Anal HPV infection & disease
Common and preventable, but hard to treat
Marra, E.
CHAPTER 1

Introduction
Introduction

What is HPV?

History
Since Harald zur Hausen discovered the link between human papillomavirus (HPV) and cervical cancer in the early 1970s, knowledge on HPV and HPV-related diseases has grown rapidly. It is now known that HPV infection can not only lead to cervical cancer, but also to anogenital warts, and to vaginal, vulvar, anal, penile, and head-and-neck cancers.

Low vs. high risk HPV
Of all known papillomavirus types, over 100 types are infectious for humans, the human papillomavirus types. These HPV types can be distinguished in mucosal and cutaneous HPV types, based on the type of epithelium they can infect. Mucosal HPV types are divided into low risk (lr) and high risk (hr) HPV types. HrHPV types are the carcinogenic HPV types. Specifically, HPV16 and HPV18 cause the highest proportion of HPV-related cancers at all anatomical locations. Low risk HPV types can cause warts, for example HPV6 and HPV11 cause genital warts or mild dysplasia, but rarely severe dysplasia.

Epidemiology and risk factors of infection
HPV is known to be the most common sexually transmitted infection (STI), with over 80% of sexually active people getting infected with HPV at least once in their lifetime. There are many similarities between HPV infection at different anatomical locations but also some differences. Since this thesis concerns mainly anal HPV infection and disease, and to a small extent cervical HPV infection, similarities and differences between these two anatomical locations will be described in this introduction.

Epidemiology of cervical HPV infection
Prevalence of cervical HPV infection generally varies by age, with a peak in prevalence around the age of 25 years, with a decreasing prevalence with increasing age.

Worldwide, with age standardized by world population, cervical HPV infection prevalence is 10% in women without cervical intraepithelial neoplasia (CIN) aged ≥15 years. The highest prevalence is found in Africa, with 22% of the women being
infected, whereas in Central America and Mexico 20% is infected, in northern America 11%, and in Europe and Asia 8% 17. High risk HPV prevalence in Europe differs widely by country and ranges between 3% to over 15% 18. In the Netherlands, based on samples of the Dutch cervical cancer screening, the prevalence of high risk HPV among women aged ≥30 years was 8% 19.

**Risk factors of cervical HPV infection**

Since HPV can be considered to be an STI, many key risk factors for cervical HPV infection concern sexual behavior. Being under 16 years of age at first sexual intercourse and an increasing number of sexual contacts during life have been identified as risk factors for cervical HPV infection 20,21. Additionally, smoking 22,23, long-term use of oral contraceptives 24, and HIV infection increases this risk 21,25.

**Epidemiology of anal HPV infection**

Less is known about anal HPV infection compared to cervical HPV infection. Additionally, when discussing anal HPV infection several distinctions should be made. For women we should separate women with and without HIV infection as well as women with and without HPV-related pathology at other anatomical locations (including vulva, vagina and cervix). Among HIV-negative women with HPV-related pathology, anal hrHPV prevalence ranged from 23-36%, while among women without HPV-related pathology hrHPV prevalence ranged from 4-22% 26. In HIV-positive women of whom HPV-related pathology is not (yet) known anal hrHPV prevalence ranged from 52-82% 26-28.

As far as we know, there are no studies that have assessed anal HPV prevalence among women in the Netherlands. Among Belgian women visiting a colposcopy clinic, of whom 23% did not have a history of abnormal cervical Pap smear, an anal HPV prevalence of 56% was found 29.

For men a distinction should be made between HIV-negative and HIV-positive men. Men who have sex with men (MSM) are at increased risk for anal HPV infection because of passive anal sexual contact. Among HIV-negative MSM the world-wide prevalence of anal hrHPV is estimated to be around 37%, while among HIV-positive MSM the prevalence is estimated around 74% 30. In a cohort of MSM recruited in 2010-2011 in Amsterdam, the prevalence of hrHPV among HIV-negative MSM was slightly higher, with 45%, and among HIV-positive MSM 65% 31.
Introduction

Risk factors of anal HPV infection

Comparable to cervical HPV infection, anal HPV infection is associated with sexual behavior. In women, the lifetime number of sexual partners and detection of HPV infection at the genitals were found to increase the risk of anal HPV infection. Furthermore, younger women (≤25 years) tend to have a higher risk of incident anal HPV infection, compared to older women (≥45 years).

