

# HPV Prevalence in HIV Infected and Healthy Rwandan Women

Degree project thesis in Medicine

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## **Abstract**

### **Degree project, Programme in Medicine**

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### **Background**

Cervical cancer is the leading cause of cancer death among women in Rwanda, thus making it essential to map out the prevalence of different human papillomavirus (HPV) types. Mass vaccination programs of school girls have been enrolled in Rwanda covering HPV16 and HPV18. In Sweden, HPV16 and HPV 18 stand for 70 % of all cervical cancers. In Rwanda, the prevalence of HPV strains in the population is not known. Here we assessed the prevalence of different HPV strains in HIV patients and a healthy control group of patients in Rwanda.

### **Aim**

To assess the prevalence of HPV infection in healthy and HIV infected women in Rwanda.

### **Methods**

Cervical specimens and data were obtained from 200 healthy and 200 HIV-infected Rwandan women. The prevalence of different HPV strains among the study participants was analysed. The patients were tested for 39 different HPV strains.

### **Results**

The prevalence of any HPV infection was higher among HIV positive-women than HIV-negative women (27.7% vs.10.5%) (P <0.001, OR 3.26, CI 1.81-5.87), and so was the

prevalence of 'high-risk'-HPV (HR-HPV) (17.5% vs. 4.3%) and the ratio of HR-HPV out of any HPV (63.2% vs. 41.2%). The prevalence of multiple HPV infections was also higher among HIV-positive women (8.7% vs. 1.2%,  $P < 0.001$ ). The most common HR-HPVs were HPV16 (29.4%) and HPV52 (11.8%) in the HIV-negative group, and HPV52 (22.8%), HPV16 (17.5%), HPV31 and HPV58 (7.0%) in the HIV-positive group. HPV52 was common as a co-infection in the HIV-positive group.

### **Conclusions**

The results show that HPV16 and HPV18 may not be as common in Rwanda as in Western countries, and that other carcinogenic HPV strains are more prevalent. Hence, the present findings should be taken into consideration for future vaccination programs in Rwanda.

**Keywords:** HPV, cervical cancer, HIV, Rwanda

## Introduction

### *The burden of cervical cancer: globally, in Sub-Saharan Africa, and in Rwanda*

Cervical cancer is the third most common cancer in women in the world accounting for 9% of all female cancers and 9% of all cancer deaths in women (1). In Sub-Saharan Africa, cervical cancer is the most common cancer form among women. Cervical cancer is caused by the sexually-transmitted human papillomavirus (HPV) (2, 3). A prevalence study in South Africa showed that 74% of all women attending a public sector in primary care were HPV positive and 54% of the infections were classified as 'high-risk' HPV (HR-HPV) infections (4).

In Rwanda, cervical cancer is the leading cause of cancer death among women. In 2008, Rwanda had 34.5 cervical cancer cases and 25.5 cervical cancer deaths per 100,000 inhabitants (5). Company-sponsored vaccination programmes have been enrolled in Rwanda since 2013. It is predicted that vaccination of young girls naive to HPV with HPV-16/18 vaccine may result in a reduction in the number of cervical cancer cases and deaths in the future. Furthermore, it may also protect against other HPV types than HPV16 and HPV18 (6, 7). However, studies show that vaccination against HPV-16/18 may not be sufficient since many other HPV strains than the HPV-16/18 are prevalent in cervical HPV infections in Africa (8). It is of utmost interest to further investigate these aspects that may have significance for primary prevention against cervical cancer, i.e. when developing a vaccination programme as well as a screening programme adjusted to Rwandan women.

### *Human papillomaviruses (HPVs) and cervical cancer*

Human papillomaviruses (HPVs) are a group of double-stranded circular DNA viruses (8).

The HPVs constitute five out of sixteen genera of papilloma viruses (PVs): alpha, beta, gamma, mu and nu (9). HPV is the etiological agent of anogenital cancers and about 30-40% of oropharyngeal cancers. There are more than 150 HPV genotypes characterized and the types associated with anogenital cancer are of alpha-PV genus (10).

The HPVs infect skin and mucosa and require the availability of a basal cell layer (cells able to proliferate), and infect the tissue commonly via microlesions of skin or mucosa. Some of the early expressed viral genes (E5, E6 and E7) stimulate proliferation in different ways. E5 stimulates cell growth and prevents apoptosis following DNA damage, E6 degrades the pro-apoptotic proteins p53 and BAK and activates telomerase, E7 degrades the retinoblastoma tumour suppressor protein (pRB) and stimulates the S-phase genes cyclin A and cyclin E. When E6 and E7 are expressed together they have a complementary and synergistic effect in immortalizing human cells (11, 12).

HPV genotypes are normally divided into three groups. The HPV types mainly found in cervical and other anogenital cancers are referred to as 'high-risk' HPVs (HR-HPVs) and those found preferentially in genital warts and non-malignant lesions are referred to as 'low-risk' HPVs (LR-HPVs) (12). The third group consists of HPV types that are considered as 'potentially high-risk' HPV genotypes and includes HPV26, 53 and 66 (3). The most prevalent HR-HPV genotypes are 16, 18, 33, 45, 31, 58, 52, 35, 59, 56 and 51 (2, 13) while the main LR-HPV genotypes are 6, 11, 40, 42, 43, 44, 54, 61, 70, 72 and 81 (3). Of the HR-HPV genotypes, HPV16 and HPV18 are responsible for 70% of all cervical cancers and about 50% of all cervical intraepithelial neoplasia grade 3 (CIN3) (12). It is common to be infected

with multiple HPV strains simultaneously (3), although this is not associated with higher risk of developing cervical dyskaryosis compared to being infected with a single HPV strain (14).

Ye et. al. observed a high prevalence of HPV52 and HPV58 in addition to HPV16 and HPV18 among women in the Zhejiang Province in southeast China (9). Data show that these strains are common also in Japan, Taiwan and Eastern Africa (15). These results show that the heterogeneity in HPV type-specific distributions needs to be considered when predicting the effects of current prophylactic vaccines as well as when forming the basis for the second-generation vaccines targeted to specific regions (9, 16).

#### *Prevention: Methods of screening and vaccination*

The two leading strategies used to lower the incidence and mortality in cervical cancer are prevention through vaccination of young girls naive to HPV, and cervical cytological screening. The currently available vaccines provide protection against the HR-HPV genotypes 16 and 18, as well as the LR-HPV genotypes 6 and 11 (Gardasil) or only 16 and 18 (Cervarix) (7). A 9-valent vaccine containing HPV6, 11, 16, 18, 31, 33, 45, 52, and 58 antigens was licensed by the US Food and Drug Administration (FDA) in 2014 (17) and by the European Medicines Agency (EMA) in June 2015 (18). HPV vaccination is an effective method for protection against HPV infection (6). Vaccination seems to induce a low degree of cross-protection between HPV types (19), making the development of a polyvalent vaccine a priority in order to decrease the burden of cervical cancer. Cervical cancer screening is used to detect and treat precancerous lesions or early stage cancer in order to prevent surgery or radiotherapy demanding, or incurable cervical cancer from developing. A population-based cohort study in Sweden from 2012 concluded that screening is associated with improved chance of cure of cervical cancer (20).

The gold standard screening method is cervical cytology or Papanicolau, “Pap” smear. It is likewise the most frequently used method (21). A smear of collected cervical cells is stained and analysed so that lesions can be detected and staged through microscopy. In low-income countries, however, it is difficult to perform cytology due to the requirement of trained personnel and certified laboratories that can analyse the samples. Another method is to use HPV-DNA testing for cervical screening, thereby detecting high-risk strains of HPV using PCR. HPV-DNA testing requires laboratory access - and the analysis is expensive and takes a minimum of seven hours to process. An alternative in low-income countries is careHPV which is a modified HPV test that is less resource demanding and more rapid. The least complicated screening method is visual inspection with acetic acid (VIA). Diluted acetic acid or vinegar is applied to the cervical mucosa. Abnormal cells, e.g. dysplastic cells and cancer cells, turn white while normal cells are unaffected. Using this inexpensive method, abnormal cells can be detected and treated almost immediately. Cytology is the method with the highest specificity while HPV-DNA has been shown to have the highest level of sensitivity (22). The World Health Organization (WHO) has presented guidelines for cervical cancer in a “screen-and-treat programme”. The WHO expert panel has concluded that HPV-DNA testing followed by VIA and then treatment should be the standard method in resource-constrained settings. Where this is not available, VIA followed by treatment is the preferred screening approach (23).

### *HPV prevention in Rwanda*

The Department of Obstetrics and Gynecology of Kigali University Teaching hospital, Kigali, Rwanda has recently implemented an online cervical cancer prevention course for all the resident doctors in Gynecology. The course is provided through an internet-based virtual training project specializing in cancer, called E-oncologia. Its main objective is to spread

knowledge about HPV and cervical cancer to health care professionals involved in cervical cancer prevention (24).

