IMMUNE STATUS AND TUMOUR LOCALIZATION IN THE DEVELOPMENT AND PROGNOSIS OF GENITAL (PRE)MALIGNANCIES IN WOMEN

FLOOR HINTEN
IMMUNE STATUS AND TUMOUR LOCALIZATION IN THE DEVELOPMENT AND PROGNOSIS OF GENITAL (PRE)MALIGNANCIES IN WOMEN

Thesis, Radboud university medical center, Nijmegen, the Netherlands

The financial support of this thesis was kindly provided by Chipsoft, DDL Diagnostic Laboratory, Nierstichting Nederland, Pfizer B.V., Radboud university medical center, Rovers Medical Devices, Will Pharma

For reasons of consistency, terminology may be changed throughout this thesis when compared to the original publications.

ISBN: 978-94-028-0673-1

Design and lay-out:
Mikka Art, Den Bosch
Print
Ipskamp Printing, Enschede

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IMMUNE STATUS AND TUMOUR LOCALIZATION IN THE DEVELOPMENT AND PROGNOSIS OF GENITAL (PRE)MALIGNANCIES IN WOMEN

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Radboud Universiteit Nijmegen op gezag van de rector magnificus prof. dr. J.H.J.M. van Krieken, volgens besluit van het college van decanen in het openbaar te verdedigen op woensdag 5 juli 2017 om 10.30 uur precies

doors

FLOOR HINTEN
geboren op 8 november 1985
te Tilburg
PROMOTOREN
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GENERAL INTRODUCTION
GENERAL INTRODUCTION

Genital (pre)malignancies in women comprise all (pre)malignancies found in the female genital tract: ovary, tuba, uterus, cervix, vagina and vulva. This thesis mainly focuses on the lower genital tract, namely cervix and vulva. Cervical cancer is a major health problem, with more than 500,000 new cases occurring each year worldwide, especially in developing countries. In 2012 about 265,000 deaths from cervical cancer were reported, making it the fourth leading cause of cancer death in women in the world (1). Virtually all cervical cancers are caused by high-risk (hr) Human Papillomavirus (HPV) infection, which implies that cervical cancer does not and will not develop in the absence of persistent HPV DNA (2). The primary site for cervical cancers is the cervical transformation zone (border between the glandular and squamous epithelium). The transformation zone is assumed to be more susceptible to oncogenic influences, like hrHPV infection, due to the high cell-turnover (3). Cervical intraepithelial neoplasia (CIN) is the non-invasive precursor lesion of cervical cancer. The CIN lesions are classified as CIN 1, CIN 2 and CIN 3 based on the presence of mitotic activity and nuclear atypia within respectively the basal third, two thirds and whole thickness of the epithelium (4). Since the introduction of cytological screening for cervical cancer in many developed countries, the incidence and mortality rate of cervical cancer in these countries have markedly diminished (5), despite differences in terms of invitation methodology, target population (start between 18-30 years until 59-70 years), screening intervals (once a year until once every 5 years), organization and quality assurance methodology. In the Netherlands, all women between 30-60 years of age receive an invitation from the regional screening organization or from their general practitioner for cervical screening once every five years. The participation rate of the screening programme is about 65% (6). About half of all women with newly diagnosed invasive cervical cancer never participated in any screening programme (7-9). As previously stated, HPV causes almost all cervical cancers and is therefore the main factor in the oncogenesis of cervical cancer. The sensitivity of hrHPV detection is higher than for cytology (~90% versus ~60%), but the specificity is slightly lower (70-90% versus 90-100%) (10-16). Recently, it has been decided to introduce the hrHPV test as primary screening tool in the Netherlands in 2016-2017 (17).

Vulvar cancer is a rare disease, representing approximately 3-5% of malignancies of the female genital tract (18). There are two different etiologic pathways for the development of vulvar squamous cell carcinoma (SCC) (19, 20). In the first, most common pathway, the most precursor lesion is differentiated vulvar intraepithelial neoplasia (dVIN), which often occurs in the background of lichen sclerosus. This pathway is rarely associated with a hrHPV infection. The second pathway is related to a hrHPV infection and covers about 20-30% of all vulvar SCCs (19, 21, 22). The precursor lesion in this pathway is usual vulvar intraepithelial neoplasia (uVIN). Differentiated VIN is suggested to be highly proliferative, increasing the likelihood of progression to vulvar SCC compared to uVIN (23, 24). Most vulvar SCCs are dVIN-related, while most isolated premalignant lesions are of the usual type (20, 25). In general, the possibility of uVIN progressing into vulvar SCC is low in comparison with dVIN (26), namely 9-16% in untreated patients and 3% in treated patients, compared to about 30% in patients with dVIN (27). There is no regular screening tool for VIN; symptoms are frequently
absent or variable and suspicious vulvar lesions are not always histopathologically examined. The absence of symptoms might cause this disease to be underreported.

**HUMAN PAPILLOMAVIRUS**

A common nominator in the genital (pre) malignancies is HPV; cervical cancer for approximately 100% and vulvar cancer for 20-30% HPV-related. HPV is a double stranded DNA virus that can infect basal epithelial cells of all human squamous epithelia. The HPV genome encodes for 9 viral proteins; viral proteins E6 and E7 are known cancer promoting genes. The E6 protein inactivates p53 (a tumour suppressor protein), which results in chromosomal instability and diminished apoptosis. The E7 protein suppresses the retinoblastoma protein (pRB)-pathway which leads to enhanced cell proliferation (28, 29) (Figure 1).

**Figure 1.** Human papillomavirus with the role of several proteins elucidated.

E3 protein is one of the membrane proteins; no specific role specified (not shown in figure).

HPV can be classified into two groups: cutaneous and mucosal HPV subtypes. Cutaneous subtypes are predominantly found in cutaneous verrucae and mucosal subtypes predominate in anogenital
lesions (30, 31). Mucosal HPV subtypes can be divided in high-risk (subtype 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82) and low-risk (subtype 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108) subtypes (10, 32-36).

Worldwide, the overall cervical HPV prevalence is around 10% (37). The prevalence of any cervical HPV infection ranges between 2 and 44%, with a peak in women aged 18-29 years old (38) and, in most regions, a subsequent peak in women aged 45 years or older (37). HPV is sexually transmitted and up to 80% of sexually active women will be genitally infected by one or more HPV subtypes during their life (39, 40). The majority of HPV infections are transient; these infections will be cleared by the immune system without causing any abnormality (41, 42). However, a part of the HPV infections will not be cleared and persist. HPV persistence is commonly defined as having two or more positive tests (43, 44), time to clearance (45-47) or proportion of HPV-positive visits (48, 49). Persistent HPV infections are strongly associated with the development of CIN 2-3 and are considered to drive progression of CIN into invasive cervical cancer (50). Laboratory evidence shows that persistent HPV oncogene expression is critical for the maintenance and progression of CIN (51). The mean duration of a cervical HPV infection in the general population is thought to vary between 8 and 13 months (52). In immunocomprised patients an increased prevalence of genital HPV is observed. The largest immunocomprised patient groups are HIV-infected patients and organ transplant recipients. In this thesis we focus on the renal transplant recipients (RTRs), because little research has been performed compared to HIV-infected patients and renal transplantation comprises a large part of all solid organ transplantations.

**RENA L TRANSP LANTATION**

Renal transplantation is the preferred treatment for most patients with end stage renal disease. Yearly more than 1000 renal transplantations are performed in the Netherlands. Organ transplantation requires the lifelong administration of intense immunosuppressive therapy. Immediate post-transplantation, high doses of multi-agent immunosuppressive therapy are needed to prevent acute allograft rejection by host immune mechanisms. The current immunosuppressive regimes have led to a 1-year graft survival of more than 90% and the incidence of acute rejection has fallen to 10-15% (53). Due to the immunosuppression after organ transplantation these patients are more susceptible not only for infectious diseases but also for particularly virus-associated cancers. Immunosuppressive agents may indirectly contribute to the development of cancer, as an impaired surveillance ability of T-cells may disrupt anti-tumour immune surveillance and may potentiate oncogenic stimuli as chemical carcinogens, ultraviolet (UV) light and also viruses (54-56). Viruses that are associated with the development of cancer in RTRs are the Epstein-Barr virus which can induce PTLD and the hepatitis B/hepatitis C virus which are linked to the development of hepatocellular carcinoma (57-62). The incidence of virus-associated cancer is estimated to be between 4 and 18%, which means a 100-fold increase compared with immunocompetent people (63). HPV causes not only anogenital (pre)malignancies in females, but also anogenital (pre)malignancies in males and head and neck tumours, and is one of the viruses RTRs are more susceptible to and are more prone for progression to (pre)malignant lesions.
HPV INFECTION IN FEMALE RENAL TRANSPLANT RECIPIENTS

RTRs have a higher risk of HPV infections compared to immunocompetent women. The incidence of HPV infections, both low and high risk types, in RTRs varies between 22 and 63%. Furthermore, RTRs are more often infected with multiple HPV infections compared to the general population (64-70). A recent study among 35 RTRs found that 62.8% of the RTRs were positive for HPV-DNA in cervical scrapes and that 59% of the HPV-positive RTRs exhibited a hrHPV genotype (69). It is hypothesized that transplant recipients may not only have a higher risk to acquire an HPV infection, but that the infections may also have a higher potential to develop into (pre)malignancies in a relative short period of time (71, 72). The role of HPV in the oncogenesis of cervical cancer suggested that the immunosuppressed state of RTRs poses them at risk to HPV infection with subsequent cancer development. From 1975 on, several studies reported increased incidence and prevalence rates of CIN lesions in RTRs compared to the general population (64, 73). In 1986, Penn et al. already showed that 11% of women with post-transplant malignancies had cervical cancer (74). In the following years, several other studies showed an increased incidence of cervical cancer after renal transplantation with standardized incidence rates (SIRs) described between 2.3 and 8.6 (56, 75-77). More recently, a study from our centre showed data that support the findings of earlier studies, namely a 2- to 6-fold increased risk of developing CIN (78).

Vulvar (pre)malignancies in RTRs are less frequently described in the current literature. Several studies show that 100% of the vulvar lesions among RTRs are HPV-positive, compared to approximately 25-30% of the vulvar carcinomas in the general population (22, 65, 79). Vulvar cancer has a relatively high prevalence in RTRs compared to the general population and belongs to the predominant malignancies in RTRs (63, 77, 80). Several studies found a 50-fold increased risk to develop vulvar cancer in female RTRs compared to the general population (78, 81, 82).

This introduction describes the most common lower genital (pre)malignancies in females, the role of HPV in the oncogenesis of these (pre)malignancies in general and the implications of an HPV infection in an immunocomprised patient group like the female RTRs. The main objective of this thesis is to get insight in the tumour localization of vulvar cancer and especially the role of HPV in the oncogenesis in vulvar cancer. Furthermore, the behaviour of genital HPV infection in female RTRs and its implications on post transplantation follow up is assessed.

AIMS AND OUTLINES OF THIS THESIS:

This thesis firstly addresses vulvar cancer and especially the role of the tumour localization. The main objective of the next chapter is the influence of tumour localization on the prognosis of vulvar cancer (Chapter 2). The localization of all vulvar cancers was assessed retrospectively and related to the disease free survival. The localization of the vulvar tumour is also of great importance in the following chapter (Chapter 3). The relation between HPV and vulvar cancer is well established over the years, but the exact oncogenesis remains unknown. Other anogenital (pre)malignancies, namely cervical- and anal (pre)malignancies, are located at a transformation zone. However, no transformation zone has ever been described in the vulvar area. Micro-traumata caused by sexual intercourse might function as a porte d'entree for HPV and the perineum could be prone for these micro-traumata. This chapter might provide more insight in the predilection site of HPV-
related vulvar cancer and clarify the oncogenesis of vulvar cancer further. Next to the localization of the tumour, the differences in patient- and tumour characteristics, p16 expression and overall-, disease free- and disease specific survival between HPV-related and non HPV-related vulvar cancer patients are assessed. The next part of this thesis focuses mainly on HPV infections and genital (pre) malignancies in female RTRs. **Chapter 4** provides an extensive overview of HPV-related anogenital (pre)malignancies in female RTRs and the consequences for treatment in case of an anogenital malignancy. The literature on HPV infections in female RTRs consists primarily of retrospective studies, which show an increased risk of developing HPV-related genital (pre)malignancies. The renal transplantation and related immunosuppressant regime are considered as main risk factor for obtaining an HPV infection. However, no research has been executed on the prevalence of HPV infections in patients before and after transplantation. The exact behaviour of an HPV infection in female RTRs before and after renal transplantation is unclear. **Chapter 5** shows the HPV prevalence of female RTRs closest to the transplantation in a six month period before and furthest from transplantation in a three to 12 month period after renal transplantation. Furthermore, sexual behaviour of the female RTRs and cytological abnormalities are described. The increased risk of developing genital (pre)malignancies after renal transplantation has prompted several European and American guideline committees to recommend annual cervical cancer screening in female RTRs (83-85). Meeuwis et al. (78) and Courtney et al. (86) showed that a considerable proportion of the female RTRs does not follow this advice. The reasons for not participating in the gynaecological screening are unclear. Of the Dutch female RTRs, 63.4% (128/202) underwent at least one cervical smear after their renal transplantation and 36.6% (74/202) were screened once every five years according to the national screening programme (78). In the general population the barriers for participating in cervical screening programme lay mainly in the organisational domain, for example planning an appointment (87). So, to improve the participation rate of female RTRs it is essential to first investigate the barriers and facilitators in gynaecological screening of female RTRs. **Chapter 6** shows the results of focus group interviews with female RTRs and nephrologists from which barriers and facilitators for gynaecological screening were identified. Ultimately, the aim of this study is to present suggestions for increasing the participation rate. In the general population, a self-sampling method was introduced to reach women not participating in the national screening programme. Self-sampling is a cervico-vaginal specimen collection method that is highly accepted by women and the sensitivity of self-sampling is sufficient to be used as an appropriate alternative for physician sampling for HPV testing in low-resource settings or to increase screening rates (88-91). The next chapter (Chapter 7) focuses on self-sampling in gynaecological screening of female RTRs. This article assesses the clinical applicability and acceptability of HPV testing on self-sampling material in female RTRs. In **Chapter 8** the data are put into perspective and the implications for the female RTRs are discussed. Future perspectives on optimizing post transplantation care in female RTRs, especially in the area of gynaecological screening, will be given. Next to these perspectives on post transplantation care, the future in diagnosing and treating vulvar cancer will be discussed. A summary follows this general discussion.


CLITORAL INVOLVEMENT OF SQUAMOUS CELL CARCINOMA OF THE VULVA: LOCALIZATION WITH THE WORST PROGNOSIS

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Loes C.G. van den Einden,
Maartje Cissen,
Joanna IntHout,
Leon F.A.G. Massuger,
Joanne A. de Hullu

European Journal of Surgical Oncology 2015; 41(4): 592-8
ABSTRACT

Objective: The overall 5-year survival of patients with vulvar squamous cell carcinoma (SCC) is 70%. The clinical impression is that localization of SCC on the clitoris may lead to worse prognosis. The aim of this study is to assess the disease specific survival (DSS) in patients with clitoral SCC compared to patients with SCC without clitoral involvement.

Methods: All consecutive patients with primary vulvar SCC treated with surgery at the Department of Gynaecologic Oncology at the Radboud university medical center (Radboudumc) between March 1988 and January 2012, were analyzed. The clinical and histopathological characteristics and DSS rates of patients with (N=72) and without clitoral SCC (N= 275) were compared. Furthermore, patients with clitoral involvement were compared to patients with perineal SCCs (N= 52) and other central SCCs without clitoral and/or perineal involvement (N= 117).

Results: Patients with clitoral SCC more often had larger and deeper invaded tumours, lymphovascular space involvement (LVSI), positive surgical margins and a higher percentage of positive lymph nodes. Kaplan-Meier survival analyses showed worse DSS in patients with a clitoral SCC compared to patients without clitoral involvement. Multivariable analysis showed that not clitoral involvement, but invasion depth, differentiation grade and lymph node status are independent prognostic factors.

Conclusions: Patients with clitoral SCC have worse survival compared to patients without clitoral involvement. This is probably caused by unfavourable histopathological characteristics of the tumour rather than the localization itself. Prospective studies are needed to further assess the influence of localization of the vulvar SCC on prognosis.
INTRODUCTION

Vulvar cancer is rare with a worldwide incidence of 2-3 per 100,000 women, increasing with age (1-3). It represents 1% of all malignancies in women and about 3-5% of all gynaecological malignancies (4). Approximately 80% of vulvar malignancies are squamous cell carcinomas (SCC). Two different types of SCC are described, namely a type caused by an infection with high risk Human Papillomavirus (HPV) via usual vulvar intraepithelial neoplasia (uVIN) that primarily affects younger women and the second type SCC is the most common type, which often occurs in a background of lichen sclerosus (LS) and/or differentiated VIN (dVIN), especially in elderly women (5). Taking a biopsy for histopathological examination is the gold standard for diagnosing vulvar SCC. Nowadays, the triple incision technique, consisting of wide local excision (WLE) with uni- or bilateral inguinofemoral lymphadenectomy (IFL) via separate incisions, is the standard treatment for vulvar SCC with > 1 mm invasion (6). Early stage vulvar SCC patients can be treated by WLE and a sentinel lymph node (SLN) procedure, preferentially within the protection of a clinical trial such as the GROINSS VII study (7).

Several studies show that age, tumour size, invasion depth, extranodal growth, and differentiation grade are independent prognostic factors in vulvar SCC (8-11); lymph node status being the most important prognostic factor (11-14). The overall 5-year survival rate is 70% and in patients with negative inguinofemoral lymph nodes (stage I and II) this reaches 90%, but diminishes in case of positive inguinofemoral lymph nodes (15, 16). In several other malignancies, an association between the anatomic localization and the prognosis has been described. For example, patients with cutaneous melanomas have a worse prognosis when the lesions are located on the head, neck and/or trunk compared to lesions on the extremities (17). It has been hypothesized that the location of the primary tumour in vulvar SCC may also influence prognosis. Magrina et al. (18) showed that invasion of the urethra had a significant disadvantageous impact on the 5-year survival. Boyce et al. (19) and Andreasson et al. (20) showed the unfavourable impact of clitoral involvement on the prognosis, while Masak et al. (21) on the other hand determined that clitoral involvement did not predict poor prognosis. Although the literature does not provide unambiguous evidence, we hypothesize that primary tumour localization on the clitoris correlates with an unfavourable prognosis in patients with vulvar SCC, based on our clinical experience. Therefore, the aim of this study is to assess the disease specific survival (DSS) of clitoral SCC compared to SCC of the vulva without clitoral involvement.

MATERIALS AND METHODS

PATIENTS AND DATA

Data of 385 consecutive patients with primary vulvar SCC who were primarily surgically treated at the Department of Gynaecological Oncology at the Radboud university medical center (Radboudumc) between March 1988 and January 2012 (follow up until 1st of August 2012), were selected from the local patient registry of vulvar SCC patients. Data were collected after consultation of medical files
and pathological reports. A total number of 38 patients were excluded from the analysis; these patients did not receive surgical treatment (N=30) and/or received incomplete surgical treatment (N=2) and/or the exact localization of the tumour was unclear (N= 6). The variables extracted from the database included patient characteristics (age at diagnosis and time of follow up) and histopathological characteristics (tumour size, focality, depth of invasion, surgical margin status (positive, ≤8mm or > 8mm). Furthermore, lymphovascular space involvement (LVSI), lymph node status, differentiation grade, the FIGO stage at time of diagnosis (staging system of 1988 or 2009), adjuvant radiotherapy and the recurrence rate (vulvar -, groin - and/or distant) were extracted from the database.

Within the total group of 347 vulvar cancer patients, two different groups were defined: the clitoral group consisted of patients with a clitoral SCC (N=72) and the non-clitoral group (≥ 1mm from the clitoris) (N= 275) consisted of patients with tumours without clitoral involvement. The non-clitoral SCC group was divided in three different groups, namely perineal SCCs (all tumours with perineal involvement, except the tumours with simultaneous clitoral involvement) (N= 52), other central SCCs (≤ 1 cm from the midline (22)) without clitoral and/or perineal involvement (N= 117) and lateral SCCs (> 1 cm from the midline) (N= 106).

STATISTICAL METHODS

Patient and histopathological characteristics and adjuvant radiotherapy were compared between groups using Chi-square tests and t-tests for independent samples. Disease specific survival (DSS) was defined as survival from the date of diagnosis to the date of death due to vulvar SCC or the date of last follow-up. Censoring was applied to patients alive at last follow-up, patients who were lost to follow-up and patients who died of another disease. The DSS and recurrence rates were estimated according to the Kaplan-Meier method and were compared using the log-rank test. Univariate Cox regression was used to assess the prognostic value of patient and histopathological characteristics on the prognosis of vulvar cancer. Based on the subset of statistically significant variables, multivariable Cox regression with a forward stepwise procedure based on likelihood ratio statistics was used to identify those characteristics that independently contributed to the prognosis of vulvar SCC patients. Hazard ratios (HR) with 95% confidence intervals (CIs) are presented. A p-value of <0.05 was considered statistically significant. Analyses were performed using SPSS (PASW statistics, version 20).

RESULTS

Three hundred and forty-seven patients were included in this retrospective study; 72 patients had a clitoral SCC (21%, Group 1) and 275 patients had a tumour without clitoral involvement (79%, Group 2). The median follow up time of the Clitoral group was 32 months (range 0-259) and the median follow up of the Non clitoral group was 49 months (range 0-276). The baseline characteristics of the study population are listed in Table 1. Patients in the Clitoral group had larger tumours, deeper tumour invasion, more LVSI, more often positive surgical margins and surgical margins < 8 mm, a
higher percentage of positive lymph nodes, and a higher percentage of a high FIGO stage (FIGO staging valid in year of diagnosis), compared to patients in the Non clitoral group. Compared to the Perineal group (N= 52), the group had more LVSI, a higher percentage of positive lymph nodes and a higher percentage of a high FIGO stage. The Central group with central SCCs without clitoral- and perineal involvement (N= 117) was also compared to the Clitoral group. The Clitoral group had larger tumours, deeper tumour invasion, a higher percentage of positive lymph nodes, and a higher percentage of a high FIGO stage. Finally, compared to the Lateral group, the Clitoral group had larger tumours, deeper tumour invasion, more often positive surgical margins and surgical margins < 8 mm, more often multifocal tumours, and a higher percentage of a high FIGO stage.

Total number of patients that received adjuvant radiotherapy (location was not specified) was 24 (33%) in the Clitoral group and 60 (22%) in the Non clitoral group (p-value: 0.04).

The estimated 5-, 10-year and overall recurrence rates (vulvar/groin/distant) did not significantly differ between the Clitoral and the Non clitoral group; 5 year recurrence rate was, respectively, 51% and 42% (p-value: 0.10), the 10 year recurrence rate 63% and 59% (p-value: 0.13) and the overall recurrence rate 78% and 92% (p-value: 0.16). The adjuvant radiotherapy did not correlate with the recurrence rate.

The 5-year and the 10-year DSS rates of clitoral SCC were estimated 73% and 64%, respectively, and subsequently compared to DSS rates of perineal SCC (estimated 87% for 5- and 10-year DSS). The 10-year DSS rate of perineal SCC was significantly (p-value: 0.04) lower (Figure 1). The 5- and 10-year DSS rates of the Clitoral group were lower, although not significantly (p-value: 0.09 and 0.07, respectively), compared to the Non clitoral group (estimated 5- and 10-year DSS rates of 79% and 76%). Figure 2 displays the 10-year DSS.

The influence of all variables on the prognosis (5- and 10-year DSS) of vulvar SCC was assessed. The results of the univariate analyses are displayed in Table 2. Notably, clitoral involvement was not a significant prognostic factor: the 5-year DSS HR is 1.61 (95% CI 0.92-2.80), and the 10-year DSS HR is 1.63 (95% CI 0.96-2.77). The multivariable analysis showed that invasion depth, moderately and poorly differentiated tumours, and presence of positive lymph nodes, especially those with extranodal growth, were independent prognostic factors (Table 3).
Table 1. Baseline characteristics of patients with primarily surgically treated vulvar SCC.

