Anal HPV infection & disease
Common and preventable, but hard to treat
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CHAPTER 11

Discussion
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In this thesis we assessed several epidemiological aspects of human papillomavirus (HPV) infection and disease, as well as HPV vaccination intention among key populations. Included key populations were men that visit the STI clinic in Amsterdam, the Netherlands, men who have sex with men (MSM), both HIV-negative and HIV-positive, as well as female sex workers (FSW). The epidemiological studies aimed to gain insight in and increase the understanding of the natural history of anal HPV infection and disease and to try to predict HPV-related anal disease in MSM, and to understand the relationship between HPV detection at multiple anatomical locations in FSW. Results from the socio-psychological studies on HPV vaccination intention aimed to understand HPV vaccination intention and be therefore informative for possible introduction of HPV vaccination for these key populations.

Part 1: Anal HPV infection and HIV infection among men who have sex with men

Anal cancer incidence is much higher among HIV-positive compared to HIV-negative MSM. Since HPV infection is the cause of anal cancer in approximately 88% of all anal cancer cases, differences in the epidemiology and natural history of anal HPV infection could be expected between HIV-positive and HIV-negative MSM. This increased risk is confirmed by previous studies that found that HIV-negative and HIV-positive MSM differ in anal HPV prevalence of infection, incidence of infection, persistence of infection, clearance of high-risk HPV infection, and HPV16 and -18 seropositivity. All these parameters show more and longer infection and seropositivity, and less clearance in HIV-positive compared to HIV-negative MSM. In Chapter 2 we showed that HPV viral load, the proportional amount of HPV virus particles, in the anal canal does not differ between HIV-positive and HIV-negative MSM for the two most carcinogenic HPV types, HPV type 16 and HPV type 18. Among both HIV-positive and HIV-negative MSM, HPV viral load was an independent determinant of HPV persistence in the anal canal.

These results allow us to conclude that the mechanism of a productive HPV infection leading to persistence, measured based on HPV viral load, does not differ by HIV-status. As this association between HPV viral load and persistence has previously also been found in penile HPV infection and in cervical HPV infection, it can be expected that this mechanism does not differ by gender nor by anatomical location of infection. This results in the conclusion that a higher viral load cannot explain
increased HPV persistence in HIV-positive MSM, mostly using combination antiretroviral therapy, compared to HIV-negative MSM. This suggests that there are other factors, viral-host interactions, that explain differences in HPV persistence between HIV-positive and HIV-negative MSM. Future studies should focus on the effectiveness of immune responses after HPV infection in HIV-negative MSM and HIV-positive MSM on combination antiretroviral therapy (cART).

MSM are at increased risk for HPV-associated anal lesions, anal HSIL. HIV-positive MSM are at highest risk for these lesions. Much is known about HPV incidence and clearance among HIV-positive MSM, but less is known for HIV-negative MSM, despite their increased risk. In Chapter 3 we assessed transition rates and risk factors for transition between infected and uninfected among HIV-negative MSM over a follow-up period of 5 years. We found the HPV16 had the highest incidence and the lowest clearance rate compared to other hrHPV types. Furthermore, having had over 100 lifetime sex partners was significantly associated with incident anal hrHPV infection.

These results confirm the importance of HPV16 in carcinogenesis and emphasize the importance of HPV16 in anal dysplasia. The fact that having had over 100 lifetime sex partners being significantly associated with incident infection, but the number of sex partners in the preceding 6 months was not suggests reactivation of latent infections. The concept of latency has previously been described in cervical HPV infections related to immunosuppression by HIV infection or immunosuppressive medication. Recently potential latency of HPV infection has also been shown independent of immunosuppression by HIV infection or organ transplantation for anal HPV infection among HIV-positive and HIV-negative men who have sex with men. Future studies should focus on strengthening the evidence of the existence of latency in anal HPV infection, as well as the transmission risk of reactivated HPV infection and the carcinogenic impact of reactivated HPV infections.