Also in MSM, sexual behavior is associated with anal HPV infection, specifically (condomless) receptive anal sex and a higher number of sexual partners. Both number of recent and lifetime partners were found to be associated. Additionally, HIV-positivity is a risk factor for anal HPV in MSM. In HIV-positive MSM, time since HIV-diagnosis, a higher nadir CD4+ count, a younger age, and higher number of recent sexual partners have been identified as risk factors of anal HPV infection.

From infection to clearance/HPV-disease

HPV requires micro-abrasions or wounds in order to reach the basal layer of the epithelium. Once the basal layer is infected, a productive infection can be established in which viral particles will be shed at the epithelial surface. Most HPV infections are cleared by the immune system within 2 years after infection. However, if an HPV infection persists, dysplasia can develop which can progress to malignancy. The pathway from infection to cancer is long: for example, it takes approximately 10-20 years to develop cervical cancer after cervical HPV infection.

Figure 1. Natural history of HPV and HPV-related diseases in humans. Steps in carcinogenesis of HPV-related cervical and anal cancer. Figure from Schiffman.

Persistent HPV infection does not directly lead to cancer. First precursor lesions develop, which can be detected through histological assessment of taken biopsies. Cervical precursor lesions are called cervical intraepithelial neoplasia (CIN); anal precursor lesions are called anal intraepithelial neoplasia (AIN). These precursor...
lesions have three stages: mild cellular abnormalities (CIN1/AIN1), and severe cellular abnormalities (CIN2/AIN2/CIN3/AIN3) (Figure 1) 39. Both high-grade CIN (CIN2/CIN3) and high-grade AIN (AIN2/AIN3) are also known as high-grade squamous intraepithelial lesions (HSIL).

**Persistence and clearance**

Persistence and clearance rates in cervical HPV infection differ by HPV type. Cervical HPV16 tends to persist most often with approximately 34% persistence approximately 12 months after incident infection 40. After 36 months of HPV16 infection, the progression rate to CIN1 is 18%, to CIN2 8% and to CIN3 2% 41. Of all women with CIN3, approximately 30% will develop invasive cervical cancer if left untreated 42.

For anal HPV infection persistence and clearance rates also differ by HPV type, sex and HIV-status 39,43. Anal HPV16 persistence over 21 months among HIV-positive men who have sex with men (MSM) was approximately 56%, and 53% among HIV-negative MSM 43. Persistence of anal HPV over 36 months among women is approximately 44% 44. Progression rates of anal HPV infection to AIN1/AIN2/AIN3/anal cancer are currently studied by the SPANC 45 and ANCHOR study 46. Annual progression rates from anal HSIL to anal cancer are estimated to be 1 in 377 patients in HIV-positive MSM, and 1 in 4196 patients in HIV-negative MSM 30.

**Serology**

After most viral infections antibodies are developed. However, not everyone seroconverts after HPV infection and there is a large difference in seroconversion rates between men and women. Approximately 20% of all HPV DNA positive men seroconvert within 13 to 24 months after genital infection 47, while approximately 60% of all HPV DNA positive women seroconvert after cervical infection in the same timespan 48. This difference in seroconversion rates might be caused by anatomical site of infection 49. Type-specific HPV antibodies acquired after natural infection do not seem to be protective against reinfection with this HPV type among both men and women 50,51.

**Epidemiology of HPV-related cervical and anal cancer**

Globally, approximately 630,000 cases of HPV-related cancer per year are diagnosed in men and women (Table 1), which is 9% of all cancers in women and 1% in
men\textsuperscript{10}. It should be noted that the number of cancer cases attributable to HPV differs by region. This HPV attributable fraction is highest in Sub-Saharan Africa, where 26\% of all cancers diagnosed in women are being caused by HPV, which is mainly driven by cervical cancer. In Europe, 3\% of all diagnosed cancers are HPV-driven: 1\% in men and 4\% in women\textsuperscript{10}.

**Cervical cancer**

Worldwide, 530,000 cervical cancer cases are diagnosed yearly, of which most are diagnosed in low-income regions, like Sub-Saharan Africa and India\textsuperscript{10,52}. In Europe, 58,000 cervical cancer cases are diagnosed yearly\textsuperscript{10}, in the Netherlands 847 cases were diagnosed in 2016\textsuperscript{53}.