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<tr>
<th></th>
<th>Clitoral N</th>
<th>n/N(%)</th>
<th>Non clitoral N</th>
<th>n/N(%)</th>
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<tr>
<td><strong>Patient characteristics</strong></td>
<td></td>
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<tr>
<td>Age (years) (mean (SD))</td>
<td>72</td>
<td>68.7 (14.4)</td>
<td>275</td>
<td>67.3 (15.6)</td>
</tr>
<tr>
<td><strong>Tumour characteristics</strong></td>
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<tr>
<td>Tumour size (mm) (mean (SD))</td>
<td>72</td>
<td>34.8 (19.4)</td>
<td>273</td>
<td>27.6 (21.7)*</td>
</tr>
<tr>
<td>Focality</td>
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<td>Unifocality</td>
<td>55/72(76)</td>
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<td>224/274(82)</td>
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<td>Multifocality</td>
<td>17/72(24)</td>
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<td>50/274(18)</td>
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<tr>
<td><strong>Depth of invasion (mm) (mean (SD))</strong></td>
<td>69</td>
<td>9.0 (7.2)</td>
<td>255</td>
<td>6.2 (5.6)*</td>
</tr>
<tr>
<td>Surgical margins</td>
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<tr>
<td>≥8 mm</td>
<td>37/72(51)</td>
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<td>191/273(70)&quot;</td>
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<td>&lt;8 mm</td>
<td>26/72(36)</td>
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<td>Positive</td>
<td>9/72(13)</td>
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<td>15/273(5)</td>
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<td>Well</td>
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<td></td>
<td>55/244(23)</td>
<td></td>
</tr>
<tr>
<td>Moderately</td>
<td>44/69(64)</td>
<td></td>
<td>127/244(52)</td>
<td></td>
</tr>
<tr>
<td>Poorly</td>
<td>13/69(19)</td>
<td></td>
<td>62/244(25)</td>
<td></td>
</tr>
<tr>
<td><strong>LVSI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>48/71(68)</td>
<td></td>
<td>213/271(79)&quot;</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>23/71(32)</td>
<td></td>
<td>58/271(21)</td>
<td></td>
</tr>
<tr>
<td><strong>Lymph node status</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Lymph node metastases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>35/72(49)</td>
<td></td>
<td>180/275(65)&quot;</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22/72(31)</td>
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<td>59/275(22)</td>
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<tr>
<td>Yes; extranodal</td>
<td>15/72(20)</td>
<td></td>
<td>36/275(13)</td>
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</tr>
<tr>
<td>Stage of disease</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>33/72(46)</td>
<td></td>
<td>179/274(65)&quot;</td>
<td></td>
</tr>
<tr>
<td>III-IV</td>
<td>39/72(54)</td>
<td></td>
<td>95/274(35)</td>
<td></td>
</tr>
</tbody>
</table>

* Without perineal or clitoral involvement, # p-value <0.05; Non clitoral, perineal, central and lateral were all compared to the clitoral group.
Table 1. Baseline characteristics of patients with primarily surgically treated vulvar SCC.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Perineal</th>
<th>Central *</th>
<th>Lateral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N/ n/N(%)</td>
<td>N/ n/N(%)</td>
<td>N/ n/N(%)</td>
</tr>
<tr>
<td>Age (years) (mean (SD))</td>
<td>72</td>
<td>68.7 (14.4)</td>
<td>275</td>
</tr>
<tr>
<td>Tumour size (mm) (mean (SD))</td>
<td>72</td>
<td>34.8 (19.4)</td>
<td>273</td>
</tr>
<tr>
<td>Focality</td>
<td>55/72(76)</td>
<td>17/72(24)</td>
<td>224/274(82)</td>
</tr>
<tr>
<td>Depth of invasion (mm) (mean (SD))</td>
<td>72</td>
<td>9.0 (7.2)</td>
<td>255</td>
</tr>
<tr>
<td>Surgical margins</td>
<td>37/72(51)</td>
<td>26/72(36)</td>
<td>9/72(13)</td>
</tr>
<tr>
<td>Differentiation grade</td>
<td>12/69(17)</td>
<td>44/69(64)</td>
<td>13/69(19)</td>
</tr>
<tr>
<td>LVSI</td>
<td>48/71(68)</td>
<td>23/71(32)</td>
<td>213/271(79)</td>
</tr>
<tr>
<td>Lymph node metastases</td>
<td>35/72(49)</td>
<td>22/72(31)</td>
<td>15/72(20)</td>
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<tr>
<td>Stage of disease</td>
<td>33/72(46)</td>
<td>39/72(54)</td>
<td>129/274(65)</td>
</tr>
<tr>
<td></td>
<td>17/52(33)</td>
<td>22/52(42)</td>
<td>11/52(21)</td>
</tr>
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<td></td>
<td>4/52(8)</td>
<td>8/52(15)</td>
<td>1/52(2)</td>
</tr>
</tbody>
</table>

* Without perineal or clitoral involvement, # p-value <0.05; Non clitoral, perineal, central and lateral were all compared to the clitoral group.
Figure 1. Disease specific survival of clitoris SCC compared to perineal SCC.

Figure 2. Disease specific survival of clitoral SCC compared to vulvar SCC without clitoral involvement.
Table 2. Hazard ratios with 95% confidence interval of patient- and histopathological characteristics for 5- and 10 year disease specific survival in vulvar cancer patients using univariate Cox regression.

<table>
<thead>
<tr>
<th>Variables</th>
<th>5-year DSS N</th>
<th>HR (95% CI)</th>
<th>10-year DSS N</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>347</td>
<td>1.03 (1.01-1.05)*</td>
<td>347</td>
<td>1.03 (1.01-1.05)*</td>
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<tr>
<td>Tumour size (mm)</td>
<td>345</td>
<td>1.03 (1.02-1.04)*</td>
<td>345</td>
<td>1.03 (1.02-1.04)*</td>
</tr>
<tr>
<td>Focality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uni</td>
<td>279</td>
<td>1.00 (ref)</td>
<td>279</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Multi</td>
<td>67</td>
<td>1.09 (0.60-1.97)</td>
<td>67</td>
<td>1.05 (0.59-1.86)</td>
</tr>
<tr>
<td>Depth of invasion (mm)</td>
<td>324</td>
<td>1.11 (1.07-1.14)*</td>
<td>323</td>
<td>1.10 (1.06-1.13)*</td>
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<td>Surgical margins</td>
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<tr>
<td>0 mm</td>
<td>24</td>
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<td>24</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>&lt; 8mm</td>
<td>93</td>
<td>0.31 (0.15-0.65)*</td>
<td>93</td>
<td>0.32 (0.15-0.66)*</td>
</tr>
<tr>
<td>≥ 8mm</td>
<td>228</td>
<td>0.21 (0.10-0.41)*</td>
<td>228</td>
<td>0.24 (0.13-0.48)*</td>
</tr>
<tr>
<td>Differentiation grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Well</td>
<td>66</td>
<td>1.00 (ref)</td>
<td>66</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Moderately</td>
<td>171</td>
<td>7.21 (1.74-29.93)*</td>
<td>171</td>
<td>5.42 (1.68-17.52)*</td>
</tr>
<tr>
<td>Poorly</td>
<td>75</td>
<td>13.28 (3.14-56.20)*</td>
<td>75</td>
<td>9.08 (2.73-30.19)*</td>
</tr>
<tr>
<td>LVSI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>260</td>
<td>1.00 (ref)</td>
<td>260</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Yes</td>
<td>81</td>
<td>2.23 (1.34-3.73)*</td>
<td>81</td>
<td>1.92 (1.16-3.17)*</td>
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<td>Lymph node metastases</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>215</td>
<td>1.00 (ref)</td>
<td>215</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Yes</td>
<td>81</td>
<td>2.71 (1.45-5.08)*</td>
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<td>2.48 (1.34-4.40)*</td>
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<td>9.30 (5.13-16.82)*</td>
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<td>8.74 (5.00-15.33)*</td>
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<tr>
<td>FIGO stage</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (I-II)</td>
<td>212</td>
<td>1.00 (ref)</td>
<td>212</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>High (III-IV)</td>
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<td>4.78 (2.80-8.18)*</td>
<td>134</td>
<td>4.27 (2.60-7.04)*</td>
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<tr>
<td>Clitoral involvement</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>275</td>
<td>1.00 (ref)</td>
<td>275</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Yes</td>
<td>72</td>
<td>1.61 (0.92-2.80)</td>
<td>72</td>
<td>1.63 (0.96-2.77)</td>
</tr>
</tbody>
</table>

HR= hazard ratio, CI= confidence interval, DSS = disease specific survival, N = total number of patients in analysis, LVSI= lymphovascular space invasion, * p-value <0.05
Table 3. Hazard ratios with 95% confidence interval of patient- and histopathological characteristics for 5- and 10 year disease specific survival in vulvar cancer patients using multivariable Cox regression.

<table>
<thead>
<tr>
<th>Variables</th>
<th>5-year DSS N= 257</th>
<th>10-year DSS N= 253</th>
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<tbody>
<tr>
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<td>HR (95% CI)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tumour size in mm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Depth of invasion in mm</td>
<td>1.06 (1.02-1.10)</td>
<td>1.05 (1.01-1.09)</td>
</tr>
<tr>
<td>Surgical margins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>&lt; 8mm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>≥ 8mm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Differentiation grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Moderately</td>
<td>8.70 (1.18-64.29)</td>
<td>5.09 (1.21-21.41)</td>
</tr>
<tr>
<td>Poorly</td>
<td>13.22 (1.74-100.34)</td>
<td>7.22 (1.65-31.60)</td>
</tr>
<tr>
<td>LVSI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lymph node metastases</td>
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<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
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<td>1.55 (0.80-3.03)</td>
<td>1.43 (0.75-2.73)</td>
</tr>
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<td>4.53 (2.43-8.43)</td>
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<tr>
<td>FIGO stage</td>
<td></td>
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</tr>
<tr>
<td>High (III-IV)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Clitoral involvement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Yes</td>
<td>1.19 (0.66-2.14)</td>
<td>1.13 (0.64-2.00)</td>
</tr>
</tbody>
</table>

HR= hazard ratio, CI= confidence interval, DSS = disease specific survival, N = total number of patients in analysis, LVSI= lymphovascular space invasion
DISCUSSION

In this retrospective study we showed that patients with vulvar SCC with clitoral involvement have a worse prognosis compared to patients without clitoral involvement. This is probably due to unfavourable characteristics of the clitoral SCCs (larger diameter of the tumour, deeper invasiveness with more LVSI resulting in a higher percentage positive lymph nodes) more than to the localization itself. Thereby, the 5- and 10-year DSS rates of patients with clitoral involvement were lower compared to non clitoral SCCs. Multivariable analysis showed that invasion depth, differentiation grade and lymph node status were independent prognostic factors in patients with vulvar SCCs. Vulvar SCCs with clitoral involvement were larger and deeper invaded compared to vulvar SCCs without clitoral involvement. An explanation might be the patients’ and doctors’ delay in the diagnostic process. A Danish study showed that the diagnostic delay in vulvar cancer exceeds that in ovarian, endometrial and cervical cancer, mainly caused by patients’ delay (23). Fear of impairing the clitoris function might be an explanation of patients’ delay. Fons et al. showed that “the mean waiting time of four weeks before treatment of vulvar cancer in our clinic resulted in an average increase in tumour size of 50%”. This illustrates that vulvar tumours are very fast-growing tumours as soon as they are clinically detectable. However, this does not explain larger tumours in clitoral SCC. Diagnostic delay in clitoral SCC, specifically, might be caused by the difficulty to biopsy the lesion, especially for inexperienced doctors.

Another hypothesis for the increased depth of invasion could be that clitoral tissue is more prone for tumour invasion. There are no comparative studies addressing this hypothesis. However, clitoral tissue is comparable to the tissue of the glans penis in men, and are both characterized by high circulation and a large number of nerves. It could be that the vascularization of these tumours is more optimal and stimulates faster and deeper tumour growth. There is no literature available to support this hypothesis.

Furthermore, the group of patients with a clitoral SCC had more dVIN than uVIN adjacent to the tumour (results not shown). Differentiated VIN is highly proliferative and might be more likely to progress to invasive SCC than lichen sclerosus and uVIN. Furthermore, dVIN is frequently found adjacent to rapidly growing invasive vulvar SCC (24, 25). Specifically, patients with clitoral SCC had significant more dVIN compared to patients with a perineal SCC, who had more uVIN adjacent to the tumour. The uVIN related pathway in vulvar cancer is associated with better prognosis compared to the LS/dVIN pathway (26).

Our finding of more positive groin lymph nodes in patients with clitoral involvement is likely due to the size and invasion depth of the clitoral tumour (27). Another explanation could be a more direct lymphatic drainage from clitoris to groin nodes. However, Iversen et al. (28) assessed lymph drainage from different vulvar areas to pelvic lymph nodes and found no difference in uptake of 99mTc-colloid between different injection sites (including clitoris) (28). The bilateral drainage from the clitoris (28) might explain the higher percentage of positive nodes. However, our study did not find
a difference in positive groin nodes between central and lateral tumours (not displayed in results). Our study found extranodal growth to be a prognostic factor and to be more often present in vulvar SCC with clitoral involvement. Fons et al. (8) also found positive groin nodes with extranodal growth to decrease survival. They did not find bilateral groin nodes to worsen the prognosis, as did our study. Furthermore, our results showed a lower percentage of positive groin nodes in patients with a perineal SCC (31%) compared to a clitoral SCC (51%). It might be that the lymph drainage from the perineum differs from the other tumour localizations of the vulva and is more comparable to anal tumours. In anal cancer, Biliomoria et al. (29) and Gerard et al. (30) found that respectively 13% and 19.5% of patients have positive lymph nodes. As in vulvar SCC, inguinal groin node status is an important prognostic factor in anal cancer (30). Thereby, the treatment of the groins in anal cancer is radiotherapy, instead of lymphadenectomy in vulvar SCC. It would be interesting to assess whether perineal tumours behave more like anal cancer.

The patients with a clitoral SCC in our study more often had positive surgical margins or margins < 8 mm. Several studies show that close surgical margins give a higher local recurrence rate (11, 14), but the influence on survival remains a subject of debate. However, our results did not show significant differences in total recurrence rate and local recurrence rate between the clitoral and the non clitoral group (not shown in results). Furthermore, the gynaecologic oncologists did not experience more difficulty in the surgical removal of the clitoris in case of clitoral involvement.

The literature on clitoral SCC is scarce, however, three studies investigated the prognosis of different localizations of vulvar SCC. Boyce et al. (19) and Andreasson et al. (20) also found that clitoral SCCs had a higher percentage of positive groin nodes, which is consistent with our findings. However, it is difficult to compare these data to our own data, because these studies comprise a limited number of patients compared to our study population. The overall finding of decreased DSS can be explained by the higher percentage of positive nodes in the group of patients with clitoral SCC, which is also defined a prognostic factor in our study (9, 11-14).

Magrina et al. (18) mentioned urethral involvement as a prognostic factor, nevertheless, we did not analyse this factor in our study population, because only two patients had urethral involvement. Also, several studies showed age being an independent prognostic factor in vulvar cancer patients. However, in our study age was only found to be significantly correlated in the univariate analysis.

Our study comprised a large number of patients with vulvar SCC and we showed that patients with clitoral involvement tend to have a worse prognosis. Nevertheless, this study analyzed the data retrospectively. The precise tumour localization was not always exactly described in patients’ charts. Consequently, the group of patients without clitoral involvement could also comprise tumours localized 1 mm from the clitoris. Our study could not make clear from which distance from the clitoris the survival rates increased. A prospective study on the precise localization of the primary tumour (in mm from clitoris, perineum and midline) may give us more insight in the reason for the possible differences in survival of the different localizations of vulvar SCC. This may help us in optimizing the counselling of vulvar cancer patients.
Furthermore, gynaecologists should realize that patients with vulvar SCC with clitoral involvement tend to have a worse prognosis. Advisory, to avoid diagnostic delay, patients with vulvar complaints should be seen on a vulvar outpatient clinic. In case of a clitoral SCC, patients should be prepared for positive groin nodes as well as its influence on the prognosis.

**CONCLUSION**

Optimal management and follow-up of patients with primary vulvar SCC require understanding of the factors associated with prognosis. Quick referrals of patients with suspicion of vulvar cancer may prevent delay in diagnosis. Vulvar SCCs with clitoral involvement tend to have worse prognosis compared to tumours without clitoral involvement. This is probably caused by unfavourable histopathological characteristics of the clitoral SCCs more than the localization itself. A large prospective study is necessary to assess the exact influence of localization of the primary tumour on prognosis.
REFERENCE LIST

VULVAR CANCER: TWO PATHWAYS WITH DIFFERENT LOCALIZATION AND PROGNOSIS

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Laurie Eckhardt,
Leon F.A.G. Massuger,
Wim Quint,
Peter Bult,
Hans Bulten,
Willem J.G. Melchers,
Joanne A. de Hullu

Submitted
ABSTRACT

Purpose: Two etiologic pathways for vulvar squamous cell carcinoma (SCC) are described: in a background of lichen sclerosus and/or differentiated vulvar intraepithelial neoplasia and related to high-risk human papillomavirus (HPV) infection with high grade squamous intraepithelial lesion (HSIL) as precursor. The aim was to compare the predilection site and survival of HPV-related to non HPV-related vulvar SCCs.

Patients and Methods: Data of patients treated for primary vulvar SCC at the Radboudumc between March 1988 and January 2015 were analyzed. All histological specimens were tested on HPV with the SPF10/DEIA/LiPA25 system assay and p16INK4a immunohistochemical staining was performed using CINtec® histology kit. Vulvar SCCs were considered HPV-related in case of either >25% p16INK4a expression and HPV positive or >25% p16INK4a expression, and HSIL next to the tumour. The tumour localization, disease specific survival (DSS), disease free survival (DFS) and overall survival (OS) of patients with HPV-related and non HPV-related vulvar SCC were compared.

Results: In total 318 patients were included: 55 (17%) patients had an HPV-related vulvar SCC (Group 1) and 263 (83%) patients had a non HPV-related vulvar SCC (Group 2). The tumours in Group 1 were significantly more often located at the perineum compared to Group 2, 30% and 14%, respectively (p = 0.001). The DSS, DFS and OS was significantly better in the HPV-related vulvar SCC patients.

Conclusion: HPV-related vulvar SCCs are more frequently located at the perineum and have a favourable prognosis compared to non HPV-related vulvar SCCs. Both localization of the tumour and the HPV-related pathway could explain the favourable prognosis. HPV-related vulvar malignancies seem to be a separate entity within vulvar SCC.
INTRODUCTION

Vulvar cancer is a rare disease, representing approximately 3-5% of the malignancies of the female genital tract (1), but with an increasing incidence rate from 2002 onwards (2). Around 80% of the malignant tumours of the vulva are squamous cell carcinomas (SCCs). Most SCCs of the vulva occur on the labia majora, but the labia minora, clitoris and perineum may also be primary sites.

There are two different etiologic pathways for the development of vulvar SCC. The first pathway is the most common pathway, with its precursor lesion differentiated vulvar intraepithelial neoplasia (dVIN) and often occurs in the background of lichen sclerosus (LS). The second pathway is related to the high-risk human papillomavirus (hrHPV) infection and covers about 25-30% of all vulvar SCCs (3, 4). High grade squamous intraepithelial lesion (HSIL) of the vulva, formerly known as usual vulvar intraepithelial neoplasia, is the precursor lesion (5). Differentiated VIN is suggested to be highly proliferative and therefore more likely to progress to vulvar SCC compared to HSIL (6, 7). Remarkably, most vulvar SCCs are dVIN-related but most isolated premalignant lesions are HSILs (4, 8). In general, the possibility of HSIL progressing into vulvar SCC is low in comparison to dVIN (9), namely 9-16% in untreated patients and 3% in treated patients, compared to about 30% in case of dVIN (10).

The role of HPV in cervical cancer is clearly established: an infection with hrHPV is the most important etiological step in the pathogenesis of cervical carcinoma (11-13). Most cervical (pre) malignancies occur at the transformation zone: an area where squamous epithelium merges into columnar epithelium. The cervical transformation zone is most susceptible for an HPV infection because of the high cell turnover and close relation to the basal cell layer (14). The anus also has a similar transformation zone where hrHPV associated anal intraepithelial lesions (AIN) and anal cancer occur. The incidence of vulvar SCC is similar to that of anal cancer, however only a minority of all vulvar SCCs is caused by hrHPV infection. Nevertheless, the oncogenic pathway of how HPV causes vulva SCC remains unclear. Our hypothesis states that micro-traumata, due to, for example, friction caused by sexual intercourse, might facilitate access of HPV to the basal cell layer, may result in integration and finally cause vulvar SCC. A previous study by our study group investigated the influence of localization on the prognosis of vulvar SCC. Tumours with clitoral involvement had worse prognosis compared to other localizations on the vulva. Several tumour characteristics such as depth of invasion were less favourable and these characteristics were more often present in vulvar SCC located at the clitoris (15).

Several studies have evaluated the relationship between HPV infection and disease specific survival (DSS) in vulvar SCC. These studies showed contradictory results. The studies of Monk et al. (16) and Ansink et al. (17) suggest that HPV-positive vulvar SCC (established by HPV PCR only) has a better DSS, where patients with HPV negative vulvar SCC have an increased risk of recurrence and death from vulvar SCC. Van de Nieuwenhof et al. found dVIN-associated vulvar SCC to have a significantly worse DSS (18). However, Alonso et al. (19) retrospectively evaluated 98 patients and
found HPV-positive and negative vulvar SCCs to have a similar overall survival and disease free survival. Furthermore, Pinto et al. (20) reported HPV status in the tumour as not important in terms of prognosis. Two other studies found that not HPV positivity, but the overexpression of p16<sup>INK4A</sup> might be associated with higher survival rates and better prognosis in vulvar SCC (21, 22).

The aim of the current study was to investigate the predilection site of HPV-related compared to non HPV-related vulvar SCC to better understand the oncogenesis of HPV-related vulvar SCC. Secondly, we assessed the disease specific survival (DSS), disease free survival (DFS) and overall survival (OS) in patients with HPV-related and non HPV-related vulvar SCC.

**MATERIAL & METHODS**

**PATIENTS AND DATA**

Data of all consecutive patients diagnosed with vulvar SCC who were primarily surgically treated at the Department of Gynaecologic Oncology of the Radboud university medical center, The Netherlands, were prospectively collected and stored in our local vulvar SCC database. Data were collected by consulting the medical files and pathology reports of the patients (N = 520). For the current study we used data of patients who have been treated between March 1988 and January 2015. In total 232 patients were treated between 1988 and 2006; we used the data of 130 patients, because these data were available and complete (18). From 2006 on, all vulvar SCC patients were included. All data were anonymously processed so no ethical approval was necessary.

Parameters extracted from the database included: patient characteristics (age at diagnosis, history of tobacco use, immune status, history of LS), histopathological characteristics (localization of the tumour, tumour diameter, dept of invasion, presence of dVIN or HSIL, type of VIN, focality, lymphovascular space invasion (LVSI), differentiation grade, presence of positive groin nodes, extra nodular growth in positive lymph nodes, and International Federation of Gynecology and Obstetrics (FIGO) stage 2009), treatment characteristics (adjuvant treatment, recurrences, and cause of death) and survival (DSS, DFS, and OS). The localization of the tumour was divided in four groups: clitoris, labium, perineum, and both clitoral and perineal involvement.

**HISTOLOGY**

All histological specimens were collected from the archives of the department of Pathology at the Radboudumc. All slides were evaluated by two pathologists (JB and PB) on the presence of vulvar SCC. The histological specimens which contained vulvar SCC were used to determine the presence of HPV and to assess p16<sup>INK4A</sup> expression. The above mentioned pathologists also interpreted the degree of p16<sup>INK4A</sup> expression.
**HPV PRESENCE AND GENOTYPING**

DNA was isolated from formalin fixed paraffin-embedded tissue sections (4µm) with 250µl Proteinase K solution and incubated overnight at 70°C. This was followed by heat inactivation of Proteinase K at 95°C for 10 minutes. RNaseP/IC qPCR was used to evaluate DNA quality and PCR inhibition (23). Each isolation and PCR run contained HPV positive and HPV negative controls. Specimens were tested for HPV DNA by PCR amplification/detection/typing using the HPV SPF\textsubscript{10} PCR-DEIA-LiPA\textsubscript{25} version 1 assay (Labo Bio-medical Products, Rijswijk, The Netherlands). Briefly, the broad-spectrum HPV SPF\textsubscript{10} PCR amplifies a 65 bp fragment of the L1 open reading frame and recognizing a broad spectrum of at least 65 different HPV genotypes. SPF\textsubscript{10} DEIA was used for detection of generated amplimers. All SPF\textsubscript{10} PCR DEIA-positive samples were used for subsequent genotyping by reverse hybridisation line probe assay (LiPA), allowing simultaneous typing of the 25 mucosal HPV types (HPV6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68 or 73, 70 and 74). The combined HPV SPF\textsubscript{10} PCR-DEIA-LiPA\textsubscript{25} system for detection and genotyping of HPV has been described in detail elsewhere (24-26). Only the hrHPV positive vulvar SCC samples were considered HPV positive.

**P16\textsuperscript{INK4A} IMMUNOHISTOCHEMICAL EXPRESSION**

Tissue sections (4 µm) of the archival paraffin-embedded tissue samples of vulvar SCC were mounted onto SuperFrost glass slides (Menzel-Gläser, Braunschweig, Germany) and dried overnight at 37°C. In total 379 samples were stained. One hundred and thirty samples were stained earlier in light of previous conducted research (18). Immunohistochemistry for p16\textsuperscript{INK4A} of the remaining 249 samples was performed using the automated Ventana XT system (Ventana Medical Systems, Inc., Tucson, AZ). The reaction was developed using CINtec® p16 histology kit (Ventana Medical Systems, Inc.). All sections were then counterstained with hematoxylin, dehydrated, and cover-slipped. Interpretation of p16\textsuperscript{INK4A} nuclear and cytoplasmic p16\textsuperscript{INK4A} staining were both considered as a positive reaction. The results were reported in a semi quantitative fashion: negative (-) if <5% of the cells had nuclear or cytoplasmic staining, slightly positive (1+) if 5 to 25% of the cells were stained, moderately positive (2+) if staining was present in 25 to 75% of the cells, and markedly positive (3+) if >75% of the cells showed nuclear or cytoplasmic staining (27, 28).