Persistent HPV infection leads to precursor lesions of anal cancer (anal high-grade squamous intraepithelial lesions; anal HSIL) before eventual transformation into anal cancer. In order to prevent anal cancer it is expected that treatment of anal HSIL may prevent development into anal cancer, despite high recurrence rates of anal HSIL. For the treatment of anal HSIL, detection of these lesions is required through
screening. For screening of cervical lesions a highly efficient algorithm based on HPV testing and cervical cytology is available \(^{26,27}\). Unfortunately, this algorithm is not applicable to anal HSIL screening in HIV-positive MSM, as the pooled-prevalence of high-risk HPV in this group is very high (estimated at 74%, 95%CI 64–83%) \(^1\). Based on HPV testing the large majority of HIV-positive MSM should still undergo anoscopy, thus hrHPV positivity would be an inefficient screening tool. Anal cytology is also not an appropriate method because of its limited sensitivity (30%; 95%CI 19–44%) and specificity (93%; 95%CI 90%–95%) \(^{28-30}\).

The gold standard for screening for anal HSIL is high-resolution anoscopy (HRA). HRA is costly, time-consuming, cumbersome for the patients, and it is a difficult procedure, which requires extensive training and experience \(^{31}\). To facilitate quality assurance in HRA practices, we showed the potential of the anal HSIL detection rate of individual anoscopists as a quality metric in Chapter 4. This quality assurance is needed, since we observed a learning curve for HRA and the anal HSIL detection rate appears to stabilize after approximately 200 anoscopy sessions.

Conflicting prevalence figures of anal HSIL are found between studies as well as within studies, including in Chapter 4 \(^{1,32-34}\). A meta-analysis showed a pooled prevalence of histologically proven anal HSIL among HIV-positive MSM of 29% (95%CI 23–35%), however an increase over time was seen in the prevalence of anal HSIL \(^1\). It is expected that the true prevalence among HIV-positive MSM in the Netherlands is somewhere around 40%, based on the anal HSIL detection rates found in Chapter 4.

Based on the combination of incidence of anal cancer and the prevalence of anal HSIL, we must conclude that many of these anal HSIL lesions will not progress to cancer. Up to now we are unable to predict which lesions will progress to anal cancer and which will not. Therefore, all anal HSIL lesions should at least be monitored and preferably treated in order to try to prevent anal cancer. Being able to predict which anal HSIL requires follow-up and treatment would be useful. In addition, it would be useful to distinguish those at high risk for the presence of HSIL from those at low risk, through identifying risk factors of anal HSIL. Previous data on these risk factors are somewhat ambiguous. We therefore studied risk factors of anal HSIL among 1678 HIV-positive MSM from 3 different clinics in Chapter 5. We demonstrated that young HIV-positive MSM and those with no or a short time living with HIV viral suppression are
Discussion

statistically at higher risk for presence of anal HSIL. However, only 14% of participants was virally suppressed for less than one year, and the prevalence of anal HSIL was also high (31%) among participants that were virally suppressed for more than a year. This supports the current advice that all HIV-positive MSM should be screened for anal HSIL.

Prioritizing HIV-positive MSM for anal HSIL screening could potentially be done with biomarkers. In Chapter 6 we showed that type-specific anal hrHPV persistence was strongly associated with anal HSIL caused by the same HPV type. But we also showed that persistent hrHPV infection did not have enough discriminatory power to be a good predictor for the presence of anal HSIL, irrespective of the causative type.

These data thus confirm that a persistent HPV infection precedes the development of anal HSIL. However, these results do not suggest that repeated anal hrHPV testing can be used to triage patients for HRA screening. For patients and clinicians it is less relevant to know that the odds of having anal HSIL that is caused by HPV16 is much higher if you have an HPV16 persistent infection; it is relevant for patients to know whether they have anal HSIL or not. Unfortunately, the studied biomarkers could not predict the presence of anal HSIL on a patient level.

Up to now, no other prediction models for anal HSIL have been developed, and the search for potential predictive biomarkers needs to continue. Possibly our study had too limited power to identify weak predictors. However, it is debatable whether weak predictors are useful to select patients for screening, especially since the aim of this screening is to prevent a serious event, anal cancer. The results from the Study on the Prevention of ANal Cancer (SPANC study) from Australia could potentially contribute to the prediction of anal HSIL among MSM. The SPANC study is a prospective cohort study on the epidemiology of low-risk and high-risk anal HPV infection and related cytological and histological abnormalities in HIV-negative and HIV-positive homosexual men aged 35 years and over. In this study, much larger than our cohort of 193 men, many potential predictors of anal HSIL can be studied.