<table>
<thead>
<tr>
<th>HPV-related cancer site</th>
<th>Number of incident cases</th>
<th>Number of cases attributable to HPV</th>
<th>Number attributable to HPV by gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervix</td>
<td>530 000</td>
<td>530 000</td>
<td>Males: 0, Females: 530 000</td>
</tr>
<tr>
<td>Anus</td>
<td>40 000</td>
<td>35 000</td>
<td>Males: 17 000, Females: 18 000</td>
</tr>
<tr>
<td>Vulva</td>
<td>34 000</td>
<td>8 500</td>
<td>Males: 0, Females: 8 500</td>
</tr>
<tr>
<td>Vagina</td>
<td>15 000</td>
<td>12 000</td>
<td>Males: 0, Females: 12 000</td>
</tr>
<tr>
<td>Penis</td>
<td>26 000</td>
<td>13 000</td>
<td>Males: 13 000, Females: 0</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>96 000</td>
<td>29 000</td>
<td>Males: 24 000, Females: 5 500</td>
</tr>
<tr>
<td>Oral cavity</td>
<td>200 000</td>
<td>4 400</td>
<td>Males: 2 900, Females: 1 500</td>
</tr>
<tr>
<td>Larynx</td>
<td>160 000</td>
<td>3 800</td>
<td>Males: 3 300, Females: 460</td>
</tr>
<tr>
<td>Total</td>
<td>1,101 000</td>
<td>630 000</td>
<td>Males: 60 000, Females: 570 000</td>
</tr>
</tbody>
</table>

In total, 265,672 deaths occurred from cervical cancer worldwide in 2012, making cervical cancer worldwide the 4\textsuperscript{th} leading cause of cancer deaths in women. In Europe, cervical cancer mortality was 24,404 cases, of which 3,479 occurred in Western Europe\textsuperscript{54}. In the Netherlands 207 women died from cervical cancer in 2015\textsuperscript{53}.

Both worldwide, and in Europe, HPV infection with HPV type 16 is the leading cause of cervical cancer incidence and mortality, followed by HPV 18\textsuperscript{7,55}. HPV16/18 prevalence in invasive cervical cancers is 70\%, but is slightly higher in Western countries, including Europe, with 74-77\%\textsuperscript{7}. In Europe the most common HPV types in cervical cancer are HPV16 (63\%), HPV18 (15\%), and HPV45 (5\%)\textsuperscript{55}.
Introduction

Anal cancer

Anal cancer is less common than cervical cancer, with approximately 35,000 HPV-related anal cancers per year globally. Also for anal cancer, regional differences are seen, with also a higher number of diagnoses in low-income countries. In Europe, 6,900 anal cancers are diagnosed yearly, of which 2,700 in men and 4,200 in women. In the Netherlands 242 cases of anal cancer were diagnosed in 2015, of which approximately 194 may be attributed to HPV. Of these 242 cases, 117 were men and 116 were women; of 9 cases gender was unknown.

When reviewing incidence rates of anal cancer in women, three groups should be distinguished: HIV-negative women, HIV-positive women, and women with a history of genital HPV-related pathology. The incidence rate of anal cancer among HIV-negative women is estimated to range from 1-2 per 100,000 person-years. Among HIV-positive women this incidence rate is estimated to range between 4-30 per 100,000 person-years, and among women with a history of genital HPV-related pathology between 1-64 per 100,000 person-years. A meta-analysis including the highest male risk group for anal cancer, MSM, showed that proportionally HIV-positive MSM bear the highest burden of HPV-related anal cancer. The incidence is approximately 78 per 100,000 person-years in HIV-positive MSM and 5 per 100,000 person-years in HIV-negative MSM.

No worldwide anal cancer mortality rates have been estimated up to now. However, country-specific anal mortality rates are available. Both in the United States and the United Kingdom anal cancer mortality rates are <1 per 100,000 person-years, which are approximately 1,100 deaths in the United States (in 2016) and 358 in the United Kingdom (in 2014).

In the Netherlands 34 people died from anal cancer in 2015, of which approximately 30 may be attributed to HPV. Of these 34 deaths, 19 were men, and 15 were women.

HPV16 (81%) is also the most frequent HPV type found in anal cancers worldwide and in Europe, followed by HPV18 (4%). In the Netherlands, HPV type distribution was studied among anal cancer cases from 1986 to 2010 in Amsterdam, showing that
HPV16 was the causative HPV type in 61% of the cases among HIV-positive MSM, and 100% of the cases among HIV-negative men and women \(^{58}\).  

**Prevention and control of HPV-related diseases**  
HPV infection, and therefore HPV-related diseases, can be prevented both by primary prevention (preventing infection) and secondary prevention (preventing disease/cancer).

