Background
Anal human papillomavirus (HPV) infection and its related diseases are relatively common in men who have sex with men (MSM), especially in those HIV positive. In China, molecular epidemiology of anal HPV infection among HIV-positive MSM has been sparsely studied.

Methods
A cross-sectional study was conducted among HIV-positive MSM in Xi’an, China between April and July 2014. Anal swabs were collected for HPV genotyping.

Results
A total of 195 HIV-positive MSM were included in this study. HPV genotyping showed that 99.0% (191/193) of participants were positive for at least one of the targeted 37 HPV genotypes. 183 (94.8%) of them were infected with multiple high-risk types and 154 (79.8%) of them with low-risk HPV types. HPV 18 was the most frequently identified high-risk type, followed by HPV 16 and HPV 51. As for low-risk types, HPV11, HPV 6 and HPV 81 were most commonly observe. High-risk HPV infection was found to be associated with the status of antiretroviral therapy (ART), the distribution of low-risk types was observed to be varied by CD4+ T cell level.

Conclusion
Almost all HIV-positive MSM were anal HPV infected in our study. It is highly recommended to consider regular active screening and preventive intervention of HPV infection among this high risk population.
Introduction

Human papillomavirus (HPV) infection is one of the most common sexually transmitted infections worldwide, representing a significant health problem due to its high prevalence and transmissibility [1]. The human immunodeficiency virus (HIV) infection has been suggested to make humans more susceptible to HPV infection because of the attenuated immune system [2,3]. It has been widely accepted that men have sex with men (MSM) is a high-risk population for both HPV and HIV infection. A recent systematic review and meta-analysis included 53 studies reported that the prevalence of the HPV co-infection was 89–93% in HIV infected MSM [4]. Additionally, anal HPV infection is one of the main causes of anal cancer, and the incidence of anal cancer is substantially higher in MSM than general population, especially in HIV positives [5–8]. It is worth to notice that the prevalence of HIV infection in MSM is increasing in China in recent years. Until 2013, it was estimated that nearly 63,730 MSM were living with HIV infection in China. The control of HPV infection and its related diseases is very important for improving the living quality of HIV-positive MSM. However, anal HPV infection and genotype distribution in the HIV-positive MSM has not been widely studied in China.

Our previously studies reported around 60% HIV negatives and 90% HIV positives were anal HPV infected in MSM from China [9,10]. To improve our understanding of anal HPV infection and its related pre-cancerous diseases, we conducted a pilot study among 95 HIV-positive MSM in Beijing, and the prevalence of abnormal anal cytology was found to be 37.9% [11]. Based on such previous work, we expanded the sample size of HIV-positive MSM in Xi’an city to explore the prevalence and distribution of anal HPV genotypes. Xi’an is the capital city of Shaanxi province, more than 1700 HIV infections had been reported in Xi’an until the end of 2012. Between 2007 and 2012, the proportion of homosexual transmission in the total HIV infections was increased from 14.3% to 56.7%, which had become the major route of transmission in Xi’an [12].

Materials and Methods

Ethic statement

The study was approved by the Ethics Committees of the Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College. Written informed consent was obtained from each study participant before the interview and testing.

Study population

The study was conducted in the Eighth People’s Hospital in Xi’an from April to July 2014. Study participants were recruited through a local nongovernment organization (Xi’an Tongkang Volunteers Workstation). Multiple methods were used for recruitment including website advertisements, distributing flyers with study-related information at MSM frequented venues (e.g., MSM clubs, bars, parks and bathhouses), and eligible study participants were also encouraged to refer their peers to attend the study. Those eligible participants were HIV-seropositive males, at least 18 years old, ever had homosexual behaviors, willing to provide anal swabs and blood for the test, and physically able and willing to provide written informed consent. Study participants who were tested HPV positive were informed by study personnel confidentially and referred to treatment at the Institute of STD/AIDS Prevention and Treatment, Xi’an District Center for Disease Prevention and Control and the STD/AIDS clinic of Xi’an eighth hospital.
Data collection
Self-reported socio-demographic characteristics (e.g., age, income, education, employment, and marriage status), antiretroviral therapy (ART) status and sexual behaviors in the past 6 months data were collected through one-to-one interviews by the trained interviewer in a separate room using a standardized questionnaire. Each study participant was assigned a unique code that was used to link the questionnaire and specimens. Personal contact information, which was blinded to researchers, was kept by the Xi’an Tongkang Volunteers Workstation for test results feedback and data validation. CD4+ T cell counts and HPV genotypes were collected for blood test and anal swabs test, respectively.