**HPV-RELATED AND NON HPV-RELATED**

Based on the existing literature on HPV-related malignancies, we believe that p16\textsuperscript{INK4A} expression in the tumour is essential in the subdivision of HPV-related and non HPV-related vulvar SCC. P16\textsuperscript{INK4A} expression is a surrogate marker of HPV infection. HPV infection leads to the inhibition of retinoblastoma protein pRB via HPV E7 protein which then leads to increased p16\textsuperscript{INK4A} expression through a feedback mechanism. Based on this mechanism an HPV-related vulvar SCC is not likely to have a negative p16\textsuperscript{INK4A} expression. We discussed the subdivision of the vulvar SCCs in a group of experts on HPV-related (pre)malignancies consisting of a medical microbiologist (WM), pathologists (PB, HB) and gynaecologic oncologists (JdH, LM) and decided on the following: Vulvar SCCs with >25% p16\textsuperscript{INK4A} expression and HPV presence, and with >25% p16\textsuperscript{INK4A}, HPV absence,
but HSIL presence were considered HPV-related (Group 1). Group 2 consisted of patients with non HPV-related vulvar SCC, including tumours with negative/≤25% p16\textsuperscript{INK4A} expression independent of HPV status, and with >25% p16\textsuperscript{INK4A} expression, HPV absence, dVIN presence and a history of LS. The cut-off point used for the p16\textsuperscript{INK4A} expression was comparable to work of Van den Nieuwenhof et al. (18) and more recently Halec et al. (29).

**STATISTICAL ANALYSIS**

All statistical analysis were performed using IBM SPSS Statistics version 20 (Armonk, NY: IBM Corp). A p-value of <0.05 was considered to be statistically significant. Patient, histopathological and localization characteristics were compared between the two groups using Chi-square tests and the independent samples t-tests. Disease specific survival (DSS) was defined as survival from the date of diagnosis to the date of death due to vulvar SCC or the date of last follow-up. Censoring was applied to patients alive at last follow-up, patients who were lost to follow-up and patients who died of another cause. Disease free survival (DFS) defined as disease free from date of treatment to the date of recurrence or death due to vulvar SCC or the date of last follow-up. Censoring was applied to patients recurrence free at last follow-up, patients who were lost to follow-up and patients who died of another cause. Overall survival (OS) was defined as survival from the date of diagnosis to the date of death (any cause) or the date of last follow-up. Censoring was applied to patients alive at last follow-up and patients who were lost to follow-up. The DSS, DFS and OS were estimated according to the Kaplan-Meier method and were compared using the log-rank test.

Multivariable Cox regression with a forward stepwise procedure based on likelihood ratio statistics was used to identify those characteristics that independently contributed to the prognosis of vulvar SCC patients. Hazard ratios (HR) with 95% confidence intervals (CIs) are presented.

**RESULTS**

Of the 520 patients in our database, 367 patients were included in this study. The remaining 153 patients were excluded because the data of patients treated between 1988-2006 were not complete (N=102), they did not have a SCC (N=8), did not undergo surgery (diagnosed elsewhere) (N=10), and because no sufficient histological material was available for further research (N=33).

Of the 367 patient included, 49 patients could not be divided into groups, because p16\textsuperscript{INK4A} staining could not be performed or properly be evaluated, because no histological material was left, the material was misplaced in the archive or the tumour was cut out of the paraffin-embedded tissue (N=20) and no clear division could be made based on p16\textsuperscript{INK4A} expression, HPV status, premalignancy present, and history of LS (N=29). Of the remaining 318 patients, 55 patients had an HPV-related vulvar SCC (Group 1, 17%) and 263 patients had a non HPV-related vulvar SCC (Group 2, 83%). The baseline characteristics are shown in Table 1.

In Group 1 51 patients (94%) tested hrHPV positive with HPV 16 and 33 as the predominant genotypes. In Group 2 241 patients (92%) tested hrHPV negative and 22 patients hrHPV positive with HPV 16 as predominant genotype. Figure 1 shows the HPV status, p16\textsuperscript{INK4A} expression and
premalignancy present for the HPV-related and the non HPV-related vulvar SCC group. The 7.2 percent hrHPV negative samples in the HPV-related group had >75% p16\textsuperscript{INK4A} expression and all had HSIL next to the tumour. High-risk HPV negativity could be explained by false negative test result or the material did not contain HPV DNA anymore. In the non HPV-related group 20/22 (91%) patients, who tested hrHPV positive had no p16\textsuperscript{INK4A} expression. Two patients were hrHPV positive and had >25% p16\textsuperscript{INK4A} expression in the non HPV-related group, but due to a history of LS and dVIN next to the tumour, the non HPV-related pathway was more likely in these cases. HPV positivity in the non HPV-related group may be explained by an additional transient HPV infection next to the tumour. The median follow-up of patients in Group 1 was 58 months (range 0-250) and in Group 2 was 37 months (range 0-293). Patients in Group 1 were significantly younger, more often immunocomprised and smoked/had a history of smoking more often than patients in Group 2. Patients in Group 2 more often had LS in their history, had larger and deeper invading tumours, higher percentage of LVSI, a higher percentage of positive lymph nodes consequently leading to a higher FIGO stage, and more recurrences compared to patients in Group 1.

**Figure 1.** HPV status, p16\textsuperscript{INK4A} expression and premalignancy present in 318 patients with vulvar squamous cell carcinoma.
Table 1. Overview of patient, tumour, and treatment characteristics of 318 patients with HPV-related (Group 1) and non HPV-related (Group 2) vulvar squamous cell carcinomas

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (N=55)</th>
<th>Group 2 (N=263)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (in years)</td>
<td>55</td>
<td>263</td>
</tr>
<tr>
<td>Median (range)</td>
<td>58 (33-90)</td>
<td>72 (30-95)</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Non smoking</td>
<td>33/51 (65)</td>
<td>18/51 (35)</td>
</tr>
<tr>
<td>- Smoking or stopped</td>
<td>18/51 (35)</td>
<td>33/51 (65)</td>
</tr>
<tr>
<td><strong>Immune status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Not disturbed</td>
<td>49/55 (89)</td>
<td>231</td>
</tr>
<tr>
<td>- Disturbed</td>
<td>6/55 (11)</td>
<td>231</td>
</tr>
<tr>
<td><strong>History of lichen sclerosus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Yes</td>
<td>49/55 (89)</td>
<td>262</td>
</tr>
<tr>
<td>- No</td>
<td>6/55 (11)</td>
<td>262</td>
</tr>
<tr>
<td><strong>Tumour</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Largest tumour</td>
<td>51</td>
<td>242</td>
</tr>
<tr>
<td>diameter (in mm)</td>
<td>20 (2-120)</td>
<td>29.5 (2-150)</td>
</tr>
<tr>
<td>Invasion depth (in mm)</td>
<td>53</td>
<td>4 (0.8-21.5)</td>
</tr>
<tr>
<td><strong>Focality</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Unifocal</td>
<td>40/54 (74)</td>
<td>212</td>
</tr>
<tr>
<td>- Multifocal</td>
<td>14/54 (26)</td>
<td>212</td>
</tr>
<tr>
<td><strong>LVS1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Yes</td>
<td>9/55 (16)</td>
<td>212</td>
</tr>
<tr>
<td>- No</td>
<td>46/55 (84)</td>
<td>212</td>
</tr>
<tr>
<td><strong>Positive groin nodes</strong></td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>- Yes</td>
<td>11/52 (21)</td>
<td>212</td>
</tr>
<tr>
<td>- No</td>
<td>41/52 (79)</td>
<td>212</td>
</tr>
<tr>
<td><strong>Positive groin nodes with extranodal growth</strong></td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>- Yes</td>
<td>3/11 (27)</td>
<td>212</td>
</tr>
<tr>
<td>- No</td>
<td>8/11 (73)</td>
<td>212</td>
</tr>
<tr>
<td><strong>Differentiation grade</strong></td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>- Well</td>
<td>6/45 (13)</td>
<td>212</td>
</tr>
<tr>
<td>- Moderate</td>
<td>25/45 (56)</td>
<td>212</td>
</tr>
<tr>
<td>- Poor</td>
<td>14/45 (31)</td>
<td>212</td>
</tr>
<tr>
<td><strong>FIGO stage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- I-II</td>
<td>41/52 (79)</td>
<td>212</td>
</tr>
<tr>
<td>- III-IV</td>
<td>11/52 (51)</td>
<td>212</td>
</tr>
</tbody>
</table>
Vulvar cancer: two pathways with different localization and prognosis

Table 1. Overview of patient, tumour, and treatment characteristics of 318 patients with HPV-related (Group 1) and non HPV-related (Group 2) vulvar squamous cell carcinomas

<table>
<thead>
<tr>
<th>Group 1 (n=55)</th>
<th>Group 2 (n= 263)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong> Median (range)</td>
<td><strong>n</strong> Median (range)</td>
</tr>
<tr>
<td>Patient</td>
<td>Smoking</td>
</tr>
<tr>
<td>263</td>
<td>72 (30-95)</td>
</tr>
<tr>
<td>231</td>
<td>18/51 (35)</td>
</tr>
<tr>
<td>262</td>
<td>177/231 (77)</td>
</tr>
<tr>
<td>256</td>
<td>18/51 (35)</td>
</tr>
<tr>
<td>256</td>
<td>256/262 (98)</td>
</tr>
<tr>
<td>238</td>
<td>6/262 (2)</td>
</tr>
<tr>
<td>242</td>
<td>97/238 (41)</td>
</tr>
<tr>
<td>250</td>
<td>141/238 (59)</td>
</tr>
<tr>
<td>250</td>
<td>72/113 (64)</td>
</tr>
<tr>
<td>251</td>
<td>52/250 (21)</td>
</tr>
<tr>
<td>251</td>
<td>137/251 (55)</td>
</tr>
<tr>
<td>251</td>
<td>114/251 (45)</td>
</tr>
<tr>
<td>Treatment</td>
<td>Group 1 (N=55)</td>
</tr>
<tr>
<td>---------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
</tr>
<tr>
<td>Adjuvant radiotherapy</td>
<td>55</td>
</tr>
<tr>
<td>- Yes</td>
<td>9/55 (16)</td>
</tr>
<tr>
<td>- No</td>
<td>46/55 (84)</td>
</tr>
<tr>
<td>Recurrence</td>
<td>55</td>
</tr>
<tr>
<td>- Yes</td>
<td>11/55 (20)</td>
</tr>
<tr>
<td>- No</td>
<td>44/55 (80)</td>
</tr>
<tr>
<td>Deceased</td>
<td>55</td>
</tr>
<tr>
<td>- Alive</td>
<td>41/55 (74)</td>
</tr>
<tr>
<td>- Deceased</td>
<td>14/55 (26)</td>
</tr>
<tr>
<td>Cause of death</td>
<td>11</td>
</tr>
<tr>
<td>- Vulvar cancer</td>
<td>7/14 (50)</td>
</tr>
<tr>
<td>- Intermittent disease</td>
<td>6/14 (43)</td>
</tr>
<tr>
<td>- Unknown</td>
<td>1/14 (7)</td>
</tr>
</tbody>
</table>

*LVSI = lymphovascular space invasion, FIGO = International Federation of Gynecology and Obstetrics*

**Figure 2.** Disease specific survival (5-year and total) in patients with HPV-related and non HPV-related vulvar squamous cell carcinoma

![5-year disease specific survival](image_url)
### Group 1 (n=55)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Median (range) N</th>
<th>n/N (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjuvant radiotherapy</td>
<td>55</td>
<td>9/55 (16)</td>
<td>0.141</td>
</tr>
<tr>
<td></td>
<td>46/55 (84)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Group 2 (n=263)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Median (range) N</th>
<th>n/N (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrence</td>
<td>263</td>
<td>76/263 (29)</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>186/263 (71)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deceased</td>
<td>263</td>
<td>94/263 (36)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>169/263 (64)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>260</td>
<td>135/260 (52)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>125/260 (48)</td>
<td>0.230</td>
<td></td>
</tr>
<tr>
<td>Cause of death</td>
<td>14</td>
<td>7/14 (50)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6/14 (43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/14 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>127</td>
<td>90/125 (72)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>29/125 (23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6/127 (5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2.** Continued

![Total disease specific survival](image)

- **non HPV-related**
- **HPV-related**

**P-value = 0.001**

Follow-up in months

Cum survival
Figure 3. Disease free survival (5-year and total) in patients with HPV-related and non HPV-related vulvar squamous cell carcinoma.
Figure 4. Overall survival (5-year and total) in patients with HPV-related and non HPV-related vulvar squamous cell carcinoma

**5-year overall survival**

- P-value = 0.001

**Total overall survival**

- P-value = 0.001
Table 2 shows the localization of the vulvar tumour in patients with HPV-related and non HPV-related vulvar SCCs. HPV-related vulvar SCCs were more often located on the perineum than non-HPV-related vulvar SCCs, 37% and 13.3%, respectively. Non HPV-related vulvar SCCs were more often located on the clitoris.

Table 2. Tumour localization compared between HPV-related and non HPV-related vulvar SCCs.

<table>
<thead>
<tr>
<th>HPV-related (N=54)*</th>
<th>Non HPV-related (N=263)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour localization</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Perineum</td>
<td>20 (37.0)</td>
<td>35 (13.3)</td>
</tr>
<tr>
<td>Clitoris</td>
<td>7 (13)</td>
<td>81 (30.8)</td>
</tr>
<tr>
<td>Labium</td>
<td>26 (48.1)</td>
<td>146 (55.5)</td>
</tr>
<tr>
<td>Clitoris and perineum</td>
<td>1 (1.9)</td>
<td>1 (0.4)</td>
</tr>
</tbody>
</table>

*in 1 patient the localization of the tumour is unknown

The 5-year and total DSS rates significantly differed between the HPV-related and non HPV-related vulvar SCC patients, 89% versus 63% and 72% versus 35%, respectively (Figure 2). Furthermore, the DFS also significantly differed between the two groups. The 5-year and total DFS rates were 76% versus 46% and 28% versus 13%, respectively (Figure 3). The 5-year and total OS also significantly differed between the two groups: 85% versus 57% and 53% versus 16%, respectively (Figure 4). To distinguish between the influence of HPV relation and localization, survival rates between tumours with (N = 55) and without perineal involvement (N = 262) were compared. The 5-year and total DSS rates, 80% versus 65% (p-value: 0.036) and 61% versus 37% (p-value: 0.029), respectively, and 5-year and total OS rates, 77% versus 59% (p-value: 0.025) and 44% versus 23% (p-value: 0.029), respectively, were significantly better in the patients with vulvar SCCs with perineal involvement. The DFS rates were also better in the group of patients with vulvar SCCs with perineal involvement: 61% versus 50% (p-value: 0.058) and 22% versus 14% (p-value: 0.035), respectively. Comparing the HPV-related with perineal involvement (N = 20) to the non HPV-related vulvar SCCs with perineal involvement (N = 35) showed significantly better survival rates for the HPV-related vulvar SCCs. Multivariable Cox regression including the variables perineal involvement and HPV-related vulvar SCC, showed that HPV relation is an independent prognostic factor for 5-year and total DSS, DFS, and OS.
DISCUSSION

Our study has shown that HPV-related vulvar SCCs were more often located on the perineum compared to the non HPV-related SCCs and the DSS and DFS was significantly better in hrHPV-related vulvar SCC patients compared to patients with non HPV-related vulvar SCC. Not the localization of the tumour, but the HPV-related pathway appeared to be the explanation for a favourable prognosis.

The last decade the incidence of vulvar SCC has increased significantly. The two main causes are the ageing of the population and HPV infections. In our study 17% of the vulvar SCCs were considered HPV-related. This is a lower percentage than described in previous publications (3, 4). An explanation for this difference might be that we consider p16INK4A expression playing a key role in dividing the groups in our study, where in previous studies only the presence of HPV was assessed. HPV positivity itself is not proving a causal relationship between HPV and the vulvar SCC. If we would divide our groups only based on hrHPV presence, eight percent of the samples would be "misplaced". A similar distribution considering p16INK4A expression was made in a recent study by Halec et al. (29) and they found an HPV-attributable fraction in vulvar SCC of 21% worldwide. Our results show that patients with HPV-related vulvar SCC tend to be younger and have a history of smoking more often than patients with non HPV-related vulvar SCC. This is in concordance with the existing literature (30-32). The occurrence of HPV-related vulvar SCC at relatively young age may be explained by a higher prevalence of HPV infection at a young age (33, 34). Smoking is described as possible cofactor for HPV infection, but the data on the influence of smoking on HPV-related vulvar SCC are limited (35-37). Associations between smoking and anal and head and neck cancer have been established (38-42).

In our study, non HPV-related tumours were larger and deeper invading, more positive lymph nodes were found and the positive nodes more often showed extranodal growth resulting in higher FIGO stages and more often adjuvant radiotherapy. This finding might be explained by diagnostic delay (both patients' and doctors') or more aggressive growth pattern of non HPV-related tumours (43-45). Patients' and doctors' delay in vulvar SCCs is known to be notorious prolonged (43, 44). Patients often are ashamed and inexperienced doctors might not recognize vulvar SCC. Patients with non HPV-related tumours are older and might feel more ashamed or hesitate to talk about vulvar complaints. It is shown that diagnostic delay is associated with an age above 60 years (45). Non HPV-related tumours are often preceded by dVIN. Compared to HSIL, dVIN more often develops into cancer probably because dVIN is more difficult to diagnose. Furthermore, p53 positivity is more often found in non HPV-related vulvar SCC and is associated with poor prognosis and postulated as a marker for more aggressive biologic behaviour (46).

The predilection site of HPV-related tumours in our study was the perineum. This might be explained by micro-traumata due to sexual intercourse. The perineum may function as a transformation zone, which facilitates access of HPV to the basal cell layer, HPV ultimately integrates and may cause
vulvar SCC. It may be difficult to know where the tumour originates exactly: in some cases there is a debate whether the tumour is an anal or vulvar cancer. The distinction between anal and vulvar cancers is important since the treatment modalities are different; vulvar SCC is primarily surgically treated and anal cancer with chemoradiation. However, in case of an anal tumour <2 cm and with limited invasion (<1 cm), and patients with poor general condition, or HIV positive patients with impaired immune system, radiotherapy alone or surgery are treatments of choice (47).

Patients with HPV-related vulvar SCC in our large study showed better DSS, DFS and OS rates, which is in concordance with only part of the literature (21, 46, 48). There are studies on vulvar SCC with controversial results regarding the relationship between HPV infection and prognosis in vulvar SCC (16-20), however none of these studies included p16INK4A expression as a criterion to divide vulvar SCCs into two oncogenetic pathways. In our study p16INK4A expression was essential in classifying a tumour as HPV-related or non HPV-related, because we stated that a tumour with negative p16INK4A expression was not caused by hrHPV. P16INK4A expression was used as a surrogate marker of HPV infection, because HPV infection leads to the inhibition of the retinoblastoma protein pRB via HPV E7 protein which then leads to increased p16INK4A expression through a feedback mechanism. In several other areas, such as the head and neck region (oropharyngeal and sinonasal tumours (49-51)), HPV-related tumours have consistently shown a better prognosis than non HPV-related tumours (15-17, 52). Furthermore, studies show HPV-related head and neck SCCs to have an improved response to conventional radiotherapy (49, 53, 54). The biologic basis for the improved response is not totally clear, but most likely based on an intact p53 protein. Novel treatment options like HPV vaccination, targeting viral oncoproteins, and immune therapy are currently investigated (55). Thereby, studies show that cell expression of p16INK4A is an independent prognostic factor for treatment response to radiotherapy (15,40). Lee et al. (48) found similar results in patients with vulvar SCC. HPV DNA positivity and p16INK4A positivity were significantly associated with better DFS and lower rates of in-field relapse in patients treated with radiotherapy. In the future, the localization of the tumour, HPV status and p16INK4A expression might be useful for the therapeutic process of vulvar SCC, especially in determining the role of radiotherapy.

CONCLUSION

This study shows that there are two distinctly different pathways in vulvar SCC with different survival rates and localizations. HPV-related vulvar SCCs were more often located on the perineum and patients with HPV-related vulvar SCC have a better prognosis. Not the localization on the perineum but the HPV infection seems to be the explanation for a more favourable prognosis. More insight in the precise role of HPV in the oncogenesis of vulvar SCC is necessary to ultimately apply changes in the therapeutic possibilities of HPV-related vulvar SCC.
Vulvar cancer: two pathways with different localization and prognosis

REFERENCE LIST


Vulvar cancer: two pathways with different localization and prognosis


HPV-RELATED (PRE)MALIGNANCIES OF THE FEMALE GENITAL TRACT IN RENAL TRANSPLANT PATIENTS

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Critical Reviews in Oncology and Hematology 2012; 84(2): 161-180
ABSTRACT

Renal transplantations (RTs) are performed routinely in many countries. After RT, the administration of lifelong immunosuppressive therapy is required. As a consequence, renal transplant recipients (RTRs) have a high risk to develop virus-associated (pre)malignancies, such as Human papillomavirus (HPV) related anogenital (pre)malignancies. It is known that the majority of the RTRs are infected with HPV and that these women have a 14-fold increased risk of cervical cancer, up to 50-fold of vulvar cancer and up to 100-fold of anal cancer. Often, treatment of these lesions requires concessions and may be suboptimal as radiation therapy and extensive surgery may damage the renal transplant. Therefore, prognosis may be compromised due to inadequately treated malignancies. Especially for these immunocompromised patients prevention is of utmost importance. Yearly cervical cancer screening for RTRs is advised, but appears to be executed poorly. For the future, optimizing screening and prevention of anogenital (pre)malignancies is an important issue for women after RT. This review gives a broad overview of all aspects regarding HPV-related (pre)malignancies of the female anogenital tract in RTRs.
INTRODUCTION

RENAI TRANSPALNTATION
In 1954 the first renal transplantation (RT) was performed between identical twins. Five years later, a successful RT was made between non-identical twins. Following this, promising attempts to transplant other organs, such as pancreas, liver, heart and lung were made. At present, solid organ transplantations are performed routinely in many countries: about 18,000 RTs are performed each year in the European Union (500 million inhabitants) according to the European Committee on Organ Transplantation (CD-P-TO) (1). After solid organ transplantation, the administration of lifelong intense immunosuppressive therapy is required.

IMMUNOSUPPRESSIVE THERAPY
Immunosuppressive therapy has developed over the years from whole-body irradiation, splenectomy, thymectomy, and thoracic duct drainage towards combination therapy with several immunosuppressive drugs. Most post-RT regimens now combine a corticosteroid, a calcineurine inhibitor (cyclosporine or tacrolimus) and an adjunctive agent (azathioprine, mycophenolatomefetil or sirolimus), resulting in a 1-year patient and renal transplant survival exceeding 90%. In the last two decades, the incidence of acute rejection has fallen towards 10-15% (2). Initially, the clinical management of organ transplant recipients was dominated by acute post-operative problems, acute rejection, infection and cardiovascular disease. However, the improvement of immunosuppressive protocols and anti-infectious therapy has led to a decrease of these problems (3, 4).

MALIGNANCIES AFTER RENAI TRANSPALNTATION IN GENERAL
EPIDEMIOLOGY
As a consequence of the improved long term survival of renal transplant recipients (RTRs), there is a tremendous increase in the incidence of post-transplant malignancies. Although the late consequences of RT are likely to be underreported, it is known that the overall incidence rate of cancer in RTRs is at least three- to fivefold increased compared to the general population with similar age and gender distribution (5-8). However, the elevated risk for specific cancers, such as nonmelanoma skin cancer (NMSC), cancer of the lip, Kaposi sarcoma, post-transplant lymphoproliferative disease (PTLD) and Human Papillomavirus (HPV) related anogenital malignancies is known to be even higher (5-11). Besides NMSC, which is extremely common in RTRs, the cumulative risk to develop any malignancy has been estimated at 20% after 10 years of chronic immunosuppression. Over the next 20 years, mortality from cancer may exceed that from cardiovascular disease among transplant recipients (4).

AETIOLOGY
The aetiology of post-transplant malignancies is multifactorial, with a main role for the use of immunosuppressive medication. The duration and dose of immunosuppressants is clearly linked to the appearance of post-transplant cancer (4, 12, 13). Various immunosuppressants (e.g. azathioprine and cyclosporine) may have direct carcinogenic effects by inhibiting DNA-repair
capacity or by the production of growth factors, which may lead to irreversible DNA alteration and subsequent carcinogenesis (6, 14-17). In addition, immunosuppressive agents may indirectly contribute to the development of cancer, as an impaired surveillance ability of T-cells may disrupt anti-tumour immune surveillance and may potentiate oncogenic stimuli as chemical carcinogens, ultraviolet (UV) light and viruses (4, 18, 19). Viruses that are associated with the development of cancer in RTRs are the Epstein-Barr virus which can induce PTLD and the hepatitis B/hepatitis C virus which are linked to the development of hepatocellular carcinoma (20-25). Besides, various subtypes of HPV are associated with squamous cell carcinomas (SCCs) of the cervix, vulva, perineum and anus (18, 26). It is also suggested that HPV infection may act as cofactor with UV-radiation in the carcinogenesis of extragenital NMSC, but the exact mechanism remains a subject for debate (27-32). There appears to be no correlation between patients who develop anogenital cancer and those who develop extragenital NMSC after RT and vice versa (33). Therefore, assuming that HPV plays a role in the carcinogenesis of NMSC, it is very probable that the HPV subtypes which are responsible for the occurrence of NMSC are other subtypes than the oncogenic high-risk HPV subtypes which are causally linked to the development of anogenital cancers.