A different approach to predict the presence of high-grade lesions is studying methylation markers, that are already proven successful in predicting high-grade
Discussion

cervical lesions \(^{38-45}\). Methylation of DNA is one of the mechanism in which the expression of specific genes can be inhibited and it can increase the probability of mutations in the genes. Methylation is a normal process, however distinct and abnormal patterns of methylation are observed in cancer \(^{38}\). Therefore, methylation markers can show the methylation state of lesions, correlating with the progression towards cancer, as is proven in cervical lesions \(^{39-45}\). For anal HSIL, the first promising results of the potential of DNA methylation as a screening tool to detect HSIL at high risk for development of cancer have been shown. Up to now, six genes have been studied that all showed significantly increased methylation levels with the increase of the severity of anal lesions, up to 95% in anal squamous cell carcinoma. The combination of these markers expressed in biopsy samples resulted in an area under the ROC curve of 0.85 for the detection of high-risk AIN3 or worse \(^{46}\).

**Future research on anal HPV infection and anal HSIL**

Following the studies in this thesis, as described above, future research on anal HPV infection should focus on the effectiveness of immune responses after anal HPV infection in HIV-negative MSM and HIV-positive MSM, as well as on the extending the evidence of the existence of latency in anal HPV infection. Also transmission risk of reactivated anal HPV infection and the carcinogenic impact of reactivated anal HPV infections is not yet known. Future studies on predicting anal HSIL, like the SPANC study \(^{36,37}\), should further assess potential biomarkers of anal HSIL.

Indirectly following the studies in this thesis, other questions remain. HPV can infect the epithelium of a host during direct contact. The prevalence of hrHPV infection is high with 65% in HIV-positive and 45% in HIV-negative MSM having at least one hrHPV infection. Also persistent hrHPV infections are common in these groups \(^{9}\). Based on the high sexual exposure among MSM, and taking into account natural biological processes of transmission, transmission risk may be HPV disease-stage dependent. Although the precise mechanisms are unclear up to now, a combination of virological as well as host characteristics determines the dynamics of infection \(^{47}\). This interaction is confirmed by the higher HPV prevalence and incidence, and lower clearance rate, among immunosuppressed persons \(^{1,35,48}\).
Although the current knowledge on infectiousness and susceptibility is limited, some knowledge is already gathered. Infectiousness may depend on the productivity of the lesion, i.e. the number of viral particles produced. HPV E4 is an immunohistochemical marker, and has a role in virus release and transmission\textsuperscript{49}. It has been shown that HPV E4 can be found in reproductive infections like anal low-grade SIL (AIN1), as well as in some AIN2 lesions, but it is only occasionally found in AIN3 lesions\textsuperscript{50}. Therefore E4 positivity might be an indicator for infectiousness. However, in Chapter 6 we found that the HPV viral load, the number of viral particles in the anal canal was not predictive of anal HSIL, but Jin and colleagues found that HPV viral load was associated with anal HSIL\textsuperscript{37}. Further research should assess this association between HPV viral load and anal HSIL, as well as the association between HPV viral load and infectiousness.

Concerning susceptibility, it is known that naturally acquired type-specific HPV antibodies do not seem to be protective against anal reinfection with the same HPV type\textsuperscript{51,52}. Humoral immunity might not be protective of new HPV infections, but we know that incidence, prevalence and persistence is higher among HIV-positive people as well as among otherwise immunocompromised persons. Furthermore, in Chapter 3 we found that HPV viral load could also not explain persistence differences between HIV-positive and HIV-negative MSM. Cellular immunity might play an important role in HPV susceptibility. A potential explanation for the higher HPV incidence and persistence in HIV-infected MSM may be that the T-cell receptor repertoire diversity after cART therapy in HIV-positive MSM is still diminished compared to HIV-negative MSM, thereby contributing to delayed clearing of HPV\textsuperscript{53}. Furthermore, although limited proof is available up to now, host genetic factors, human anogenital microbiota, anogenital mucosal immunology and hormone concentrations might affect HPV susceptibility\textsuperscript{47}.

