Anal Human Papillomavirus Infection, and associated Neoplastic Lesions in Homosexual Men: Systematic Review and Meta-Analysis

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Summary

Background: Homosexual men are at greatly increased risk of human papillomavirus (HPV) associated anal cancer. Screening for the presumed cancer-precursor, high-grade anal intraepithelial neoplasia (AIN) followed by treatment in a manner analogous to cervical screening, has been proposed. An understanding of the natural history of cervical HPV infection has informed pre-cancer screening, but fewer data are available for anal HPV disease.

Methods: We conducted a systematic review of published, peer-reviewed studies reporting prevalence and incidence of anal HPV detection, AIN, and anal cancer in homosexual men. Summary estimates were calculated using random-effects meta-analysis.

Findings: Fifty three studies met the inclusion criteria, including 31 estimates of HPV prevalence, 19 estimates of cytological abnormalities, 8 estimates of histological abnormalities, and 9 estimates of anal cancer incidence. Data on incident HPV and high-grade AIN were sparse. Among HIV-positive men, the pooled prevalence of anal HPV-16 was 35·4% (95% CI 32·9%-37·9%). In the only published estimate, incidence and clearance of anal HPV-16 occurred in 13·0% (95% CI 9·6-17·6), and 14·6% (95% CI 10·2-21·2) of men per year, respectively. The pooled prevalence of histological high-grade AIN was 29·1% (95% CI 22·8%-35·4%) with incidence of 8·5% (95% CI 6·9-10·4), and 15·4% (95% CI 11·8-19·8) per year, in two published estimates. The pooled anal cancer incidence was 45·9 per 100,000 (95% CI 31·2-60·3). Among HIV-negative men, the pooled prevalence of anal HPV-16 was 12·5% (95% CI 9·8%-15·4%). Incidence of HPV-16 occurred in 11·8% (95% CI 9·2-14·9), and 5·8% (95% CI 1·9-13·5) of men per year, in two published estimates. The pooled prevalence of histological high-grade AIN was 21·5% (95% CI 13·7-29·3), with incidence of 3·3% (95% CI 2·2%-4·7%), and 6·0% (95% CI 4·2-8·1) per year, based on two estimates. Anal cancer incidence was 5·1 per 100,000 (95% CI 0-11·5) based on two estimates. There were no published estimates of high-grade AIN regression.

Interpretations: Anal HPV and anal cancer precursors were extraordinarily common in homosexual men. However, based on limited data, rates of progression to cancer appeared to be substantially lower than for

cervical pre-cancerous lesions. Large, high quality prospective studies are needed to inform the development of anal cancer screening guidelines for homosexual men.

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Introduction

Infection with high-risk types of human papillomavirus (HPV) causes over 80% of anal cancer[1], and is recognised as a necessary cause of virtually 100% of cervical cancer[2]. Over the last 20-30 years, the incidence of anal cancer has been increasing. Populations at increased risk include women with prior cervical HPV-related disease[3], immune suppressed transplant recipients, and the HIV positive[4]. Incidence is highest among homosexual men, who are about 20-fold more likely than other men to develop the disease[5]. HIV positive homosexual men are at even greater risk[6]. Given the increasing health burden of anal cancer in homosexual men and its similarities to cervical cancer, an anal cancer screening program has been proposed for this population[7]. It would be based on cytological detection of HPV-related abnormalities, or possibly by direct detection of HPV-related biomarkers, followed by histological confirmation of the presumed cancer-precursor lesion high-grade intra-epithelial neoplasia (AIN), and treatment. Since the implementation of population based screening programs for cervical cancer, there has been a substantial reduction in the incidence and mortality from this malignancy[8]. Recently, HPV vaccination has been introduced for females in several countries, with the aim of further reducing cervical cancer incidence. In Australia, where there has been a rapid and widespread uptake of the quadrivalent HPV vaccine by women aged less than 27, dramatic reductions in cervical high-grade pre-cancerous lesions in young women have recently been described, in addition to a rapid decline in genital warts[9]. A parallel, although lesser, decline in genital warts in young heterosexual men [9] suggests that female vaccination will ultimately lead to a reduction in HPV-related morbidity in heterosexual men through herd immunity. Unfortunately, homosexual men will not benefit from such herd immunity, so other approaches are required to reduce HPV-related morbidity in this population. Universal vaccination of adolescent males has enormous potential to prevent HPV-related morbidity in the future[10], but for current generations, alternative approaches such as screening are required.

Cervical screening based on detection of cytological abnormalities has existed for 60 years[11]. Since the discovery of HPV, a large number of observational studies have described the natural history of cervical HPV infection and

of cytological abnormalities[12]. While there have been a number of studies which have estimated the prevalence and incidence of anal HPV infection, anal cytological abnormalities and anal intra-epithelial neoplasia (AIN) in homosexual men, the natural history of progression of anal HPV infection to anal cancer in homosexual men is unclear. There is no consensus as to how common anal high-risk HPV infection is; the prevalence and significance of AIN, and the rate of progression of AIN to anal cancer.

This systematic review and meta-analysis presents a summary of all published estimates of data on anal HPV detection, cytological and histological abnormalities and anal cancer in HIV positive and HIV negative homosexual men.

Materials and Methods

Search strategy and selection criteria

A systematic review was conducted without language restrictions for all peer-reviewed, published studies on the prevalence and incidence of anal HPV infection, anal cytological and histological neoplastic abnormalities and the incidence of anal cancer in homosexual men published until the 1st of November 2011. Relevant studies were identified from Pubmed, OVID Medline and Embase databases using the following combined heading search strategy: "anal intraepithelial neoplasia" OR "AIN" AND "men who have sex with men" OR "MSM" OR "homosexual men." For the review of HPV prevalence and incidence, literature searches were performed using the combined heading search strategy "human papillomavirus" OR "HPV" AND "men who have sex with men OR "MSM"OR "homosexual men." For the review of anal cancer incidence, literature searches were performed using the combined heading search strategy "anal cancer" AND "men who have sex with men" OR "MSM" OR "homosexual men." Reference lists of selected articles were also reviewed to identify other relevant studies. Data from abstracts and unpublished studies were not included.

Studies were reviewed by two study authors (DM and IMP). Studies were included in the review if they had quantitative estimates of the variables of interest and were undertaken for populations that included HIV positive and/or negative men who were described as homosexual, bisexual or MSM. In those studies where data were not stratified by sexual orientation or HIV status, data were requested from the corresponding author via email, and a follow up email was sent within 3-4 weeks of initial contact. Additional data were obtained from six of 17 authors contacted.

Studies which sampled in a manner which would clearly over- or under-represent the prevalence of anal HPV-related conditions were excluded. Studies reporting anal HPV infection were included if they measured anal canal HPV DNA by polymerase chain reaction (PCR) based technologies (including commercial and in-house assays) or by Hybrid Capture (HC) and Hybrid Capture 2 (HC-2). Studies using older non-amplification methods such as in situ or dot blot hybridization assays were also included, but were analyzed separately due to their limited sensitivity for HPV DNA detection[13]. Studies were included if they reported on anal cytological and/or histological findings in all participants. Studies in which HRA examination was performed only on participants with abnormal cytology were excluded from the meta-analysis. This is because anal cytology is substantially less than 100% sensitive in the detection of anal histological abnormalities, and thus such studies are likely to under-estimate the true prevalence of AIN[14]. Studies which only presented a composite anal diagnosis comprising the most severe of the cytological and histological results were not included.

Data extraction

For each study, data on first author, publication date, source of recruitment, study location, sample size, and participant age range, and HIV status were recorded. For anal HPV prevalence studies information on sample collection and detection methods were also recorded. Studies were stratified by HIV status, source of participants (community or clinic based recruitment) and method of diagnosis of disease endpoints (cytology or histology).

Data on anal HPV prevalence was extracted for “any HPV type”, “any low-risk HPV types”, “any high-risk HPV type,” “HPV-16” and “HPV-18”. If more than one type of assay was used to detect the presence of HPV and the results of both were reported (e.g. studies reporting HPV results using a PCR based assay and Hybrid Capture), both results were recorded separately. Studies were stratified by HIV status and source of participants (community or clinic based recruitment). Prevalence of anal HPV was expressed as a percentage of all participants tested for anal HPV. For PCR based detection, results from samples negative for beta-globin, which were considered inadequate for analysis, were excluded.