This review will provide an overview of different aspects of various HPV-related (pre)malignancies of the female anogenital tract in RTRs. It will include cervical, vulvar and anal (pre)malignancies in general and in RTRs and additionally, the difficulties in treatment of these lesions in RTRs. Finally, our vision to the future regarding screening and prevention of anogenital (pre)malignancies in RTRs will be marked.

**DATA SOURCES**

Relevant publications were identified by an extensive literature search in the computerised bibliographical database PubMed (date of last search June 1st 2011). As keywords we used ‘renal’ or ‘kidney’, combined with ‘transplantation’, ‘graft’ or ‘transplant recipient’. Subsequently, these terms were combined with cancer, neoplasm, malignancy, intraepithelial neoplasia or carcinoma in situ. Thereafter, a combination with Alpha papillomavirus, Human Papillomavirus, vulva, vulvar, cervix, cervical, uterine cervical, cervix uteri, anus, anal canal, anal, urogenital, anogenital, female genital tract, female genitalia, gynaecologic, gynaecology, vaccine or vaccination was made. Additional papers were identified using the ‘related articles’ button in PubMed and reference lists from selected articles were scrutinised in order to identify other relevant articles that had been overlooked in the database search. Table 1 gives an overview of the articles used in the paragraphs on cervical, vulvar and anal (pre)malignancies in RTRs.
Table 1. Overview of studies concerning HPV infections and HPV-related anogenital malignancies in RTRs

<table>
<thead>
<tr>
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<th>Year</th>
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<th>Number of patients</th>
</tr>
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<tr>
<td>Buell [4]</td>
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<td>Review</td>
<td>-</td>
</tr>
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<td>Meeuwis [33]</td>
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<td>2006</td>
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<td>Cohort (RTR)</td>
<td>21</td>
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<tr>
<td>Alloub [99]</td>
<td>1989</td>
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<tr>
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<td>Meeuwis [113]</td>
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**HUMAN PAPILLOMA VIRUS**

**VIRAL CHARACTERISTICS**
HPV is a double stranded DNA virus that can infect basal epithelial cells of all human squamous epithelia. The HPV genome encodes for 9 viral proteins; the specific early (E)-region encodes for regulatory, transforming and replication viral proteins which are expressed in un- to moderately differentiated basal replicating cells. The late (L)-region encodes for viral capside proteins which are only expressed in highly differentiated epithelial cells, resulting in a high number of HPV copies in superficial epithelial layers (see Figure 1) (34).

Viral proteins E6 and E7 are known cancer promoting genes. The E6 protein may inactivate p53 (a tumour suppressor protein), which results in chromosomal instability and diminished apoptosis. The E7 protein may suppress the retinoblastoma protein (pRB)-pathway which leads to enhanced cell proliferation (34, 35).

**EPIDEMIOLOGY OF HPV INFECTIONS**
Worldwide, the overall HPV prevalence is around 10% (36). Up to 80% of sexually active women have been genitally infected by one or more HPV subtypes during their life (37, 38). The majority of HPV infections are transient; these infections will be cleared by the immune system without causing any abnormality (39, 40). The mean duration of a cervical HPV infection in the general population is thought to vary between 8 and 13 months (41).

The prevalence of any HPV infection in the general population ranges between 2 and 44%, with a peak incidence in the young age groups (42) and, in most regions, a subsequent peak in women aged 45 years or older (36). Recently, HPV point prevalence in women between 18 and 29 years of age showed to be 19% (42). Prevalence in women between 40 and 50 years of age, which is the average age for RT, is about 10% (43). The HPV prevalence is not only age dependent, but also country dependent. Africa registers the highest prevalence (32%), Asia the lowest (6%) and...
Southern Europe registers the second lowest prevalence. An HPV infection with subtype 16 occurs most frequently (41, 44-46).

**Figure 1.** HPV-mediated progression to cervical cancer


Basal cells in the cervical epithelium rest on the basement membrane, which is supported by the dermis. Human Papillomavirus (HPV) is thought to access the basal cells through micro-abrasions in the cervical epithelium. Following infection, the early HPV genes E1, E2, E4, E5, E6 and E7 are expressed and the viral DNA replicates from episomal DNA (purple nuclei). In the upper layers of epithelium (the midzone and superficial zone) the viral genome is replicated further, and the late genes L1 and L2, and E4 are expressed. L1 and L2 encapsidate the viral genomes to form progeny virions in the nucleus. The shed virus can then initiate a new infection. Low-grade intraepithelial lesions support productive viral replication. The progression of untreated lesions to micro invasive and invasive cancer is associated with the integration of the HPV genome into the host chromosomes (red nuclei), with associated loss or disruption of E2, and subsequent up regulation of E6 and E7 oncogene expression.

**LCR = Long control region**
**TRANSMISSION**

HPV is mainly transmitted by sexual intercourse (47). However, as HPV has rarely been detected in the cervix of virgins, other ways of transmission e.g. through contaminated hands, might play a role (48-50). Winer et al. showed that about 60% of type-specific HPV detected in fingertips was detected in a concurrent genital sample. As redetection of the HPV-DNA in fingertips at a subsequent visit was low, this finding pleads for deposition of HPV-DNA from the genitals rather than true fingertip infection (51). Besides, several studies suggest a perinatal mechanism of viral transmission; one study showed that 38% of the infants were HPV-DNA positive at 24 hours after birth (48). Furthermore, another study showed 33% presence of HPV in the oral pharyngeal cavity of the neonates (49). Incidence of HPV infection in neonates of HPV-positive women is significantly higher after vaginal birth compared to birth after caesarean section. However, as the incidence after caesarean section is also fairly high (52), it has been postulated that intrauterine transmission of HPV is possible. This is stated in previous studies which found HPV-DNA in the amniotic fluid and in blood from the umbilical cord (49, 53). So, until now there is no evidence that newborn infants of HPV-positive women would be protected by Caesarean section (49, 53).

**HPV SUBTYPES**

Since the improvement of DNA technology, over 100 distinct HPV subtypes have been described and probably more will follow. HPV can be classified into two groups: cutaneous and mucosal HPV subtypes. Cutaneous subtypes are predominantly found in cutaneous verrucae and mucosal subtypes predominate in anogenital lesions (28, 54).

Mucosal HPV subtypes can be divided in high-risk (subtype 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82) and low-risk (subtype 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108) subtypes (55-60). Persistent infection with high-risk HPV (hrHPV), especially subtype 16 and 18, has an essential role in the carcinogenesis of anogenital malignancies (61). The low-risk HPVs, especially HPV 6 and 11, are responsible for about 90% of the condylomata acuminata (61, 62), which are not considered as precursor lesions of cervical cancer.

**PERSISTENCE, LATENCY, INTEGRATION AND VIRAL LOAD**

HPV persistence is commonly defined as having two or more positive tests (63, 64), time to clearance (65-67) or proportion of HPV-positive visits (68, 69). Defining HPV persistence is complicated by differences in HPV tests, testing intervals, HPV categorization and status for analysis. However, the most important difficulty in defining HPV persistence is the lack of knowledge on the behaviour of an HPV infection. The magnitude of the effect of HPV persistence seems to be stronger with longer duration of the infection, wider HPV testing interval, and higher-grade cervical disease (70). Persistent HPV infections are strongly associated with the development of cervical intraepithelial neoplasia (CIN) 2-3 and are considered to drive progression of CIN into invasive cervical cancer (71). Laboratory evidence shows that persistent HPV oncogene expression is critical for the maintenance and progression of CIN (72).

As mentioned earlier, the prevalence of HPV shows a second peak in women aged over 45 years (36). This phenomenon could be attributed to several mechanisms, such as reactivation of latent
infections acquired earlier in life due to gradual loss of type-specific immunity, acquisition of new infections due to new sexual partners later in life or a cohort-effect (45). Latency implies that no HPV-DNA is detectable by conventional molecular tests, but that very small foci of cells maintain infection at low DNA copy numbers (63). Several animal studies showed that papillomaviruses have the ability to persist in the absence of clinical signs of disease. For example, infection of the skin of domestic rabbits with cottontail rabbit papillomavirus can induce an asymptomatic infection, which can be activated by exposure to exogenous factors, such as ultraviolet light, and leads to lesions/symptoms (73, 74). Furthermore, respiratory papillomatosis (RRP) is associated with benign papillomas of the larynx, which can recur rapidly. A latent infection has been accounted responsible for the rapid recurrence, because viral transcripts were often found in clinically normal tissue of RRP patients (75-79). Moreover, studies in immunocompromised patients hint also to the possibility of latent HPV infections. Theiler et al. (80) conducted a study in a group of immunocompromised patients, namely HIV positive women, where the prevalence of HPV infections is very high. The results suggest that HIV infected women with a previous HPV infection that was presumably cleared, may still develop an active HPV infection in the absence of further sexual exposure. It remains unclear if this theory applies to all immunocompromised patients such as transplant patients. Figure 2 shows a potential model of HPV infection in immunocompromised patients.

Trottier et al. (81) analyzed the association between latent/new infection and new sexual partners at the time the infections were detected. They found that the magnitude of the association in older women with new partners was comparable between putatively latent infections (same HPV subtypes) and new infections (with different HPV subtypes), which favours the notion of true transmission rather than reactivation. Muñoz et al. (82) showed that new sexual partners increased the incidence of HPV infections in older women. Finally, a cohort-effect needs to be taken into account concerning the second peak in HPV prevalence, meaning that age-related variations in prevalence may reflect the diverse HPV exposure of successive birth cohorts.
So far, a definitive conclusion on whether reactivation of latent HPV infections or newly acquired HPV infections causes the second prevalence peak in women over 45 years has not yet been made and the matter needs to be elucidated more in future studies. Furthermore, it is not clear whether the differences between a latent and active cervical infection are qualitative or quantitative (34).

A persistent infection with hrHPV may lead to integration of the viral DNA into the human genome. Integration of HPV-DNA in the human genome is not a normal part of the HPV cycle and is characterized by deletion of viral genes that are essential for synthesis of an infectious virus. In general, only integration of hrHPV subtypes leads to increased expression and stability of the
transcripts encoding for the E6 and E7 oncoproteins, which may bind and disrupt the function of key cellular proteins like p53 and pRb. High-risk HPV-DNA integration causes cellular immortality, deregulated proliferation and increased genomic instability of host cells (35). Integrated HPV-DNA is often found in high-grade pre-invasive lesions and even more frequent in cervical cancer (83-86). These findings show that integration can be postulated as a possible driving factor in the transition of high-grade intraepithelial lesions to invasive disease. Lesions containing HPV in its episomal form are thought to pursue a less aggressive course (85, 87-89). On the other hand, Pirami et al. (89) showed that in cervical cancers not all the HPV was present in integrated form, which suggests that integration is certainly fundamental, but not obligatory in the development of cervical cancer. Unpublished data from our research group showed that HPV-DNA integration is significantly higher in cervical glandular lesions (93%) than in squamous cell lesions (37%), particularly obvious for HPV 16. Figure 1 presents a clear overview of the process of HPV-DNA integration.

After infecting the basal epithelial layer, the virus replicates and produces viral particles. The amount of viral particles can be measured in a cervicovaginal sample and is defined as viral load. It was hypothesized that a higher viral load would be associated with more severe cervical disease. However, it is now clear that the relationship between viral load and disease is more complex. Cross-sectional studies reported an increase in viral load with increasing disease severity, while others found either no association or a higher viral load in women with low-grade squamous intraepithelial lesions than in those with high-grade squamous intraepithelial lesions (90-94). A measurement of the viral load is therefore not yet clinically relevant to determine the severity of the disease (95).

To recapitulate, persistence is defined as having two or more positive tests, time to clearance or proportion of HPV-positive visits. Latency implies that no HPV-DNA is detectable, but very small foci of cells maintain infection. Integration is characterized by deletion of viral genes leading to disruption of the function of p53 and pRb, eventually causing cellular immortality, deregulated proliferation and genomic instability.

**HPV AND RENAL TRANSPLANT RECIPIENTS**

RTRs have a higher risk of HPV infections compared to immunocompetent women. The question rises whether this group of patients has a higher incidence of HPV infections or not. HPV is sexually transmitted, but whether the HPV infections in RTRs are newly acquired or reactivated latent infections is unknown and has not been studied yet. Two studies suggest a more conservative sexual behaviour in RTRs than the general population, which may plead against newly acquired infections (96, 97). Hence, the hypothesis of a latent state of HPV infection seems more plausible. Unlike in HIV positive women, the transplant recipients have not been studied extensively on the latent state of HPV infections yet. It is hypothesized that the behaviour of HPV infections is quite similar to that in HIV positive women; however, this has to be further examined.

The incidence of mucosal HPV infections in RTRs varies between 22 and 63% (98-104). Furthermore, RTRs are more often affected with multifocal HPV infections compared with the general population (98-104). A recent study among 35 RTRs found that 62.8% of the RTRs were positive for HPV-DNA and that 59% of the HPV-positive RTRs exhibited a hrHPV genotype (104). It is hypothesized that transplant recipients may not only have more risk to develop an HPV infection, but that the
infections may also have a higher aggressiveness and that the evolvement to (pre)malignancies may also go faster (105, 106).

In the general population, it is known that a patient with a hrHPV-related (pre)malignant anogenital lesion frequently develops other lesions of the vulva, cervix or anus; metachronously or synchronously. Accordingly, up to 71% of patients with vulvar dysplasia or cancer have a previous concomitant or subsequent history of vaginal intraepithelial neoplasia, CIN or cervical cancer (107-110). Those multifocal lesions frequently contain identical HPV subtypes even after an interval of more than 10 years (107). In contrast, it has recently been shown that multifocal anogenital lesions in RTRs frequently contain different HPV subtypes, including subtypes that are not typical for high-grade lesions in immunocompetent patients (111, 112). Therefore, it is hypothesized that RTRs would be targets for repeated or recurrent infections with various HPV subtypes that induce independent lesions (111, 112).

CERVICAL (PRE)MALIGNANCIES

EPIDEMIOLOGY
Cervical cancer is a major health problem, with more than 500,000 new cases occurring each year worldwide, especially in developing countries. In 2008 about 250,000 deaths from cervical cancer were reported, making it the third leading cause of cancer death in women in the world (113). In developed countries, cervical cancer has been considered a preventable cancer because of its long pre-invasive state, cervical screening cytology programmes to detect pre-invasive lesions at an early stage and the possibility to treat these premalignancies. Virtually all cervical cancers are caused by hrHPV infection, which implies that cervical cancer does not and will not develop in the absence of the persistent presence of HPV DNA (114). HPV 16 accounts for 50 – 60% of the cervical cancer cases in most countries, followed by HPV 18 (10 – 20%), HPV 45 (4-8%) and HPV 31 (1-5%) (43, 114, 115). Only the minority of patients with hrHPV infections will develop cervical cancer eventually. Probably other factors (e.g. viral persistence, viral load, integration, genetic predisposition and smoking) exert influence on the development into cervical cancer (98, 99, 116).

ONCOGENESIS
The primary site for cervical cancers is the cervical transformation zone (border between the glandular and squamous epithelium). The transformation zone is assumed to be more susceptible to oncogenic influences, like hrHPV infection, due to the high cell-turnover (117). Virtually all cases of cervical cancer develop through the following distinct and sequential steps: acute infection with hrHPV followed by detectable viral persistence linked to the development of CIN and subsequent invasion (118) (Figure 3). About 10% of the hrHPV infections that persist for several years is linked to a high risk of CIN. Furthermore, the lag time between infection and histological evidence of CIN can be short, often within 5 years in immunocompetent female patients (119).
Cervical intraepithelial neoplasia is the non-invasive precursor lesion of cervical cancer. The CIN lesions are classified as CIN 1, CIN 2 and CIN 3 on the basis of the presence of mitotic activity and
nuclear atypia within respectively the basal third, two thirds and whole thickness of the epithelium (120). Table 2 gives an overview of the different terms that are used to describe the histological and cytological abnormalities of premalignant stages of cervical cancer.

**Table 2.** Different cytology nomenclature


<table>
<thead>
<tr>
<th>Papanicolaou</th>
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<th>Bethesda</th>
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<tr>
<td>Pap 1</td>
<td>Normal</td>
<td>Normal</td>
<td>Within normal limits</td>
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<td></td>
<td>Benign atypia</td>
<td>Inflammatory atypia</td>
<td>Benign cellular changes</td>
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<td>Pap 2</td>
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<td>Squamous atypia</td>
<td>ASC-US</td>
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<td></td>
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<td>Pap 3A1</td>
<td>Mild dysplasia</td>
<td>CIN 1</td>
<td>Low-grade SIL</td>
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<td>Pap 3A2</td>
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<td>CIN 3</td>
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<tr>
<td>Pap 5</td>
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</table>

*CIN: Cervical intraepithelial lesion, CIS: Carcinoma in situ, Pap: Papanicolaou classification of cervical scrapes, ASC-US: Atypical squamous cells of undetermined significance, ASC-H: Atypical squamous cells cannot exclude high-grade SIL, AGC (NOS): Atypical glandular cells (not otherwise specified), SIL: Squamous intraepithelial lesion*

Squamous and glandular cells from the transformation zone of the cervix may be collected with cervical scrapes. In several review articles, the risk of eventually developing invasive cervical cancer was estimated to be 1% in patients with CIN 1, 5% in patients with CIN 2, and 15% in patients with CIN 3. Fortunately, a great part of CIN lesions regresses spontaneously in time; 30-62% of the CIN 1 lesions, in 17-54% of the CIN 2 lesions and in up to 30% of the CIN 3 lesions (121-123). It has been estimated that 10-20% of the CIN 3 lesions will finally progress to invasive disease when left untreated (123). Therefore, CIN 3 lesions are always treated and patients with CIN 1 and/or CIN 2 lesions preferably end up in follow-up care. Currently, CIN 3 lesions are mostly treated by large loop excision of the transformation zone (LLETZ).
Eighty-five percent of the cervical cancers are squamous cell carcinomas (SCCs) and a small group (10-20%) consists of adenocarcinomas. The incidence of cervical adenocarcinoma has been increasing in the past decades (124-130). The overall prevalence of HPV infection in cervical adenocarcinomas ranges between 40-100% (131-137). Several studies show a predominance of HPV subtype 18 (133, 135, 138), others an equal distribution between HPV subtype 16 and 18 (131, 134, 136) and there are studies that show a predominance of HPV subtype 16 (132, 137), though not in the same proportion as in SCC. As the treatment of cervical SCCs and adenocarcinomas is identical and the majority of cervical cancers are SCCs the following sections of this review will only focus on cervical SCCs.

After the histologically confirmed diagnosis of cervical SCC, usually clinical staging is performed. The stage of the tumour is determined following the FIGO clinical staging for cervical cancer (139). Examination under anaesthesia is generally performed to be informed on possible invasion of parametria and vagina.

**Figure 3.** Risks of Human papillomavirus (HPV) persistence and progression and cervical cancer progression model

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**Left graph:** Proportion of prevalent carcinogenic HPV infections that clear, persist, or progress to cervical intraepithelial neoplasia grade 3 (CIN3) in the first 3 years after first detection, based on all infections found at baseline screening in the Guanacaste Natural History Study. The great majority represented “new” infections. Persistence without CIN3 is surprisingly uncommon. Uncommon reappearances of HPV types following clearance did not predict risk of CIN3. **Right graph:** Proportion of untreated CIN3 lesions that invade to cancer within 30 years following the initial diagnosis (based on data from New Zealand).
Figure 3. Continued

In the top of the figure the three steps in cervical carcinogenesis are described, namely acute Human papillomavirus (HPV) infection, HPV persistence associated with the development of cervical precancer (cervical intraepithelial neoplasia grade 3 [CIN3] in particular), and invasion. The graph shows that infection and clearance are extremely common and together contribute to the sharp peak of HPV prevalence in the years following average age of first sexual intercourse in the population. The average age at CIN3 diagnosis depends on the intensity of the screening effort in the population. Typically, it takes decades for a CIN3 lesion to grow and eventually invade, although there are rare and rapid exceptions.

Treatment of cervical cancer consists primarily of surgery or (chemo)radiation therapy. In tumours limited to the cervix or with minimal expansion to the proximal vagina, radical surgery will be performed with post-operative radiotherapy in about 20%; based on lymph node metastases and/or unfavourable tumour parameters. In case of expansion outside the cervix the primary treatment consists of radiotherapy combined with chemotherapy and/or hyperthermia. In general, the prognosis of cervical cancer is good with an overall 5-year survival of 65% and for stage I disease a 5-year survival up to 97.5% (140). On the other hand, the morbidity after treatment is impressive with infertility, ovarian dysfunction, micturation- and defecation problems and sexual discomfort as the most imposing complications (141).
All women between 30 and 60 years of age in the Netherlands receive an invitation from the regional screening organization or from their general practitioner for cervical screening, once every five years. The participation rate of the screening programme is about 65% (142). About half of all women with newly diagnosed invasive cervical cancer never participated in any screening programme (143-145). As previously stated, HPV causes almost all cervical cancers and is therefore the main factor in the oncogenesis of cervical cancer. The clinical sensitivity of hrHPV detection is higher than the cytology (~90% versus ~60%), but the specificity is slightly lower (70-90% versus 90-100%) (56, 146-151). Recently, it has been decided to introduce the hrHPV test as primary screening tool in the Netherlands (152).

Currently, physician-collected cervical specimens are considered as the gold standard collection method for HPV detection (153). Recently, a self-sampling method was introduced to reach also the non-responder group of the population screening. Self-sampling is a specimen collection method that is highly accepted by women and the sensitivity of self-sampling is sufficient to be used as an appropriate alternative for physician sampling in low-resource settings or to increase screening rates (154-157). The implementation of self-sampling in the Netherlands in a recent study showed a response rate between 27.5-31.3% in the non-responders of the standard population screening (158-160).

Cervical (pre)malignancies in renal transplant recipients

At the age of 29 years, a female patient underwent a renal transplantation, because of loss of renal function due to IgA-nephropathy. After seven years, she was diagnosed with hrHPV 16 positive cervical cancer stage IB2 which was treated with neoadjuvant chemotherapy and surgery (without the removal of the left pelvic lymph nodes because of the location of the renal transplant in the pelvis). Radiotherapy was contraindicated, because of the localisation of the renal transplant in the pelvis. The tumour board chose, in consultation with the patient, for no adjuvant treatment. The cancer recurred after one year (see Figure 4) and the patient died of disease at the age of 38 years.

The role of HPV in the oncogenesis of cervical cancer has urged investigators to theorize that the immunosuppressed state poses transplant patients at risk to HPV infection with subsequent cancer development. Already in 1975, Porreco et al. (22) described a 14-fold increased incidence of intraepithelial lesions of the cervix in RTRs compared with an age matched group in the general population. Also, Alloub et al. (98) showed a significant higher prevalence rate of CIN lesions between women with renal transplants (49%) compared with controls (10%). More recently, we published data that support the findings of these earlier studies. We detected CIN lesions in 8 of 224 patients (3.6%). When compared with the general population, these RTRs had at least a 2- to 6-fold increased risk of developing CIN (33).

In 1986, Penn already showed that 11% of women with post-transplant malignancies had cervical cancer (161). In the following years, several other studies showed an increased incidence of cervical cancer after renal transplantation with standardized incidence rates (SIRs) described between 2.3 and 8.6 (4, 7, 162, 163).
Cervical cancer in RTRs may lead to major dilemmas regarding treatment decisions. As described earlier, there are several treatment options, but in RTRs the renal transplant is a barrier for many of these treatments. The dilemma rises whether to treat the patient optimally for cervical cancer or to maintain the function of the renal transplant. For example, radiotherapy of the pelvic area is not possible without compromising renal function leading to a dysfunctional renal transplant. Furthermore, radical surgery is difficult because the pelvic nodes can hardly be reached.

**Figure 4.** Recurrent disease of cervical cancer in a renal transplant patient

The transversal section of a T2-weighted MRI shows recurrent disease of cervical cancer on the vaginal vault. MRI: Magnetic resonance imaging, 1: Vagina filled with contrast fluid, 2: Recurrent disease

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**VULVAR (PRE)MALIGNANCIES**

**EPIDEMIOLOGY**

Vulvar cancer is a rare disease which accounts for approximately 3-5% of all gynaecological cancers, with an incidence of about 2/100,000 (110, 164). In the Netherlands, about 300 new cases of vulvar SCC are diagnosed per year (110, 164). In the general population, 54% of patients with vulvar SCC are above the age of 70 years and only 15% of the patients are under the age of 50 years (110). About 80% of vulvar malignancies are SCCs. The remaining 20% comprise a variety of tumours ranging from mainly basal cell carcinoma to more rare tumours such as malignant melanoma, adenocarcinoma of Bartholin’s gland and Paget’s disease (165). In this paper we will focus on the SCC and its precursor lesions of the vulva. There has long been a three-grade system to define these vulvar cancer precursers (vulvar intraepithelial neoplasia [VIN] grade 1-3), analogous with CIN lesions. However, as clinicopathological data did not support the concept of a continuum spectrum
of VIN lesions leading to vulvar cancer, the International Society for the Study of Vulvovaginal Disease (ISSVD) modified the classification of VIN. VIN 1 was abandoned and VIN 2 and 3 were consolidated into one category simply termed VIN. Subsequently, as the single diagnostic category VIN included two different vulvar premalignancies that have a different malignant potential, the classification was modified into VIN usual type (uVIN) and VIN differentiated type (dVIN) (166).