To conclude, transmission of HPV infection is a combination of infectiousness and susceptibility, but specific transmission and infection risks need more exploration. Gaps in knowledge that should be considered for future research are the effect of host genetic factors, human microbiota, mucosal immunology, hormone concentrations as well as the interaction between HPV, HIV and other sexually transmitted infections on HPV transmission risk\textsuperscript{47}.
Discussion

**Part 2: HPV vaccination for boys or men**

HPV vaccination prior to or early after sexual exposure has been known to be safe and effective in the prevention of HPV infection of the cervix among girls. As a result, HPV vaccination for girls has been introduced in many countries, including the Netherlands, to prevent cervical cancer. Following studies on safety and effectiveness of HPV vaccination for girls or young women, it was found that the vaccine was not only safe and effective in preventing HPV infection and persistence among girls, but also among boys and young men. Some countries, like Australia, the United States and Austria, currently offer gender-neutral HPV vaccination. A study on the effect of gender-neutral HPV vaccination in the Netherlands suggested that it could be cost-effective to offer HPV vaccination to boys and girls in the year they turn 13 years of age. An alternative strategy would be to start with targeted vaccination, in which the HPV vaccination is offered to men at Sexually Transmitted Infections (STI) clinics, like is done in the United Kingdom. HPV vaccination for MSM who visit STI clinics has been found to be cost-effective in the United Kingdom.

Gender neutral HPV vaccination is still being discussed in the Netherlands and future implementation is unclear. HPV vaccination for a target population, for example men that visit the STI clinic, could be a realistic policy change, achievable on relatively short term. However, the success of vaccination depends on the willingness of men to get vaccinated against HPV. In Chapter 7 we showed that HPV vaccination intention among male clients of the STI clinic in Amsterdam is very high. Most of the variance in HPV vaccination intention among men could be explained by socio-psychological factors and these determinants were largely similar in MSM and in men who have sex with women. Out-of-pocket payment had a strong negative impact on HPV vaccination intention.

These results suggest that if HPV vaccination would be offered to men at STI clinics free of charge, uptake of the HPV vaccination would be high. This would especially be important for MSM, since it has been modeled that the high burden of anal cancer among MSM will not be prevented even if a high HPV vaccination coverage among girls would be reached. Increase in uptake of HPV vaccination among girls does not protect MSM, since most MSM do not, or barely, interact with the sexual network of women.
and therefore do not profit from herd-immunity \(^{63}\). Increasing the HPV vaccination coverage to 80% would yield the same gain in life-years as vaccinating 40% of boys and 60% of girls \(^{67}\). The current coverage among girls is 53% and has been declining recently \(^{57}\). Additionally, males are increasingly affected by HPV-related diseases in the Netherlands: the male proportion of the total burden of HPV-related diseases increased from 9.8% in 1989 to 26% in 2014, and this proportion keeps increasing \(^{68}\). The above mentioned evidence, as well as additional current knowledge on HPV-related disease among men and knowledge on HPV vaccination for boys or men are discussed in Chapter 8. Combining those data also showed the relevance for HPV vaccination among boys and allows several conclusions. First, HPV causes a substantial burden of disease among men. This burden of disease is preventable by offering gender-neutral HPV vaccination to boys and girls, or to some extend offering HPV vaccination to adolescent/adult men. Second, gender neutral HPV vaccination was shown to be cost-effective in the Netherlands. Targeted HPV vaccination of MSM was shown to be cost-effective in the United Kingdom. Therefore, HPV vaccination of boys and/or men could substantially and cost-effectively reduce the burden of HPV-related diseases, like penile, anal and oropharyngeal cancers, among men in the Netherlands.

**Future research on HPV vaccination for boys or men**

A currently ongoing HPV vaccine trial tests safety and efficacy of HPV vaccination among men over 26 years of age with more than 5 lifetime sex partners \(^{69}\). The mid-adult male vaccine trial among males 27-40 years of age has already shown safety and immunogenicity of HPV vaccination, showing immune responses comparable with males up to 26 years of age \(^{69}\). Efficacy of HPV vaccination to prevent HPV infections in mid-adult men is currently being studied.

Long-term safety of HPV vaccination has been monitored and debated since the introduction of HPV vaccination. A recent review on HPV vaccination-related serious adverse events concluded that HPV vaccination caused serious adverse events \(^{70,71}\), however this review had strong methodological weaknesses. This review contrasts to reviews and results from randomized controlled trials that show widely proven safety for short and long-term follow-up for available HPV vaccines \(^{61,72}\). HPV can cause cancer 10 to 20 years after infection \(^{73,74}\), and we do not yet have follow-up of safety and effectiveness of HPV vaccines for a period of 20 years. Ten years after HPV
vaccination started, the HPV vaccine has been found safe with strong immunogenicity resulting in an effective long-term protection\textsuperscript{75-77}. Although no changes on safety and effectiveness to persistence of HPV infection are expected in 20 years of follow-up, the debate will at least remain until this follow-up period is completed.