### *Rwandan HPV vaccination and screening programme*

Rwanda is a low-income country in Sub-Saharan Africa with a population of 11.2 million and has become the first African country to develop and implement a national strategic plan for cervical cancer prevention, care and control. Through donation and concessional pricing of the HPV vaccine Gardasil, integration of the HPV vaccination has been enrolled into Rwanda's national immunization programme starting 2011. All girls in the 6<sup>th</sup> grade received the vaccine, covering more than 93% and also reaching out-of-school girls. Beside the vaccination programme, Rwanda has initialized a screening programme which begun in 2013. The intention is that the detection pathway should include testing for HPV DNA followed by visual inspection with acetic acid (VIA) and, when necessary, colposcopy and biopsy. However, at present the screening method used in the clinical practice is cytology. The cytology screening is free and includes HIV-positive women aged 30-50 years and HIV-negative women aged 30-45 years recruited to health centres. Development of the screening programme also relies on training of doctors and specialized nurses to perform the test and interpret the samples, a very important step in order to expand the screening process nationwide (5, 22, 25).

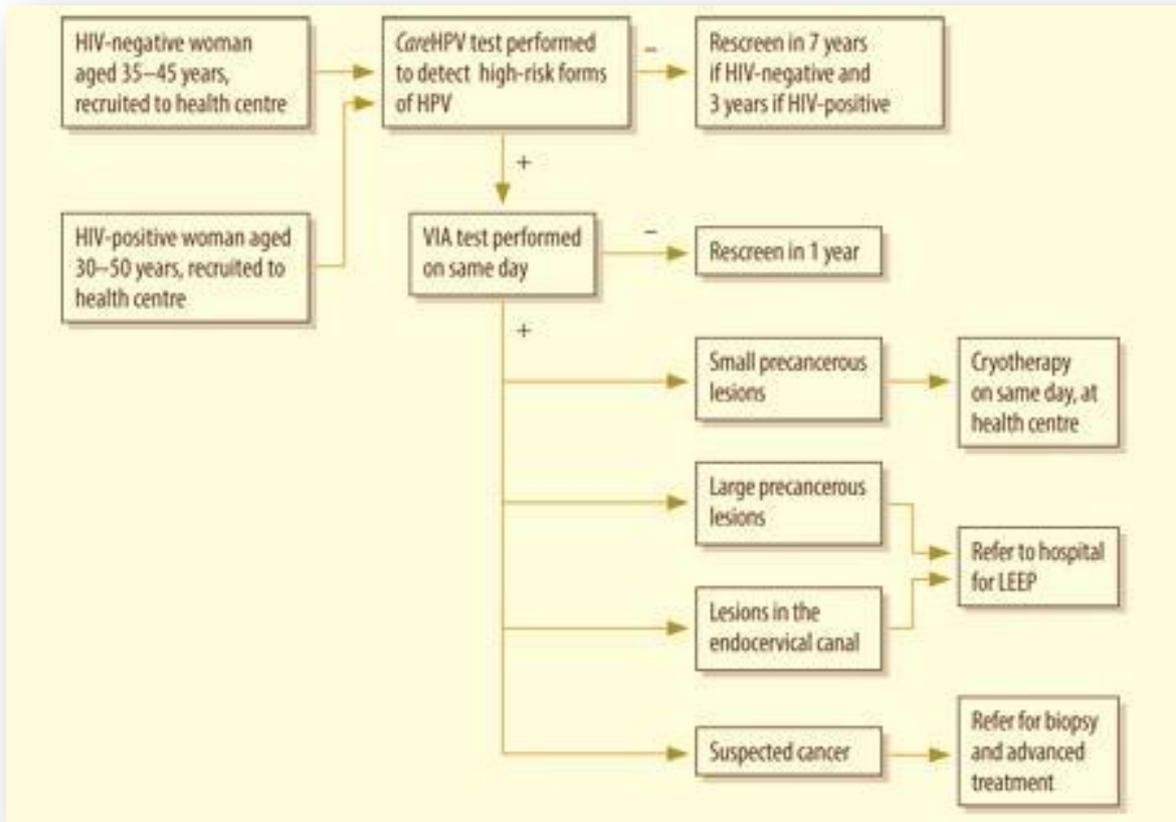


Figure 1. Cervical cancer screening and treatment in Rwanda, 2013. Figure originally from Binagwaho et. al. (5) with kind permission.  
 HIV= human immunodeficiency virus; HPV=human papillomavirus; LEEP= loop electrosurgical excision procedure; VIA= visual inspection with acetic acid.

### *Challenges for cervical cancer prevention in Rwanda*

The HPV vaccination and screening programme is an important step on the way to prevention, care and control of cervical cancer in Rwandan women. Nevertheless, a considerable amount of work remains before similar results when it comes to incidence and mortality can be seen in Rwanda as in industrial countries. In order for a nationwide vaccination programme to be successful it requires collaboration between public and private institutions as well as a well-established vaccine delivery system (25). A company-sponsored vaccine and screening programme is also fragile due to the dependence on the company's good will. In 2012, the Global Alliance for Vaccination and Immunization (GAVI) initialized

funding of the HPV vaccine in some countries, and in 2014, Rwanda switched from manufacturer- to GAVI-support (17).

A limiting factor for a nationwide implementation of the cervical screening programme is the lack of pathologists in Rwanda, with only three general pathologists in 2012 for a population of 11 million (26). In comparison, there were 189 fulltime pathologists in Sweden in 2010 for a population of 9.4 million (27), a number regarded as insufficient (28). The present screening programme for cervical cancer in Rwanda relies on women seeking health care for gynaecological examination, which results in a vulnerable system where only a minority of the population participates in the screening programme. In order for a screening programme to be successful it must be affordable and have accessible referral sources, treatment and follow-up (29) in addition to increased knowledge in the population.

### *Risk factors and limitations for optimal management of cervical cancer*

In addition to HPV infection, other risk factors for developing cervical cancer have been identified and include high parity, oral contraceptives (30) and smoking (31). Further risk factors identified in African settings are genital hygiene, alcohol use and male sexual behavior (32).

It is common that women in Sub-Saharan Africa who develop cervical cancer do not receive treatment, which is primarily due to financial and/or geographical lack of access to health care (33). The mortality in cervical cancer is significantly higher in Sub-Saharan Africa than in high-income countries, i.e. in 2002 the survival rate was 21% compared with 66% in Western Europe and 70% in the United States (34). A number of reasons for the low survival rate have been listed: poor access to medical facilities, poor nutrition and co-morbidities, e.g. malaria, anemia (35) and HIV-infection, late presentation with the disease, large tumours at diagnosis,

poor quality health care service, high rate of loss of follow-up and women not completing treatment due to barriers imposed by poverty (33).

### *HPV under concomitant HIV infection*

HIV infection increases the risk of developing cervical cancer. In fact, it has been shown that HIV alters the natural history of HPV infection with decreased regression rates of cervical lesions and more rapid progression to high-grade and invasive lesions (36). Alternatively, HPV infection may predispose an individual to HIV infection and facilitate the progression of HIV (37). In Rwanda, the official numbers on prevalence of HIV-infection is estimated to 2.8% (38). Among HIV-positive women in Rwanda, 46% are positive for carcinogenic HPV subtypes (39). Studies in Rwanda show that 9% of HIV+/HPV+ women are diagnosed with CIN grade 3 (40). Studies have observed a significantly higher prevalence of CIN in HIV-infected than in uninfected women, identifying both HPV infection and the immunodeficiency as strong independent risk factors for abnormal cytology (41). HIV infected women also tend to develop cervical cancer at an earlier age than uninfected women (42). The importance of cervical screening and management guidelines for HIV positive women is evident (43). HPV genotypes 16 and 18 are responsible for 70% of cervical cancers; however, this distribution is somewhat different in HIV-infected women, with other HR-HPVs more prevalent than in the general population. Furthermore, the HIV-positive women are more often presenting with multiple HPV strings. A study on HIV-positive women living in Europe, the majority of whom were of African descent, observed a high prevalence of HPV52 (19.8%) and HPV31/35/51/58 (12.1%), compared to HPV18 (14.6%) and HIV16 (9.5%). The same study concluded that 79% of the women in the study would be covered by the 9-valent vaccine against HPV (44).

## Aim

The present Master thesis was performed within the project “Immunological responses in the cervix in response to HPV infection in a cohort of HIV infected and non-infected Rwandan women” sponsored partly by SIDA. The overall aim of the SIDA-sponsored project is to assess whether immunological differences exist between women that develop chronic HPV infection and women who eradicate their HPV infection. The aim of the present Master thesis was to assess the prevalence of different HPV infections in a cohort of HIV-infected women and a cohort of healthy women in Rwanda.

## Material and Methods

### *Study description*

A cohort of women seeking voluntarily for ThinPrep Pap (“Pap”-smear) test (cohort A; 200 patients) were recruited at the Department of Obstetrics and Gynaecology of Kigali University Teaching Hospital, Kigali, Rwanda and Butare University Teaching Hospital, Butare, Rwanda, where patients with cervical cancer are generally admitted. Additionally a cohort of HIV infected women (cohort B; 200) were recruited at the HIV-clinics of the two hospitals. The nurse who normally receives patients was the one to recruit the patients according to the study protocol. The recruitment process was performed continuously and for the voluntary cohort it relied to a great extent on information spread by word of mouth. Additionally, recruitment was supplemented by women who sought health care at the gynaecological clinics in Butare or Kigali for other reasons than for cervical cancer screening. The data used for the master thesis was collected during the period of July to October 2015 at the time of enrolment in the larger study. The study participants did not have to finance partaking in the study; however, they had to cover other costs related to the first consultation. The study participants financed the prescribed additional laboratory tests, e.g. HIV test, performed at the first visit, and were later compensated with 5000 RWF for time and travel costs related to the study.