Sample collection and laboratory tests
Blood samples were collected for testing CD4+ T cell counts (BD FACSCountsystem). Trained personnel collected anal samples by rotating a saline water moistened nylon flocked swab in the anal canal for about 2 minutes. The swab was then kept in 3 mL of sample transport medium for Hybribio HPV DNA Test. Hybribio HPV DNA Test is based on flow-through hybridization to identify HPV types. The denatured DNA was placed into sample wells containing specific probes on the Hybrimem HPV-37 membrane to determine HPV types. The final results were detected by colorimetric change on the membrane under direct visualization. Positive and negative controls were included in the GenoArray test kit in every PCR analysis as well as during the hybridization process for quality. Mixtures of different specific probes can be used in the same well of a 42-well plate format allowing for multiplex analysis. HPV were classified as low-risk (LR) type and high-risk (HR) type according to the kit instruction [11,13]. Hybribio HPV DNA Test provides identification of 21 recommended HR subtypes (16, 18, 26, 31, 33, 34, 35, 39, 45, 51, 52, 53, 55, 56, 58, 59, 66, 68, 69, 82, and 83) which are associated with cervical cancer. Besides that, the kit also provides detection of 16 LR subtypes (6, 11, 40, 42, 43, 44, 54, 57, 61, 67, 70, 71, 72, 73, 81, and 84).

Statistical analysis
Questionnaires and laboratory results were double entered and compared with EpiData software (EpiData 3.02 for Windows, The Epi Data Association Odense, Denmark). After cleaning, the data was analyzed using SPSS (version 15.0 for Windows; SPSS Inc., Chicago, IL, USA). The characteristics of study population were showed by age, education, marriage status, self-reported sexual orientation, ever had sex with women, age at the first homosexual act, whether anal sex is a regular sex behavior or not. Based on CD4+ T cell level (divide by the median value of 394 cells/μL) or ART status (started or not), subjects were classified into two categories, respectively. Differences between HPV-types in these variables were assessed with Pearson chi-square test. Frequency distributions are presented for qualitative variables, medians, and inter-quartile ranges (IQRs) are presented for quantitative variables with asymmetrical distributions. The general description of the principal variables including central tendency and dispersion (mean, standard deviation, median, percentiles) for the quantitative variables was performed. To identify potential variables related with HPV infection, univariate analysis were performed using Pearson’s chi-square test. All variables with p-values < 0.05 in univariate analysis were entered into the unconditional multiple logistic regression analyses, and the associations were then assessed by means of odds ratios (OR) and 95% confidence intervals. Age, ART status and CD4 level were fixed in the multiple logistic regression models.
Results

Participant Characteristics

A total of 195 eligible HIV-positive MSM were enrolled. HPV genotyping results and current CD4+ T cell counts were available for 193 (99.0%) and 170 (87.2%), respectively. The major characteristics of the study participants are listed in Table 1. Overall, the mean (standard deviation, SD) age was 34±9 years with a range of 18–60 years. More than a third of them (38.5%) were 20–29 years old. Around half of the participants were single (52.3%) and 64.1% of them have received education more than 12 years. The median current CD4+ T cell count was 394 cells/μL (range, 259–517 cells/μL). A vast majority (86.2%) of them ever had started ART.

Anal HPV-types Distribution

As shown in Table 2, the genotyping results of HPV were available for 193 participants. 99.0% (191/193) of them were positive for at least one of the targeted 37 HPV types. 94.8% (183/193) of them were multiple HR-types infected, and 79.8% (154/193) were LR-HPV infected.

Fig 1A showed the prevalence of type-specific HPV infection. HPV 11 (59.7%) and HPV 6 (39.6%) were found to be the most frequently identified LR-HPV. The following prevalent LR-HPV genotypes were: HPV 81 (23.4%), HPV 61 (18.2%), HPV 84 (11.0%), HPV 73 (10.4%), and HPV 70 (9.1%). As depicted in Fig 1B, among the HR-HPV types, HPV 18, HPV 16, and HPV 51 were most frequently identified (40.4%, 33.9% and 31.7%, respectively). HPV 33, along with HPV 39, HPV 58, HPV 52, HPV 53, HPV 68, HPV 66, HPV 31, and HPV 59 were the following common detected HR-HPV types, with prevalence of 26.2%, 24.6%, 24.6%, 24.0%, 20.2%, 19.7%, 19.1%, 15.3%, and 11.5%, respectively.