\textbf{Part 3: HPV infection and vaccination intention among female sex workers}

Some studies have shown a small protective effect of condom use against HPV infection, but most show a lack of protective effect against HPV acquisition\textsuperscript{78-80}. Therefore, people with a high number of sexual partners are at increased risk of HPV infection regardless of condom use. Due to their profession, female sex workers (FSW) are at increased risk of HPV infection. Currently, women who are now FSW have not benefitted from the national HPV vaccination program, because they were older than 16 years when vaccination was introduced. Additionally, a large proportion has a migration background, coming from countries without a comprehensive HPV vaccination program. In \textbf{Chapter 9} we showed that vaginal and anal hrHPV infection and hrHPV seropositivity are very common among FSW in Amsterdam, the Netherlands. No protective effect of condom use was found in this study. Additionally, a high concordance between vaginal and anal hrHPV infection was found in the absence of reported anal sex.

The high prevalences indicated that FSW are indeed a risk group for HPV-related diseases. The observed high concordance between HPV types found in the vagina and those found in the anus, without report of anal sexual contact, indicates smear infections. This might occur for example by sexual contact using only fingers, anal-penile contact without penetration, or post-toilet front-to-back wiping behavior\textsuperscript{81}. Among women, potential smear infections were previously described for other sexually transmitted diseases, for example vaginal and anal chlamydia infection\textsuperscript{82}. Based on this high risk for both cervical and anal HPV-related diseases, the limited protective effect of antibodies induced by natural infection\textsuperscript{83-85}, and the proven safety and effectiveness of HPV vaccination in women aged up to 45 years\textsuperscript{86,87}, HPV vaccination of FSW, preferably at the beginning of the sex work career, may be a useful protective method against hrHPV infection and it sequelae.
Based on the risk of FSW and safety and effectiveness of the HPV vaccination, offering HPV vaccination would be the best choice. The next question is whether FSW are willing to get vaccinated against HPV? In Chapter 10 we assessed HPV vaccination intention and determinants of HPV vaccination intention among FSW in Amsterdam, the Netherlands. In this study we found a high HPV vaccination intention. This HPV vaccination intention was determined by different socio-psychological factors, but most strongly by attitude towards HPV vaccination, normative beliefs and self-efficacy. Out-of-pocket payment of HPV vaccination had a negative effect on the HPV vaccination intention, but remained (slightly) positive even at a required payment of €350.

Based on our study, we conclude that a high uptake can be expected if HPV vaccination is offered free of charge by the Prostitution & Health Center 292, a specialized STI clinic for sex workers in Amsterdam. In the prevalence study we found that 36% of the included FSW had any indication of a current or previous HPV16 infection. Since HPV vaccination is safe and effective up to the age of 45 years, this suggests that at least 64% of all included FSW could possibly benefit from HPV vaccination as far as HPV16 is concerned. The proportion of FSW benefitting from vaccination would even be larger if they would be vaccinated early in their sex (work) career. Based on their profession, prevention of genital warts is very important for these women. As only 8% of FSW was seropositive for all HPV types included in the 4-valent vaccine, the 4-valent vaccine (also offering protection against genital warts caused by HPV types 6 and 11) should be considered for FSW in the Netherlands to prevent HPV-related diseases in this group.

Future research on HPV vaccination for female sex workers

HPV vaccination safety, efficacy and immunogenicity was demonstrated in a study in women up to the age of 45 years, but in that study only approximately 4% of included women had had ≥ 20 sex partners during their lifetime. The FSW in our studies, had a median number of 250 sex partners in the preceding 6 months. So in terms of degree of exposure, these women are not comparable. It could be argued that in order to know the magnitude of the effect of HPV vaccination on HPV infection and diseases for this group, it should be assessed in a group with more than 20 lifetime sex partners. A problem with these kind of studies is the number of FSW, and even
more so the number of FSW that is located in a specific area for a sufficient time period in order to be able to follow them up. I would not recommend starting such a study. Despite the high exposure, “only” 36% of FSW in our study showed any sign of current or previous HPV16 infection, indicating potential protective effect of HPV16 infection for 64% of the included FSW. In my consideration this should be enough proof to offer HPV vaccination to FSW in the Netherlands without an additional study.
Discussion

References


Discussion


Discussion


Discussion

Discussion

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Discussion


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270