After having answered a questionnaire, the participants underwent a pelvic examination including a ThinPrep Pap test. The gynaecological examination and sampling was performed by a physician at respective clinic. The ThinPrep tests were then analysed in Sweden for cytology, identifying patients with CIN or cancer and additionally screened for HR-HPVs using RT-PCR. Patients that were positive for HPV were classified as HPV-positive and other

patients were classified as HPV-negative. The HPV types tested for are listed in Table 1 (3, 45). During the same visit the participants were requested having additional laboratory exams performed by a laboratory technician, including tests for HIV and other sexually transmitted diseases (STDs) such as Chlamydia, Gonorrhoea, Hepatitis B and C, Trichomonas and Herpes Simplex 2 (HSV-2).

Table 1. HPV strains tested for.

HPV class	HPV genotype
High risk	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82
Low risk	6, 11, 30, 40, 42, 43, 54, 61, 70, 74, 81, 86, 87, 89, 90, 91
Potentially high risk	26, 53, 66, 67, 69, 70, 83

\*In this study, HPV74, 86, 87, 89, 90, and 91 were considered ‘low-risk’ HPVs -however, more studies are required to evaluate the cancerogenicity of these HPV genotypes.

The master thesis includes compiled data from the two cohorts where an analysis of the available data was performed regarding the patients’ HPV status, i.e. if infected with HPV and which HPV strain/-s. The women from the two cohorts were divided into two new groups for comparison and statistical analyses based on the HIV test results: HIV-positive and HIV-negative. Thirty-two participants from the voluntary cohort were excluded because they were not tested for HIV at the time of the cervical screening. Furthermore six patients first belonging to the voluntary cohort were diagnosed as HIV positive and were therefore transferred to the HIV-positive group.

### *Participants – inclusion and exclusion criteria*

Women visiting gynaecologic outpatient departments at Kigali or Butare Teaching Hospitals were invited to participate in the study. Women agreeing to participate signed an informed

consent form and were informed about the study both orally and by written information. If a candidate met the inclusion criteria, a pelvic examination was performed and a cervical sample was taken for cytology and HPV testing.

Participants in the voluntary cohort were women considered eligible if they were 17 years of age or older, were voluntarily seeking cervical cancer screening service, were literate in Kinyarwanda, English or French, had completed and signed the Informed Consent Form and had no plan to relocate in the next two years. Participants in the HIV cohort had the same inclusion criteria as those in the voluntary cohort with the additional requirement of being HIV infected.

Patients were excluded from either cohort if they had any known concurrent disease likely to limit life expectancy to less than 24 months, had any other factors suggesting inability to comply with the study protocol, were diagnosed with cervical cancer before or at the time of inclusion, had known or visible present vaginal or cervical infection besides HPV at the time of inclusion, or had any condition or major comorbidity that the study investigators believed would compromise the patient's ability to comply with the requirements of the study.

#### *Data handling*

The main identifier (the National Identification number and names) was hidden in a database only accessible to the investigators and personnel within the project. All samples were coded so that the biomarker data and analysis of the patients could not be connected to an individual patient.

#### *Clinical examination and laboratory analyses*

A standard cervical specimen was collected. Smears were obtained as standard procedures by inserting a cytobrush in the endocervical canal and rotating it twice at 360°. Some of the

samples were aliquoted for storage at  $-80^{\circ}\text{C}$  and then transported to the University of Gothenburg, Sweden, for further cytology, and to Karolinska Institutet, Stockholm, Sweden, for HPV DNA and genotyping test. The remaining analyses for sexually transmitted diseases were performed at the Kigali University Teaching Hospital (KUTH) clinical laboratory. HPV expression was analyzed with RT-PCR.

## **Ethics**

The study has been approved by the Ethical Review Board of the University of Gothenburg and the University of Rwanda. It was possible for the patient to withdraw from the study at any time without stating a reason for it, and this was explained before inclusion in the study. We also informed the patients about confidentiality and the handling of collected data.

## **Data collection procedures/Variable analyses/Statistical methods**

All data was collected in Rwanda during 2015 as described above. Analyses were done in SPSS Version 21 and Microsoft Excel 2010. Statistical analyses for P-value, odds ratio and 95 % confidence limits were done by Chi-square test, Fisher's Exact test, binary logistic regression analysis, ordinal scale regression and t-test. To test for differences between HIV-positive and HIV-negative patients a Chi-square test and Fisher's Exact test was performed. Binary logistic regression analysis was used to calculate odds ratio and confidence intervals. Ordinal scale regression was used to calculate p-value and confidence intervals for multiple HPV infections in the groups. A p-value of 0.05 was considered statistically significant.

## Results

Cytological and HPV data were available for all 400 women in the study. Missing data for HIV status was the reason for excluding thirty-two of the women in the voluntary cohort. Furthermore six of the women in the voluntary cohort tested positive for HIV, and were thus included in the HIV-positive group when analysing the data. This resulted in 162 women included in the HIV-negative group and 206 women in the HIV-positive group. HIV-positive women were older, i.e. the mean age was 45.6 compared with 39.9 for HIV-negative women ( $P < 0.001$ ), HIV-positive patients had undergone cervical cancer screening to a greater extent than voluntary patients, i.e., 27.7% vs. 7.4% ( $P < 0.001$ ) (Figure 3). HIV patients had their sexual debut at an older age than HIV-negative patients, i.e., 22.6 vs. 19.9 ( $P < 0.001$ ), and had had a higher number of sexual partners, 3.8 vs. 2.04 partners ( $P < 0.002$ ). Characteristics for the participants are displayed in Table 2.

The prevalence of HPV infection was higher among HIV-positive women than HIV-negative women (27.7% vs. 10.5%) ( $P < 0.001$ , OR 3.26, CI 1.81-5.87) (Figure 2), and so was the prevalence of HR-HPV (17.5% vs. 4.3%) ( $P < 0.001$ , OR 4.69, CI 2.03-10.84) (Figure 6, 7, 8). The percentage of HR-HPV out of any HPV was also higher, although not statistically significant (63.2% vs. 41.2%,  $P = 0.248$ ). The prevalence of multiple HPV infections was higher among HIV-positive women than HIV-negative women (8.7% vs. 1.2%,  $P < 0.001$ ) (Figure 9, 10). The most common HR-HPVs were HPV16 (29.4%) and HPV52 (11.8%) in the HIV-negative group (Figure 11), and HPV52 (22.8%), HPV16 (17.5%), HPV31 and HPV58 (7.0%), and HPV18, HPV39, HPV68 (3.5%) in the HIV-positive group (Figure 13). The distribution of HR-HPV strains is shown in Figure 15 and Figure 17. In the HIV-positive group, seven out of the thirteen patients (54%) infected with HPV52 were also infected with

other strains of HPV, in comparison to the patients infected with HPV16 where five out of ten patients (50%) were also infected with other strains of HPV.

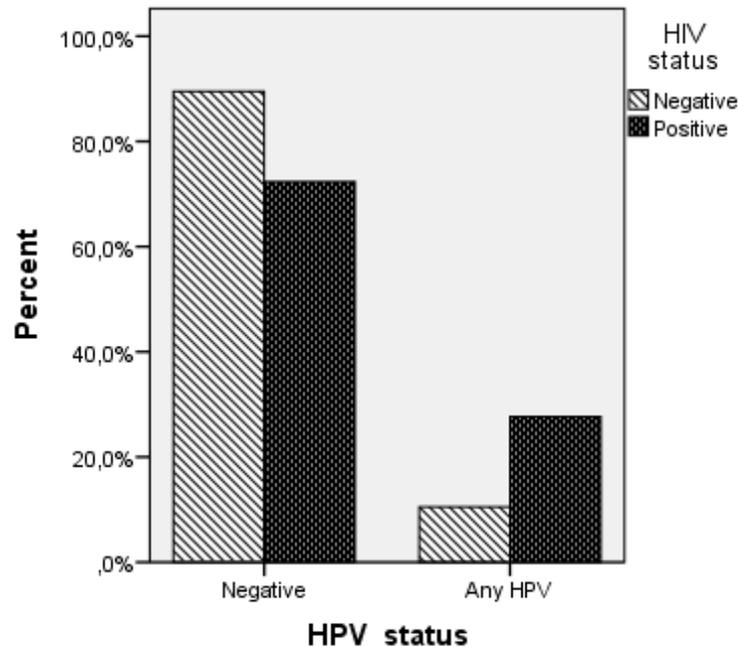


Figure 2. HPV status (any HPV or negative) stratified by HIV status. HPV; human papillomavirus. The total number of patients in each group was 162 in the HIV-negative group and 206 in the HIV-positive group. Any HPV includes all of the HPV strains tested for, explained in Table 1. The prevalence of any HPV was 10.5% (17/162) in the HIV-negative group, and 27.7 % (57/206) in the HIV-positive group. Binary scale regression analysis was used to calculate p-value, confidence interval (CI) and odds ratio (OR) (P <0.001, OR 3.26, CI 1.81-5.87).

## Discussion with Conclusions and Implications

The present study shows that HPV infections are common among women, particularly among HIV patients. In contrast to the situation in Europe, the prevalence of HPV16 and HPV18 is not as high in comparison to other HPV strains when it comes to HPV-related cervical infections in the Rwandan women in this study. Especially HPV18 was not as frequent as the prevalence observed in Western countries (46). According to the results from this study, 86% of the women with HR-HPVs would be covered by the new 9-valent vaccine. Similar tendencies have been observed in other studies, with HPV16 and HPV18 being underrepresented in HIV-positive women compared to the general population, and HPV52 being more frequent, especially in women from Africa (47).