**Primary prevention: using condoms**  
Since HPV is a sexually transmitted infection, condom use as a primary prevention method immediately comes to mind. Some studies found indeed that condom use can prevent HPV infection, but other studies found only limited or no protective effect of condom use on HPV infection \(^{59-61}\).

**Primary prevention: HPV vaccination**  
Currently three vaccines are registered to prevent persistent HPV infection. Cervarix\(^{®}\) consists of virus-like particles of HPV-16 and -18; Gardasil\(^{®}\) consists of virus-like particles of HPV6, 11, 16, 18. In December 2014, the FDA approved a new 9-valent vaccine protecting against nine HPV types: HPV6, 11, 16, 18, 31, 33, 45, 52, 58. Gardasil 9\(^{®}\) has the potential to protect against 90% of the cervical-, vulva-, vaginal- and anal cancers caused by HPV infection \(^{62}\). HPV vaccination has been shown to be safe and effective for the prevention of HPV infection and many of its precancerous and cancerous sequelae among women younger than 25 years \(^{63-70}\), as well as among men under the age of 26 years \(^{71,72}\). Although HPV vaccines have the highest efficacy among HPV-naïve people, efficacy is also proven for non-HPV-naïve men and women, for women up to the age of 45 years \(^{73-76}\).

HPV vaccination coverage differs across the globe, with a total of 64 countries offering HPV vaccination, in which approximately 118 million women have been targeted, and 47 million women have received a full course of the vaccine in 2014. However, this only covers 1% of low-/lower-middle-income countries \(^{77}\). HPV vaccination requires multiple vaccinations over time, 2 or 3 doses dependent on the type of vaccine and the age of the girl/woman \(^{67,78-81}\). Therefore, a full course of vaccine is not always achieved. A single dose was administered to approximately 59 million women, which is 1.7% of the world population \(^{77}\).
In the Netherlands HPV vaccination was added to the National Vaccination Program in 2009 offering girls HPV vaccination in the year they turn 13 years of age. In the National Vaccination Program Cervarix® is used. Vaccination coverage among these Dutch girls is relatively low, and declined from 61% to 53% between 2014 and 2016.

A study among Dutch mothers of girls who were offered HPV vaccination found that the attitude towards HPV vaccination, beliefs about HPV vaccination, subjective norm and habit strength significantly determined HPV vaccination intention. HPV vaccination intention was found to be a strong determinant for HPV vaccination uptake among Dutch mothers. Specific determinants of HPV vaccination uptake differed by ethnic group in the Netherlands, for example among Surinamese, Netherlands Antillean and Aruban parents, HPV vaccination uptake was determined by HPV vaccination intention and habit strength.

Secondary prevention: screening and treatment
The last decades millions of lives have been saved globally by cervical cancer screening, using the Pap (Papanicolaou) smear. For this Pap smear, cells are collected from the transformation zone of the cervix, which are cytologically tested to detect CIN/cervical cancer. If abnormal cytology is found, a woman is referred to a gynecologist for further assessment and treatment of precancerous lesions.

In the Netherlands a nationwide screening program was set up in 1989. The current cervical cancer screening program (2017) includes women aged 30 to 60 years who are screened every 5 years at their general practitioner.

Screening for anal HPV-related disease is more complicated than screening for cervical HPV-related disease, since anal cytology shows limited sensitivity and specificity (30% sensitivity and 93% specificity). Currently, the gold standard for screening for anal dysplasia/cancer is a high-resolution anoscopy (HRA). HRA is to some extent comparable to colposcopy: during HRA the transformation zone in the anal canal is visualized after acetic acid application. However, it should be noted that HRA is a cumbersome procedure due to the extensive folding of the anal canal, including the transformation zone. Therefore, extensive training is required for accurate performance. If during HRA suspicious lesions are identified at the
transformation zone or perianally, biopsies are taken and pathologically graded, according to the previously explained AIN-system, of no dysplasia/AIN1/AIN2/AIN3/anal cancer. If a diagnosis of AIN2/3 is made, the abnormal tissue is treated with electrocautery or cryotherapy.

Aim of this thesis

The aim of this thesis is to provide insights in the epidemiology of anal HPV infection in MSM and female sex workers, and to some extent in the epidemiology of cervical HPV among female sex workers. Additionally, this thesis provides insight into socio-psychological determinants of HPV vaccination intention among these risk-groups as well as among heterosexual men.