Table 1. Characteristics of the study population.

| Variables                      | n (%)     |
|--------------------------------|-----------|
| Total                          | 195       |
| **Age**                        |           |
| Mean ± SD (range)              | 34±9 (18–60) |
| ≤ 20 years                     | 3 (1.5)   |
| 20–29 years                    | 75 (38.5) |
| 30–39 years                    | 61 (31.3) |
| ≥ 40 years                     | 56 (28.7) |
| **Education level**            |           |
| ≤ 9 years                      | 25 (12.8) |
| 9–12 years                     | 45 (23.1) |
| > 12 years                     | 125 (64.1)|
| **Current marital status**     |           |
| Single                         | 102 (52.3)|
| In marriage                    | 65 (33.3)|
| Divorced/widowed               | 28 (14.4)|
| **Antiretroviral treatment (ART)** |       |
| Started                        | 168 (86.2)|
| Not started                    | 27 (13.8)|
| **Median CD4+ T cell counts (IQR)#(cells/μL)** | 394 (259, 517) |

#23 subjects' blood samples were unavailable for testing CD4+T cell counts.

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As shown in Table 2 and Table 3, ART and condom using were significantly associated with any-type HPV infection ($p = 0.019$ and $p = 0.044$, respectively). ART status, role of sexual behaviors and un-preferred respective sexual behavior were associated with HR-HPV infection ($p = 0.036$, $p = 0.004$ and $p = 0.031$, respectively), CD4$^+$ T cell level ($p = 0.037$) and having a fixed sexual partner ($p = 0.019$) were related with LR-HPV infection in univariate analysis. In multiple logistic regression analysis, as shown in Table 4, higher CD4$^+$ T cell level was found to be associated with significantly increased rate of LR-HPV infection (adjusted OR: 2.99, 95%CI: 1.26–7.08) and started ART was associated with decreased risk of HR-HPV infection (adjusted OR: 0.13, 95%CI: 0.02–0.71).

HPV types distribution stratified by median CD4$^+$ T cell counts (Fig 2A) showed that distribution of HPV 84 was significantly associated with CD4$^+$ T cell level ($p = 0.015$). As shown in the Fig 2B, for participants with lower level of CD4$^+$ T cell, HPV 18, HPV 51, and HPV 16 were the most frequently identified HR-HPV types, but HPV 16, HPV 18, and HPV 33 were found to be the most frequently identified types among subjects with higher CD4$^+$ T cell counts $\geq$ 394 cells/µL.

**Table 2.** Association between characteristics and HPV types distribution in HIV-positive MSM.

|                        | Any HPV n(%) | High-risk HPV n(%) | Low-risk HPV n(%) |
|------------------------|--------------|--------------------|-------------------|
| **Total** ($N = 193$)  | 191 (99.0)   | 183 (94.8)         | 154 (79.8)        |
| **Age**                |              |                    |                   |
| < 20 years             | 3/3 (100.0)  | 3/3 (100.0)        | 1/3 (33.3)        |
| 20–29 years            | 72/74 (97.3) | 69/74 (93.2)       | 57/74 (77.0)      |
| 30–39 years            | 60/60 (100)  | 58/60 (96.7)       | 49/60 (81.7)      |
| $\geq$ 40 years        | 56/56 (100)  | 53/56 (94.6)       | 47/56 (83.9)      |
| **p value**            | 0.355        | 0.712              | 0.186             |
| **Education level**    |              |                    |                   |
| $\leq$ 9 years         | 25/25 (100.0)| 25/25 (100.0)     | 22/25 (88.0)      |
| 9–12 years             | 44/45 (97.8) | 42/45 (93.3)       | 39/45 (86.7)      |
| $>12$ years            | 122/123 (99.2)| 116/123 (94.3)   | 93/123 (75.6)     |
| **p value**            | 0.595        | 0.570              | 0.185             |
| **Current marital status** |            |                    |                   |
| Single                 | 99/101 (98.0)| 96/101 (95.0)     | 77/101 (76.2)     |
| In marriage            | 65/65 (100.0)| 62/65 (94.5)      | 55/65 (84.6)      |
| Divorced/widowed       | 27/27 (100.0)| 25/27 (92.6)      | 22/27 (81.5)      |
| **p value**            | 0.646        | 0.811              | 0.409             |
| **Antiretroviral treatment (ART)** |        |                    |                   |
| Started                | 166/166 (100.0)| 160/166 (96.4) | 134/166 (80.7)   |
| Not started            | 25/25 (92.6) | 23/27 (85.2)       | 20/27 (74.1)      |
| **p value**            | 0.019        | 0.036              | 0.286             |
| **CD4$^+$ T cell level** |            |                    |                   |
| $\leq$ 394 cells/µL    | 84/85(98.8)  | 80/85(94.1)        | 73/85(85.9)       |
| $\geq$ 394 cells/µL    | 84/85(98.8)  | 82/85(96.5)        | 62/85(72.9)       |
| **p value**            | 1.000        | 0.720              | 0.037             |

*2 anal samples were unavailable for HPV genotyping.
*23 subjects’ blood samples were unavailable for testing CD4$^+$ T cell counts.