There are few available data on HPV-DNA prevalence in African women, and the populations studied have not been comparable. The lowest prevalence was observed in a study on women without cervical cancer in South Africa, with a prevalence of 12% (48), while the highest prevalence, 46%, was observed in women attending clinics for antenatal care or general genital symptoms in Gabon (49). A study on Rwandan women from 2009 observed a prevalence of HPV infection of 13% in HIV-negative women. This study showed a HPV-DNA prevalence of 10.4% in the HIV-negative group. This prevalence number is slightly lower than what has been observed in previous studies on women in Sub-Saharan Africa. Potential reasons for this discordance are discussed further down. The result of the present study is an important contribution to the mapping of HPV-DNA prevalence in women in Rwanda. However, it is still essential with larger studies to assess the HPV-DNA prevalence in Rwandan women, as well as in African women in general.

The results from this study are in line with previous studies, demonstrating differences in prevalence of HPV genotypes in HIV patients compared to HIV-negatives. The most common HR-HPVs were HPV16 (29.4%) and HPV52 (11.8%) in the HIV-negative group, while HPV52 (22.8%) and HPV16 (17.5%) were the most common HPV strains in the HIV-positive group. Other common HPV infections were HPV31 and HPV58 in the HIV-positive group. At present, the Rwandan vaccination programme includes Gardasil only covering HPV16 and HPV18 among HR-HPV, making the coverage incomplete. The HR-HPVs that we have identified as the most common among Rwandan women in this study would all be covered by the 9-valent vaccine. The 9-valent vaccine has been shown to have an efficacy of 96.7% against HPV 31, 33, 45, 52 and 58 associated high-grade cervical, vulvar, and vaginal lesions. The vaccine demonstrated also an equivalent level of antibody response to HPV 6, 11, 16, and 18 as the quadrivalent vaccine Gardasil (17, 18).

Noteworthy is that the division of HPV genotypes into groups is based on results from previous studies examining HPV genotypes present together with cervical cancer or precancerous lesions (3). The cancerogenicity of some of the most common HPV strains is well-established; however, further studies are required in order to distinguish the cancerogenicity of some of the less frequently occurring HPV strains (45). First when this is clarified, it is possible to fully evaluate the effect of new broader vaccines.

The study observed a significantly higher HPV-DNA prevalence in the HIV infected women compared to the uninfected women, which is consistent with previous findings (47). The prevalence was 27.7% for any HPV, 17.5% for HR-HPV, and 8.7% for multiple HPV types in the HIV positive group. The aforementioned 2009 Rwandan study showed a higher prevalence of infection with HPV among HIV-positive women than this study (39). The

difference in prevalence could result from differing CD4-cell counts, HIV viral loads, age distributions, sexual behaviours etc. The observed higher prevalence of multiple HPV-infections among HIV-positive women than among HIV-negative women is in line with results from previous studies from Rwanda (8). This finding indicates that in addition to alteration of the natural history of HPV infection with decreased regression rates of HPV infection and more rapid progression to high grade and invasive lesions, HIV infection might facilitate multiple HPVs to infect the cervical mucosa, possibly by affecting the immune response in the cervix.

One interesting finding in the HIV cohort was that seven out of the thirteen patients infected with HPV52 were also infected with other strains of HPV. This finding could potentially indicate that an HPV52 infection causes vulnerability in the infected woman which facilitates further HPV infections of the cervical mucosa. This finding suggests a need for future studies designed to assess the characteristics of different HPV strains.

Studies acknowledge several obstacles to cervical cancer prevention and treatment in Sub-Saharan countries, including low level of cervical cancer awareness and understanding, lack of effective and high quality screening, financial constraints and poor knowledge of risks and treatment options (50). In Rwanda, the resources for treatment of advanced cervical cancer are limited, thus making it even more important with a well-functioning prevention strategy through vaccination and screening. Furthermore it is of utmost importance to target the above mentioned obstacles to cervical cancer prevention and treatment in order to optimize the Rwandan cervical cancer prevention programme.

The results on HPV prevalence observed in this study are representable for the HIV-infected female Rwandan population, and can be used to help evaluate which approach to cervical cancer prevention among HIV-positive women is most effective. Regarding the HIV-negative group, the study population did not provide a large enough material to be able to evaluate the prevalence of different HPV strains in the general population. Furthermore, the participants cannot be considered representative for the entire population due to the design of the study. The recruitment process of the voluntary cohort was carried out to a great extent through word of mouth, and through women who had already sought care at a gynaecological clinic. It is possible that the knowledge about HPV infection and cervical cancer is higher in a group of women who voluntarily seek health care at a gynaecological clinic than the average in the population. The fact that the study was performed at University Hospitals in the two largest cities in Rwanda, Kigali and Butare, in addition to the inclusion criteria of being literate in either Kinyarwanda, English or French, may also have contributed to a selected group of women with a lower prevalence of HPV infection than the average in the female Rwandan population. The HIV cohort was recruited to the study during planned visits to the HIV-clinic which postulates a better generalisability for the female HIV population in Rwanda.

In addition to the possibility that the recruited women in the voluntary cohort are more likely to have a higher awareness about cervical cancer and screening than the general population, the aforementioned aspects might also affect factors that may have an impact on the risk for HPV-infection and cervical cancer. Potential affected aspects include propensity to seek health care, age at first sexual intercourse, number of sexual partners, plus co-factors for developing cervical cancer, e.g. smoking, use of hormonal contraceptives, and other STIs.

This study did not examine the potential influence of other sexually transmitted diseases (STDs) in addition to HIV on HPV infection and cervical cancer. For future studies it would be of great interest to investigate the connection between HPV infection and additional STDs other than HIV, e.g. Chlamydia, Gonorrhoea, HSV-2, Hepatitis B and C, and Trichomonas. It would also be interesting to assess whether sexual behaviour has the most significant impact on the prevalence of HPV infection-, or if concomitant STDs may facilitate the transmission and/or persistence of an HPV infection by altering the immune response.

It would be desirable to perform larger studies on HPV-DNA prevalence in the female Rwandan population, as well as studies on HPV prevalence in the male population, since HPV infection also causes the majority of anal cancers, penile cancers, oropharyngeal cancers, and genital warts. The chain of transmission is also maintained if boys are not included in the vaccination programme (51). Including men in a HPV-DNA prevalence study would be helpful when evaluating the cost-effectiveness of the potential inclusion of boys in the Rwandan vaccination programme (52). The European Centre for Disease Prevention and Control (ECDC) has concluded that universal coverage of both males and females would be the most effective strategy to prevent HPV-related morbidity, since it both prevents HPV-related conditions in men as well as provides protection against cervical cancer in women through herd immunity (53).

The aim of the larger study, to assess the immunological response of the cervix in response to HPV infection, can contribute to the understanding of the natural course of HPV infection, and possibly highlight potential risk groups in the female Rwandan population that might need to be more intensely examined in the recently initiated cervical cancer screening

programme. Individualised treatment and screening procedures could potentially be realised within a foreseeable future based on the patients' immunological profiles.

This study has identified HIV-infected women as one of the risk groups that would most likely benefit from more intense cervical cancer prevention strategies. Considering the high prevalence of HPV52, HPV31 and HPV58 in the HIV-positive group, a majority of the cancerogenous HPV infections are not covered by the vaccine currently used in the Rwandan vaccination programme. However, the prevalence of HIV in the Rwandan adult population is only 2.8% (38). This implies that further studies and analyses are required in order to conclude if implementation of a new broader vaccine for all young girls would be the most cost-efficient alternative for cervical cancer prevention among HIV-positive women, or if more intense screening for this risk group is a more efficient approach.

## Populärvetenskaplig sammanfattning

### HIV-prevalens hos HIV-infekterade och friska kvinnor i Rwanda

Livmoderhalscancer orsakas av det sexuellt överförbara viruset humant papillomvirus (HPV). I Rwanda är livmoderhalscancer den främsta orsaken till cancerdöd bland kvinnor. 2011 inleddes ett företagsfinansierat vaccinationsprogram i Rwanda där unga flickor vaccineras med Gardasil som skyddar mot de mest cancerframkallande HPV-typerna HPV16 och HPV18. Detta förväntas resultera i en minskning av förekomsten av livmoderhalscancer i framtiden. Studier har dock visat att vaccination mot HPV-16/18 kan vara otillräckligt eftersom flera andra cancerframkallande HPV-typer är vanligt förekommande vid HPV-infektion i livmoderhalsen hos kvinnor i Afrika.

Den aktuella studien utfördes i Rwanda där vi undersökte förekomsten av HPV-infektion och cellförändringar i livmoderhalsen hos 200 kvinnor med HIV och 200 friska frivilliga kvinnor. Våra resultat visar att HIV-positiva kvinnor oftare var infekterade med HPV än HIV-negativa patienter och andelen med mer cancerframkallande HPV-arter var högre bland patienter med samtidig HIV-infektion. Dessutom var det vanligare bland HIV-infekterade än bland HIV-negativa att vara infekterad av flera HPV-typer samtidigt.