ONCOGENESIS
Vulvar SCC originates from two separate pathways. The first is an HPV-independent pathway and occurs in a background of non-neoplastic epithelial disorders (e.g. lichen sclerosus). This form is usually seen in older women and encompasses the majority of vulvar SCCs in the general population (~80%) (167, 168). Differentiated VIN is presumed to be the precursor lesion of the HPV-negative vulvar SCC (110, 169).

The second pathway is an HPV-dependent pathway, caused by a persistent infection with hrHPV. hrHPV subtypes 16 and 33 are the most commonly seen genotypes in vulvar lesions present in about 60% and 20% of HPV-dependent vulvar carcinomas in the general population, respectively. Other less common hrHPV subtypes like 18, 52 and 58 have also been reported (107, 167, 170, 171). The premalignant stage for this tumour is uVIN (167, 172). See Figure 5 for an example of uVIN of the clitoris. Figure 6 represents clinical pictures of a uVIN lesion in a female RTR with widespread condylomata acuminata. The oncogenesis of this type of vulvar SCC has a remarkable resemblance to the development of CIN into cervical cancer. The immune system seems to play an important role in the progression of uVIN to invasive disease (173). HPV-dependent vulvar SCCs primarily affect younger women and comprehend the minority of vulvar malignancies in the general population (in about 20% of all vulvar SCCs there appears to be a causal relation between hrHPV and vulvar SCC carcinogenesis) (167, 171). Oppositely, HPV-dependent vulvar SCCs seem to comprehend the majority of vulvar carcinomas in immunocompromised women.

VULVAR (PRE)MALIGNANCIES IN RENAL TRANSPLANT RECIPIENTS
At the age of 32 years, a female patient underwent a renal transplantation because of chronic pyelonephritis with negligible renal function. After an interval of 12 years after transplantation, she was diagnosed with HPV 16 related uVIN that recurred after two years. Both lesions were treated with laser therapy. Thereafter, she was lost for follow up and 4 years later she presented with vulvar cancer. She had a radical vulvectomy and bilateral inguinofemoral lymphadenectomy. All lymph nodes were negative so no radiotherapy was indicated. There was no evidence of disease after 5 months of follow up.

Vulvar (pre)malignancies in RTRs are only sparsely described in the current literature. However, it is known that RTRs have an obviously increased risk for (HPV-related) vulvar (pre)malignancies. Several studies show that 100% of the vulvar lesions among RTRs are HPV-positive, compared to approximately 20% of the vulvar carcinomas in the general population (99, 112, 171). Figure 7 shows an example of a vulvar SCC in a RTR.
Figure 5. Clinical picture of usual VIN of the clitoris in a female RTR (indicated by the arrow).

VIN: vulvar intraepithelial neoplasia, RTR: renal transplant recipient

Figure 6. Clinical pictures of widespread vulvar and perineal condylomata acuminata in a female RTR (A). During inspection of the affected area, usual VIN was detected at the commissura posterior of the vulva (B).

RTR: renal transplant recipient, VIN: vulvar intraepithelial neoplasia
There is no regular screening tool for VIN; symptoms are frequently absent or variable and suspicious vulvar lesions are not always histopathologically examined. Therefore, this disease is likely to be underreported; both in the general population and in RTRs. A recent retrospective cohort study on 224 female RTRs detected four patients with dysplasia of the vulva (33). The fact that all of the lesions were HPV-related (uVIN) types underlines the preponderance of the HPV-dependent pathway of the development of vulvar SCC in RTRs.

Vulvar cancer has a relatively high prevalence in RTRs compared to the general population and belongs to the predominant malignancies in RTRs (7, 9, 10). The ANZDATA Registry, which contains data of more than 13,000 RTRs (110,395 person years) transplanted between 1980 and 2003, reported SIRs varying between 45.6 and 55.8 for vulvar cancer (8, 174). Correspondingly, a recent comparison of the standardized age-adjusted prevalence rates of vulvar cancer in a cohort of 224 female RTRs and in the general population of the Netherlands showed a 50-fold increased risk to develop vulvar cancer (33). Adami et al. showed a standardized incidence rate of 26.2.

In general, vulvar SCCs develop rather late after transplantation with an average time to cancer ranging between 10 and 20 years, although one case is reported of vulvar cancer even 33 years after RT (7, 33, 112, 175).

High-risk HPV 16 and 33 are most frequently detected in vulvar cancers of RTRs, but also less common hrHPV subtypes are seen which is comparable to the general population (99, 112). Multicentric lesions (involving perineum, anus, uterine cervix or vagina) are frequently seen in RTRs with HPV-dependent vulvar disease. Those multicentric lesions either originate synchronously or metachronously and are probably the result of incapacity to clear the HPV infection and higher
susceptibility for newly acquired infections (11, 112, 176). Immunocompromised patients tend to be much younger than patients in the general population with vulvar malignancies (10). The median age at diagnosis of vulvar cancer appeared to be approximately 40 years in a Dutch cohort of female RTRs, which is remarkably young compared to the median age at diagnose of the general Dutch population (~ 70 years) (110, 112). Additionally, vulvar cancer in RTRs may behave more aggressively and may be associated with a worse prognosis compared to the general population, but this should be further studied (177).

Just as in immunocompetent patients, surgery is the cornerstone of treatment for vulvar cancer in RTRs, sometimes combined with radio- and/or chemotherapy. However, one should realise that standard treatment may harm the renal transplant which may result in an alternative treatment schedule to save the function of the transplant kidney.

**ANAL (PRE)MALIGNANCIES**

**EPIDEMIOLOGY**

Tumours of the anal region can be divided into several distinctive histological types, according to the anatomy of different zones of the anal canal. The anal canal extends from the perianal skin (squamous mucosa) via the transitional zone (transition from squamous to non squamous mucosa) to the rectal mucosa. Tumours arising above the dentate line (the line which indicates the end of the squamous mucosa and the beginning of the transitional zone) are usually mixed adenosquamous carcinomas.

Carcinoma of the anus is a rare disease in the general population and accounts for less than 1.5% of all gastrointestinal tract malignancies (178). The incidence of anal cancer is rising and is now about 1.5 – 2.0 per 100,000 in the general population (179, 180). However, this incidence is higher for high-risk populations such as men who practice anoreceptive intercourse and immunosuppressed patients such as HIV-infected patients and solid organ transplant recipients (178, 181, 182). For example, the incidence in HIV-negative men who have sex with men is about 40 per 100,000 and for HIV-positive men who have sex with men the incidence is about 80 per 100,000 (183). Women with a history of HPV related vulvar or cervical cancer also have substantially higher incidences of anal cancer (184, 185). The incidence of invasive cancer is consistently higher for women (about 66% of cases) compared to men (about 34% of cases) (179, 180, 186).

The most frequently diagnosed anal tumours are SCCs, which account for 70-85% of all anal cancers (180, 187). In general, mixed adenosquamous carcinomas and SCCs have similar biology and prognosis. On the opposite, anal adenocarcinomas behave like rectal cancer (178, 187). In a case-control study, Frisch et al. (188) detected HPV-DNA in 88% of 388 patients with anal cancer, but in none of the control patients with rectal adenocarcinoma. Another case-control study by Daling et al. (189) showed only 40% of anal adenocarcinomas positive for HPV versus 92.2% of anal SCCs. Similarly, although a role for HPV in rectal cancer is suggested, a causal relationship has never been established (190-193). Therefore, anal adenocarcinomas and rectal cancer beyond the scope of this chapter.
**ONCOGENESIS**

Anal SCC has, just as cervical cancer, a strong etiologic association with HPV infection. The precursor lesion for anal cancer is anal intraepithelial neoplasia (AIN). Natural history studies adapted similar histology grades for AIN as for CIN. AIN 1 is an indication of active HPV infection and not a cancer precursor as is CIN1. AIN 2 mimics CIN 2 and is unpredictable for cancer progression. AIN 3 lesions have a nearly complete association with HPV infection, but have, unlike CIN 3, relatively low rate of malignant transformation in immunocompetent patients (183).

In general, malignant progression from high-grade AIN (AIN2/3) to anal SCC is reported in about 10% of the cases. However, progression is more likely in the presence of immunosuppression; about 50% within 5 years (194, 195).

The association between HPV and anal cancer is established in various population-based epidemiological studies. Of all anal cancers 71 – 88% is attributable to HPV, with a preponderance of hrHPV subtypes (188, 189, 196, 197). These population-based studies, including specific high-risk patients such as HIV-positive patients or men who practice anoreceptive intercourse, showed that HPV subtype 16 seems to be the predominant genotype associated with anal cancer, detected in 66 – 73% of HPV-positive anal cancer cases; followed by HPV subtype 18 in 5.2 – 6.9% (188, 189, 196, 197) and HPV subtype 33 in 3.3 – 5.9% (188, 196, 197).

**ANAL (PRE)MALIGNANCIES IN RENAL TRANSPLANT RECIPIENTS**

A female patient with end stage renal disease due to chronic membranous glomerulonephritis received a renal allograft at the age of 34 years. Since then, she was maintained on oral prednisolone and azathioprine daily. Almost 4 years later, she was diagnosed with an HPV 6 & 16 positive CIN3 lesion, which was managed by diathermic stripping (ablatio cervicis). Seven years thereafter, she was found to have a moderately differentiated, HPV 58 positive SCC of the anus which was treated with radiation therapy. Multiple cutaneous SCCs developed before and after the diagnosis of anal cancer. Six months after initial treatment, the anal cancer recurred and was surgically treated. Nevertheless the patient died of recurrent disease 1.5 years later, at the age of 47 years.

Ogunbiyi et al. (198) were the first who established that RTRs are at significant higher risk of developing AIN and having anal HPV infection compared with the general population (20% versus 1% and 47% versus 12% respectively). Comparably, AIN was diagnosed in 18.3% of RTRs in another cohort (199). It is established that AIN in RTRs is significantly associated with anal hrHPV infection, duration of immunosuppression, previous condylomata acuminata and anoreceptive intercourse (200).

A few studies have been conducted on the appearance of anal cancer following RT, showing an obviously elevated risk for the development of these tumours. Reported risk ratios have a broad range, varying between 10 and 100-fold (163, 201-204). In Figure 8, clinical pictures of a female RTR who synchronously developed uVIN and anal SCC are shown.

The optimal treatment for AIN is difficult to determine as large series comparing treatments with prolonged follow up are lacking (205). Different strategies can be employed, for example expectant management for low-grade AIN, imiquimod 5% cream, infrared coagulation, or local resection (205-
Also, early stage cancer is generally treated with local resection. In case of more advanced stage disease, local resection is usually replaced by or combined with radiation therapy with or without chemotherapy. However, obtaining complete remission of anal cancer in RTRs is challenging as radiation therapy may have toxic effects on the renal transplant and therapeutic compromises are frequently needed.

**Figure 8.** Clinical pictures of usual VIN (A) and anal SCC (B) that were diagnosed in a female RTR at the same moment.

VIN: vulvar intraepithelial neoplasia, SCC: squamous cell carcinoma, RTR: renal transplant recipient

**PREVENTION**

The increased risk for anogenital malignancies that is associated with long term immunosuppressive therapy use and the difficulties in treatment of these lesions have consequences for the long term survival of RTRs. Therefore, prevention of these cancers and their precursors should be given high priority.

**PRIMARY PREVENTION**

Currently, two HPV vaccines that may prevent a majority of cases of HPV 16 and 18 related anogenital (pre)malignancies are available in many countries. These vaccines have been shown to be highly effective in the general population and also seem promising for RTRs. However, there are no publications about effectiveness or safety of these HPV vaccines among patients with end stage...
renal disease and RTRs until now. Those patients may have a decline in vaccine-induced immunity (reduced response and/or rapid decline of antibody levels), which is perceived by vaccination against hepatitis B (208, 209). Currently, several studies are conducted on the safety and immunogenicity of the HPV vaccines in immunocompromised patients (www.clinicaltrial.gov). Besides, it may be possible that there is also room for therapeutic HPV vaccination for RTRs with high-grade anogenital lesions in the future. Kenter et al. (210) recently showed promising results of such vaccination for the treatment of high-grade VIN lesions in immunocompetent individuals and more studies on this theme are in progress. The value of therapeutic HPV vaccination in immunocompromised patients should be topic of further investigation.

At this moment, organized prophylactic vaccination in women before RT under a conceptual setting of annual cervical screening is unlikely to be cost effective in preventing cervical lesions (209). Nonetheless, the benefits of the quadrivalent vaccine may also be directed at HPV 6 and 11-related condylomata acuminata and vaccine-subtype specific lesions of the vulva, vagina and anus. When including these data, cost effectiveness analyses would possibly be more favourable (209). The content of this discussion will definitely change in a few years, as organised HPV vaccination programmes for young adolescents will become implemented in a growing number of countries. However, vaccination will not control anogenital cancer completely. Therefore, a proactive policy regarding screening of the anogenital tract will remain important.

SECONDARY PREVENTION

Based on the evidence of the higher risk for malignancies, several publications have suggested intensifying the cervical cancer screening in the transplantation population, although there is no consistent evidence that this will lead to a reduction in cervical cancer incidence and subsequent mortality (22, 33, 99, 103). By now, several European and American guidelines (e.g. the European Best Practice Guidelines, the “Kidney Disease: Improving Global Outcomes”-foundation and the American Society of Transplantation) recommend to perform at least annual cervical cancer screening with pelvic examination and Pap smear in female RTRs (211-213). According to Wong et al. (209), annual screening for cervical cancer using conventional cytology in female RTRs is cost effective when compared with no screening.

Until now, there is only limited evidence regarding the implementation of this policy. Courtney et al. (214) showed that the uptake of annual cervical cancer screening in the RTR population of Northern Ireland is low. Of the 173 female RTRs eligible for cervical cancer screening, only 10% undertook the advised number of screening procedures and 32% had never had a cervical smear performed at all. Additionally, we demonstrated that the mean number of cervical smears per RTR per year in the Netherlands was 0.2, which is significantly less than the recommended yearly interval. Even 37% of the RTRs who should undergo yearly cervical smears had never had a cervical screening after their transplantation (33). A reasonable explanation for the discordance between the recommendations and the observed practice might be that both patients and healthcare professionals underestimate the importance of anogenital screening and focus primarily on the preservation of renal function. Additionally, regular surveillance of the anogenital region of female RTRs is important (212, 213). Annual screening for cervical cancer provides an excellent opportunity to perform a thorough external examination of the anal and vulvar regions, for example with digital photo’s during follow-up.
CONCLUSION / FUTURE PERSPECTIVES

This review focused on hrHPV infections and anogenital (pre)malignancies in female RTRs. These immunocompromised patients have a high incidence of anogenital hrHPV infections and it is known that they have a 14-fold increased risk for cervical cancer, up to 50-fold increased risk for vulvar cancer and up to 100-fold increased risk for developing anal cancer compared to the general population.

Unfortunately, the treatment options in this particular population are limited due to the localization of the renal transplant. To spare the renal transplant, concessions in the treatment plan are necessary although this may lead to an elevated mortality rate due to suboptimal treatment.

As a consequence of the obviously elevated risk and the restrictions in the treatment of RTRs, additional attention to screening and prevention of anogenital (pre)malignancies is required. At present we have to tighten up the annual screening with external anogenital inspection, pelvic examination and cervical smear. It would be worthwhile to evaluate the feasibility of gynaecological examination and treatment before transplantation to diminish the early development of anogenital (pre)malignancies after transplantation. Furthermore, it is a challenging task for further research to get more insight in the incidence, prevalence and genotype of genital HPV infection before and after RT in women with end stage renal disease in the setting of a prospective study. Herewith, a base for additional research may be found in the field of prophylactic hrHPV vaccination in patients who are waiting for transplantation.
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REACTIVATION OF LATENT HPV INFECTIONS AFTER RENAL TRANSPLANTATION

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American Journal of Transplantation 2016 Dec 23. [Epub ahead of print]
ABSTRACT

Female renal transplant recipients (RTRs) have an increased risk for developing human papillomavirus (HPV) related (pre)malignant lesions of the genital tract. This study aims to assess the genital prevalence of HPV before and after renal transplantation (RT). In female patients who were counselled for renal transplantation at the Radboud university medical center Nijmegen, the Netherlands, gynecological examination was performed at first visit, and 1 and 2 years later. HPV self-sampling and questionnaires on sexual behaviour were performed every 3 months. In 65 patients who underwent RT, the hrHPV prevalence as assessed with the highly sensitive SPF10-LiPA25 test, increased significantly from 19% before to 31% after RT (p=0.045). Based upon the clinically validated Cobas 4800 HPV test, the hrHPV prevalence increased from 10% before to 14% after RT (p=0.31). During follow-up no changes in sexual behaviour were reported. Thirty-three patients who did not undergo RT showed a hrHPV prevalence of 21% at study entry and of 27% after 12 months with the sensitive test, and a stable prevalence of 16% with the clinically validated test. The results of this study indicate that activation of latent HPV infections may contribute to the increased risk of HPV-related (pre)malignant lesions in female RTRs.
INTRODUCTION

Organ transplantation is the treatment of choice for patients with failing kidneys, heart, lungs, pancreas or liver. To prevent rejection, organ transplant recipients have to use lifelong immunosuppressive therapy. This makes them more susceptible for the development of malignancies with standardized incidence ratios (SIRs) ranging from 2.1 to 4.0 (1-6).

It is well known that high-risk human papillomaviruses (hrHPVs) are causative in the development of genital premalignant or malignant lesions ((pre)malignancies), mainly due to HPV 16 and HPV 18. In fact, 95% of all cervical malignancies and about 20% of the vulvar malignancies are hrHPV-related (7). We previously showed that using a highly sensitive detection assay, the point prevalence of genital HPV infections in women aged 18-29 in the Netherlands is 19%, with an HPV 16 and HPV 18 prevalence of 2.8% and 1.4%, respectively (8). The majority of hrHPV infections in the general population will be cleared within one year. It is thought that only those women in whom the virus is persistently present over a longer period, have an increased risk of genital (pre)malignancies.

HPV positive anogenital tract (pre)malignancies (e.g. cervix, vulva and anus) are found at a significantly higher rate in renal transplant recipients (RTRs) than in immunocompetent individuals with SIRs between 1.0-2.5 for cervical cancer and between 7.3 and 23.9 for vulva/vaginal cancer (1, 3, 5, 6, 9), and belong to the most frequent (pre)malignancies in RTRs (10-12). In observational and other studies on posttransplant malignancies in female RTRs, 11% developed cervical cancer and 4% developed vulvar cancer (13). Another study of our group showed that (pre)malignancies are primarily diagnosed early (within 3 years) or late (>10 years) after transplantation (14).

Data on the prevalence of genital HPV infections in RTRs are scarce. One study showed a prevalence of HPV of 62.8% in 35 female RTRs (15). In contrast with the general population in whom about 20% of the vulvar lesions is HPV positive, Brown et al. found that all vulvar lesions in transplant patients were HPV positive (16). Recently, Meeuwis et al. (17) showed a cervico-vaginal HPV prevalence of 27.1% in a large cohort of female RTRs after renal transplantation (RT).

The HPV prevalence in female RTRs has been mainly assessed after the transplantation. The natural course of hrHPV infections from the time before to the period after RT is unknown. Therefore, the aim of this study was to assess the prevalence of genital HPV in female RTRs before and after transplantation. More specifically, we were interested to investigate whether the use of immunosuppressive therapy has an effect on the prevalence of hrHPV. Knowledge with respect to the biological behaviour of genital HPV infections might provide a scientific basis for rational means to prevent anogenital malignancies in the future, such as HPV vaccination and screening.
PATIENTS AND METHODS

STUDY POPULATION AND IMMUNOSUPPRESSIVE TREATMENT
Between February 28th, 2012 and April 1st, 2015, female patients with end-stage renal disease (ESRD), who had an appointment at the outpatient clinic of the Department of Nephrology at the Radboud university medical center (Radboudumc) to judge whether they were appropriate candidates for RT were invited to participate in this study. See Figure 1 for inclusion and exclusion criteria. All patients underwent renal transplantation in the Radboudumc. After transplantation, patients were treated with tacrolimus, mycophenolate mofetil and prednisone as immunosuppressive therapy. From August 2014, induction therapy with basiliximab was added to the immunosuppressive therapy. Acute rejection episodes were treated with methylprednisolone followed by antithymocyte globulin in case of steroid resistant rejection. This study was approved by the local medical ethics committee [protocol number: 2011/425].

GYNAECOLOGICAL EXAMINATION
Patients who returned the informed consent form were examined by the clinical researcher of the department of Obstetrics & Gynaecology. This first appointment with the researcher included: detailed medical history, dermatological and gynecological examination with inspection of the external genitalia and perianal area, inspection of the vagina and cervix (vaginal vault in absence of the uterus), and collection of cytology by a smear of the cervix or the vaginal vault. This complete gynecological examination was repeated at 1 and 2 years after inclusion in the study.

CYTOLOGY
The Bethesda system 2001 was used for cytological classification. In case of normal cytology no further tests or treatments were necessary. When the cytology showed atypical squamous cells of undetermined significance (ASC-US) or low-grade squamous intraepithelial lesion (LSIL), the smear was repeated after 6 months according to hospital protocol. In case of high-grade squamous intraepithelial lesion (HSIL) a colposcopic examination was performed and patients were treated according to current guidelines.
Figure 1. Scheme of the inclusion

**Inclusion criteria:**
- Female ≥ 18 years
- Eligible for RT at Radboudumc
- Mentally capable
- Sufficient knowledge of the Dutch language
- Signed informed consent

**Exclusion criteria:**
- Not willing to undergo gynaecological examination
- Pregnant
- Within 3 months from delivery/miscarriage

**Patients summary:**
- 351 patients referred to the Radboudumc
- 302 patients received study information
- 130 patients signed informed consent
- 123 patients were examined by the researcher
- 65 patients underwent renal transplantation
  - One patient died after transplantation due to brain tumour
- 43 patients were not transplanted, because:
  - Donor not approved
  - No replacement therapy necessary
  - Waiting list
  - Eurotransplant
  - Other reasons
- 15 patients were found unfit for transplantation and excluded.
- 36 patients were HPV tested every 3 months
- 7 patients were examined once at the first visit to the outpatient clinic and only more if they would undergo RT
SELF-SAMPLING
The patient was asked to self collect a cervico-vaginal sample with a collection device (a small brush packaged in an individual sterile cover, Rovers Evalyn brush®, Rovers medical Devices B.V. Oss, the Netherlands (18)) at the first visit and at home every 3 months during follow-up. Therefore, patients were sent an HPV self-sample kit every three months. This kit contained a collection device, detailed instructions on how to perform the cervico-vaginal self-sampling (written and in cartoon) and a return package consisting of a leak-proof seal bag, absorption sheet and an easy-slider plastic return envelope.

SPECIMEN PREPARATION AND DNA EXTRACTION
The dry Evalyn Brush was resuspended in 1·5 ml Thin Prep. The vials were vortexed for 3x15s, stored overnight at 4°C, and again vortexed for 2x15s. From each resuspended dry specimen, 200 μl was used for DNA extraction with the MagNaPure 96 (Roche Molecular Diagnostics, Indianapolis, Indiana, USA). The purified DNA was eluated in 50μl TE-buffer.

HPV DETECTION AND GENOTYPING
For the HPV detection on the collected self-sampling material, two HPV detection methods were used. The first is the highly analytical sensitive short-PCR-fragment assay (SPF_{10} - LiPA_{25}; Labo Biomedical Products B.V., Rijswijk, The Netherlands). This assay has been widely used for epidemiological and vaccination studies, because of its very high analytical sensitivity (19). This assay was included to identify the smallest foci of detectable HPV. However, because of its high analytical sensitivity, the SPF_{10} - LiPA_{25} assay has a very low clinical specificity (19). As a second test we therefore used the clinically validated Cobas 4800 HPV test (Roche), which enables to detect chronic productive infections that are potentially related to cervical abnormalities (20). The data obtained with this test may guide future clinical management of transplant patients.

The SPF_{10} - LiPA_{25} assay amplifies a small fragment of 65-bp from the L1 open reading frame and allows detection of a broad range of HPV genotypes (21-23). Next, HPV DNA enzyme immunoassay (DEIA) was used, which comprises a defined cocktail of digoxigenin-labelled probes to detect a broad spectrum of HPV mucosal genotypes. All HPV DNA-positive samples were genotyped subsequently with a line probe assay (LiPA_{25}) by reverse hybridization with type-specific probes for mucosal HPV genotypes 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, 74 (22). The LiPA strips were interpreted visually, using the standard reference guide. When samples tested positive using the DEIA, but showed no results on the LiPA strip, the SPF_{10} amplimer was analyzed by direct sequence analysis with the BigDye Terminator cycle sequencing kit (Applied Biosystems, Nieuwerkerk a/d Ijssel, The Netherlands). The sequences were used as a query for screening in the GenBank database (www.ncbi.nlm.nih.gov) with BLAST software (24). HPV genotypes were assigned when a complete match between the 22-bp interprimer region and an HPV sequence in GenBank was found.