Cytological data were extracted if results were reported according to either the 1988 or the 2001 Bethesda System terminology. Categories are: negative, atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesions (LSIL), and high-grade squamous intraepithelial lesions (HSIL)[15]. The 2001 Bethesda system also includes the category atypical squamous cells – cannot exclude HSIL (ASC-H). ASC-H is likely to predict histological high-grade disease more often than ASC-US[16]. Cytological predictions were expressed as a percentage of the total samples tested after excluding samples which were inadequate for cytology.

Histological data were reported as low-grade AIN (LGAIN or AIN1) and high-grade AIN (HGAIN or AIN2/3). AIN prevalence by histological diagnosis was expressed as a percentage of the total number of HRA examinations performed.

For anal cancer incidence studies, the observed number of cancer cases and the person-years of follow up were abstracted. When not presented, the number of person-years was calculated by dividing the number of observed cases by the reported incidence rate. The diagnosis of invasive anal cancer was made using the International Classification of Disease (ICD) codes ICD9 prior to 1997 and ICD10 thereafter[17]. Carcinoma *in situ* was not included when presenting estimates of anal cancer incidence.

Incidence and regression of high-grade AIN and anal HPV-16 and HPV-18 infection were reported as percentage (%) per year, whereas anal cancer incidence was reported per 100 000 person-years. We calculated a theoretical

rate of progression from high-grade AIN to anal cancer, based on the assumption that all anal cancer arises in a person with previous high-grade AIN, by dividing the prevalence of high-grade AIN by the incidence of invasive anal cancer.

Statistical Analysis

Due to the expected heterogeneity in populations sampled, screening technique and study method we calculated pooled estimates and 95% confidence intervals (CI) using the random effects models[18]. Heterogeneity was measured with the I^2 statistic (values of less than 25%, between 25 and 75%, and over 75% representing low, medium, and high heterogeneity, respectively)[19]. Meta regression and subgroup analysis were performed to investigate potential sources of heterogeneity, and p values for linear trend calculated using random effects meta regression[18]. The effect of individual studies on the meta-analysis pooled estimates was assessed by re-estimating the overall effect after omitting each study. The potential presence of publication bias was assessed using funnel plots and statistical test for asymmetry was performed by regressing the estimate by the sample size, weighted by the reciprocal of the standard error (1/se)[20,18]. Analyses were performed using STATA version 12 (Stata Corporation, College Station, Texas, USA).

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Results

A total of 729 abstracts were identified in Pubmed, Embase and OVID Medline databases (Figure 1) of which 352 (48%) articles were excluded based on abstract alone. A further 324 articles (44%) were excluded after review of the manuscript. Three studies were excluded from the analysis of anal HPV prevalence as their recruitment

strategies resulted in non-representative samples (Supplementary Table 7). One of these studies was limited to homosexual men with less than five lifetime sexual partners, while two clinic based studies only recruited patients with evidence of HPV-associated anal lesions. Three studies in which HRA was only performed on men with abnormal cytology were excluded from the histology meta-analysis (Supplementary Table 8). Thus 53 studies were included in the meta-analysis (Table 1).

The majority of studies identified were cross sectional studies and the vast majority were based in North America. For the detection of anal HPV, 86% and 64% of the sample size came from North America in HIV positive and HIV negative men respectively. For cytological abnormalities, just under 90% of the total sample size came from North America for both HIV positive and HIV negative men. For studies of histologically confirmed abnormalities, 79% and 94% of total sample size was from HIV positive and HIV negative men, respectively. Only six reports from four longitudinal cohort studies of incident HPV infection or high-grade AIN were identified[10, 21-24]. Information specific to individual studies included in the meta-analysis are summarized in Table 1 and Supplementary Tables 1-8.

Detection of anal HPV

Thirty-one studies were included in the meta-analysis of anal HPV. HPV prevalence was described in 29 studies for HIV positive homosexual men (4868 samples) and 18 studies for HIV negative homosexual men (4487 samples). The majority of HIV positive men were recruited from clinics (68%) and the majority of HIV negative men recruited from the community (68%). Meta-analytic results for PCR-based HPV detection are presented in Figure 2 and Figure 3, and HC/HC- 2 and other non-amplification methods are presented in Supplementary Figure 1.

Data were reported on detection of any HPV type by PCR in 2718 HIV positive and 3246 HIV negative men (Figure 2a). The pooled prevalence was substantially higher in HIV positive than in HIV negative men (92·6%, 95% CI 90·8-94·5 versus 63·9%, 95% CI 55·2-72·6; p=0·0050). There was no association between year of publication, number of HPV types detected or the source of recruitment and reported HPV prevalence.

For any high-risk HPV, PCR data were reported on a total of 1352 and 2103 samples in HIV positive and HIV negative men respectively (Figure 2b). Again, the pooled prevalence was substantially higher in HIV positive than in HIV negative men (73·5%, 95% CI 63·9-83·0 and 37·2%, 95% CI 27·4-47·0; p=0·010). Studies published in recent years reported higher prevalence of high-risk HPV than earlier studies, after accounting for the number of HPV types detected, both in HIV positive (p=0·012), and HIV negative men (p=0·054). There was no association between source of recruitment and reported high-risk HPV prevalence.

When HC or HC-2 was used, the pooled prevalence of any HPV type and any high-risk HPV type varied slightly from PCR but remained higher in HIV positive compared with HIV negative men (89·0%, 95% CI 84·7-93·3 versus 53·6%, 95% CI 21·6-80·6; p=0·047 for any HPV type, and 79·1%, 95% CI 70·0-88·2 versus 31·5%, 95% CI 7·2-55·8; p=0·034 for any high-risk type, respectively). The pooled prevalence for any HPV type using other non amplification detection methods was much lower than for PCR and HC/HC-2, however the statistically higher HPV prevalence among HIV positive, compared with HIV negative, men was still observed (54·2%, 95% CI 50·9-57·5 versus 21·6%, 95% CI 16·8-26·6; p<0·0001, Supplementary Figure 1).

Data were reported on detection of HPV-16 in 1468 HIV positive and 2687 HIV negative men and for HPV-18 in 1328 and 1283 men, respectively. Again, the prevalence HPV-16 was much higher among HIV positive than HIV negative men (35·4%, 95% CI 32·9%-37·9% and 12·5%, 95% CI 9·8-15·4; p=0·0027, Figure 3a). Similar results were found for HPV-18 (18·6%, 95% CI 12·8-24·4 and 4·9%, 95% CI 2·7-7·1; p=0·019, Figure 3b). There was no association between year of publication and source of recruitment with either HPV-16 or HPV-18 detection.

Incidence and clearance of anal HPV-16 and HPV-18 infection

Three studies of anal HPV incidence and clearance were identified; one in HIV positive men in the HAART era[25] and two in HIV negative homosexual men[10, 21] (Supplementary Table 2). In HIV positive men, the incidence rates of HPV-16 and HPV-18 were 13·0% (95% CI 9·6-17·6; 316·2 person-years) and 5·3% (95% CI 3·5-8·0; 415·8 person-years) per year, and the clearance rates were 14·6% (95% CI 10·2-21·2; 190·5 person-years) and 24·5% (95% CI 16·9-35·4; 114·6 person-years) per year, respectively. In HIV negative men, annual HPV-16 incidence in

the two studies was 11·8% (95% CI 9·2-14·9; 599·9 person-years)[10] and 5·8% (95% CI 1·9-13·5; 86·5 person-years)[21], and annual HPV-18 incidence rate was 6·1% (95% CI 4·3-8·3; 641·3 person-years)[10] and 4·5% (95% CI 1·2-11·6; 88·3 person-years)[21]. Clearance rates were not presented but in one study with only 6 months follow up[21] clearance of HPV-16 and HPV-18 occurred in 27% (3 of 11)and 62·5% (5 of 8) respectively.

Prevalence of Cytological abnormalities

Nineteen studies reporting the prevalence of anal cytological abnormalities were included in the meta-analysis (Figure 4, Supplementary Table 3), comprising 17 studies in HIV positive men (2791 samples), and 6 studies in HIV negative men (1827 samples). The majority of HIV positive men were recruited from clinics (84%) and the majority of HIV negative men from the community (87%). Approximately 70% of the HIV negative men analyzed came from a single community based study in the United States[26].

