infection to seroconversion for either HPV 16/18. “HPV16/18-” refers to men with neither incident anal HPV16 nor 18. “HPV16/18+” refers to men with either incident anal HPV16 or 18.

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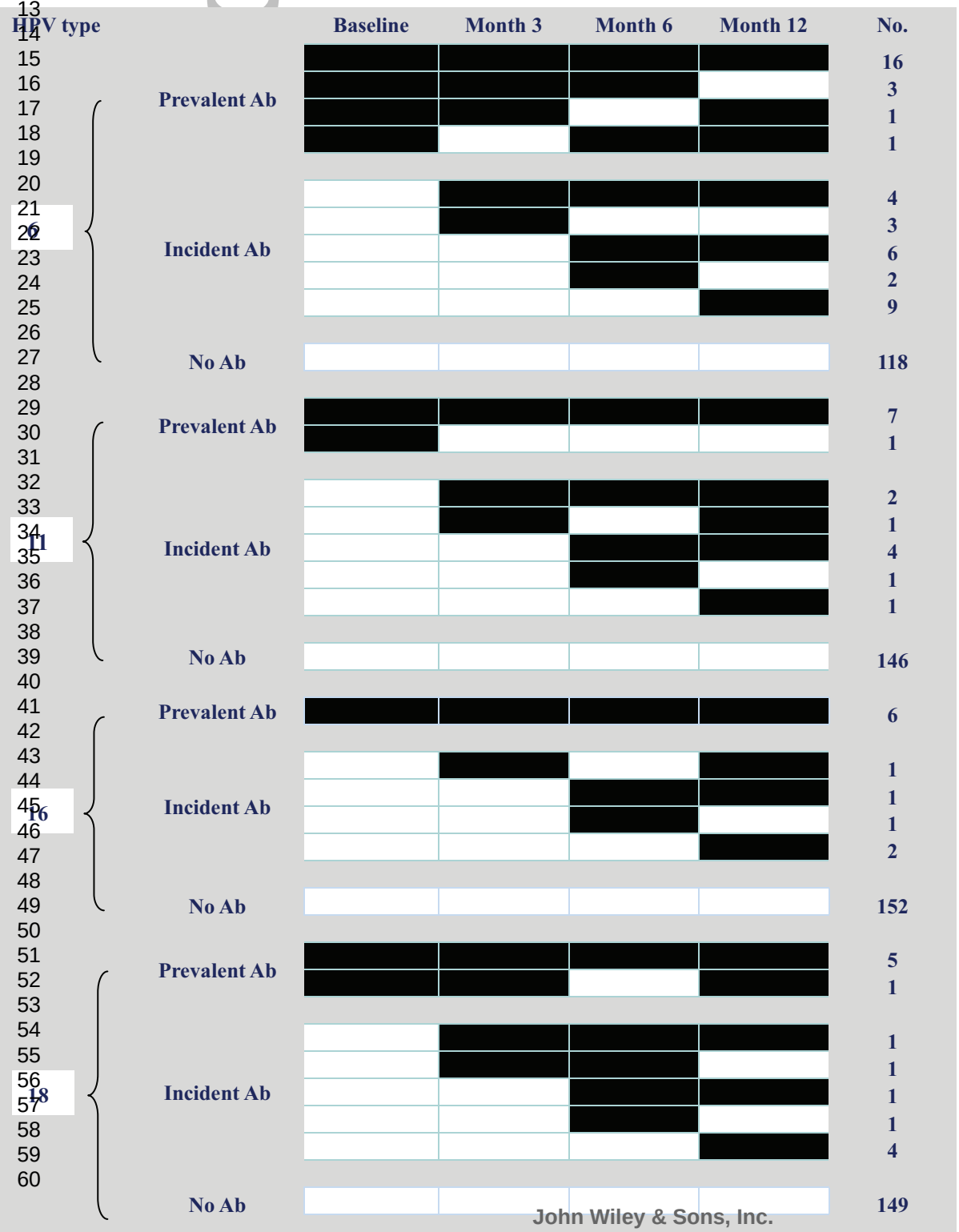
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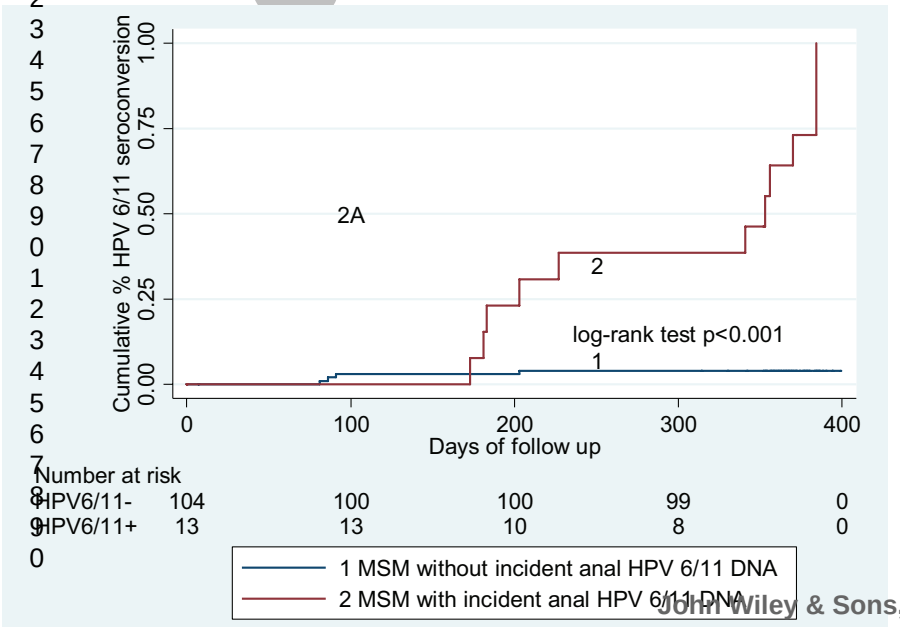
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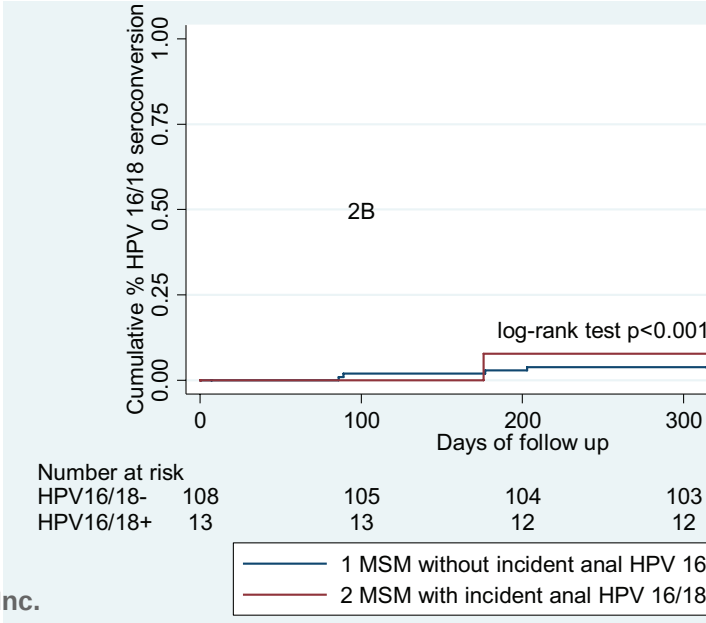
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