Genotypes not available on the LiPA strip and without a complete match in GenBank were considered HPV genotype ‘X’. Low risk HPV (lrHPV) genotypes were defined as genotype 6, 11, 34,
40, 42, 43, 44, 54, 74, 84, 89, 90 and 'X'. High risk HPV genotypes were defined as genotype 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59. HPV genotypes 53, 66, 68, 69, 70 and 73 are nowadays classified as possible/probable high risk HPV (phrHPV) genotypes (25). For analytical purposes, they were defined as hrHPV genotypes in this study. HPV genotypes 3, 12, 20 and 76 were identified by sequencing as cutaneous genotypes. These HPV genotypes were regarded as contamination during sample handling (self-sample) and were not taken into account in the calculation of HPV prevalence rates.

The self-sampling material of all included patients at first examination and of all the patients who underwent a RT, before and after transplantation, was also tested with the clinically validated Cobas 4800 HPV test (Roche) according to the manufacturer’s recommendations in the laboratory of the Department of Medical Microbiology, Radboudumc (20). The Cobas 4800 HPV test provides separate results for HPV 16, 18, and a pool of 12 other hrHPV types (31, 33, 39, 45, 51, 52, 56, 59, 66, and 68) (26).

**QUESTIONNAIRES**

Patients filled out questionnaires on relations/sexual behaviour at start of the study and at every three-monthly self-sampling moment, and they were specifically asked whether there were any changes in their relationship and/or sexual behaviours. The questionnaires are available as Supplemental material.

**STATISTICAL ANALYSIS**

Data of all women who gave informed consent and underwent gynecological examination, including an HPV test, were included in the analyses. The prevalence of HPV infection after transplantation was only assessed in patients who underwent a transplantation within three years after inclusion in the study. In the primary analysis we compared the HPV prevalence before (measurement closest to RT in 6 month period preceding RT) and after (latest measurement in the period between month 3 and month 12 after RT) transplantation in patients with both measurements available. In a secondary analysis we used a scoring system to quantify the degree of HPV positivity in multiple measurements before and after RT; a score of 0 corresponding with 100% negative measurements and a score of 100 with 100% positive measurements. Patients who were included in the study, but did not undergo a RT during the follow-up period were used as a control group. In these patients, HPV prevalence at first examination and the latest measurement in 12 month period were used in the analysis.

A model based on the generalized estimating equations (GEE), with a binomial distribution and identity link was used to analyze the pre- and posttransplant prevalence and the change in prevalence. The mean scores calculated from multiple measurements before were compared with the mean scores after RT by a paired samples t-test. Continuous variables were described by medians (and range) or means (and standard deviation). Discrete variables were described by frequencies and percentages. The genotype of each HPV infection was assessed and described per time point. The results from the questionnaires asking for sexual behaviour were summarized according to the transplantation status of the patient. All data were anonymously stored in an
electronic database. Statistical analysis was performed using IBM SPSS Statistics 20 (Armonk, NY: IBM Corp). Two-sided p-values <0.05 were regarded as statistically significant.

**RESULTS**

During the study period, 351 patients were referred to the outpatient clinic of the Radboudumc to judge whether they were appropriate candidates for RT. During the study period, 65 patients underwent a RT. The remaining 58 patients did not receive a kidney graft for various reasons; 15 patients were found unfit for transplantation and were excluded. See Figure 1 for an overview of the inclusions. In total 13 patients (three underwent RT) were lost to follow up: four patients died (three patients due to cardiac problems and one patient developed a brain tumour), five patients left the study because it was too time consuming or their physical condition was impaired, three patients did not respond to reminders, and one patient due to psychiatric problems. The general patient characteristics, details on their renal disease and treatment, and gynecological characteristics at first contact are summarized in Table 1.

The median follow up after transplantation (last follow up date 12th of October 2015) was 15 months (range 0-29 months). Five patients did not have any measurement after transplantation and one patient did not have any measurements before transplantation. Finally, HPV prevalence before and after transplantation was determined in 59 patients with both the clinically validated HPV test and the highly sensitive HPV test.

As assessed with the highly sensitive HPV test, the overall HPV prevalence increased from 25% (95% CI 14-37%) before transplantation to 39% (95% CI 27-51%) after transplantation using the definition of the primary outcome measure (p= 0.04; Table 2). The hrHPV prevalence increased from 19% (95% CI 9-29%) before transplantation to 31% (95% CI 19- 42%) after transplantation (p=0.045). The hrHPV prevalence before transplantation (median time to transplantation: 30 days (1 to 180 days)) determined with the clinically validated HPV test, was 10% (95% CI 2-18%). With this test, the hrHPV prevalence after transplantation (median time from transplantation: 300 days (120 -360 days)), was 14% (95% CI 5-22%) (p=0.31). Four patients already used immunosuppressive drugs for chronic renal disease at the time of transplantation. Exclusion of these four patients did not change the results. Since there was a variable number of measurements per patient before as well as after transplantation, we used a score system to quantify the degree of HPV positivity in multiple measurements.
**Table 1.** Patient characteristics of female patients with end stage renal disease at first contact at the outpatient clinic

<table>
<thead>
<tr>
<th></th>
<th>Transplanted patients (N=65)</th>
<th>Not transplanted patients (N=43)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (range)</td>
<td>% (n/N)</td>
<td>Median (range)</td>
</tr>
<tr>
<td><strong>General characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.0 (19-73)</td>
<td>54.0 (21-70)</td>
<td>0.54</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Yes</td>
<td>28 (18/65)</td>
<td>23 (10/43)</td>
<td>0.66</td>
</tr>
<tr>
<td>- No</td>
<td>72 (47/65)</td>
<td>74 (32/43)</td>
<td></td>
</tr>
<tr>
<td>- Unknown</td>
<td>0 (0/65)</td>
<td>2 (1/43)</td>
<td></td>
</tr>
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<td><strong>Renal Disease</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Polycystic kidney disease</td>
<td>23 (15/65)</td>
<td>28 (12/43)</td>
<td></td>
</tr>
<tr>
<td>- Chronic interstitial nephritis</td>
<td>6 (4/65)</td>
<td>2 (1/43)</td>
<td></td>
</tr>
<tr>
<td>- Focal segmental glomerulosclerosis</td>
<td>6(4/65)</td>
<td>2 (1/43)</td>
<td></td>
</tr>
<tr>
<td>- Chronic glomerulonephritis</td>
<td>22(14/65)</td>
<td>12 (5/43)</td>
<td></td>
</tr>
<tr>
<td>- Urinary tract anomaly</td>
<td>6 (4/65)</td>
<td>12 (5/43)</td>
<td></td>
</tr>
<tr>
<td>- Haemolytic uremic syndrome</td>
<td>5 (3/65)</td>
<td>2 (1/43)</td>
<td></td>
</tr>
<tr>
<td>- Chronic pyelonephritis</td>
<td>6 (4/65)</td>
<td>0 (0/43)</td>
<td></td>
</tr>
<tr>
<td>- Diabetic nephropathy</td>
<td>3 (2/65)</td>
<td>2 (1/43)</td>
<td></td>
</tr>
<tr>
<td>- Renovascular disease</td>
<td>5 (3/65)</td>
<td>19 (8/43)</td>
<td></td>
</tr>
<tr>
<td>- Other</td>
<td>12 (8/65)</td>
<td>16 (7/43)</td>
<td></td>
</tr>
<tr>
<td>- Unknown</td>
<td>6 (4/65)</td>
<td>5 (2/43)</td>
<td></td>
</tr>
<tr>
<td><strong>Use of iSD in the past</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- No</td>
<td>72 (47/65)</td>
<td>84 (36/43)</td>
<td></td>
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<tr>
<td>- Yes</td>
<td>28 (18/65)</td>
<td>16 (7/43)</td>
<td></td>
</tr>
<tr>
<td><strong>Current use of iSD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- No</td>
<td>92 (60/65)</td>
<td>95 (41/43)</td>
<td></td>
</tr>
<tr>
<td>- Yes</td>
<td>8 (5/65)</td>
<td>5 (2/43)</td>
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<td></td>
<td>Transplanted patients (N=65)</td>
<td>Not transplanted patients (N=43)</td>
<td>p-value</td>
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<tr>
<td>------------------------------</td>
<td>------------------------------</td>
<td>----------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>Median (range)</td>
<td>% (n/N)</td>
<td>Median (range)</td>
</tr>
<tr>
<td>Interval last ISD treatment to first examination (months)</td>
<td>31.0 (0-115)</td>
<td>27.5 (0-76)</td>
<td>0.92</td>
</tr>
<tr>
<td>Previous RT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- No</td>
<td>88 (57/65)</td>
<td>88 (38/43)</td>
<td>0.92</td>
</tr>
<tr>
<td>- Yes</td>
<td>12 (8/65)</td>
<td>12 (5/43)</td>
<td></td>
</tr>
<tr>
<td>Dialysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Haemodialysis</td>
<td>52 (34/65)</td>
<td>42 (18/43)</td>
<td>0.29</td>
</tr>
<tr>
<td>- Peritoneal dialysis</td>
<td>41 (14/34)</td>
<td>33 (6/18)</td>
<td></td>
</tr>
<tr>
<td>- Both</td>
<td>6 (2/34)</td>
<td>6 (1/18)</td>
<td></td>
</tr>
<tr>
<td>Duration of dialysis (months)</td>
<td>12.5 (3-96)</td>
<td>14.0 (1-120)</td>
<td>0.33</td>
</tr>
<tr>
<td>Gynaecological</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical smear</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- WNL</td>
<td>90 (59/65)</td>
<td>91 (39/43)</td>
<td>0.13</td>
</tr>
<tr>
<td>- ASC-US/LSIL</td>
<td>5 (3/65)</td>
<td>2 (1/43)</td>
<td></td>
</tr>
<tr>
<td>- HSIL</td>
<td>5 (3/65)</td>
<td>0 (0/58)</td>
<td></td>
</tr>
<tr>
<td>- No uterus:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>not performed</td>
<td>0 (0/65)</td>
<td>7 (3/43)</td>
<td></td>
</tr>
</tbody>
</table>

ISD = immunosuppressive drugs, RT = renal transplantation, WNL = within normal limits, including normal vaginal cytology in absence of uterus, ASCUS-US/LSIL = atypical squamous cells of undetermined significance/low-grade squamous intraepithelial lesion, HSIL = high-grade squamous intraepithelial lesion
Table 2. HPV prevalence before and after transplantation in 59 female renal transplant recipients

<table>
<thead>
<tr>
<th>Comparison</th>
<th>SPF$<em>{10}$-LiPA$</em>{25}$ system</th>
<th>Cobas 4800</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N % (95% CI)</td>
<td>N % (95% CI)</td>
</tr>
<tr>
<td>Overall (ir + hr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- All</td>
<td>59 25(14-37)</td>
<td>59 10(2-18)</td>
</tr>
<tr>
<td>- No ISD use at time of RT</td>
<td>55 25 (14-37)</td>
<td>55 11(3-19)</td>
</tr>
<tr>
<td>hrHPV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- All</td>
<td>59 19 (9-29)</td>
<td>59 10(2-18)</td>
</tr>
<tr>
<td>- No ISD use at time of RT</td>
<td>55 18 (8-28)</td>
<td>55 11(3-19)</td>
</tr>
</tbody>
</table>

RT = renal transplantation, ir = low risk, hr = high risk, ISD = immunosuppressive drugs, CI = confidence interval

The mean score for overall HPV positivity before transplantation was 25.5 (mean number of measurements before was 2.26 (range 1-9) in a time period of 1 day to 2 years before transplantation) and the mean score after transplantation was 32.9 (mean number of measurements after was 4.14 (range 1-8) in a time period of >1 month to 2 years after transplantation) with a mean difference of 7.4 (95% CI -3.1-17.9; p=0.163). For hrHPV positivity the mean score before transplantation with the sensitive HPV test was 17.9 and after transplantation 22.0 with a mean difference of 4.1 (95% CI -4.6-12.9; p=0.349). With the clinically validated HPV test the mean score was 10 before transplantation and 12.5 after transplantation with a mean difference of 2.5 (95% CI -3.5-8.5) (p=0.41).

In 12/59 patients (20%) the HPV status changed (tested with SPF$_{10}$-LiPA$_{25}$ assay) from negative before transplantation to positive after transplantation. We specified the characteristics of these patients in Table 3. This change in HPV status was not associated with treatment for acute rejection after transplantation. In none of the 12 women who went from HPV negative to HPV positive we found SIL after transplantation. Notably, lrHPV was detected in two of these women and follow-up time was relatively short for development of (pre)malignant lesions. In 4/59 patients (7%) the HPV status changed from positive before RT to negative after RT. Furthermore, a wide range of HPV types were found: hrHPV types 16, 31, 52, 66, 45, 68, 53, 58, 18, 51, 34, 56 and 59 and lrHPV types 6, 11, 40, 42, 89, 61, 84, 54, 74, and 44 and type X. None of these HPV subtypes was predominantly present. As assessed with the highly sensitive HPV test, the overall HPV prevalence in the patients who did not undergo RT (N= 33), was 27% (95% CI 12-42%) at first examination and 33% (95% CI
Table 3. Patient- and test characteristics of newly HPV-positive patients after renal transplantation

<table>
<thead>
<tr>
<th>Age</th>
<th>RD</th>
<th>Previous Tx</th>
<th>Cytology pre-RT</th>
<th>Pre-RT HPV status</th>
<th>Post-RT HPV status</th>
<th>Bethesda ≤6 months pre-RT*</th>
<th>3-12 months post-RT</th>
<th>SPF</th>
<th>Cobas</th>
<th>SPF</th>
<th>SPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53 Other</td>
<td>WNL</td>
<td>neg</td>
<td>neg</td>
<td>pos X</td>
<td>neg</td>
<td></td>
<td>SPF</td>
<td>Cobas</td>
<td>SPF</td>
<td>SPF</td>
</tr>
<tr>
<td>2</td>
<td>51 Diabetic nephropathy</td>
<td>1x 2010</td>
<td>WNL</td>
<td>neg</td>
<td>neg</td>
<td>pos 45</td>
<td>neg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>28 Urinary tract anomaly</td>
<td>WNL</td>
<td>neg</td>
<td>neg</td>
<td>pos X</td>
<td>neg</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>4</td>
<td>40 Polycystic kidney disease</td>
<td>WNL</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>pos 68</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>5</td>
<td>58 Polycystic kidney disease</td>
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<td>neg</td>
<td>neg</td>
<td>pos 68</td>
<td>x</td>
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<td></td>
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<tr>
<td>6</td>
<td>57 Polycystic kidney disease</td>
<td>WNL</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>7</td>
<td>47 FSGS</td>
<td>WNL</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>57 Chronic glomerulonephritis</td>
<td>WNL</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>48 Other</td>
<td>WNL</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10*</td>
<td>52 Chronic glomerulonephritis</td>
<td>WNL</td>
<td>neg</td>
<td>pos</td>
<td>pos 16/59</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>34 Other</td>
<td>1x 1997</td>
<td>WNL</td>
<td>neg</td>
<td>pos 51</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>61 Chronic pyelonephritis</td>
<td>NA</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td></td>
<td></td>
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</tbody>
</table>

* Only patient who used Basiliximab, # = measurements used in primary analysis, bold results = last measurement in period 3-12 months after RT and used in primary analysis, RD = renal disease, FSGS = focal segmental glomerulosclerosis, Tx = transplantation, RT = renal transplantation, Bethesda = Bethesda system 2001 was used for cytological classification, SPF = SPF10-LiPA® system assay, Cobas = Cobas 4800 assay, WNL = within normal limits, NA = not available, x = self-sample not available
### Table 3.

<p>| Patient- and test characteristics of newly HPV-positive patients after renal transplantation |
| --- | --- | --- | --- | --- | --- | --- | --- |</p>
<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Previous tx</th>
<th>Cytology pre-rt</th>
<th>Pre-rt HPV status</th>
<th>Change sexual behaviour</th>
<th>≥ 3-12 months post-RT</th>
<th>Bethesda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beth</td>
<td>SPF</td>
<td>Cobas SPF</td>
<td>SPF</td>
<td>SPF</td>
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<tr>
<td>≤6 months pre-rt#</td>
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<td>3-12 months post-rt</td>
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<tr>
<td>≥3-12 months post-RT</td>
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<td>1</td>
<td>53</td>
<td>Other</td>
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<td>neg</td>
<td>pos</td>
<td>X</td>
</tr>
<tr>
<td>2</td>
<td>51</td>
<td>Diabetic nephropathy</td>
<td>WNL</td>
<td>neg</td>
<td>pos</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>Urinary tract anomaly</td>
<td>WNL</td>
<td>neg</td>
<td>pos</td>
<td>X</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>Polycystic kidney disease</td>
<td>WNL</td>
<td>neg</td>
<td>neg</td>
<td>pos</td>
</tr>
<tr>
<td>5</td>
<td>58</td>
<td>Polycystic kidney disease</td>
<td>NA</td>
<td>neg</td>
<td>neg</td>
<td>pos</td>
</tr>
<tr>
<td>6</td>
<td>57</td>
<td>Polycystic kidney disease</td>
<td>WNL</td>
<td>neg</td>
<td>neg</td>
<td>pos</td>
</tr>
<tr>
<td>7</td>
<td>47</td>
<td>FSGS</td>
<td>WNL</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>8</td>
<td>57</td>
<td>Chronic glomerulonephritis</td>
<td>WNL</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>9</td>
<td>48</td>
<td>Other</td>
<td>WNL</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>10*</td>
<td>52</td>
<td>Chronic glomerulonephritis</td>
<td>WNL</td>
<td>neg</td>
<td>pos</td>
<td>16/59</td>
</tr>
<tr>
<td>11</td>
<td>34</td>
<td>Other</td>
<td>1x 1997</td>
<td>WNL</td>
<td>neg</td>
<td>pos</td>
</tr>
<tr>
<td>12</td>
<td>61</td>
<td>Chronic pyelonephritis</td>
<td>NA</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
</tbody>
</table>

* Only patient who used Basiliximab, # = measurements used in primary analysis, bold results = last measurement in period 3-12 months after RT and used in primary analysis, RD = renal disease, FSGS = focal segmental glomerulosclerosis, Tx = transplantation, RT = renal transplantation, Bethesda = Bethesda system 2001 was used for cytological classification, SPF = SPF10-LiPA25 system assay, Cobas = Cobas 4800 assay, WNL = within normal limits, NA = not available, x = self-sample not available.
17-49%) approximately 12 months later (median time 360 days (range 270-360 days)) \( (p=0.41) \). The hrHPV prevalence in these patients was 21% (95% CI 7-35%) at first examination and 27% (95% CI 12-42%) approximately 12 months later \( (p=0.41) \). The hrHPV prevalence determined with the clinically validated HPV test remained stable over this period and was 16% (95% CI 3-29%).

During the study period eight recipients of a transplant kidney had abnormal cytology: three patients with ASC-US/LSIL and five patients with HSIL. Six of these patients had abnormal smears before transplantation and two patients developed cytological abnormalities after transplantation (2 HSIL (one preceded by ASC-US/LSIL)). All patients with HSIL tested HPV positive with the highly analytical sensitive HPV test as well as with the clinically validated test. They all underwent colposcopy with tissue sampling for histology. One patient was diagnosed with cervical intraepithelial neoplasia (CIN) 1, one patient with CIN2, one patient with CIN3 and one patient ultimately developed a stage IBI cervical cancer. The patient who developed the cancer underwent her second RT during the study period. Both patients who developed HSIL after transplantation were hrHPV positive when the cytological abnormality was diagnosed (type 16 and type 18). None of the patients developed vulvar abnormalities during the follow-up period.

At their first visit, patients received a questionnaire about their relationships and sexual behaviour. The answers on this questionnaire are summarized in Table 4. Every 3 months the patients were asked whether their relationships and/or sexual behaviour had changed. Of the 65 patients in whom a transplantation was performed, 11 patients (16.9%) reported a change in sexual behaviour after transplantation: eight became more sexually active while three patients reduced their sexual activities. Only one of the eight female RTRs who reported an increase in sexual activities converted from HPV negative to HPV positive after transplantation.
Table 4. History of- and current sexual behaviour of patients with end stage renal disease at first contact

<table>
<thead>
<tr>
<th></th>
<th>Transplanted patients N=65</th>
<th>Not transplanted patients N=43</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median (range) % (n)</strong></td>
<td><strong>Median (range) % (n)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
<td>0.43</td>
</tr>
<tr>
<td>- Married</td>
<td>69 (45)</td>
<td>63 (27)</td>
<td></td>
</tr>
<tr>
<td>- Living together</td>
<td>12 (8)</td>
<td>16 (7)</td>
<td></td>
</tr>
<tr>
<td>- Relationship</td>
<td>3 (2)</td>
<td>5 (2)</td>
<td></td>
</tr>
<tr>
<td>- Divorced</td>
<td>2 (1)</td>
<td>5 (2)</td>
<td></td>
</tr>
<tr>
<td>- Widow</td>
<td>0 (0)</td>
<td>5 (2)</td>
<td></td>
</tr>
<tr>
<td>- Single</td>
<td>14 (9)</td>
<td>7 (3)</td>
<td></td>
</tr>
<tr>
<td><strong>Age first intercourse (years)</strong></td>
<td>17 (13-24)</td>
<td>18 (14-26)</td>
<td>0.64</td>
</tr>
<tr>
<td><strong>Sexually active &lt;16 years</strong></td>
<td></td>
<td></td>
<td>0.72</td>
</tr>
<tr>
<td>- Yes</td>
<td>19 (12)</td>
<td>16 (7)</td>
<td></td>
</tr>
<tr>
<td>- No</td>
<td>63 (41)</td>
<td>67 (29)</td>
<td></td>
</tr>
<tr>
<td>- Unknown</td>
<td>19 (12)</td>
<td>16 (7)</td>
<td></td>
</tr>
<tr>
<td><strong>Lifetime sex partners</strong></td>
<td>3 (1-14)</td>
<td>3 (0-55)</td>
<td>0.41</td>
</tr>
<tr>
<td><strong>Gender sex partner</strong></td>
<td></td>
<td></td>
<td>0.47</td>
</tr>
<tr>
<td>- Men</td>
<td>77 (50)</td>
<td>77 (33)</td>
<td></td>
</tr>
<tr>
<td>- Women</td>
<td>3 (2)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>- Both</td>
<td>3 (2)</td>
<td>5 (2)</td>
<td></td>
</tr>
<tr>
<td>- Unknown</td>
<td>17 (11)</td>
<td>19 (8)</td>
<td></td>
</tr>
<tr>
<td><strong>Condom use</strong></td>
<td></td>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td>- No</td>
<td>69 (45)</td>
<td>72 (31)</td>
<td></td>
</tr>
<tr>
<td>- Yes</td>
<td>14 (9)</td>
<td>19 (8)</td>
<td></td>
</tr>
<tr>
<td>- Unknown</td>
<td>17 (11)</td>
<td>9 (4)</td>
<td></td>
</tr>
<tr>
<td><strong>Ever diagnosed with STD</strong></td>
<td></td>
<td></td>
<td>0.68</td>
</tr>
<tr>
<td>- Yes</td>
<td>8 (5)</td>
<td>5 (2)</td>
<td></td>
</tr>
<tr>
<td>- No</td>
<td>75 (49)</td>
<td>77 (33)</td>
<td></td>
</tr>
<tr>
<td>- Unknown</td>
<td>17 (11)</td>
<td>19 (8)</td>
<td></td>
</tr>
<tr>
<td><strong>Sex partners last 6 months</strong></td>
<td>1 (0-1)</td>
<td>1 (0-10)</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>Sexual contact last 6 months (per month)</strong></td>
<td>1 (0-12)</td>
<td>1 (0-20)</td>
<td>0.41</td>
</tr>
</tbody>
</table>

STD = sexually transmitted disease
DISCUSSION

This study is the first longitudinal investigation of the cervico-vaginal hrHPV prevalence in RTRs before and after RT. Although the study population was limited, we made a number of interesting observations. First, the pre-transplant hrHPV prevalence of 10% (measured with the clinically validated Cobas 4800 HPV test) in our cohort was higher than the age-specific hrHPV prevalence in the general population. After transplantation, the hrHPV prevalence was 14%. When an analytically more sensitive HPV test was used, the hrHPV prevalence increased significantly from 19% before to 31% after transplantation without changes in sexual behaviour. Apparently there was reactivation of latent HPV infections after RT.