Vår studie visar också att HPV16 och HPV18 är betydligt ovanligare i Rwanda än i Västvärlden. Enligt våra resultat var HPV52 och HPV16 de cancerframkallande HPV-arter som förekom mest frekvent, och bland HIV-infekterade var även HPV31 och HPV58 vanligt förekommande. Detta skulle kunna innebära att det vaccin som används idag i Rwanda ger ett inkomplett skydd mot livmoderhalscancer. På marknaden finns idag ett nytt vaccin

tillgängligt som skyddar mot HPV16, HPV18, HPV31, HPV33, HPV45, HPV52 och HPV58 vilket skulle kunna ge ett skydd mot de vanligast förekommande HPV-arterna i Rwanda.

Det är dock av stor vikt att undersöka om man i framtiden kan införa vaccinationer som bättre täcker de vanligaste HPV-arterna som förekommer i Rwanda. På detta sätt kan man på sikt minska dödligheten i livmoderhalscancer i ett land där terapi mot sjukdomen också är mycket begränsad. För att ytterligare minska förekomsten av långt gången livmoderhalscancer behövs ett fungerande screeningprogram som möjliggör tidig upptäckt och behandling av förstadier till cancer och behandlingsbar cancer.

## Acknowledgement

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## Tables and Figures

Table 2. Baseline characteristics of the participants stratified by HIV status.

HIV status		N*	Minimum	Maximum	Mean	Std. Deviation	P-value
<b>Positive</b>	Age at first sexual intercourse	205	7	33	<b>19,87</b>	4,156	<b>P&lt;0.001</b>
	Number of sexual partners	206	1	55	<b>3,80</b>	6,565	<b>P=0.002</b>
	Number of spontaneous or voluntary abortions	206	0	5	,48	,807	P=0.397
	Number of live births	206	0	8	<b>3,56</b>	1,909	<b>P=0.023</b>
	Participant age in years	206	26	66	<b>45,59</b>	7,826	<b>P&lt;0.001</b>
<b>Negative</b>	Age at first sexual intercourses	159	12	42	<b>22,55</b>	5,473	<b>P&lt;0.001</b>
	Number of sexual partners	159	1	43	<b>2,04</b>	3,555	<b>P=0.002</b>
	Number of spontaneous or voluntary abortions	159	0	8	,57	1,117	P=0.397
	Number of live births	159	0	9	<b>3,07</b>	2,179	<b>P=0.023</b>
	Participant age in years	162	23	70	<b>39,94</b>	9,648	<b>P&lt;0.001</b>

\*The total number of patients (N) in each row is based on the number of patients for whom there were data on the questions at enrolment. T-test was used to calculate P-values on the difference between the HIV-negative and HIV-positive group.

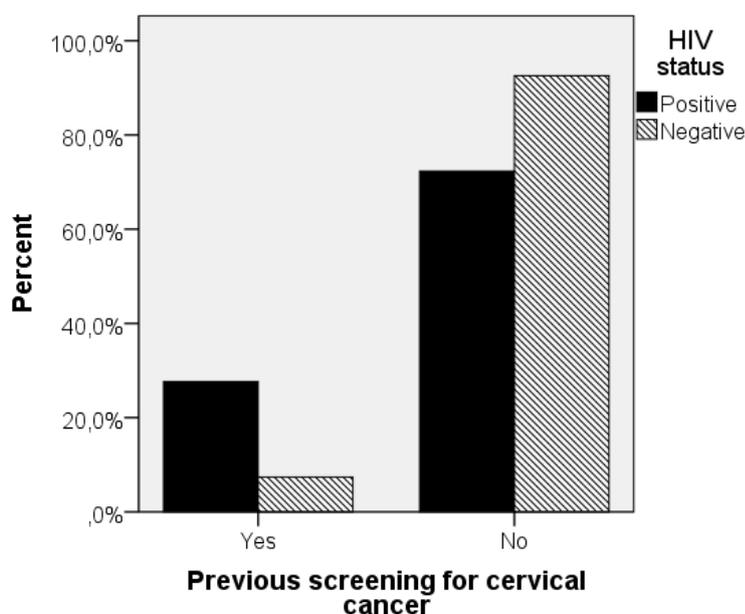


Figure 3. Previous screening for cervical cancer stratified by HIV status.

The total number of patients in each group was 162 in the HIV-negative group and 206 in the HIV-positive group. 27.7% had previously been screened for cervical cancer in the HIV-positive group, vs. 7.4% in the HIV-negative group. ChiSquare test was used to calculate a P-value (P<0.001).

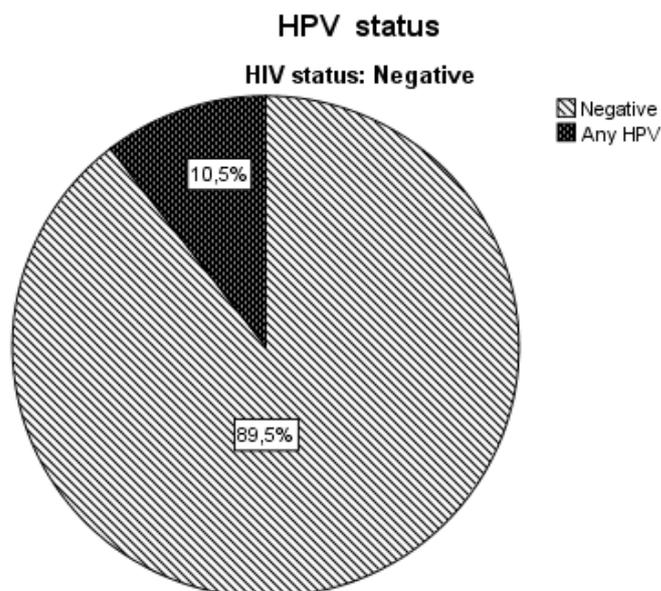


Figure 4. HPV status among HIV-negative.

HPV; human papillomavirus. The total number of patients in each group was 162 in the HIV-negative group and 206 in the HIV-positive group. Any HPV includes all of the HPV strains tested for, explained in Table 1. The prevalence of any HPV was 10.5% (17/162) in the HIV-negative group, and 27.7% (57/206) in the HIV-positive group. Binary logistic regression analysis was used to calculate p-value, confidence interval (CI) and odds ratio (OR) (P <0.001, OR 3.26, CI 1.81-5.87).

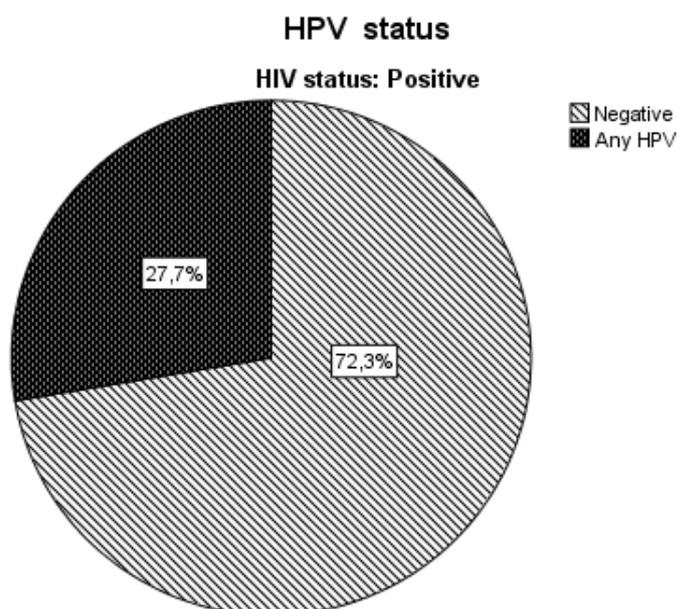


Figure 5. HPV status among HIV-positive.

HPV; human papillomavirus. The total number of patients in each group was 162 in the HIV-negative group and 206 in the HIV-positive group. Any HPV includes all of the HPV strains tested for, explained in Table 1. The prevalence of any HPV was 10.5% (17/162) in the HIV-negative group, and 27.7% (57/206) in the HIV-positive group. Binary logistic regression analysis was used to calculate p-value, confidence interval (CI) and odds ratio (OR) (P <0.001, OR 3.26, CI 1.81-5.87).