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**HPV Distribution by CD4$^+$ T cell Level or ART Status**

As shown in Table 2 and Table 3, ART and condom using were significantly associated with any-type HPV infection ($p = 0.019$ and $p = 0.044$, respectively). ART status, role of sexual behaviors and un-preferred respective sexual behavior were associated with HR-HPV infection ($p = 0.036$, $p = 0.004$ and $p = 0.031$, respectively), CD4$^+$ T cell level ($p = 0.037$) and having a fixed sexual partner ($p = 0.019$) were related with LR-HPV infection in univariate analysis. In multiple logistic regression analysis, as shown in Table 4, higher CD4$^+$ T cell level was found to be associated with significantly increased rate of LR-HPV infection (adjusted OR: 2.99, 95%CI: 1.26–7.08) and started ART was associated with decreased risk of HR-HPV infection (adjusted OR: 0.13, 95%CI: 0.02–0.71).

HPV types distribution stratified by median CD4$^+$ T cell counts (Fig 2A) showed that distribution of HPV 84 was significantly associated with CD4$^+$ T cell level ($p = 0.015$). As shown in the Fig 2B, for participants with lower level of CD4$^+$ T cell, HPV 18, HPV 51, and HPV 16 were the most frequently identified HR-HPV types, but HPV 16, HPV 18, and HPV 33 were found to be the most frequently identified types among subjects with higher CD4$^+$ T cell counts $\geq$ 394 cells/µL.
When stratified by ART status, HPV 11, HPV 6, and HPV 81 were the most frequently identified types among LR-HPV types regardless of ART status. As for HR-HPV types group, HPV 18, HPV 16, and HPV 51 were most frequently detected among those who had started ART. But for those who had not started ART, HPV 18, HPV 52, and HPV 51 were most frequently detected among those who had not started ART. HPV 11 and HPV 6 were found to be the most prevalent low-risk types, HPV 18 and HPV 16 were most common high-risk types among the study population.

Fig 1. Prevalence of HPV types in 193 HIV-positive MSM. The numbers above the bars indicate the proportion of the patients in each category. HPV 11 and HPV 6 were found to be the most prevalent low-risk types, HPV 18 and HPV 16 were most common high-risk types among the study population.

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frequently identified. No significant different distribution of LR-HPV types was found by ART status as shown in Fig 3.

Discussion

The present study aims to investigate the HPV genotype distribution among HIV-infected MSM in China. Overall, we found a high prevalence of any-HPV (99.0%), HR-HPV (94.8%), and LR-HPV (79.8%) infection in our study participants. The prevalence of any-HPV in the
present study was close to a meta-analysis (96.2%), Lei Gao et al. (96.0%) and Xj Zhang et al. (96.6%) reported [9,10,14], but was higher than other studies (65.5%-82.1%) [11,13,15] in China. Among HR-HPV types, HPV18 was the most common, followed by HPV16, HPV51. As for LR-HPV types, HPV11, HPV6 and HPV81 were the most frequently detected. The distribution of HPV11/6/18/16 were basically in line with other studies, but other HPV types distribution were different in different regions and studies [9–11,13,15]. As for the discordant correlations between anal HPV infection and CD4 count or ART in different researches [16–19], our findings showed that HIV-positive MSM with a CD4+ T cell level higher than 394 cells/μL may be more susceptible to infection by LR-HPV than those with less than that and ART might be a protective factor for HR-HPV infection.