The Cobas 4800 HPV test will be used in the coming Dutch national screening program. In our study all women with clinically relevant cytological abnormalities tested positive with the Cobas 4800 HPV test. However no significant increase in hrHPV prevalence was found with this test during the first year after transplantation. It is possible that the hrHPV infections due to increased replication after transplantation is likely to result in an increased detection rate with the Cobas 4800 HPV test after a longer follow-up. On the other hand, using the SPF10-LiPA25 assay, which has a higher analytical sensitivity, we found a significant increase in the prevalence of hrHPV (from 19% to 31%). The prevalence of hrHPV found with this assay after RT is comparable with earlier findings of our study group with the same test in a cohort of 218 female RTRs at different time intervals after transplantation, where a prevalence of 27.1% was observed (17). The increase of hrHPV prevalence after transplantation was not associated with changes in relationships or sexual behaviour. Moreover, the hrHPV prevalence did not change significantly in the control group of patients who did not undergo a RT. Therefore, it is likely that latent HPV infections were reactivated because of immunosuppressive therapy.

Latent viral infections are characterized by a state of reversible non-productive infection of individual cells (27). Although, the existence of HPV latency is still under debate; epidemiological studies convincingly showed that in women who cleared a specific HPV type (tested HPV negative), a recurrent detection of the same genotype can occur without report of (new) sexual contacts (28-31). Several studies suggested a role for host immunity in reactivating HPV infections in patients with HIV and in patients who use immunosuppressive drugs (32-35). The clinical implications of a reactivation as compared to a recently acquired HPV infection are not clear. However, the increased risk of high grade neoplasia among HIV-infected and organ transplant recipients, suggests that an HPV reactivation carries similar or increased risk of disease development as compared to a de novo infection (36). The clinical relevance of our findings is supported by several studies that show an increase in cervical- and vulvar cancer rate in RTRs 10 years after the transplantation (14).

A remarkable finding was the high overall HPV prevalence in patients with ESRD, irrespective of receiving a transplant kidney or not. We had anticipated an overall HPV prevalence of around 10%, according to the overall HPV prevalence in women around 50 years of age (based on HPV testing in
140,000 women with different HPV assays) in the general population (37, 38). A similar observation with an high overall HPV prevalence was made by Fairley et al. (39), who showed an overall HPV prevalence of 20% in women on maintenance dialysis and of 22% in renal transplant recipients compared to 4.5% in women with moderately impaired renal function (serum creatinine 150-390 µmol/L). This may be explained by the fact that cellular immunity is frequently impaired in patients with severe renal failure (40). Other known risk factors for obtaining an HPV infection, such as smoking (41-44) and promiscuous sexual behaviour (8), were not more prevalent in our patients as compared to the general population. In accordance with the higher prevalence of hrHPV, it has been shown that dialysis patients have an increased risk of cancer, including cervical- and vulvovaginal cancer (45).

In our study approximately 9% of the patients, who underwent transplantation, had cytological abnormalities at first examination. The incidence of cytological abnormalities in the general population is 7%, but in women aged 50-59 years, which is comparable to the median age of our study population, it is only about 3.5% (46). At first examination approximately 50% of the patients with cytological abnormalities were on dialysis. This could be an explanation, but the number of patients was too low to assess dialysis as a risk factor. There is no clear explanation for the relatively high percentage of cytological abnormalities in RT patients compared to the group of patients, who did not undergo RT.

The results of our study suggest that the start of intensive therapy with immunosuppressive drugs increases the HPV prevalence after transplantation. The higher prevalence during immunosuppression can be explained by several non mutually exclusive factors, such as a higher susceptibility to become infected with HPV, a higher risk on persistence of the HPV infection or reactivation of a latent HPV infection. In a small study on HPV subtypes (6, 11, 16 and 18) in female RTRs (16), hrHPV 16 and 18 were found at a higher rate in transplant recipients (55%) compared with their immunocompetent counterparts (15%), placing these patients at increased risk for aggressive anogenital tract (pre)malignant lesions (16). Notably, at first examination in our study no difference in HPV prevalence was observed between the patients with current or prior use of immunosuppressive drugs compared to those who did never use immunosuppressive drugs. Furthermore, Meeuwis et al. (17) showed that the duration of immunosuppressive treatment and the experience of an acute rejection with subsequent intensifying of immunosuppressive therapy were not related to the risk of HPV positivity. The increased hrHPV prevalence in female patients with ESRD raises the question whether screening for HPV in this patient group would be useful.

Prophylactic vaccines against hrHPV genotypes 16 and 18 are highly effective for the prevention of persistent infections and anogenital cancer. However, recent data showed suboptimal immunogenicity of the quadrivalent HPV vaccine in patients who were treated with immunosuppressive drugs after organ transplantation (47). Vaccination in patients with renal failure prior to transplantation could be more successful. However, a barrier for implementing HPV vaccination in these women is the wide variety in HPV types found in patients with ESRD. Our study
showed 24 different HPV types, which is in accordance with a recent cohort study on HPV infections in female RTRs (17). Recently, a nonavalent HPV vaccine has been developed to protect against an additional five oncogenic types (31, 33, 45, 52, and 58 next to 6, 11, 16, and 18) and showed an overall vaccine efficacy of 96.7% in women aged 16-29 years in the general population (48). Notably, approximately 60% of the HPV positive patients were infected with genotypes not covered by the nonavalent vaccine. The protective effect of this vaccine in patients with ESRD, especially in sexually active patients, has yet to be demonstrated in prospective studies.

Nowadays, several European and American guidelines (49-51) recommend intensified cervical cancer screening in female RTRs (14, 16, 52, 53). High-risk HPV testing will take an important role in the new cervical screening program in the Netherlands, which will be implemented in 2017. If women test hrHPV negative, no cytological evaluation is necessary and they will receive another invitation in five years. In case of hrHPV positivity, there will be cytological evaluation. Cervical cytology will be repeated after six months in case of normal cytology and women will be referred to the gynaecologist in case of abnormal cytology. If the cytology is normal after six months, women return to the regular program (interval of five years). A five year screening interval in hrHPV positive RTRs seems relatively long in comparison to existing guidelines. The results of our study emphasize the importance of hrHPV testing in the follow up of patients who underwent a RT. However, the precise design of gynecological screening in female RTRs needs to be further discussed.

Our study is the first to prospectively assess HPV prevalence before and after transplantation, which has given us more information on the natural behaviour of HPV in RTRs. We used a highly sensitive test to detect HPV DNA in cervico-vaginal samples. We did not assess the HPV serology before transplantation because the use of HPV serology to detect naturally acquired HPV infections is not widely accepted. The presence of serum antibodies indicates past infection, but estimates of proportions of women who seroconvert after infection range from 55- to 85%, depending on study design and methods employed. Antibody formation in response to natural infection is a slow process that could extend to over a year (54-56). Consequently, the pre-transplant serology does not allow reliable interpretation of type-specific acquisition of HPV after transplantation. A limitation of our study is the relatively short follow up. Longer follow-up of the hrHPV positive patients is necessary to determine if reactivation of latent hrHPV infections leads to dysplasia or cancer.

**CONCLUSION**

In this cohort of patients with ESRD, the hrHPV prevalence was higher than the age-specific hrHPV prevalence in the general population. An increase in hrHPV prevalence after transplantation, without any change in sexual behaviour, suggests reactivation of latent HPV infections during immunosuppression. This could contribute to the increased risk of HPV related cervical- and vulvar (pre)malignancies in female RTRs.
Reactivation of latent HPV infections after renal transplantation

REFERENCE LIST

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33. Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared


## SUPPLEMENTAL MATERIAL

**Supplement 1.** Additional information to Table 2; HPV prevalence over time in female patients with end-stage renal disease who did not undergo renal transplantation

<table>
<thead>
<tr>
<th>Assay</th>
<th>HPV prevalence at first examination</th>
<th>HPV prevalence within 12 months</th>
<th>Difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>% (95% CI)</td>
<td>% (95% CI)</td>
<td>% (95% CI)</td>
</tr>
<tr>
<td><strong>SPF10-LiPA25 system</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall (lr + hr)</td>
<td>33</td>
<td>27 (12-42)</td>
<td>33 (17-49)</td>
<td>6 (-8-20)</td>
</tr>
<tr>
<td>hrHPV</td>
<td>33</td>
<td>21 (7-35)</td>
<td>27 (12-42)</td>
<td>6 (-8-20)</td>
</tr>
<tr>
<td><strong>Cobas 4800</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>31*</td>
<td>16 (3-29)</td>
<td>16 (3-29)</td>
<td>0 (-13-13)</td>
</tr>
</tbody>
</table>

*lr = low risk, hr = high risk, CI = confidence interval, * in two patients no test results were available
QUESTIONNAIRE

“HPV INFECTIONS BEFORE AND AFTER RENAL TRANSPLANTATION”

Questionnaire study “HPV infections before and after renal transplantation”

Dear Madam,

We appreciate your participation in the study “HPV infections before and after renal Transplantation”. We would like to ask you kindly to fill out this questionnaire.

In the square below an explanation on filling out the questionnaire is given:

- The questionnaire contains two sections: one with questions on quality of life and one about sexual relations.
- We advise you to answer these personal questions in a personal space.
- For the sake of the study outcome it is mandatory that you fill out all the questions and always answer them truthfully.
- Would you please check or circle one answer per question unless indicated otherwise.
- It takes about 10 minutes to fill out the questionnaire.
- Only the researchers mentioned in the information folder have access to your personal information.

If you completely filled out the questionnaire, would you sent it to the researcher in the attached return envelope? No stamp is necessary.

Thank you for your cooperation!

Your unique code number:

In case of any questions, you can contact the researcher through the following email address: onderzoek@obgyn.umcn.nl
### Section 1: Questions on quality of life (SF-36)

In this questionnaire we will be asking about your health. Please answer each question by checking the box or circling the number. When you are not sure what to answer, try to choose the answer most applicable.

**In general, would you say your health is:**

- Excellent
- Very good
- Good
- Fair
- Poor

**Compared to one year ago, how would you rate your health in general now?**

- Much better now than one year ago
- Somewhat better now than one year ago
- About the same
- Somewhat worse now than one year ago
- Much worse now than one year ago

The following items are about activities you might do during a typical day.

**Does your health now limit you in these activities? If so, how much? (Please circle the number)**

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes, limited a lot</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, limited a little</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No, not limited at all</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Daily activities**

a. **Vigorous activities**
   - Such as running, lifting heavy objects, participating in strenuous sports
   - 0 1 2

b. **Moderate activities**
   - Such as moving a table, pushing a vacuum cleaner, bowling, or playing golf
   - 0 1 2

c. Lifting or carrying groceries
   - 0 1 2

d. Climbing **several** flights of stairs
   - 0 1 2

e. Climbing **one** flight of stairs
   - 0 1 2
During the past 4 weeks, have you had any of the following problems with your work or other regular activities as a result of your physical health?

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

a. **Cut down the amount of time** you spent on work or other activities 0 1
b. **Accomplished less** than you would like 0 1
c. Were limited in the **kind** of work or other activities 0 1
d. **Had difficulty** performing the work or other activities (for example, it took extra effort) 0 1

During the past 4 weeks, have you had any of the following problems with your work or regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

a. **Cut down the amount of time** you spent on work or other activities 0 1
b. **Accomplished less** than you would like 0 1
c. Didn’t do work or other activities as **carefully** as usual 0 1
During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

- Not at all
- Slightly
- Moderately
- Quite a bit
- Extremely

How much bodily pain have you had during the past 4 weeks?

- None
- Very mild
- Mild
- Moderate
- Severe
- Very severe

During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

- Not at all
- A little bit
- Moderately
- Quite a bit
- Extremely

These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>All of the time</td>
<td>Most of the time</td>
<td>A good bit of the time</td>
<td>Some of the time</td>
<td>A little of the time</td>
<td>None of the time</td>
</tr>
</tbody>
</table>

How much of the time during the past 4 weeks...

a. Did you feel full of pep?
   - 0
   - 1
   - 2
   - 3
   - 4
   - 5

b. Have you been a very nervous person?
   - 0
   - 1
   - 2
   - 3
   - 4
   - 5

c. Have you felt so down in the dumps that
   - 0
   - 1
   - 2
   - 3
   - 4
   - 5
nothing could cheer you up?

d. Have you felt calm and peaceful? 0 1 2 3 4 5
e. Did you have a lot of energy? 0 1 2 3 4 5
f. Have you felt downhearted and blue? 0 1 2 3 4 5
g. Did you feel worn out? 0 1 2 3 4 5
h. Have you been a happy person? 0 1 2 3 4 5
i. Did you feel tired? 0 1 2 3 4 5

During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?

- All of the time
- Most of the time
- Some of the time
- A little of the time
- None of the time

How TRUE or FALSE is each of the following statements for you.

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. I seem to get sick a little easier than other people 0 1 2 3 4
b. I am as healthy as anybody I know 0 1 2 3 4
c. I expect my health to get worse 0 1 2 3 4
d. My health is excellent 0 1 2 3 4
Section 2: Questions on sexual relations

We know that there is a causal connection between sexual relations and the prevalence of HPV infections. The information on sexual relations is mandatory for our study to differentiate between use of immunosuppressant drugs and sexual relations as a cause for obtained an HPV infection.

Definitions:
In all the below mentioned questions 'sexual contact' means:

1. **Genital contact**: contact between the genitals of 2 persons, without the penis entering the vagina or anus.
2. **Oral sex**: contact between mouth and genital.
3. **Vaginal sex**: when the penis enters the vagina.
4. **Anal sex**: when the penis enters the.

How old were you when you had your first sexual contact with another person?

………………years old
I never had sexual contact with another person (go to question 24)

How old was the person you had sexual contact with?

…………… years old

Were you the first sexual partner of your first sexual partner?

Yes
No
I don’t know

With how many different persons did you had sexual contact before your 16th birthday (including all steady partners and flings)?

Number of sexual partners: …………………
I did not have sexual contact before my 16th birthday.

With how many different persons did you had sexual contact in your life (including all steady partners and flings)?

Total number of sexual partners: …………………
Was the sexual contact only with men, women, or both?

Man/men
Woman/women
Both

In the past 6 months, with how many different persons did you have sexual contact? (including all steady partners and flings)

Number of sexual partners

In the past 6 months, how often did you have sexual contact? (Including all steady partners and flings)?

...........times a day
...........times a week
...........times a month

How often did you use condoms during sexual contact?

Never
Sometimes (less than 50%)
Often (more than 50%)
Always

Were you ever diagnosed with a sexually transmitted disease (STD) by a physician?

Yes, once
Yes, multiple times
No, go to question 24

Which STD were you diagnosed with?

Genital warts
Chlamydia
Gonorrhoea
Genital herpes
Syphilis
HIV infection (the virus that causes AIDS)
Other, namely:........................................................................................................
How long ago were you diagnosed with the STD? (in case of multiple STDs please mention the last one)

.........weeks
.........months
.........years

Which date was the first day of your last menstruation?

___ ___ - ___ ___ - ___ ___ (day – month - year)
I am not menstruating anymore, because..........................................................................................

In the past 6 weeks, did you experience any of the following complaints? (multiple answers are possible)

Blood loss between 2 menstruations
Blood loss during or after vaginal sex
More vaginal discharge than usual
Pain or burning sensation during micturation
Peeing more often than usual
Pain in your lower abdomen
None of the above mentioned complaints

This is the end of the questionnaire.

Would you please check if you filled out all of the questions?

You can return the questionnaire to us without costs in the attached return envelope. No stamp is necessary.

Thank you very much!
Dear Madam,

We appreciate your participation in the study “HPV infections before and after renal Transplantation”. We would like to ask you kindly to fill out this questionnaire.

**In the square below an explanation on filling out the questionnaire is given:**

- The questionnaire contains two sections: one with questions on quality of life and one about sexual relations.
- We advise you to answer these personal questions in a personal space.
- For the sake of the study outcome it is mandatory that you fill out all the questions and always answer them truthfully.
- Would you please check or circle one answer per question unless indicated otherwise.
- It takes about 5 minutes to fill out the questionnaire.
- Only the researchers mentioned in the information folder have access to your personal information.

If you completely filled out the questionnaire, would you sent it to the researcher in the attached return envelope? No stamp is necessary.

**Thank you for your cooperation!**

**Your unique code number:**

In case of any questions, you can contact the researcher through the following email address: onderzoek@obgyn.umcn.nl
Change in (sexual) relations

Did anything change in your relationship/sexual relations compared to the last time you filled out a questionnaire?

☐ No, you don’t need to answer any more questions
☐ Yes

In the past 3 months, with how many different persons did you have sexual contact? (Including all steady partners and flings)

Number of sexual partners……………………….

In the past 3 months, how often did you have sexual contact? (Including all steady partners and flings)?

........times a day
........times a week
........times a month

How often did you use condoms during sexual contact?

Never
Sometimes (less than 50%)
Often (more than 50%)
Always
Here you can write down any changes that were not mentioned in the previous questions.

This is the end of the questionnaire.

Would you please check if you filled out all of the questions?

You can return the questionnaire to us without costs in the attached return envelope. No stamp is necessary.

Thank you very much!
Questionnaire study “HPV infections before and after renal transplantation”

Dear Madam,

We appreciate your participation in the study “HPV infections before and after renal Transplantation”. We would like to ask you kindly to fill out this questionnaire.

In the square below an explanation on filling out the questionnaire is given:

- The questionnaire contains two sections: one with questions on quality of life and one about sexual relations.
- We advise you to answer these personal questions in a personal space.
- For the sake of the study outcome it is mandatory that you fill out all the questions and always answer them truthfully.
- Would you please check or circle one answer per question unless indicated otherwise.
- It takes about 10 minutes to fill out the questionnaire.
- Only the researchers mentioned in the information folder have access to your personal information.

If you completely filled out the questionnaire, would you sent it to the researcher in the attached return envelope? No stamp is necessary.

Thank you for your cooperation!

Your unique code number:

In case of any questions, you can contact the researcher through the following email address:
onderzoek@obgyn.umcn.nl
Section 1: Questions on quality of life (SF-36)

In this questionnaire we will be asking about your health. Please answer each question by checking the box or circling the number. When you are not sure what to answer, try to choose the answer most applicable.

In general, would you say your health is:

- [ ] Excellent
- [ ] Very good
- [ ] Good
- [ ] Fair
- [ ] Poor

Compared to one year ago, how would you rate your health in general now?

- [ ] Much better now than one year ago
- [ ] Somewhat better now than one year ago
- [ ] About the same
- [ ] Somewhat worse now than one year ago
- [ ] Much worse now than one year ago

The following items are about activities you might do during a typical day.

Does your health now limit you in these activities? If so, how much? (Please circle the number)

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes, limited a lot</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, limited a little</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No, not limited at all</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Daily activities

k. **Vigorous activities**
   Such as running, lifting heavy objects, participating in strenuous sports

l. **Moderate activities**
   Such as moving a table, pushing a vacuum cleaner, bowling, or playing golf

m. Lifting or carrying groceries

n. Climbing several flights of stairs

o. Climbing one flight of stairs
During the past 4 weeks, have you had any of the following problems with your work or other regular activities as a result of your physical health?

- p. Bending, kneeling, or stooping
  - Yes
  - No

- q. Walking more than a mile
  - Yes
  - No

- r. Walking several blocks
  - Yes
  - No

- s. Walking one block
  - Yes
  - No

- t. Bathing or dressing yourself
  - Yes
  - No

During the past 4 weeks, have you had any of the following problems with your work or regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?:

- d. Cut down the amount of time you spent on work or other activities
  - Yes
  - No

- e. Accomplished less than you would like
  - Yes
  - No

- f. Didn’t do work or other activities as carefully as usual
  - Yes
  - No
During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

- Not at all
- Slightly
- Moderately
- Quite a bit
- Extremely

How much bodily pain have you had during the past 4 weeks?

- None
- Very mild
- Mild
- Moderate
- Severe
- Very severe

During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

- Not at all
- A little bit
- Moderately
- Quite a bit
- Extremely

These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All of the time</td>
<td>Most of the time</td>
<td>A good bit of the time</td>
<td>Some of the time</td>
<td>A little of the time</td>
<td>None of the time</td>
</tr>
</tbody>
</table>

How much of the time during the **past 4 weeks**...

- Did you feel full of pep? 0 1 2 3 4 5
- Have you been a very nervous person? 0 1 2 3 4 5
- Have you felt so down in the dumps that 0 1 2 3 4 5
nothing could cheer you up?

m. Have you felt calm and peaceful?  0 1 2 3 4 5
n. Did you have a lot of energy?  0 1 2 3 4 5
o. Have you felt downhearted and blue?  0 1 2 3 4 5
p. Did you feel worn out?  0 1 2 3 4 5
q. Have you been a happy person?  0 1 2 3 4 5
r. Did you feel tired?  0 1 2 3 4 5

During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?

- All of the time
- Most of the time
- Some of the time
- A little of the time
- None of the time

How TRUE or FALSE is each of the following statements for you.

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Definitely true</strong></td>
<td>Mostly true</td>
<td>Don’t know</td>
<td>Mostly false</td>
<td>Definitely false</td>
<td></td>
</tr>
<tr>
<td>e. I seem to get sick a little easier than other people</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>f. I am as healthy as anybody I know</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>g. I expect my health to get worse</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>h. My health is excellent</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>
Section 2: Change in (sexual) relations

We know that there is a causal connection between sexual relations and the prevalence of HPV infections. The information on sexual relations is mandatory for our study to differentiate between use of immunosuppressant drugs and sexual relations as a cause for obtained an HPV infection.

Did anything change in your relationship/sexual relations compared to the last time you filled out a questionnaire?

☐ No, you don’t need to answer any more questions
☐ Yes

In the past 3 months, with how many different persons did you have sexual contact? (including all steady partners and flings)

Number of sexual partners............................

In the past 3 months, how often did you have sexual contact? (including all steady partners and flings)?

........times a day
........times a week
........times a month

How often did you use condoms during sexual contact?

Never
Sometimes (less than 50%)
Often (more than 50%)
Always
Here you can write down any changes that were not mentioned in the previous questions.


This is the end of the questionnaire.

Would you please check if you filled out all of the questions?

You can return the questionnaire to us without costs in the attached return envelope. No stamp is necessary.

Thank you very much!
BARRIERS AND FACILITATORS IN REGULAR GYNAECOLOGICAL SCREENING OF FEMALE RENAL TRANSPLANT RECIPIENTS

Floor Hinten,
Rosella Hermens,
Kim A.P. Meeuwis,
Michelle van der Linden,
Ruud L.M. Bekkers,
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Willem J.G. Melchers,
Luuk B. Hilbrands,
Joanne A. de Hullu

Submitted
ABSTRACT

Background: Renal transplant recipients (RTRs) have an increased risk of Human Papillomavirus (HPV) related anogenital (pre)malignancies. Several guidelines recommend to perform annual cervical cancer screening in female RTRs, but the participation rate is low. The aim of this study is to identify barriers and facilitators for annual gynaecological screening in female RTRs from a patient and professional perspective and to present suggestions to increase the participation rate.

Methods: A qualitative study was performed with Dutch female RTRs transplanted at the Radboud university medical center and nephrologists specialized in renal transplantation care using explorative semi-structured interviews in focus groups.

Results: In total, 14 female RTRs and 13 nephrologists participated. Both nephrologists and female RTRs mentioned similar barriers: uncomfortable and less reliable examination by a general practitioner (GP) compared to examination by a gynaecologist, limited knowledge of professionals, and limited information supply to patients on increased risk for developing HPV related genital (pre)malignancies. RTRs focused more on unpleasantness of the examination itself. RTRs and nephrologists reported the same facilitators: a reminder, a checklist, integration of gynaecological examination in annual check-up, self-sampling and information supply at the right moment.

Conclusions: Female RTRs should receive a checklist one year after transplantation mentioning all necessary future examinations. Furthermore, introducing HPV self-sampling in the annual check-up by the nephrologist might obviate barriers and meet the main facilitators. Implementing these changes might improve the participation rate of female RTRs in annual gynaecological screening.
INTRODUCTION

Each year, about 78,000 renal transplantations (RTs) are performed worldwide (1), of which more than 1,000 are performed in the Netherlands (2), requiring the lifelong administration of immunosuppressive therapy. The current immunosuppressive regimes have led to a 1-year patient and graft survival of more than 90%. Therefore, the long-term side-effects of immunosuppressive medication need more attention. It is generally accepted that renal transplant recipients (RTRs) have an increased risk of developing Human Papillomavirus (HPV) related anogenital (pre)malignancies. The prevalence of cervical HPV infections in female RTRs varies between 22 and 63% (3-9), which is higher compared to the general female population (10). In general, HPV is the main factor in the oncogenesis of cervical (pre)malignancies. In addition, HPV is responsible for about 30% of vulvar malignancies and for nearly all premalignant usual vulvar intraepithelial neoplasia lesions. In female RTRs the contribution of HPV is even more important, as nearly 100% of the cervical- and vulvar (pre)malignancies are HPV-related.

It is generally accepted that RTRs may not only have a higher risk for developing an HPV infection, but the HPV infections and/or premalignancies may also progress into malignant disease within a shorter interval (11, 12). Madeleine et al. (13) showed that time from transplantation to anogenital (pre)malignancies ranged from 2.6 to 5.3 years. Moreover, female RTRs are more difficult to treat optimally for genital (pre)malignancies (surgery more complicated; radiotherapy contraindicated) because of the renal transplant in the pelvis: there is a need for early detection of genital (pre)malignancies, which can be established by improving gynaecological screening. Several European and American guidelines (14-16) recommend to perform intensified cervical cancer screening in female RTRs (4, 8, 17, 18). Without specific screening of the vulvar area. Gynaecological screening should be defined as inspection of the vulvar- and perianal area and cervical smear. The definition of ‘intensified’ is unclear, because no consensus on the execution of cervical screening for the general population in various countries. There are differences in terms of invitation methodology, target population (start between 18-30 years until 59-70 years), screening intervals (once a year until once every 5 years), and organization.