For each cytological outcome assessed, prevalence was substantially higher in the HIV positive men. The pooled prevalence were 57·2% (95% CI 51·2-63·2) and 18·5% (95% CI 8·0-28·9) for any cytological abnormality (p=0·0051, Figure 4a), 27·5% (95% CI 21·9-33·2) and 6·6% (95% CI 1·1-12·1) for LSIL (p=0·010, Figure 4b) and 6·7% (95% CI 4·4-9·0) and 2·7% (95% CI 0-5·1) for HSIL (p=0·11, Figure 4c) in HIV positive and HIV negative men, respectively. In meta-regression, neither the year of study publication nor the source of recruitment was related to the prevalence of cytological abnormalities.

Prevalence of Histological abnormalities

Data from eight studies in which HRA was performed on all study participants regardless of their cytology findings were included in the meta-analysis. Anal histological abnormalities were described in eight studies for HIV positive (1494 samples), and four studies for HIV negative (449 samples) men. The majority of HIV positive men were recruited from clinics (88%) and the majority of HIV negative men, from the community (94%), (Figure 5, Supplementary Table 4).

The pooled prevalence of histological abnormalities tended to be higher in HIV positive men. In meta-regression, studies published in recent years reported significantly higher prevalence of histological abnormalities than earlier studies, in both HIV positive (p = 0·010) and HIV negative (p = 0·025) men. Given this association we excluded the two oldest studies[17, 27] which had markedly lower estimates, from our final the meta-analytic estimate. This removed some of the heterogeneity from the pooled estimates of AIN prevalence. After this exclusion, the pooled prevalence estimates in HIV positive and HIV negative men were 63·1% (95% CI 52·6-73·6) and 36·9% (95% CI 30·0-43·4) for any abnormality (p=0.022), 31·3% (95% CI 18·5-44·1) and 10·6% (95% CI 6·3-14·9)for low-grade AIN (p=0.056) and 29·1% (95% CI 22·8-35·4) and 21·5% (95% CI 13·7-29·3) for high-grade AIN (p=0.26).

Incidence of histologically confirmed high-grade AIN

Two longitudinal studies involving baseline and follow up anal cytology, HRA examination and data on histologically confirmed incidence of high-grade AIN on all participants were identified. Both reported estimates of high-grade AIN incidence in HIV positive men[23-24], and one[24] reported an estimate in HIV negative men (Supplementary Table 5). In HIV positive men the annual incidence of high-grade AIN (progression from a lesser diagnosis) ranged from 8·5% (95% CI 6·9-10·4) to 15·4% (95% CI 11·8-19·8) (2 estimates, based on 1108·0 and 372·3 person-years, respectively). The incidence of high-grade AIN was lower in HIV negative men, with one study reporting a rate of 3·3% (95% CI 2·2-4·7) per year (based on 884·0 person-years). In a second study in HIV negative men in which HRA was performed only in those with abnormal cytology[10], incidence of high-grade AIN occurred in 6·0% (95% CI 4·2-8·1) of men, per year (based on 655·2 person-years). There were no published estimates of high-grade AIN regression rates for either HIV positive or HIV negative men.

Incidence of anal Cancer

Nine studies reporting anal cancer incidence in homosexual men were included in the meta-analysis. Six were linkage studies based on data obtained from HIV/AIDS and cancer registries, and three were observational cohort studies. All nine reported on cancer incidence in HIV positive men (452 cases in 956,095 person-years),

and two reported incidence in HIV negative men (3 cases in 48,881 person-years), (Figure 6, Supplementary Table 6). The incidence of anal cancer was significantly higher in HIV positive men (45·9 per 100,000; 95% CI 31·2-60·3) than in HIV negative men (5·1 per 100,000; 95% CI 0-11·5), (p=0·011). In HIV positive men, the incidence of anal cancer was significantly higher in the years after introduction of highly active antiretroviral therapy (HAART), (21·8 per 100,000 before 1996, 95% CI 8·2-35·4 versus 77·8 per 100 000 after 1996, 95% CI 59·4-96·2; p=0·013, Figure 6).

Progression of high-grade AIN to anal cancer

There were no direct estimates of the progression rate from high-grade AIN to anal cancer. From the available data, we calculated a theoretical progression rate from high-grade AIN to anal cancer of one in 633 (one in 377 in the HAART era) per year, in HIV positive men, and one in 4196 per year, in HIV negative men.

Heterogeneity

There was a high degree of heterogeneity in most of the meta-analytic results. For detection of any HPV and any high-risk HPV, restricting the analysis to studies which used the most common HPV detection methods, removed a large proportion of the heterogeneity (Supplementary Table 9).

Publication bias

Assessment of funnel plots suggested significant publication bias for prevalence of LSIL in HIV negative men (p=0·032, Supplementary Figure 2), but the smaller studies tended to report lower prevalence.

Discussion

In the studies of homosexual men we reviewed, the majority of men had detectable anal canal HPV, and histologically proven high-grade AIN was present in twenty to thirty percent of all men. HIV positive men were consistently more affected by HPV and HPV-related abnormalities than HIV negative homosexual men, and were

found to have a disturbingly high incidence of anal cancer. These anal cancer rates are comparable to the incidence of cervical cancer in the general female population before the introduction of national cervical screening programs[28], and in HIV positive men in the post-HAART era, incidence rates were even higher. Longitudinal data were extremely sparse, but those available suggested an extraordinarily high annual incidence of high-grade AIN in HIV positive men (around 10% or more) and around 3% in HIV negative men. These incidence rates appear to be higher than would be consistent with our estimate of the prevalence of high-grade AIN (29% in HIV positive and 21% in HIV negative homosexual men), unless many of these high-grade lesions regress. Unfortunately, although regression of high-grade AIN has been described[29] there were no published prospective estimates of high-grade AIN regression rates from natural history studies to confirm this. There were also no published prospective estimates of high-grade AIN progression rates. We calculated a theoretical progression rate of high-grade AIN to anal cancer of approximately one in 600 per year in HIV positive homosexual men, and approximately one in 4000 per year in HIV negative homosexual men. This calculated rate of progression of high-grade AIN to anal cancer is markedly lower than that reported for cervical intraepithelial neoplasia (CIN) grade 3 to cervical cancer, which is estimated to be around 1 in 80 per year[30].

A recent global review of invasive anal cancer concluded that 84% of cases contain HPV DNA. The vast majority of cases (87% of those HPV positive) were positive for HPV-16 and a much lesser proportion (6%) for HPV-18[1]. In our meta-analysis, anal canal HPV was extremely common, and the causative agent of most anal cancer, HPV-16, was detectable in about one third (34%) of the HIV positive men but only about one in 8 (13%) of the HIV negative men. There was moderate to high heterogeneity for all of these measures not explained by the source of participant recruitment. For high-risk HPV only, prevalence increased in more recent publications, but this may be explained by the increased sensitivity of testing for high-risk HPV in more recent years[13]. Differences in the age of study populations are unlikely to explain the heterogeneity. Among homosexual men, anal HPV infection is common at all ages, regardless of HIV status, and there is no evidence of a decline with age[31-32].

Our meta-analysis found a higher prevalence of anal cytological abnormalities in HIV positive compared to HIV negative men. There were far fewer estimates of histologically-confirmed diagnoses, particularly for HIV negative men, in whom results were available for less than 500 men. The small number of cases in published studies may reflect the fact that high resolution anoscopy is a relatively invasive procedure, resulting in a reluctance to perform HRA on all participants, in the absence of cytological and clinical abnormalities. Based on these small numbers, the prevalence of all histologically confirmed lesions was higher in HIV positive than HIV negative men. However, the excess in HIV positive men was smallest for high grade AIN, and did not reach statistical significance. Random error is one possible explanation. An alternate explanation is that some of the excess of low-grade AIN in HIV positive men may simply reflect their higher risk behaviour[33], with consequent higher rates of transient HPV infection that does not lead to high-grade AIN. Certainly, in our data, HIV positive men had markedly higher rates of HPV infection than HIV negative men. Those studies which reported age-specific prevalence, reported that neoplastic lesions did not vary in prevalence by age[34].

It was striking that the prevalence of high-grade AIN was much lower when based on a cytological diagnosis than when it was based on histology. This almost certainly represents under-diagnosis of high-grade AIN by cytology, as this technique has been shown to have limited sensitivity to detect histologically proven high-grade AIN[14]. This is probably because the large and involuted surface area of the anal canal is much more technically difficult to sample with blind swabbing than is the cervix[14, 35].