Anal cancer is a relatively rare disease compared to cervical cancer. Its annual incidence is less than 2 in 100,000 persons in the general population worldwide, but is high among well-defined populations such as MSM, particularly HIV-infected MSM, in whom the incidence is up to 80-fold higher than that in the general population [20,21]. A large proportion of anal cancer is caused by anal infections with carcinogenic HPV [22], and among them, approximately 80% to 85% cases were caused by infection of HR-HPV such as HPV16 or 18 [23,24]. Even HPV infection is quite common in HIV-positive population, HPV16 is still the most frequently found HPV type related to anal intraepithelial neoplasia and anal cancer [25]. In the present investigation, a wide array of HPV types was identified and multiple infections were found to be common, it is consistent with the reports from previous studies [14,15,26]. In our study participants, HPV18 and 16 were most frequently identified HR-HPV types. High prevalence

### Table 4. Factors correlated with HPV sub-types infection among Xi’an MSM by multivariate logistic regression.

|                | High-risk HPV | Low-risk HPV |
|----------------|---------------|--------------|
|                | p             | Adjusted OR(95%CI) | p             | Adjusted OR(95%CI) |
| **Total** (N = 193) | 183 (94.8%)   | 154 (79.8%)   |
| **Age**        |               |               |               |
| < 20 years     |               |               |               |
| 20–29 years    | 0.964         | 999.9 (0.01–999.9) | 0.584         | 0.47 (0.03–6.96)   |
| 30–39 years    | 0.964         | 999.9 (0.01–999.9) | 0.549         | 0.44 (0.03–6.61)   |
| ≥ 40 years     | 0.966         | 999.9 (0.01–999.9) | 0.637         | 0.52 (0.03–8.09)   |
| **CD4+T cell level** |               |               |               |
| < 394 cells/μL |               |               |               |
| ≥ 394 cells/μL | 0.121         | 0.27 (0.05–1.42) | 0.013         | 2.99 (1.26–7.08)   |
| **Antiretroviral treatment (ART)** | | | | |
| Not started    |               |               |               |
| Started        | 0.019         | 0.13 (0.02–0.71) | 0.821         | 0.88 (0.27–2.79)   |
| **Role of homosexual behaviors** | | | | |
| Only insertive |               |               |               |
| Only receptive | 0.425         | 2.14 (0.33–14.03) |               |               |
| Insertive or receptive | 0.114         | 0.18 (0.02–1.52) |               |               |
| **Having fixed sexual partner** | | | | |
| Yes            |               |               |               |
| No             | 0.148         | 1.86 (0.80–4.28) |               |               |

*2 anal samples were unavailable for HPV genotyping.

*23 subjects’ blood samples were unavailable for testing CD4+T cell counts.

*Adjusted for age, education, and current marital status.

**Table 4. Factors correlated with HPV sub-types infection among Xi’an MSM by multivariate logistic regression.**

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of HPV 51 was also observed. Such HR-HPV types might increase the risk of HPV related anal cancer and might be targeted for the screening HPV infection and monitoring pre-cancer in HIV-positive MSM. But further analyses are necessary to confirm such distribution is common in HIV infected MSM in China.

Fig 2. The prevalence of HPV types in 193 HIV-positive MSM by CD4 level. The study population was grouped by the median values of CD4+ T cell level (394 cells/μL). The numbers above the bars indicate the prevalence of each type. #HPV84 was significantly associated with CD4+T cell count level, the prevalence in “< 394 cells/μL” group was higher than that in “≥ 394 cells/μL” group (p = 0.015).

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The presence of LR-HPVs, in particular HPV 6 and HPV 11 has been reported for HPV-related invasive anal carcinoma as well [27,28]. The prevalence of HPV 6 and/or HPV 11 in the anal canal of MSM has been shown to be 15% in HIV negatives and as high as 60% in HIV positives [29,30]. In our present study, HPV 11 and HPV 6 were also the most common LR-HPV types in HIV infected MSM. The HPV vaccine covering HPV-6, 11, 16 and 18 subtypes has
been shown to be effective in preventing anogenital warts and anal intraepithelial neoplasia in men [31–34]. While the biggest challenge for vaccination will be to identify and vaccinate before HPV acquisition, thus vaccination before sexual activity commences is the optimal strategy. There are data supporting the alternative approach of vaccinating young MSM after sexual debut [35–37]. However, the actual situation is that HPV vaccination among males has not been suggested and widely studied in China. The introduction of a routine preventive screening and treatment program of anal cancer has not been proposed to HIV infected MSM either so far.