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

In Chapter 2 through 5, various aspects of the epidemiology of anal HPV infection and anal HSIL in MSM are analysed. First, in Chapter 2, we assess differences in anal HPV viral load between HIV-negative and HIV-positive MSM and its association with anal HPV persistence. In Chapter 3, we focus on incidence and clearance of anal high risk HPV infection among HIV-negative MSM. From Chapter 4, we focus on anal HSIL in HIV-positive MSM. HRA is a cumbersome and difficult procedure. In Chapter 4, we explore differences in detection rate of anal HSIL by physician and screening hospital, in order to assess the possibility of using this detection rate as a quality assurance metric for HRA in HIV-positive MSM. In Chapter 5, we assess demographic and HIV-related risk factors of anal HSIL in the largest multi-center study on HRA in HIV-positive MSM so far, to explore if a targeted screening protocol would be possible. In Chapter 6, potential HPV-related virological and serological predictors of anal HSIL in HIV-positive MSM are analysed.

Part 2: HPV vaccination for boys/men

In Chapter 7, we present an analysis of HPV vaccination intention among male clients of a large STI outpatient clinic in Amsterdam, the Netherlands. In Chapter 8, we present current knowledge on HPV vaccination for boys and/or men and outline important considerations for the Dutch HPV vaccination program.

Part 3: HPV infection and vaccination among female sex workers
Part 3 focuses on the epidemiology of anal and cervical HPV infection and seropositivity (Chapter 9), as well as on HPV vaccination intention (Chapter 10) among female sex workers.

In Chapter 11, the results found in this thesis are discussed and future recommendations for the prevention of cervical and anal cancer among the included risk groups are made.

Table 2 presents an overview of the different study populations and their characteristics.
Table 2. Characteristics of the studies and their study populations in which research questions of this thesis were examined.

<table>
<thead>
<tr>
<th>Study name</th>
<th>Geographic location</th>
<th>Study population</th>
<th>Source</th>
<th>Study period</th>
<th>Chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2M study</td>
<td>Amsterdam, the Netherlands</td>
<td>HIV-negative and HIV-positive MSM aged ≥18 years</td>
<td>MSM recruited at the STI clinic in Amsterdam, the Amsterdam Cohort Studies or the Jan van Goyen Medical Center</td>
<td>2010 - 2013</td>
<td>2 &amp; 5</td>
</tr>
<tr>
<td>H2M3 study</td>
<td>Amsterdam, the Netherlands</td>
<td>HIV-negative MSM aged ≥18 years</td>
<td>All HIV-negative MSM included in the H2M study and HIV-negative MSM from the Amsterdam Cohort Studies</td>
<td>2010-2015</td>
<td>3</td>
</tr>
<tr>
<td>AIN cohort study</td>
<td>Amsterdam, the Netherlands</td>
<td>HIV-positive MSM aged ≥18 years</td>
<td>All HIV-positive MSM screened for anal HSIL by the Academic Medical Center, OLVG or the DC clinic, in Amsterdam</td>
<td>2010 - 2015</td>
<td>4 &amp; 5</td>
</tr>
<tr>
<td>H2M2 study</td>
<td>Amsterdam, the Netherlands</td>
<td>HIV-positive MSM aged ≥18 years</td>
<td>All HIV-positive MSM included in the H2M study and screened for anal HSIL by the Amsterdam Medical Center, OLVG or the DC clinic, in Amsterdam</td>
<td>2010-2015</td>
<td>6</td>
</tr>
<tr>
<td>HP4V men study</td>
<td>Amsterdam, the Netherlands</td>
<td>Men, aged ≥18 years</td>
<td>Men recruited from the STI outpatient clinic of the Public Health Service (GGD) of Amsterdam</td>
<td>2015</td>
<td>7</td>
</tr>
<tr>
<td>HP4V FSW study</td>
<td>Amsterdam, the Netherlands</td>
<td>Female sex workers, aged ≥18 years</td>
<td>Female sex workers recruited from the Prostitution and Health Center 292 (P&amp;G292) in Amsterdam</td>
<td>2016</td>
<td>9 &amp; 10</td>
</tr>
</tbody>
</table>

All studies are cross-sectional, except for Chapter 6, which uses longitudinal data of the H2M study.

Abbreviations: H2M= HPV & HIV in MSM; AIN= anal intra-epithelial neoplasia; HP4V= human papillomavirus preparedness for vaccination; HIV= human immunodeficiency virus; MSM= men who have sex with men; STI= sexually transmitted infection; HSIL= high-grade squamous intraepithelial neoplasia; GGD= Gemeentelijke Gezondheidsdienst/ Public Health Service; FSW= female sex workers.
Introduction

References


Introduction

Introduction

Introduction


Introduction

Introduction


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