Our meta-analytic estimate of anal cancer incidence was much higher in HIV positive (46/100,000 per year) than in HIV negative men (5/100,000 per year). Since the mid 1980's, estimates of anal cancer incidence in HIV positive homosexual men have been made possible through the existence of HIV/AIDS registries which contain basic information on risk behavior. This may explain the higher number of studies (nine) with estimates of anal cancer incidence in HIV positive, but only two studies, with only 3 incident cases of anal cancer, in HIV negative homosexual men. In a population-based case control study conducted in the early 1980's the odds ratio for anal cancer in homosexual men compared to heterosexual men was reported to be 33 (95% CI 4·0-272·1)[36], but the

odds ratio for anal cancer in confirmed HIV negative homosexual men has not been reported. Given the general population anal cancer incidence in men is approximately 1-2/100,000[37], our incidence estimate of only 5/100,000 in HIV negative homosexual men, based on two small studies, may be an under-estimate. Further direct estimates of anal cancer incidence in HIV negative men are needed, but such studies are hampered by the lack of any denominator and numerator data on homosexuality outside of dedicated cohort studies. For HIV positive men, the reported incidence of anal cancer in the HAART era (from 1996) was much higher (78/100,000) than in the pre-HAART era (22/100,000). As these incidence rates are not age-adjusted, some of the increase may be due to the ageing of the HIV positive population. However, Piketty et al have reported [38] that the average age of men diagnosed with anal cancer did not differ significantly between the pre-HAART and HAART era. The reason for the increase in anal cancer rates in HIV positive men is unclear. The immune restoration related to HAART may not be sufficient to clear persistent long standing HPV infection and the improved survival associated with HAART may allow for sufficient time for chronically HPV infected men to develop invasive anal cancer[6, 39]. Increases in screening are highly unlikely to explain trends in anal cancer incidence as screening is not routinely recommended, and is not widespread outside of a few clinics in San Francisco and New York City.

Current proposals for anal cancer screening in homosexual men are based largely on the model of cervical cancer screening. This is likely to be reasonable only if the natural history of HPV infection, and the characteristics of treatment of precursors diagnosed by screening are similar at the two sites in the two populations. With regard to natural history, prospective studies in young women conducted shortly after sexual debut have shown that approximately half of new cervical HPV infections clear within 6-12 months, and more than 90% clear within a few years[12]. HPV prevalence in women peaks at less than 25 years and then declines rapidly[12]**.** For the 5% of HPV infections that persist over a period of years, the absolute risk of CIN3 diagnosis increases greatly, to more than 40% for long standing HPV-16 infection[12]. CIN3 is strongly associated with age[12], with a peak around ten years later than that of HPV detection and cervical cancer typically emerges from CIN3 over a period of decades[12]. This meta-analysis demonstrates that a strong foundation of natural history data, akin to that of cervical cancer, is absent for anal cancer precursors. We have found that there appear to be some

important differences between the natural history of the two conditions in the two populations. With regard to treatment, the absence of data from randomised trials demonstrating that treatment of high-grade AIN removes the lesion, or reduces the incidence of anal cancer, and the morbidity associated with high-grade AIN treatment[7] are additional factors suggesting that approaches to cervical cancer prevention cannot be simply extrapolated to anal cancer prevention.

The prevalence of anal HPV and anal dysplasia in homosexual men greatly exceeds that in the cervix. In addition, in contrast to the pattern in women, the prevalence of anal HPV and anal dysplasia in homosexual men appears not to decline with increasing age[31-32, 34]. These differences in overall and age-specific prevalence are almost certainly explained by differences in sexual behavior. In a population-based survey in Australia, 38% of homosexual men but only 1% of heterosexual women reported more than 50 lifetime sexual partners[40-41]. In heterosexual women, the reporting of multiple partners is common only in teenage years, and declines rapidly after the age of 20[40], whereas a substantial proportion of homosexual men in their fifth and sixth decades continue to report multiple sexual partners[42]. Another potential explanation for higher HPV prevalence at older ages is that anal HPV clearance occurs more slowly than in the cervix, leading to a longer duration of HPV infection. This appears unlikely, as in women it has been demonstrated that anal HPV clears more rapidly than does cervical HPV[43]. Based on our data, it appears that another important difference is that progression rates to cancer may also be substantially lower in the anus compared to the cervix. There are few prospective data on rates of CIN2-3 regression and invasion in women as ablative treatment is given to all. A retrospective analysis of women with CIN3 who had treatment withheld found a crude incidence rate of progression to cervical cancer of 823 per 100 000 women-years[30] . Our calculated estimates of progression to invasion from high-grade AIN are clearly much lower than this in both HIV positive and HIV negative homosexual men. However, our estimates were based on a cross sectional comparison of high-grade AIN prevalence and anal cancer incidence, and were not based on prospective follow up. One potential explanation for a lower progression rate is that high-grade AIN may regress more commonly than CIN3. Another is that high-grade AIN includes both AIN2 and AIN3. The AIN2 category, while generally included in high-grade AIN, is probably a mixture of low and high-grade disease.

This has been shown to be the case for CIN 2[44], and there is evidence that CIN2 progresses to invasion less frequently than CIN3[45]. Unfortunately, only two studies presented data on prevalence of AIN2 and AIN3 separately. In these two studies, about 60% of high-grade AIN was AIN2 [17, 23].

This meta-analysis had several limitations. Obtaining a representative sample of homosexual men is challenging given the lack of a population register which would from the sampling frame. Nevertheless, we excluded studies which had a clearly biased sampling scheme, and we found no difference in the prevalence of outcomes between community- and clinic-based samples. The excess of HPV-related lesions in HIV positive as compared to HIV-negative men was present both in community- and clinic-recruited men. In general, data on potentially important covariates such as age, anal signs and symptoms, risk behavior, CD4 cell count, or HIV-related treatment were inconsistently collected. For this reason we did not identify predictors of prevalence beyond year of publication and whether the participants were sourced from clinics or the community. It is important to note that the large majority of all studies were cross sectional and the vast majority of data were from North America for all the study endpoints. The sparsity of longitudinal data, and the lack of geographical diversity limits the potential for drawing conclusions on the natural history of anal HPV and dysplasia in all homosexual men. Data were particularly sparse for histologically proven disease and anal cancer incidence in HIV negative homosexual men. For anal HPV detection, PCR results may not represent active or persistent HPV infection. However, the meta-analytic estimates for HPV detection by hybrid capture, which detects clinically relevant (active) infection[13] , were only slightly lower than those for PCR, indicating that true anal HPV infection was probably not greatly over-estimated.

There was a high degree of heterogeneity in most of the meta-analytic estimates. A likely source of variation lies in the different laboratory detection methodologies utilized [13]. Insufficient data existed to allow for grouping of anal HPV prevalence results by individual PCR primer type, which although extensively validated, have been shown to have varying sensitivity for individual HPV genotypes[13]. For high-risk HPV, different studies included varying numbers of HPV probes. Differences in cytology reporting systems (Bethesda 1988 and 2001) may

explain some variation in cytology reporting rates. Variation in technique and standards of obtaining, processing or analysing smears and biopsies are likely to have contributed to the heterogeneity. Given the very limited availability of clinical and pathology (cytology and histology) personnel who are trained in the detection and diagnosis of anal cancer precursors[14], a high degree of variation is not surprising, and is reflected in widely varying estimates of the sensitivity and specificity of cytology to detect high-grade AIN (42-98% and 8-96% respectively[14]). HRA is acknowledged as a more technically difficult examination than cervical colposcopy[14] and variations in the ability to biopsy appropriate lesions at anoscopy will be reflected in different histological prevalence rates[35]. The increasing prevalence of histologically confirmed abnormalties in HIV positive and HIV negative homosexual men in more recently published studies suggests improvements in the screening procedure over time. Evidence suggests that inexperienced anoscopists may be insufficiently skilled to correctly target lesions for biopsy[14], leading to impaired sensitivty. Based on this observation, some have called for a composite endpoint of a positive cytological or histological diagnosis as a more sensitive measure of true disease[35]. Research in this field would greatly benefit from standardized criteria for the reporting of incidence and regression of AIN.