In our study, we obtained a remarkable finding that HIV-positive MSM with higher CD4+ T cell level were more likely to be infected by HPV. However, by applying the Bruzzi method [38], it is estimated that avoiding having CD4+ T cell counts drop below 200 cells/μL 6–7 years prior to anal cancer diagnosis would prevent 20% of anal cancer cases, this estimate could rise to 49% or 79% by keeping CD4+ T cell counts higher than 350 cells/μL or 500 cells/μL, respectively. A cohort study from Swiss addressing the relationship between CD4+ T cell level and anal cancer risk suggested a rough estimate of the fraction of anal cancer [39]. The contradictory results compared with previous literatures urged us to further explore this finding. Otherwise, ART may not have been initiated early enough to have prevented the establishment of a large proportion of irreversible precancerous anal lesions [5,40,41]. If the risk of anal cancer could be largely attributable to HIV-related immunodeficiency as assessed by decreased CD4+ T cell counts, early diagnosis of HIV infection and early starting ART might be key point to prevent anal cancer development among HIV infections [39–43].

Several limitations of this study should be kept in mind. Firstly, potential recall bias on sensitive questions could not be excluded completely because our questionnaires were interviewer-administered. Secondly, in terms of difficult sample acquirement and the potential limitation of enrollment methods, our study participants might not represent the general HIV-positive MSM in Xi’an City. Thirdly, due to the relative small sample size, the power of our study for association analysis is limited. Last but not least, cross-sectional study design has its limitation on association analysis. Therefore, our results need confirmation by further large-scale case-control studies or prospective studies.

In conclusion, high prevalence of anal HPV infection was observed among HIV-positive MSM in our investigation. Further studies are needed to estimate the risk of HPV related cancers and to explore strategies accordingly for intervention in this population. Developing targeted vaccination based on the most prevalent carcinogenic HPV types should be considered for different population in different areas.

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Author Contributions
Conceived and designed the experiments: LG. Performed the experiments: ZL XWL HRZ MFL HNX BXF YY. Analyzed the data: LG ZL HRZ. Contributed reagents/materials/analysis tools: ZL XWL HNX MFL BXF YY HRZ. Wrote the paper: ZL HRZ LG. Performed cytology and results classification: ZL MFL.
References

1. Parisi SG, Cruciani M, Scagghiante R, Boldrin C, Andreis S, Dal Bello F, et al. Anal and oral human papillomavirus (HPV) infection in HIV-infected subjects in northern Italy: a longitudinal cohort study among men who have sex with men. BMC Infect Dis. 2011; 11: 150. doi:10.1186/1471-2334-11-150 PMID: 21612634

2. Brown B, Davtyan M, Leon SR, Sanchez H, Calvo G, Klausner JD, et al. A prospective cohort study characterising the role of anogenital warts in HIV acquisition among men who have sex with men: a study protocol. BMJ Open. 2014; 4: e5687.

3. Brown B, Davtyan M, Galea J, Chow E, Leon S, Klausner JD, et al. The role of human papillomavirus in human immunodeficiency virus acquisition in men who have sex with men: a review of the literature. Viruses. 2012; 4: 3851–3858. doi:10.3390/v4123851 PMID: 23250451

4. 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: 487–500. doi:10.1016/S1470-2045(12)70080-3 PMID: 22445259

5. Vajdic CM, van Leeuwen MT, Jin F, Prestage 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: 330–335. doi:10.1136/sti.2008.034744 PMID: 19342375

6. Pierangeli A, Scagnolari C, Degener AM, Bucci M, Ciardi A, Riva E, et al. Type-specific human papillomavirus-DNA load in anal infection in HIV-positive men. AIDS. 2008; 22: 1929–1935. doi:10.1097/QAD.0b013e32830fbd7a PMID: 18784456

7. Nyitray AG, Carvalho DSR, 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: 49–57. doi: 10.1093/infdis/jiq021 PMID: 21148496

8. Latini A, Dona MG, Ronchetti L, Giglio A, Moretto D, Colafiglì M, et al. Prevalence of anal human papillomavirus infection and cytologic abnormalities among HIV-infected and HIV-uninfected men who have sex with men. J Int AIDS Soc. 2014; 17: 19662. doi:10.7448/IAS.17.4.19662 PMID: 25397412

9. Gao L, Zhou F, Li X, Yang Y, Ruan Y, Jin Q. Anal HPV infection in HIV-positive men who have sex with men from China. PLoS One. 2010; 5: e15256. doi:10.1371/journal.pone.0015256 PMID: 21151900

10. Zhang X, Yu J, Li M, Sun X, Han Q, Li M, et al. Prevalence and related risk behaviors of HIV, syphilis, and anal HPV infection among men who have sex with men from Beijing, China. AIDS Behav. 2013; 17: 1129–1136. doi:10.1007/s10461-011-0085-x PMID: 22076229