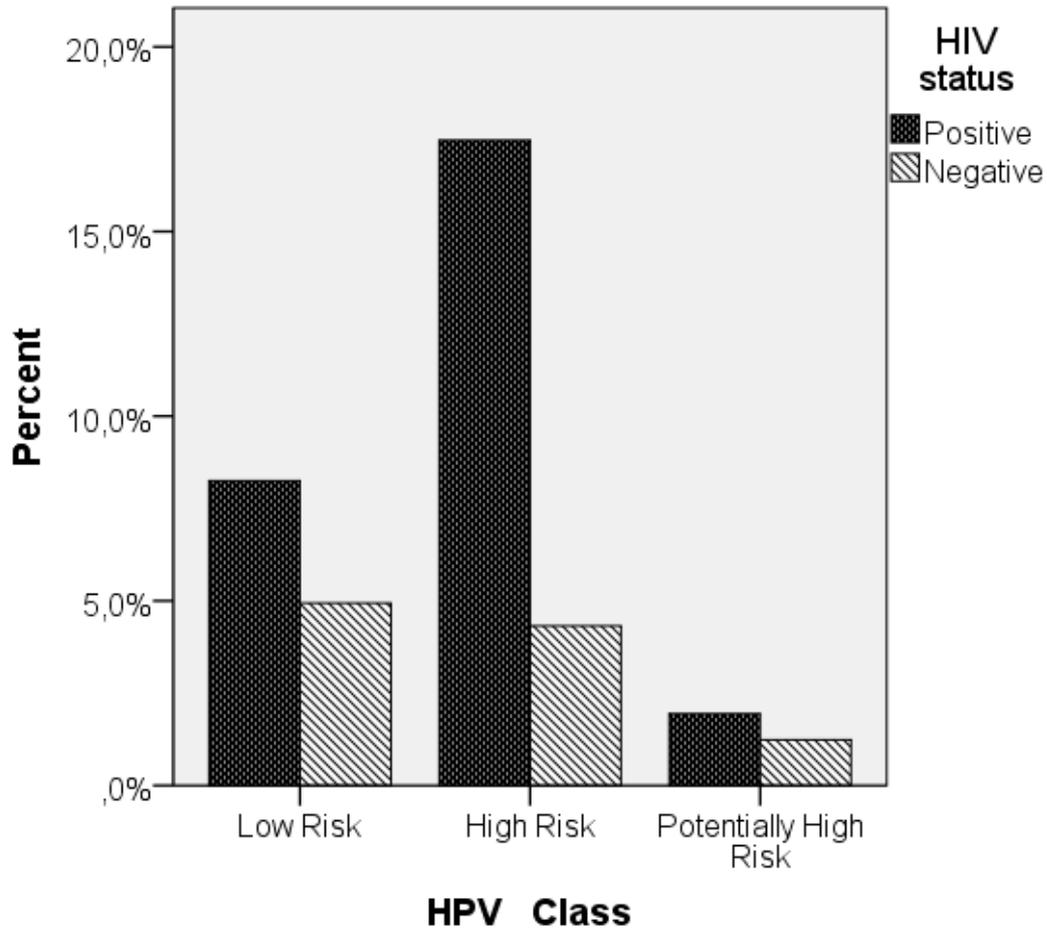


Figure 6. Comparison of human papillomavirus (HPV) class stratified by HIV-status. Low Risk Human Papillomavirus (LR-HPV) tested for were HPV6, 11, 30, 40, 42, 43, 54, 61, 70, 74, 81, 86, 87, 89, 90, 91. High Risk (HR-HPV) tested for were HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82. Potentially High Risk (potentially HR-HPV) tested for were 26, 53, 66, 67, 69, 70, 83. Not shown in the chart is the HPV negative group, which constituted 89.5% of the HIV negative women, and 72.3% of the HIV positive women. Binary logistic regression analysis was used to calculate p-value, confidence interval (CI) and odds ratio (OR) (P <0.001, OR 4.69, CI 2.03-10.84).

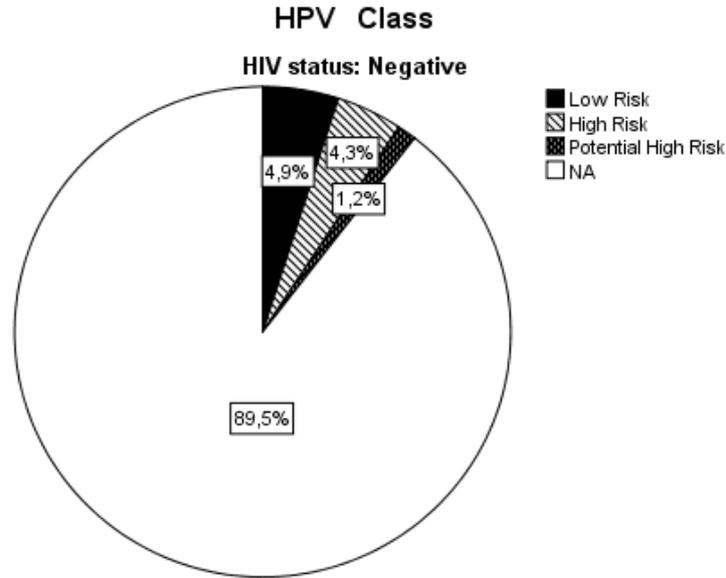


Figure 7. Comparison of human papillomavirus (HPV) class among HIV-negative women. HPV=human papillomavirus. NA=no HPV. Low Risk='low-risk' HPV. High Risk='high-risk HPV. Potential High Risk='potentially high-risk' HPV. The HPV strains tested for are displayed in Table 1. The total number of patients was 162 in the HIV-negative group. Binary logistic regression analysis was used to calculate p-value, confidence interval (CI) and odds ratio (OR) (P <0.001, OR 4.69, CI 2.03-10.84).

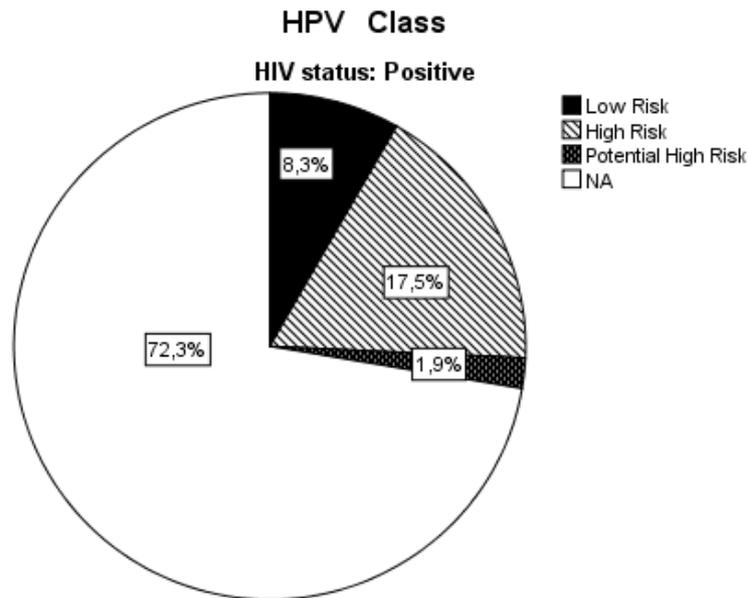


Figure 8. Comparison of human papillomavirus (HPV) class among HIV-positive women. HPV=human papillomavirus. NA=no HPV. Low Risk='low-risk' HPV. High Risk='high-risk HPV. Potential High Risk='potentially high-risk' HPV. The HPV strains tested for are displayed in Table 1. The total number of patients was 206 in the HIV-positive group. Binary logistic regression analysis was used to calculate p-value, confidence interval (CI) and odds ratio (OR) (P <0.001, OR 4.69, CI 2.03-10.84).

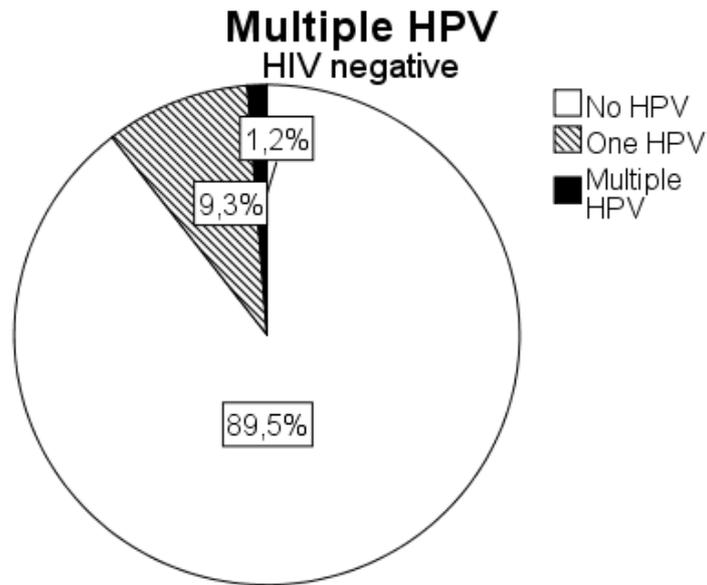


Figure 9. Distribution of HPV infection (no HPV, one HPV or multiple HPV) among HIV-negative women. HPV=human papillomavirus. Multiple HPV= infected with >1 HPV strain. The HPV strains tested for are displayed in Table 1. The total number of patients was 162 in the HIV-negative group. Ordinal scale regression analysis was used to calculate p-value ( $P < 0.001$ ).

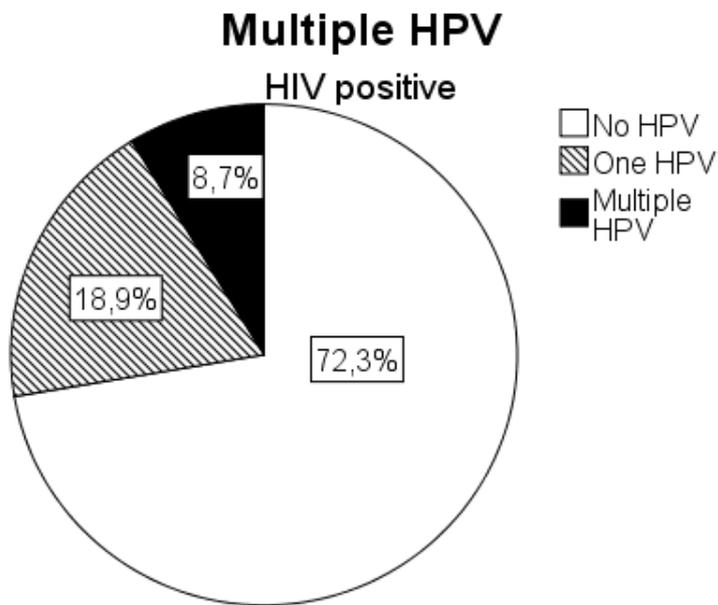


Figure 10. Distribution of HPV infection (no HPV, one HPV or multiple HPV) among HIV-positive women. HPV=human papillomavirus. Multiple HPV= >1 HPV strain. The HPV strains tested for are displayed in Table 1. The total number of patients was 206 in the HIV-positive group. Ordinal scale regression analysis was used to calculate p-value ( $P < 0.001$ ).