The data we have summarised demonstrate that the design of anal cancer screening programs is challenging. Data on the natural history of anal HPV infection in homosexual men were limited and heterogeneous. The prevalence of anal HPV infection and associated lesions was extraordinarily highProspective data were so few as to make interpretation of data on the incidence of anal HPV infection, dysplasia and cancer very difficult. A comparison of the prevalence of high-grade AIN with anal cancer incidence suggests that most high-grade AIN will never progress to anal cancer, and that progression may occur less often than for CIN3. However, the lack of data which separated AIN2 from AIN3 makes a direct comparison with data from the cervix impossible. The identification of biomarkers to establish which men with high-grade AIN are at highest risk of progression to anal cancer, and which are likely to regress, should be a research priority. The likely low progression rates of high-grade AIN to anal cancer that this review identifies provides an ethical justification for prospective studies of high-grade AIN with frequent follow up to identify which men are likely have persistent disease, and which

are likely to regress. In such studies, men with persistent AIN3 over multiple visits would be offered treatment. The substantial differences in the natural history of anal HPV infection to those of cervical HPV infection that this review has identified suggests that we cannot simply transfer cervical cancer screening strategies to anal cancer screening. Large, high quality prospective natural history studies, couple with randomised trials of treatment options, are needed to inform the development of anal cancer screening guidelines in homosexual men. Until evidence from these studies is available, screening for anal cancer and treatment of high-grade AIN should occur within a research setting.

Contributors

Dorothy Machalek co-designed the study, performed the systematic search, the statistical analysis, and drafted the paper, Mary Poynten and Fengyi Jin participated in the conception and design of the study, assisted in the systematic search, and revised the manuscript for important intellectual content. Andrew Grulich co-designed the study, and assisted in the drafting and revising of the manuscript for important intellectual content. Jennifer Roberts, Kathy Petoumenous, Annabelle Farnsworth, Sepehr Tabrizi, David Templeton, Christopher Fairley, Suzanne Garland and Richard Hillman revised the manuscript for important intellectual content.

Conflicts of interest

Professor Andrew Grulich has received honoraria and research funding from CSL Biotherapies, honoraria and travel funding from MSD, and sits on the Australian advisory board for the Gardasil. Professor Christopher Fairley has received honoraria, travel funding and research funding from CSL and MSD, sits on the Australian advisory board for the Gardasil HPV vaccine, and owns shares in CSL Biotherapies. Professor Suzanne Garland have received advisory board fees and grant support from CSL and GlaxoSmithKline, and lecture fees from Merck, GSK and Sanofi Pasteur; in addition, has received funding through her institution to conduct HPV vaccine studies for MSD and GSK and is a member of the Merck Global Advisory Board as well as the Merck Scientific Advisory Committee for HPV. Associate Professor Richard J Hillman has received support from CSL Biotherapies and MSD. All other authors have no conflicts of interest to declare.

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Table 1
Summary of identified studies addressing the selection criteria for the natural history of HPV detection and associated neoplastic lesions in homosexual men

	HIV Positive			HIV Negative		
	Number of studies/estimates	**Reference**	**Total Sample size**	**Number of studies/estimates**	**Reference**	**Total sample size**
Studies of anal HPV Prevalence (n=31	29		4868	18	31, 48-49, 51, 54, 73	4487
Estimates of any HPV type	32		4624	21	32, 55, 60, 62	4320
Estimates of any High Risk type	17	22, 31, 46-54	2448	11	63-66, 68-69	2618
Estimates of any Low Risk type only	2	55-72	53	3	70-71	510
Estimates of HPV-16	15		1468	9		2687
Estimates of HPV-18	12		1328	6		1283
Incidence of anal HPV infection (HPV-16 and 18)	1	22		2	10, 21	
Clearance of anal HPV infection (HPV-16 and 18)						
Studies of ASIL prevalence (n=19)						
all studies(community/clinic)	17 (2/15)	23, 46-47, 74-75	2791	6 (3/3)	26, 51, 66, 76	1827
Any Abnormality		50-51, 76-77	440/2351		27, 69	1596/231
LSIL		58, 78-80	440/2229			1596/231
HSIL		27, 59, 67, 69	440/2229			1596/231
Prevalence of AIN with HRA on all (community/clinic)	8 (3/5)	23, 50, 81	1494	4 (3/1)		449
Any Abnormality (com/clinic)		51, 55, 59	479/1015		27, 51, 55, 81	423/26
low-grade AIN		17, 27	479/963			423/26
high-grade AIN			479/963			423/26
Incidence of histologically confirmed AIN2-3 (from normal/lesser/AIN 1) n=3	2	23-24		2	10, 24	
Regression of histologically confirmed AIN2-3	No published estimates					
Incidence of anal cancer	9	6, 38-39, 82-87	956095	2	6, 87	48881

Figure 1: Scheme showing the study selection process for the systematic review of anal canal HPV prevalence and associated dysplasia in HIV positive and HIV negative homosexual men

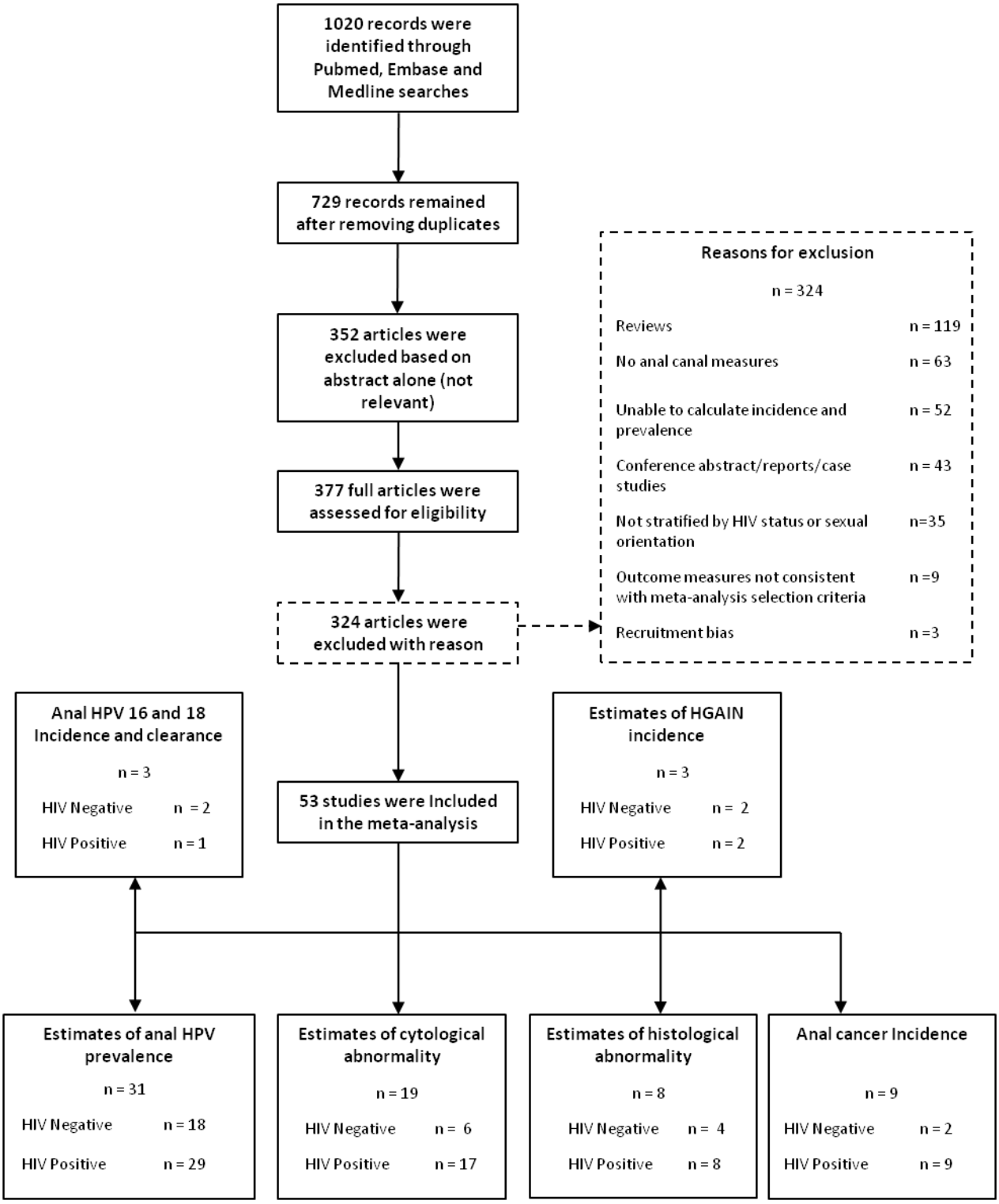
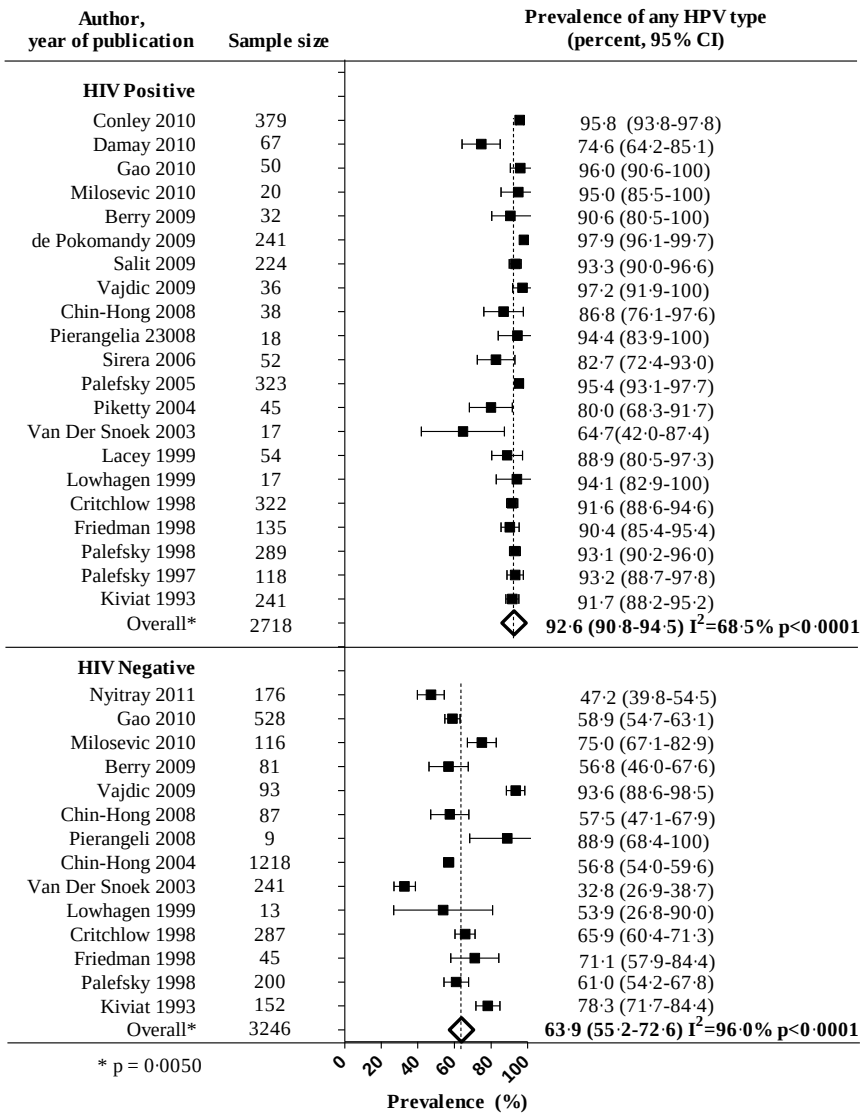