11. Yang Y, Li X, Zhang Z, Qian HZ, Ruan Y, Zhou F, et al. Association of human papillomavirus infection and abnormal anal cytology among HIV-infected MSM in Beijing, China. PLoS One. 2012; 7: e35983. doi:10.1371/journal.pone.0035983 PMID: 22558293

12. Zhang H, Yan Y, Li H, Li D, Wei X, Zheng H, et al. Molecular epidemiology related to human immunodeficiency virus type 1 infection in men having sex with men in Xian. Zhonghua Liu Xing Bing Xue Za Zhi. 2014; 35: 421–424. PMID: 25009033

13. Hu Y, Qian HZ, Sun J, Gao L, Yin L, Li X, et al. Anal human papillomavirus infection among HIV-infected and uninfection men who have sex with men in Beijing, China. J Acquir Immune Defic Syndr. 2013; 64: 103–114. doi: 10.1097/QAI.0b013e31828b6298 PMID: 23732908

14. Chow EP, Tucker JD, Wong FY, Nehl EJ, Wang Y, Wang Y, Zhuang X, et al. Disparities and risks of sexually transmissible infections among men who have sex with men in Beijing. PLoS One. 2014; 9: e89959. doi: 10.1371/journal.pone.0089959 PMID: 24587152

15. 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: e96364. doi: 10.1371/journal.pone.0096364 PMID: 24801331

16. Cingolani A, Zona S, Girardi E, Lepri AC, Monno L, Quiros Roldan E, et al. Increased incidence of sexually transmitted diseases in the recent years: data from the ICONA cohort. J Int AIDS Soc. 2014; 17: 19653. doi: 10.7448/IAS.17.4.19653 PMID: 25394157

17. Sadlier C, Rowley D, Morley D, Surah S, O’Dea S, Delamere S, et al. Prevalence of human papillomavirus in men who have sex with men in the era of an effective vaccine; a call to act. HIV Med. 2014; 15: 499–504. doi: 10.1111/hiv.12150 PMID: 24655896

18. Gonzalez C, Torres M, Benito A, Del RJ, Rodriguez C, Fontillon M, et al. Anal squamous intraepithelial lesions are frequent among young HIV-infected men who have sex with men followed up at the Spanish AIDS Research Network Cohort (CoRIS-HPV). Int J Cancer. 2013; 133: 1164–1172. doi: 10.1002/ijc.28102 PMID: 23404769
19. de Pokomandy A, Rouleau D, Ghattas G, Trottier H, Vezina S, Cote P, et al. HAART and progression to high-grade anogenital neoplasia in men who have sex with men and are infected with HIV. Clin Infect Dis. 2011; 52: 1174–1181. doi: 10.1093/cid/cir640 PMID: 21364075

20. Chaturvedi AK, Madeleine MM, Biggar RJ, Engels EA. Risk of human papillomavirus-associated cancers among persons with AIDS. J Natl Cancer Inst. 2009; 101: 1120–1130. doi: 10.1093/jnci/djp205 PMID: 19648510

21. 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: 1026–1034. doi: 10.1093/cid/cir1012 PMID: 22291097

22. Machalek DA, Grulich AE, Jin F, Templeton DJ, Poynten IM. The epidemiology and natural history of anal human papillomavirus infection in men who have sex with men. Sex Health. 2012; 9: 527–537. doi: 10.1071/SH12043 PMID: 23380235

23. Alemany L, Saunier M, Alvarado-Cabrero I, Quiros J, Shin HR, et al. Human papillomavirus DNA prevalence and type distribution in anal carcinomas worldwide. Int J Cancer. 2015; 136: 98–107. doi: 10.1002/ijc.28963 PMID: 24817381

24. De Vuyst H, Clifford GM, Nascimento MC, Madeleine MM, Franceschi S. Prevalence and type distribution of human papillomavirus in carcinoma and intraepithelial neoplasia of the vulva, vagina and anus: a meta-analysis. Int J Cancer. 2009; 124: 1626–1636. doi: 10.1002/ijc.24116 PMID: 19115209

25. 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. Int J Cancer. 2001; 129: 433–439.