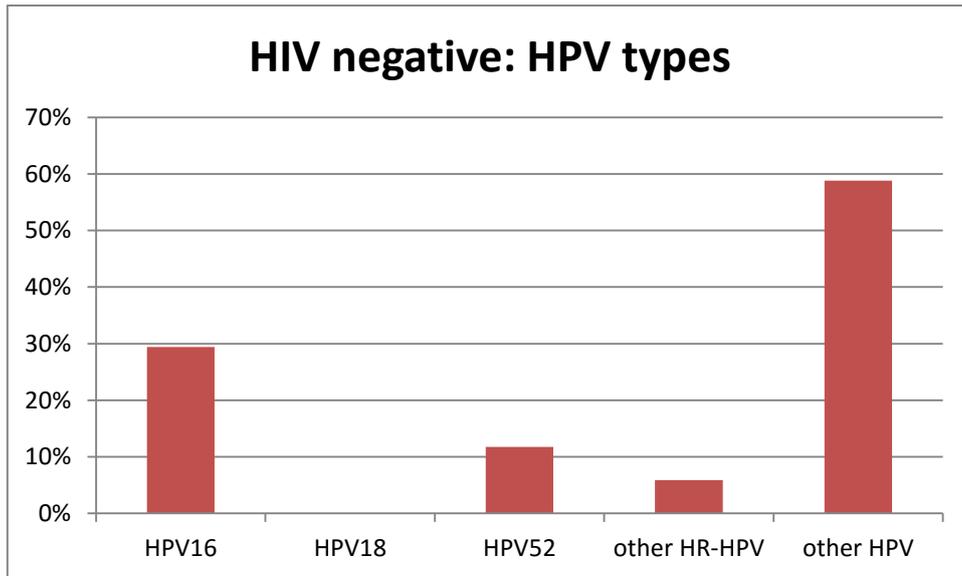


Figure 11. Prevalence of Human papillomavirus (HPV) among HIV-negative.

One patient can be infected with more than one HPV strain, hence adding up to more than 100%. The percentages were calculated as positive cases (including both single and multiple HPV infections) out of the total number of patients with HPV infection in the HIV negative group (n=17). Other HR-HPV includes HPV31, 33, 35, 39, 45, 51, 56, 58, 59, 68, 73, and 82. Other HPV includes HPV6, 11, 30, 40, 42, 43, 54, 61, 70, 74, 81, 86, 87, 89, 90, 91, 26, 53, 66, 67, 69, 70, and 73.

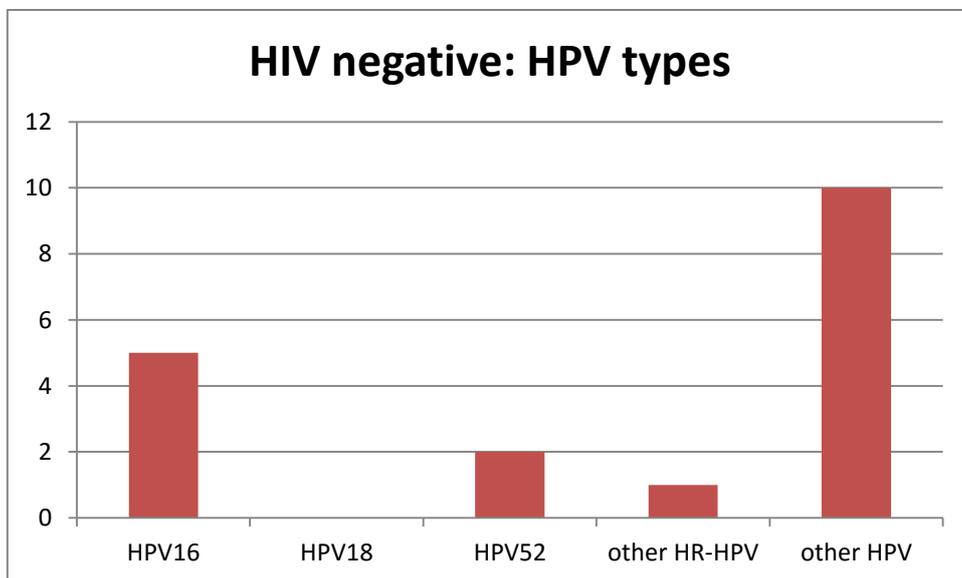


Figure 12. Prevalence (number of patients) of Human papillomavirus (HPV) among HIV-negative.

One patient can be infected with more than one HPV strain. The total number of patients with HPV infection in the HIV-negative group was 17. Other HR-HPV includes HPV31, 33, 35, 39, 45, 51, 56, 58, 59, 68, 73, and 82. Other HPV includes HPV6, 11, 30, 40, 42, 43, 54, 61, 70, 74, 81, 86, 87, 89, 90, 91, 26, 53, 66, 67, 69, 70, and 73.

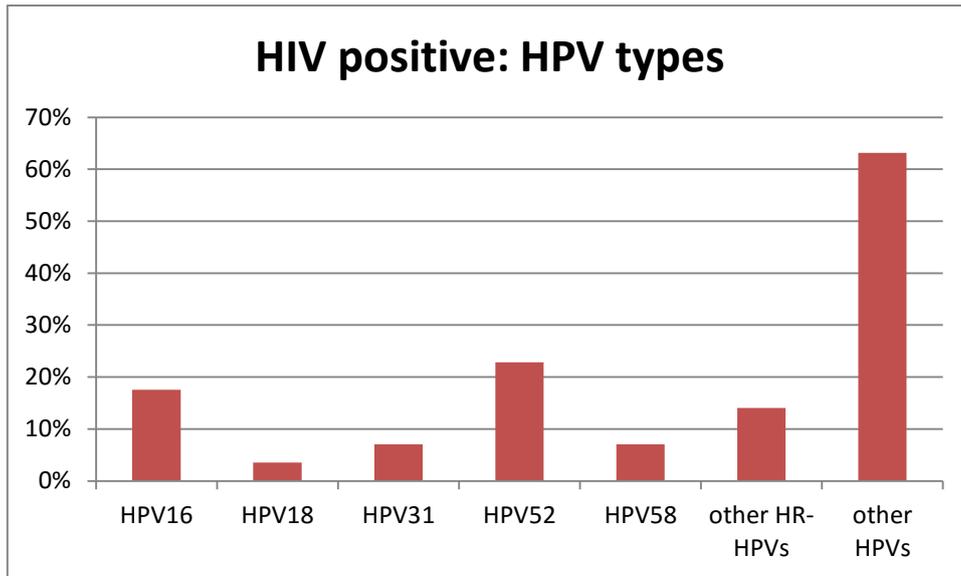


Figure 13. Prevalence of Human papillomavirus (HPV) among HIV-positive.

One patient can be infected with more than one HPV strain, hence adding up to more than 100 %. The percentages were calculated as positive cases (including both single and multiple HPV infections) out of the total number of patients with HPV infection in the HIV-positive group (n=57). Other HR-HPV includes HPV33, 35, 39, 45, 51, 56, 59, 68, 73, and 82. Other HPV includes HPV6, 11, 30, 40, 42, 43, 54, 61, 70, 74, 81, 86, 87, 89, 90, 91, 26, 53, 66, 67, 69, 70, and 73.

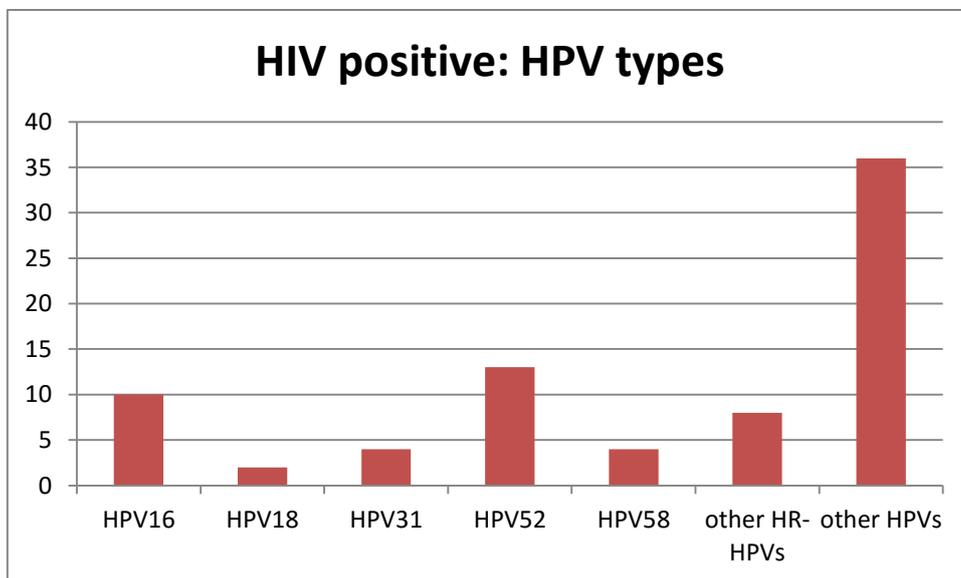


Figure 14. Prevalence (number of patients) of Human papillomavirus (HPV) among HIV positive.