Figure 2: Meta-analytic prevalence and 95% confidence intervals (CI) for the detection of any anal canal HPV (2a) and high-risk HPV (2b) by PCR in HIV positive and HIV negative homosexual men

2a. Prevalence of any anal canal HPV type



2b. Prevalence of any anal canal high risk HPV type

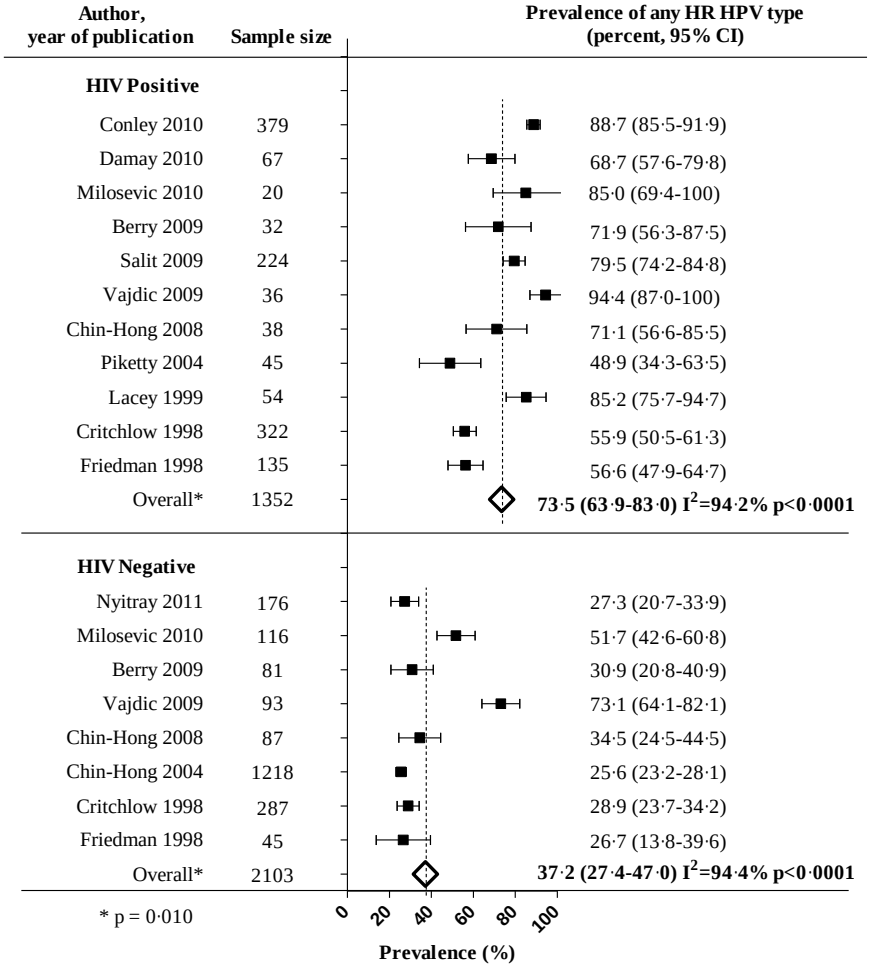
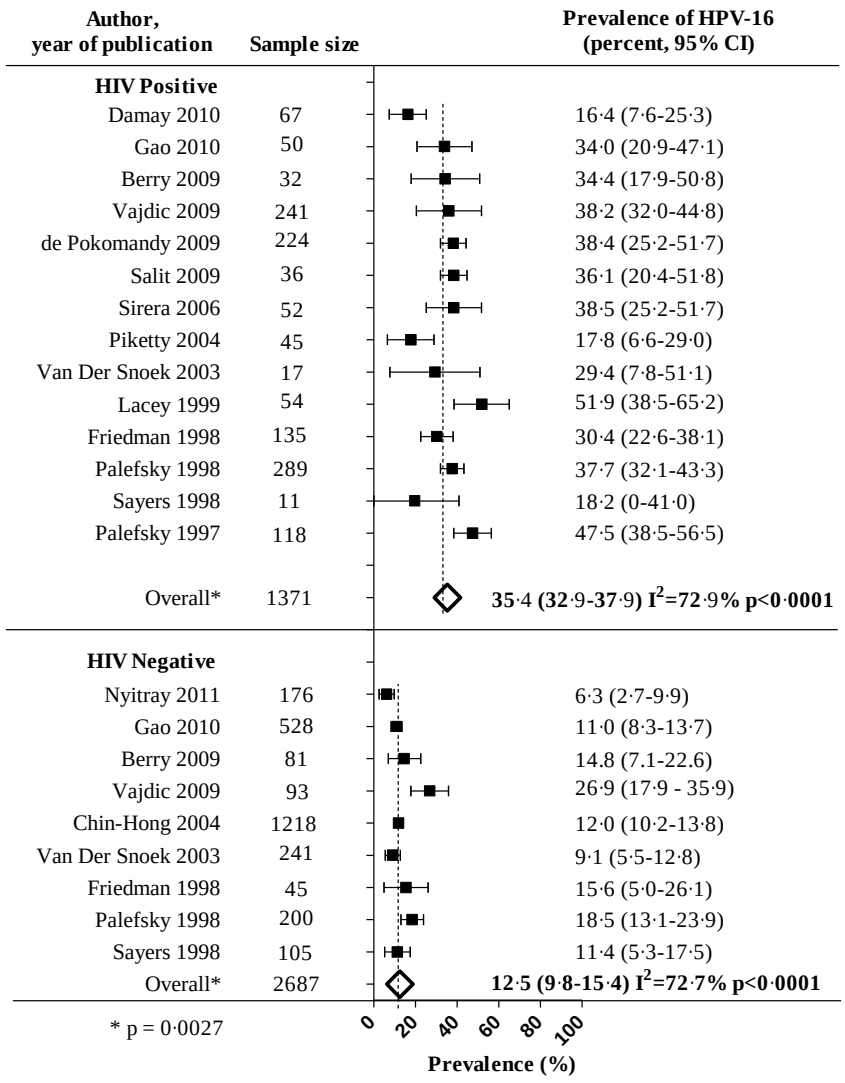