26. Mendez-Martinez R, Rivera-Martinez NE, Crabtree-Ramirez B, Sierra-Madero JG, Caro-Vega Y, Galvan SC, et al. Multiple human papillomavirus infections are highly prevalent in the anal canal of human immunodeficiency virus-positive men who have sex with men. BMC Infect Dis. 2014; 14: 671 PMID: 25510243

27. Noffsinger A, Witte D, Fenoglio-Preiser CM. The relationship of human papillomaviruses to anorectal neoplasia. Cancer. 1992; 70: 1276–1287. PMID: 1324782

28. Li N, Franceschi S, Howell-Jones R, Snijders PJ, Clifford GM. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. Int J Cancer. 2011; 128: 927–935. doi: 10.1002/ijc.25396 PMID: 20473886

29. Goldstone S, Palefsky JM, Giuliano AR, Moreira EJ, Aranda C, Jessen H, et al. Prevalence of and risk factors for human papillomavirus (HPV) infection among HIV-seronegative men who have sex with men. J Infect Dis. 2011; 203: 66–74. doi: 10.1093/infdis/jiq016 PMID: 21148498

30. de Pokomandy A, Rouleau D, Ghattas G, Vezina S, Cote P, Macleod J, et al. Prevalence, clearance, and incidence of anal human papillomavirus infection in HIV-infected men: the HIPVIRG cohort study. J Infect Dis. 2009; 199: 965–973. doi: 10.1086/597207 PMID: 19239366

31. Garland SM, Hernandez-Avila M, Wheeler CM, Perez G, Harper DM, Leodolter S, et al. Quadrivalent human papillomavirus vaccine in HIV-1-infected men. J Infect Dis. 2010; 202: 1246–1253. doi: 10.1086/656320 PMID: 20812850

32. The Future II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. N Engl J Med. 2007; 356: 1928–1943. PMID: 17494926

33. Wilkin T, Lee JY, Lensing SY, Slier EA, Goldstone BE, Berry JM, et al. Safety and immunogenicity of the quadrivalent human papillomavirus vaccine in HIV-1-infected men. J Infect Dis. 2010; 202: 1246–1253. doi: 10.1086/656320 PMID: 20812850

34. Van de Velde N, Boily MC, Drolet M, Franco EL, Mayrand MH, Kliwer EV, et al. Population-level impact of the bivalent, quadrivalent, and nonavalent human papillomavirus vaccines: a model-based analysis. J Natl Cancer Inst. 2012; 104: 1712–1723. doi: 10.1093/jnci/djs395 PMID: 23104323

35. Zou H, Grulich AE, Cornall AM, Tabrizi SN, Garland SM, Prestage G, et al. How very young men who have sex with men view vaccination against human papillomavirus. Vaccine. 2014; 32: 3936–3941. doi: 10.1016/j.vaccine.2014.05.043 PMID: 24852719

36. Meites E, Markowitz LE, Paz-Bailey G, Oster AM. HPV vaccine coverage among men who have sex with men—National HIV Behavioral Surveillance System, United States, 2011. Vaccine. 2014; 32: 6356–6359. doi: 10.1016/j.vaccine.2014.09.033 PMID: 25258097

37. Nadarzynski T, Smith H, Richardson D, Jones CJ, Llewellyn CD. Human papillomavirus and vaccine-related perceptions among men who have sex with men: a systematic review. Sex Transm Infect. 2014; 90: 515–523. doi: 10.1136/sextrans-2013-051357 PMID: 24787367

38. Bruzzì P, Green SB, Byar DP, Brinton LA, Schairer C. Estimating the population attributable risk for multiple risk factors using case-control data. Am J Epidemiol. 1985; 122: 904–914. PMID: 4050778
39. 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; 178: 877–884. doi:10.1093/aje/kwt153 PMID: 23900553

40. Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. Lancet. 2007; 370: 59–67. PMID: 17617273

41. Piketty C, Selinger-Leneman H, Grabar S, Duvivier C, Bonmarchand M, Abramowitz L, et al. Marked increase in the incidence of invasive anal cancer among HIV-infected patients despite treatment with combination antiretroviral therapy. AIDS. 2008; 22: 1203–1211. doi:10.1097/QAD.0b013e3283023f78 PMID: 18525266

42. Gage JR, Sandhu AK, Nihira M, Bonecini-Almeida MDG, Cristoforoni P, Kishimoto T, et al. Effects of human papillomavirus-associated cells on human immunodeficiency virus gene expression. Obstet Gynecol. 2000; 96: 879–885. PMID: 11084171

43. Nicol AF, Fernandes AT, Grinsztejn B, Russomano F, E SJ, Tristao A, et al. Distribution of immune cell subsets and cytokine-producing cells in the uterine cervix of human papillomavirus (HPV)-infected women: influence of HIV-1 coinfection. Diagn Mol Pathol. 2005; 14: 39–47. PMID: 15714063