One patient can be infected with more than one HPV strain. The total number of patients with HPV infection in the HIV-positive group was 57. Other HR-HPV includes HPV31, 33, 35, 39, 45, 51, 56, 58, 59, 68, 73, and 82. Other HPV includes HPV6, 11, 30, 40, 42, 43, 54, 61, 70, 74, 81, 86, 87, 89, 90, 91, 26, 53, 66, 67, 69, 70, and 73.

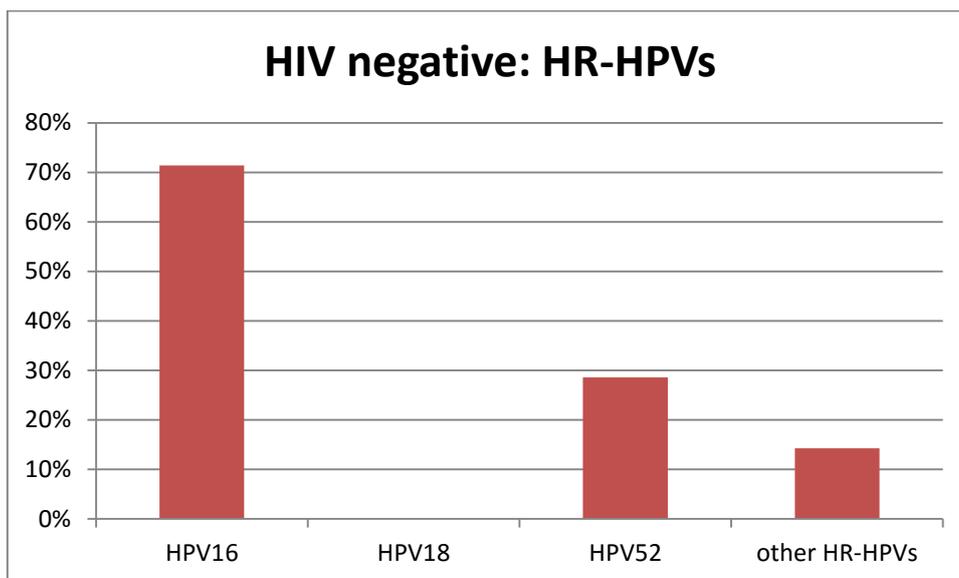


Figure 15. Prevalence of ‘high-risk’ HPV (HR-HPV) among HIV negative.

One patient can be infected with more than one HPV strain, hence adding up to more than 100 %. The percentages were calculated as positive cases (including both single and multiple HPV infections) out of the total number of patients with HR-HPV infection in the HIV-positive group (n=7). Other HR-HPV includes HPV31, 33, 35, 39, 45, 51, 56, 58, 59, 68, 73, and 82.

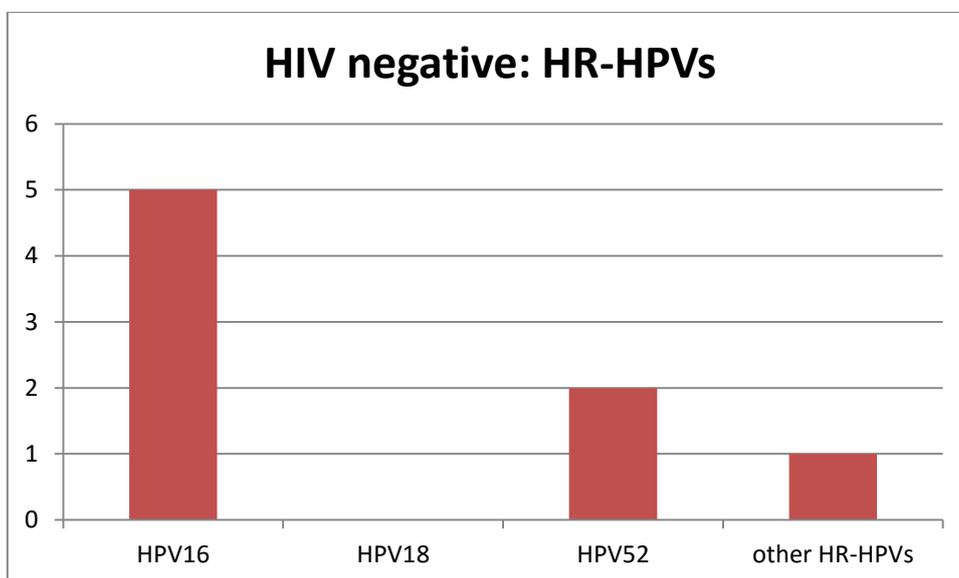


Figure 16. Prevalence (number of patients) of ‘high-risk’ HPV (HR-HPV) among HIV negative.

One patient can be infected with more than one HPV strain. The total number of patients with HR-HPV infection in the HIV-negative group was 7, and the total number of HR-HPV infections was 8. Other HR-HPV includes HPV31, 33, 35, 39, 45, 51, 56, 58, 59, 68, 73, and 82.

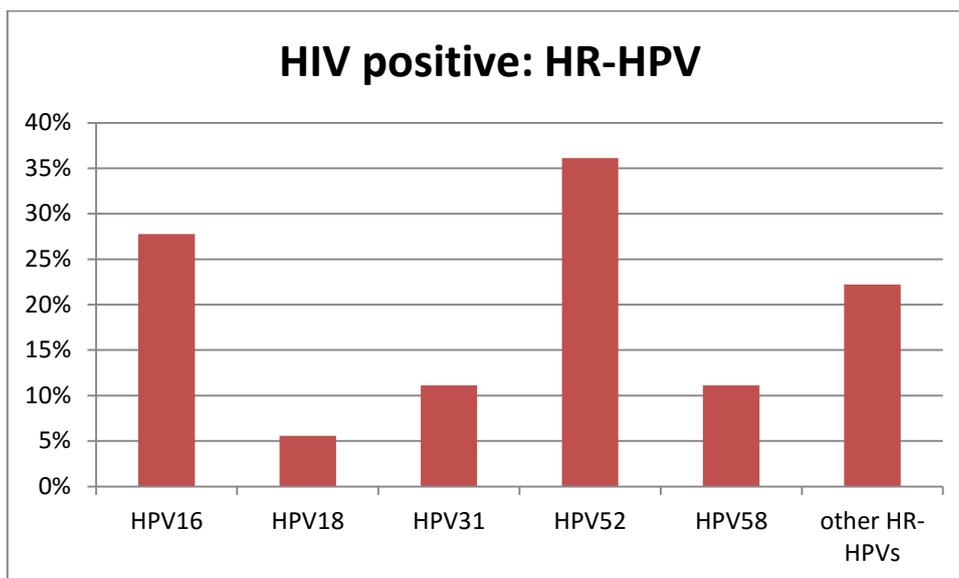


Figure 17. Prevalence of ‘high-risk’ HPV (HR-HPV) among HIV positive.

One patient can be infected with more than one HPV strain, hence adding up to more than 100 %. The percentages were calculated as positive cases (including both single and multiple HPV infections) out of the total number of patients with HR-HPV infection in the HIV-positive group (n=36). Other HR-HPV includes HPV33, 35, 39, 45, 51, 56, 59, 68, 73, and 82.

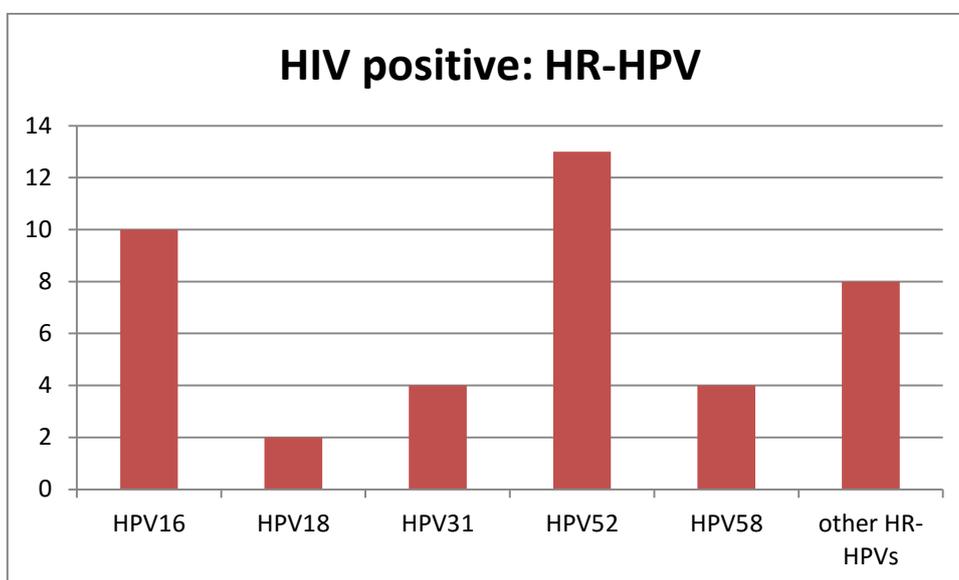


Figure 18. Prevalence (number of patients) of ‘high-risk’ HPV (HR-HPV) among HIV positive.

One patient can be infected with more than one HPV strain. The total number of patients with HR-HPV infection in the HIV-positive group was 36, and the total number of HR-HPV infections was 41. Other HR-HPV includes HPV31, 33, 35, 39, 45, 51, 56, 58, 59, 68, 73, and 82.