Figure 3: Meta-analytic prevalence and 95% confidence intervals (CI) for the detection of anal canal HPV-16 (3a) and HPV-18 (3b) by PCR in HIV positive and HIV negative homosexual men

3a. Prevalence of anal canal HPV-16



3b. Prevalence of anal canal HPV-18

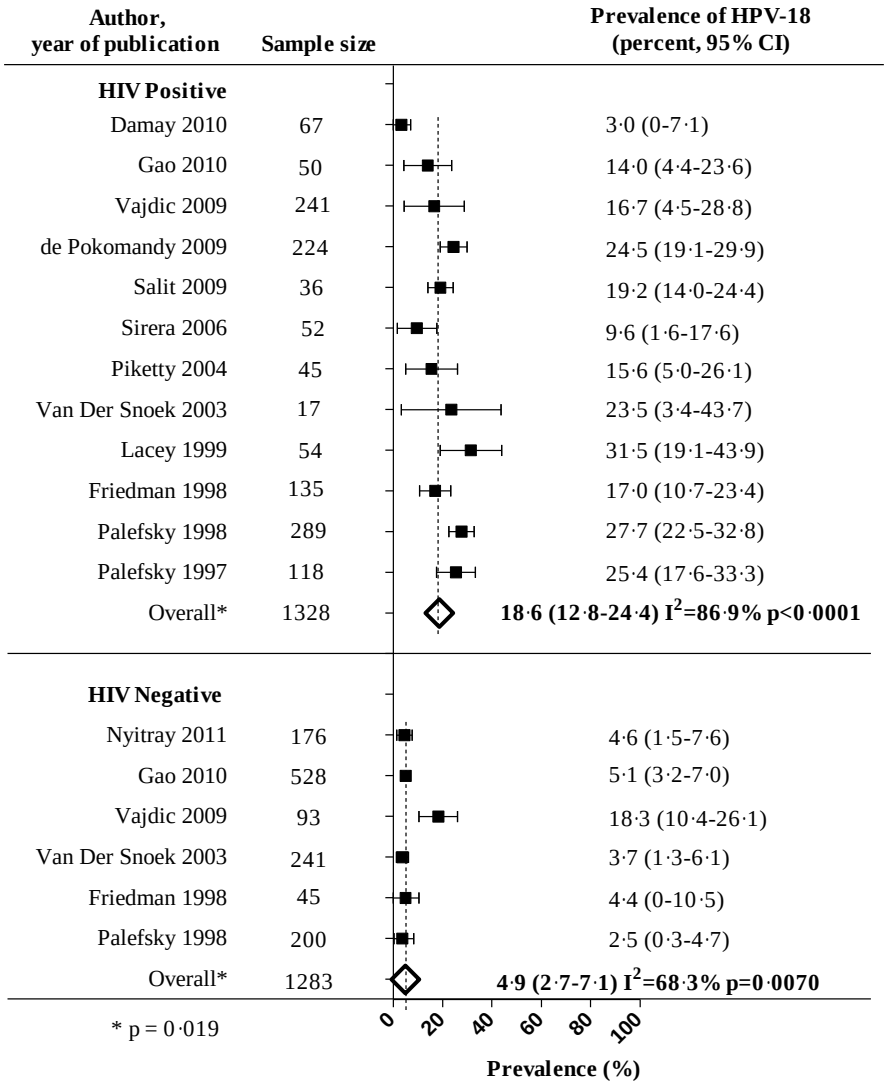
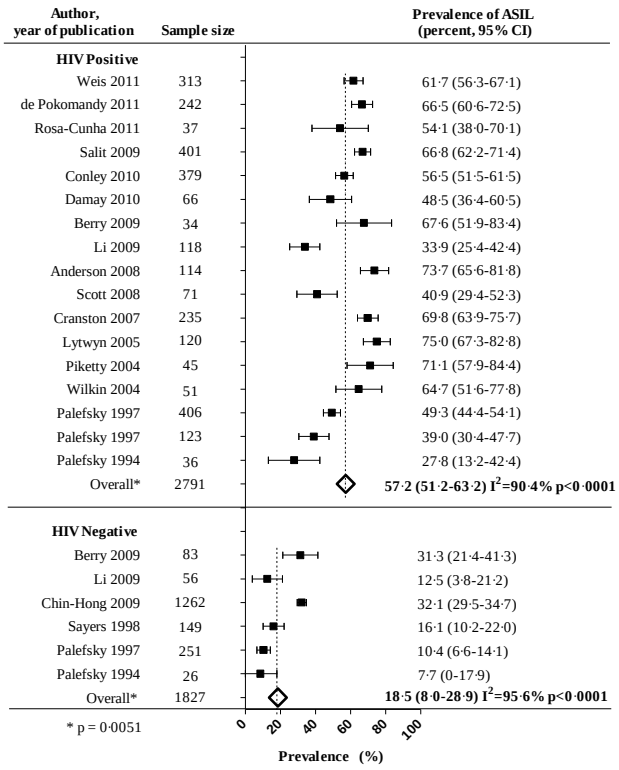
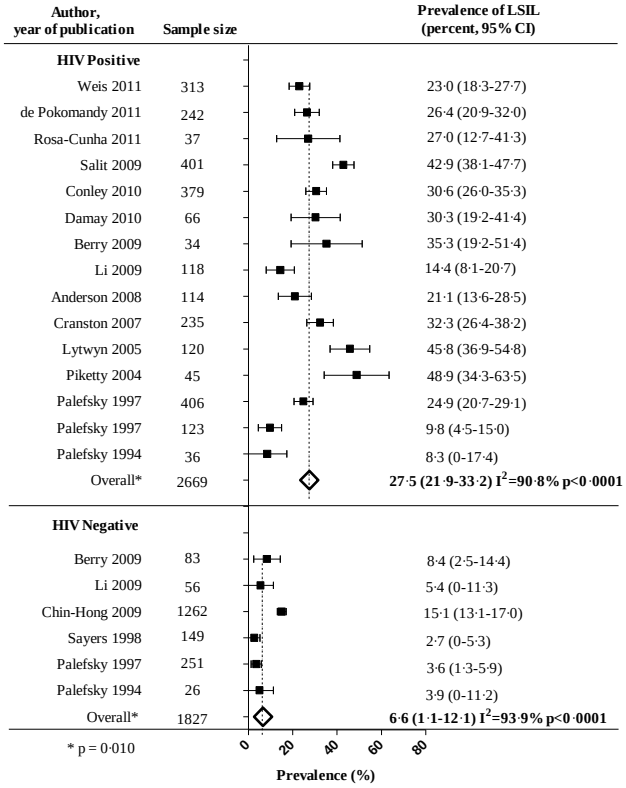


Figure 4: Meta-analytic prevalence and 95% confidence intervals (CI) of anal canal cytological abnormalities (4a), low grade cytological lesions (4b), and high grade cytological lesions (4c) in HIV positive and HIV negative homosexual men. Any cytological abnormality includes ASC-US, LSIL, ASC-H, and HSIL

4a. Prevalence of any anal cytological abnormality



4b. Prevalence of low grade anal lesions (LSIL)



4c. Prevalence of high grade anal lesions (HSIL)

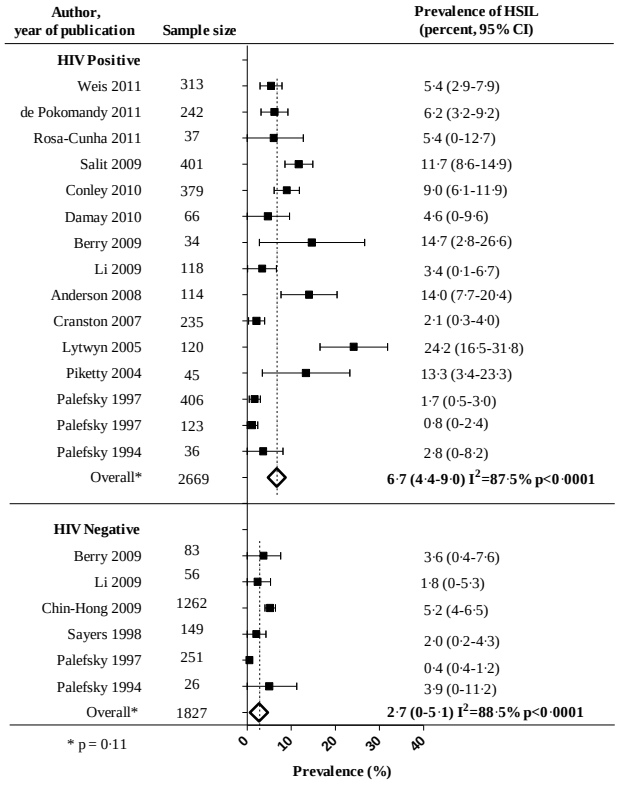
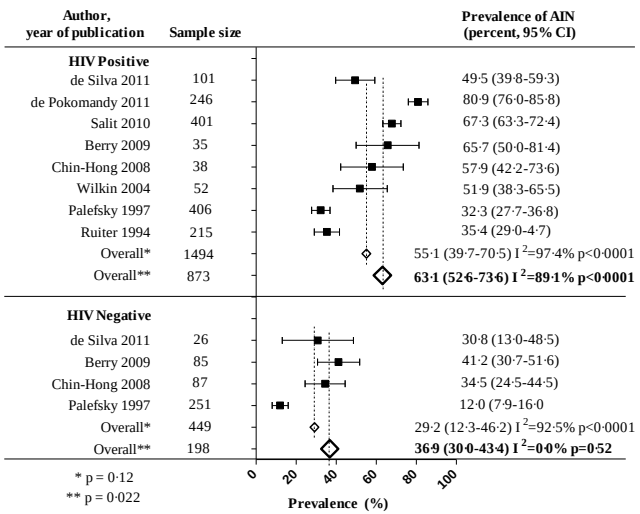
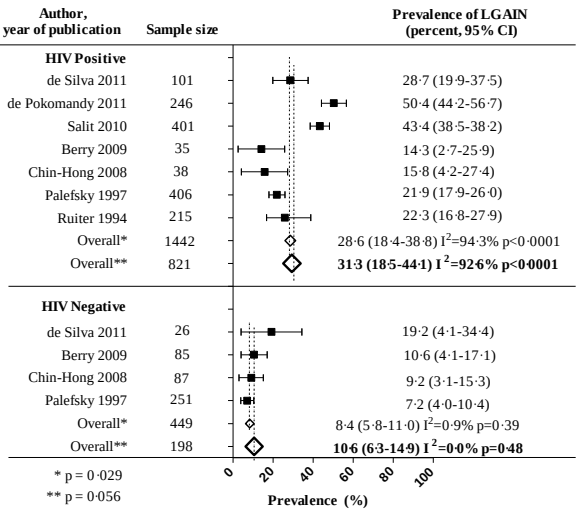


Figure 5: Meta-analytic prevalence and 95% confidence intervals (CI) of anal canal histological abnormalities (5a), low grade histological lesions (5b), and high grade histological lesions (5c) in HIV positive and HIV negative homosexual men. The large diamond indicates the meta-analytic prevalence estimates of histological abnormalities after excluding Palefsky 1997 and Ruiter 1994 studies in HIV positive, and Palefsly 1997 study in HIV negative men.

5a. Prevalence of any anal histological abnormality



5b. Prevalence of low grade anal lesions (LGAIN)



5c. Prevalence of any high grade anal lesions (HGAIN)

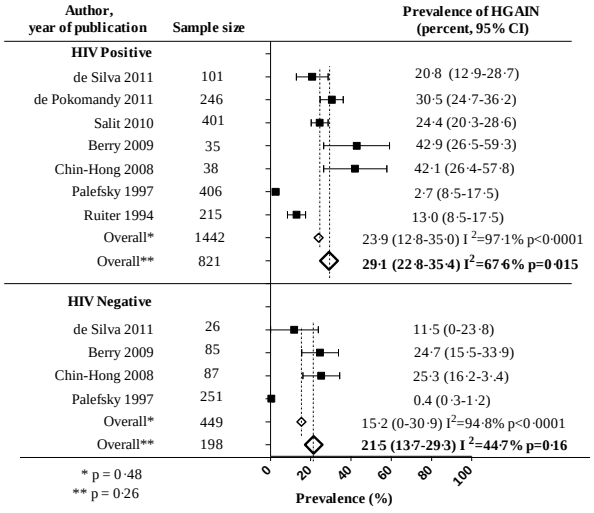
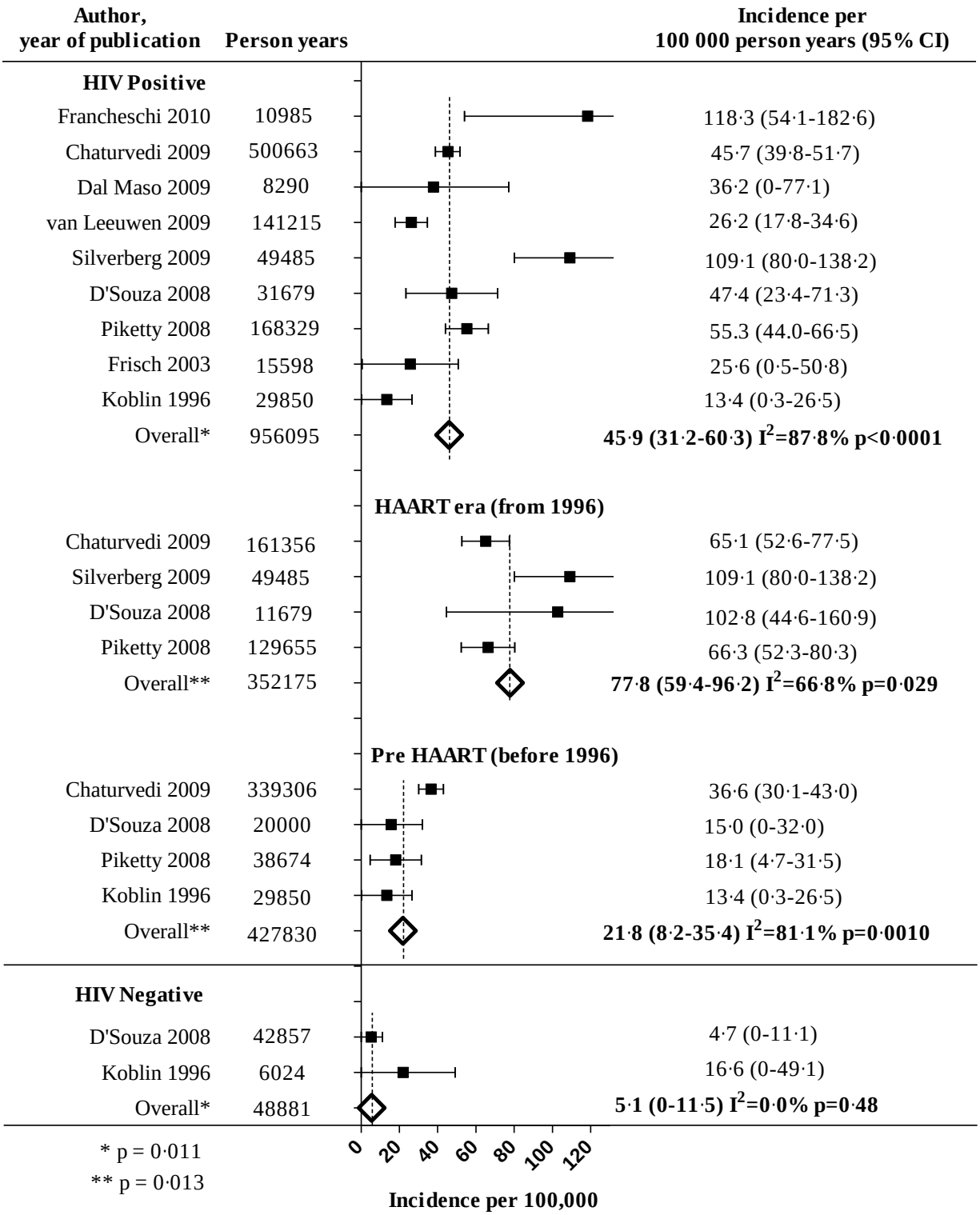


Figure 6: Meta-analytic estimate and 95% confidence intervals (CI) for the incidence of anal cancer in HIV positive and HIV negative homosexual men



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