Besides the uncertainty about the appropriate screening interval for RTRs, the participation rate of female RTRs in gynaecological screening is too low. Courtney et al. (19) showed that the uptake of the advised annual cervical cancer screening in the RTR population of Northern Ireland is low: only 10% of patients follow the advice and 32% never had a cervical smear performed at all. Meeuwis et al. (17) showed that only 38% of Dutch RTRs transplanted between 1991 and 1995 underwent cervical screening once every 5 years, and 37% never had cervical screening after their transplantation at all.

Currently, the exact reasons for this low screening uptake is unclear. A Dutch study reported that in the general population women’s beliefs about cervical screening and attendance are the best predictors of screening uptake (20, 21). One study among RTRs in Sydney showed that awareness of
increased cancer risk and cancer screening is primarily focused on skin cancer. Thereby, recipients prioritized current health issues over future risks (22). Before changes can be made to improve screening uptake, there has to be more insight in the barriers female RTRs currently experience. The aim of this study is to identify barriers and facilitators for annual gynaecological screening in Dutch female RTRs from both a patients’ and professionals’ perspective.

PATIENTS AND METHODS

STUDY DESIGN AND SETTING
A qualitative study was performed using explorative semi-structured interviews in focus groups, two with female RTRs and two with nephrologists specialized in renal transplantation care. We let the participants talk freely with structured guidance from the interviewer using an interview guide. We chose a group setting as we expected it would be useful to obtain detailed information about personal and group feelings, perceptions and opinions related to gynaecological screening. In the Netherlands, RTs are performed in eight university hospitals, including the Radboud university medical center (Radboudumc) with approximately 130 transplantations per year in adults (40% female).

STUDY POPULATION
FEMALE RENAL TRANSPLANT RECIPIENTS
Fifty women who underwent a RT between 1968 and 2013 at Radboudumc were invited for participation. The 50 female RTRs participated in previously conducted research with HPV self-sampling (self-collected cervicovaginal sample) by our study group (23) and gave permission for further research.

PROFESSIONALS
A total of 67 nephrologists in the Radboudumc (N=12) and affiliated centres (N=55) were invited by email to participate in a separate focus group interview.

DATA COLLECTION
Written informed consent was obtained from all participants and confidentiality was assured. Both focus groups with the female RTRs were guided by a chairman (K.M.), and two researchers (F.H. and R.H.) attended as observers. The focus groups with the nephrologists were performed by two chairmen (F.H. and J.H) and observed by two researchers (M.L. and F.H.). All of the focus groups were audiotaped. The chairman guided the focus groups with open-ended questions, to ensure the participants to speak freely.

INTERVIEW GUIDE
The interview guide for both the patients and professionals was based on literature and expert opinions in the fields of nephrology, gynaecology and qualitative research. This guide included
Barriers and facilitators in regular gynaecological screening of female RTRs

Questions on participation in regular gynaecological screening (particularly for patients), and on improving the participation rate. We used the modified framework of Grol & Fleuren to classify the influencing factors in the following domains: patient, professional, organisation, and finance (24-26).

INTERVIEW FEMALE RENAL TRANSPLANT RECIPIENTS
Female RTRs were asked to introduce themselves by telling their age and date of transplantation. Furthermore, a couple of questions were asked to assess the knowledge on gynaecological screening, particularly on the reasons for and content of annual gynaecological screening. The patients elaborated on their own experiences with gynaecological examination. The remaining part of the focus group targeted the bottlenecks in annual gynaecological screening and the information supply to female RTRs. The focus group ended with the question how the participation of gynaecological screening could be improved and what the role of (self-)sampling on HPV might be.

INTERVIEW PROFESSIONALS
The focus group interviews with nephrologists also started with knowledge questions about the reasons for annual gynaecological screening, the abnormalities caused by HPV and what gynaecological screening should contain. Every nephrologist was asked how and whether they inform their patients about gynaecological screening. The remaining part of the focus group targeted the bottlenecks in annual gynaecological screening and who is responsible for effectuating gynaecological screening. The focus group ended with the question how the participation of gynaecological screening might be improved and the possible role of (self-)sampling on HPV.

ANALYSES
All focus group interviews were fully transcribed and independently analyzed by two researchers (F.H. and M.L.). The analyses were conducted with the aid of the qualitative analysis tool of ATLAS.ti GmbH Version 6 (Berlin, Germany). The influencing factors at the level of patient, professional, organisation, and finance were used to distribute the relevant remarks of the female RTRs and the nephrologists. We analyzed the influencing factors on participation in annual gynaecological screening and on factors that may increase the participation rate. Furthermore, we discussed our findings until we achieved mutual agreement. After constant iteration of these steps, ‘selective coding’ led to a deep understanding of the barriers and facilitators.

RESULTS

PARTICIPANTS
FEMALE RENAL TRANSPLANT RECIPIENTS
Finally, 16/50 (32%) women gave their agreement, of which two eventually were unable to attend. One focus group contained four and the other 10 women. The group interviews took 60 – 90 minutes. Characteristics of the participating RTRs are summarized in Table 1. The median age of the female RTRs was 53.5 years (range 36-75) (at time of RT 43.5 years (21-55)). Furthermore, 64% of these RTRs underwent annual cervical screening.


**Table 1.** Characteristics of the participating female renal transplant recipients (N=14)

<table>
<thead>
<tr>
<th></th>
<th>Median (range)</th>
<th>% (n/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current age (years)</td>
<td>53.5 (36-75)</td>
<td></td>
</tr>
<tr>
<td>Age at transplantation (years)</td>
<td>43.5 (21-55)</td>
<td></td>
</tr>
<tr>
<td>Age renal transplant (years)</td>
<td>11.5 (2-23)</td>
<td></td>
</tr>
<tr>
<td>Cervical screening frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Once a year</td>
<td>64.3 (9/14)</td>
<td></td>
</tr>
<tr>
<td>- Once every 5 years</td>
<td>14.3 (2/14)</td>
<td></td>
</tr>
<tr>
<td>- Once every 4 months</td>
<td>7.1 (1/14)</td>
<td></td>
</tr>
<tr>
<td>- Once after RT</td>
<td>7.1 (1/14)</td>
<td></td>
</tr>
<tr>
<td>- Variable</td>
<td>7.1 (1/14)</td>
<td></td>
</tr>
<tr>
<td>Abnormal cytology after RT</td>
<td>35.7 (5/14)</td>
<td></td>
</tr>
</tbody>
</table>

*RT = renal transplantation*

**NEPHROLOGISTS**
A total of 13 nephrologists participated: in one focus group interview seven nephrologists (male: female ratio 5:2) working in affiliated hospitals participated; the other focus group comprised six nephrologists (male: female ratio 5:1) of the Radboudumc. Both interviews took 45 minutes.

**BARRIERS AND FACILITATORS FROM FEMALE RTRS’ PERSPECTIVE**
We have identified 21 barriers and 18 facilitators influencing participation in annual gynaecological screening. Factors cited in both focus groups are described in the text and marked in the table (**Table 2**). Some illustrative quotations from all focus groups with female RTRs are included in **Figure 1**.

**DOMAIN 1: DETERMINANTS AT PATIENT LEVEL**
In all focus groups participants emphasized that the main barrier for undergoing gynaecological examination was the painful and unpleasant examination itself. Some female RTRs are not aware of the risks of gynaecological abnormalities after transplantation. Other patients were not informed on the risks, or too preoccupied with the transplantation that they forgot this information. The following barriers were mentioned: lack of time and/or priority of gynaecological examination, laziness or negligence, being dependent on others for the hospital visits, a possible religion or language barrier or not experiencing any symptoms.

The main facilitator was internal motivation to preserve their general health. A couple of female RTRs stated that they were motivated to participate in screening because they feel a responsibility to the donor. Self-sampling was considered as a good external motivator. Female RTRs described
the HPV self-sampling as less painful, more convenient, more private and less time consuming compared to a conventional cervical smear.

**DOMAIN 2: DETERMINANTS AT PROFESSIONAL LEVEL**

Female RTRs reported insufficient information supply by professionals on the risks of HPV-related gynaecological abnormalities and the necessity of annual examination as main barriers. Some stated that they have never been informed by their nephrologist, especially the women that were transplanted more than 10 years ago. Some mentioned that screening on skin malignancies has a higher priority. Other barriers mentioned were: no initiative of the professional to remind them, inexperienced professionals performing the examination and wrong timing of information supply. The participants mentioned that the participation rate might increase by proper information at the right moment. Nowadays, patients are extensively informed about the transplantation and the impact of immunosuppressant use before transplantation. The patients mentioned that they miss some repetition of this information supply. It is suggested that the professional (nephrologist or nurse) repeats the education approximately 6 months after transplantation. Information supply by the professional after renal transplantation was stated as the main facilitator.

**DOMAIN 3: DETERMINANTS AT ORGANISATIONAL LEVEL**

Three barriers and two main facilitators were mentioned in this domain. The main barrier lies in the organizational structure of the general practitioner’s (GP’s) practice. The cervical smear is mostly performed by the GP’s assistant. Female RTRs experience the examination more painful when performed by the assistant. Other barriers were: no reminder when the gynaecological examination should be scheduled and lack of clarity where to go (GP or gynaecologist). The first main facilitator concerned the introduction of a reminder that may consist of an automatic calling system, which is set on annual appointments, and/or a reminder by the nephrologist. The second main facilitator included a checklist for (female) RTRs including all examinations they have to undergo in which timeframe after transplantation. The checklist should be integrated in (electronic) patient files and pop up when the patient visits the nephrologist. Other facilitators that were mentioned: own choice for the location of gynaecological examination, information supply through different channels including social media, an information flyer at the outpatient clinic and flexibility in making an appointment at the GPs.

**DOMAIN 4: DETERMINANTS AT FINANCIAL LEVEL**

In general, the patients mentioned the lack of reimbursement of transport to the hospital for visits for gynaecological screening as a barrier on this level. However, the participating RTRs mentioned that they already spent their own contribution on visits to the nephrologist and medication. The self-sampling method was thought to be cheaper.
Table 2. Barriers and facilitators for gynaecological screening for female renal transplant recipients (N=14)

<table>
<thead>
<tr>
<th>Barriers</th>
<th>Organisational</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Examination by GP or assistant*</td>
<td>1. Unpleasant examination*</td>
<td></td>
</tr>
<tr>
<td>2. Unclear communication after RT*</td>
<td>2. Lack of knowledge*</td>
<td></td>
</tr>
<tr>
<td>3. No reminder*</td>
<td>3. No time/priority*</td>
<td></td>
</tr>
<tr>
<td>4. Inflexible calling system GP</td>
<td>4. Laziness/negligence*</td>
<td>Dependent on others*</td>
</tr>
<tr>
<td>5. Distance to hospital</td>
<td>5. Unpleasant posture during examination</td>
<td></td>
</tr>
<tr>
<td>7. Negative first experience with gynaecological examination</td>
<td>7. Negative first experience with gynaecological examination</td>
<td></td>
</tr>
<tr>
<td>8. Fear / shame</td>
<td>8. Fear / shame</td>
<td></td>
</tr>
<tr>
<td>9. No complaints*</td>
<td>9. No complaints*</td>
<td></td>
</tr>
<tr>
<td>10. Too many examinations</td>
<td>10. Too many examinations</td>
<td></td>
</tr>
<tr>
<td>11. Denial</td>
<td>11. Denial</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Facilitators</th>
<th>Organisational</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Reminder*</td>
<td>1. General health preservation*</td>
<td></td>
</tr>
<tr>
<td>2. Checklist*</td>
<td>2. Self sampling*</td>
<td></td>
</tr>
<tr>
<td>3. Location own choice*</td>
<td>3. Kidney preservation</td>
<td></td>
</tr>
<tr>
<td>4. Information through (social) media*</td>
<td>4. Responsibility to donor* Keep control/alert*</td>
<td></td>
</tr>
<tr>
<td>5. Flexibility GP*</td>
<td>5. Material hospital more comfortable</td>
<td></td>
</tr>
<tr>
<td>6. Information flyer*</td>
<td>6. Responsibility to others</td>
<td></td>
</tr>
<tr>
<td>7. Frequent general/gyn examination (1-year)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Group education</td>
<td></td>
<td></td>
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</tbody>
</table>

* mentioned in both focus groups, GP = general practitioner, RT = renal transplantation
### Table 2.
Barriers and facilitators for gynaecological screening for female renal transplant recipients (N=14)

<table>
<thead>
<tr>
<th>Professional</th>
<th>Financial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>1. No/limited information supply to patients*</td>
<td>1. Lack of reimbursement*</td>
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<tr>
<td>2. No initiative of professional*</td>
<td>2. Expensive care*</td>
</tr>
<tr>
<td>3. Inexperienced professional performing examination*</td>
<td></td>
</tr>
<tr>
<td>4. Too much information at once</td>
<td></td>
</tr>
<tr>
<td>5. Wrong moment of information supply*</td>
<td></td>
</tr>
<tr>
<td>6. Lack of knowledge professional</td>
<td></td>
</tr>
<tr>
<td>7. Examination by a man</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Professional</th>
<th>Financial</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Right moment of education*</td>
<td>1. Self sampling cheaper</td>
</tr>
<tr>
<td>2. Information given by nephrologist*</td>
<td></td>
</tr>
<tr>
<td>3. Manner of education*</td>
<td></td>
</tr>
<tr>
<td>4. Examination by a woman</td>
<td></td>
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</tbody>
</table>
Table 3. Barriers and facilitators for gynaecological screening for nephrologists (N=13)

<table>
<thead>
<tr>
<th>Barriers</th>
<th>Organisational</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. Examination by GP or assistant*</td>
<td>1. No time/priority*</td>
</tr>
<tr>
<td></td>
<td>2. Inflexible calling system GP*</td>
<td>2. Too many examinations*</td>
</tr>
<tr>
<td></td>
<td>3. No reminder</td>
<td>3. Unpleasant examination*</td>
</tr>
<tr>
<td></td>
<td>4. Lack of communication with hospital by GP*</td>
<td>4. Laziness/negligence*</td>
</tr>
<tr>
<td></td>
<td>5. Unclear communication after RT</td>
<td>5. Lack of knowledge*</td>
</tr>
<tr>
<td></td>
<td>7. Dependent on others*</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Facilitators</th>
<th>Organisational</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. Reminder*</td>
<td>1. Self sampling*</td>
</tr>
<tr>
<td></td>
<td>2. Frequent general/gyn examination (1-year)*</td>
<td>2. General health preservation*</td>
</tr>
<tr>
<td></td>
<td>3. Information flyer</td>
<td>3. Keep control/alert</td>
</tr>
<tr>
<td></td>
<td>4. HPV status is known</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Checklist</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 Group education</td>
<td></td>
</tr>
</tbody>
</table>

* mentioned in both focus groups, GP = general practitioner, RT = renal transplantation, HPV = Human Papillomavirus
### Table 3. Barriers and facilitators for gynaecological screening for nephrologists (N=13)

<table>
<thead>
<tr>
<th>Professional</th>
<th>Financial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1.</strong> Lack of knowledge professional*</td>
<td><strong>1.</strong> Expensive specialist care*</td>
</tr>
<tr>
<td><strong>2.</strong> Inexperienced professional performing examination*</td>
<td></td>
</tr>
<tr>
<td><strong>3.</strong> No evidence for policy</td>
<td></td>
</tr>
<tr>
<td><strong>4.</strong> No/limited information supply to patients</td>
<td></td>
</tr>
<tr>
<td><strong>5.</strong> No initiative of professional*</td>
<td></td>
</tr>
<tr>
<td><strong>6.</strong> Too much information at once</td>
<td></td>
</tr>
<tr>
<td><strong>7.</strong> Wrong moment of information supply</td>
<td></td>
</tr>
<tr>
<td><strong>8.</strong> Examination by a man</td>
<td></td>
</tr>
</tbody>
</table>

* mentioned in both focus groups, GP = general practitioner, RT = renal transplantation, HPV = Human Papillomavirus

<table>
<thead>
<tr>
<th>Professional</th>
<th>Financial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1.</strong> Nephrologist know the risks*</td>
<td><strong>1.</strong> Self sampling cheaper</td>
</tr>
<tr>
<td><strong>2.</strong> Information given by nephrologist*</td>
<td></td>
</tr>
<tr>
<td><strong>3.</strong> Examination by gynaecologist*</td>
<td></td>
</tr>
<tr>
<td><strong>4.</strong> GP system accessible for hospital</td>
<td></td>
</tr>
<tr>
<td><strong>5.</strong> Right moment of education*</td>
<td></td>
</tr>
<tr>
<td><strong>6.</strong> Examination by GP is adequate</td>
<td></td>
</tr>
</tbody>
</table>
BARRIERS AND FACILITATORS FROM NEPHROLOGISTS’ PERSPECTIVE

In total, 15 barriers and 15 facilitators influencing the participation rate in annual gynaecological screening were mentioned. Factors mentioned in both focus groups are described in the text and marked in the table (Table 3). Some illustrative quotations from all focus groups with nephrologists are included in Figure 1.

DOMAIN 1: DETERMINANTS AT PATIENT LEVEL

All nephrologists mentioned that the female RTRs do not make time for gynaecological screening, because their priorities lay elsewhere. After transplantation there are more important issues such as medication, risk of rejection of the transplant graft, recovering after surgery, and a new diet. Female RTRs do not think about long-term risks. The second barrier is thought to be the quantity of examinations the patients need to undergo. RTRs have to visit different specialists a year and gynaecological screening can be one in excess. Other barriers were unpleasant examination, laziness/slackness/negligence of the patient, lack of knowledge on the risks, and dependency of others.

Overall, there were two facilitators in both focus groups: HPV self-sampling and health preservation. Female RTRs experience self-sampling as less painful and self-sampling can easily be implemented at home or in the outpatient visit. The preservation of general health was the other main facilitator mentioned by the nephrologists. The nephrologists consider prevention of a gynaecologic (pre) malignancies worthwhile because limited treatment options due to the renal transplant located in the pelvis.

DOMAIN 2: DETERMINANTS AT PROFESSIONAL LEVEL

The main barrier is the lack of knowledge of the professional, especially the GP, on the risks of gynaecological (pre)malignancies after renal transplantation. Most nephrologists know the increased risk on cervical and vulvar cancer. As GPs do not see many (female) RTRs, they are probably not aware of all associated risks after renal transplantation. Performance of gynaecological examination by an inexperienced professional was mentioned as another barrier. The quality of the examination of the vulvar area, because it is difficult for untrained professionals to recognize vulvar abnormalities, was the greatest concern.

The nephrologists mentioned their own knowledge on the risks of gynaecological abnormalities as the main facilitator. Arising from this, information given by the nephrologists to the patient should also facilitate participation in gynaecological screening. Examination performed by a gynaecologist and the right moment of education were mentioned as facilitators in both focus groups.

DOMAIN 3: DETERMINANTS AT ORGANISATIONAL LEVEL

Three main barriers were identified in this domain: the inflexibility of the calling system at the GP practice, limited communication between GP and the hospital, and lack of initiative of the professional to remind the female RTR to undergo gynaecological examination. The nephrologists feel that the organisation at the GP’s office is inflexible. The nephrologists feel that examination performed by the GP’s assistant is not reliable, because of lack of attention for the anogenital
area and lack of knowledge to recognize abnormalities. The results of the cervical smears taken by the GP are not accessible for the nephrologists and not communicated to the nephrologists either. Another barrier was the transmission of care from the transplantation centre back to the referring hospitals: especially the indistinctness of the responsibility for the participation in annual gynaecological screening. Furthermore, the lack of initiative of the professional (both nephrologist and GP) to remind the female RTRs to undergo gynaecological examination was mentioned as barrier.

Sending an annual reminder to the patients is suggested as main facilitator. Ideally, gynaecological screening should be implemented in the annual outpatient visit to the nephrologist (university or referring hospital). Patients need to undergo blood- and urine testing during this appointment and offering simultaneous gynaecological screening may increase the participation rate significantly.

**DOMAIN 4: DETERMINANTS AT FINANCIAL LEVEL**

The only barrier mentioned in this domain was that health care could become more expensive if all female RTRs will go to the gynaecologist for screening. In one of the focus groups it was suggested that self-sampling might be cheaper than conventional cervical smear.
Figure 1. Illustrative quotations from female renal transplant recipients and nephrologists

**PATIENTS’ QUOTATIONS**

- “Nowadays the assistant of the GP performs the examination and I was a little startled, because there is trust between me and my GP. And the assistant, well, she answers the phone.”

- “I do not have any reason for not undergoing a gynaecological examination...”. "Exactly"

Organisational determinant barrier

- “After six months you are a little back on track...”. “It is indeed an idea to plan an education after six months on ‘We are transplanted and what is next’”

Patient determinant barrier

Organisational determinant facilitator

**NEPHROLOGISTS QUOTATIONS**

- “When a patient is in a calling system, she does not have to think about it. In this way, a part of the slackness can be obviated.”

Organisational determinant facilitator

- “At the end of the first year after transplantation, before patients are referred back to their referring nephrologist, patients are doing well and do not have renal problems. This might be the right time for me to be more proactive in information supply”

Professional determinant facilitator

- “You need to divide your attention among different things. The thing that is important at this time has more priority than the thing you might suffer from in the future”

Patient determinant barrier
DISCUSSION

Our study provides unique perspectives on the barriers and facilitators in gynaecological screening by both female RTRs and nephrologists working in the field of transplantation. Thereby, the barriers and facilitators in this study are applicable in other developed countries as well. We found that the main barriers mentioned by female RTRs as well as nephrologists lay in the patient, professional and organisational domain. For female RTRs the main barriers were: unpleasantness of gynaecological examination especially when it is performed by GP, lack of knowledge and priority, and limited information supply by the professional. For the professionals, main barriers included: inadequate examination performed by GP, patients’ lack of time, priority or laziness/slackness/negligence and limited information supply by the professional due to limited knowledge. The main difference in barriers lay in the patient domain. The female RTRs mentioned the unpleasant gynaecological examination itself as a main barrier just as a lack of reminder, while professionals mentioned the lack of priority for the female RTR as main barrier. Total agreement was established on the facilitators, particularly reminder for the patient to undergo the examination, checklist of all examinations/visits, integration of gynaecological examination in annual check-up, self-sampling and better timed information supply.

Until now, little research has been done on the barriers for gynaecological screening in female RTRs. Most studies investigated the barriers for gynaecological screening in the general population only. The examination performed by the GP or assistant is one of the main barriers mentioned. In the general population only 2% of the women not attending regular cervical screening, did not want their own GP to perform the examination. Their main barrier was organisational; women had difficulties to schedule an appointment (27, 28). These practical barriers were found more predictable than emotional factors. However emotional barriers and a previous negative experience were also mentioned (28, 29). Female RTRs also mentioned that they forgot to make an appointment or were not aware of the risks. Furthermore, they did not receive a reminder for the gynaecological examination. In the general population research has shown that screening compliance is associated with women's knowledge of cervical cancer and their attitudes toward screening (30). Moreover, knowledge of HPV in the general population is limited (31-34). A Swedish study showed that women aged 29 years or younger were better informed than older women (30-49 years) (35). Female RTRs have a median age of 40-50 years.

The nephrologists believe that the female RTRs have other priorities than undergoing a gynaecological examination. In contrast with the general population, inspection of the vulvar area is very important in the gynaecological screening of female RTRs, because of the increased risk of vulvar (pre)malignancies. The nephrologists believe that a GP is not educated to screen for vulvar (pre)malignancies, because they diagnose 1-2 vulvar malignancies in his/her entire career due to the low incidence of vulvar cancer (36, 37). In this case, it would be more useful to inform the patients on the risks and the most common symptoms of vulvar (pre)malignancies. It also might be helpful to provide more clear information to the GPs on gynaecological screening after
renal transplantation. Female RTRs mentioned that their GPs do not know they need to undergo gynaecological examination more frequently. The nephrologists mentioned it is very difficult to change the calling system by the GP from every 5 years to every year.

Several studies have investigated the influence of a reminder letter on the participation rate in cervical cancer screening programmes in the general population. Women who are personally reminded by their GP or received a general reminder letter had the greatest likelihood of attending screening (21, 38). A pre-assigned date for screening and a personalized letter increased the coverage with 25% in women between 60-70 years in Spain (20). The influence of a reminder letter in a younger population (18-35 years) is less effective, with an increase in attendance of only 7.9% to 12.2% (39, 40). The female RTRs and nephrologists pointed out that a reminder would encourage the attendance of gynaecological screening. Nephrologists in referring hospitals might have a role in reminding their patients, since they see them regularly.

Furthermore, female RTRs pointed out that a checklist would be very helpful. This would provide an overview of all the necessary examinations they have to undergo after transplantation. Gill et al. (41) showed in a pilot study that the use of a checklist by professionals before transplantation was useful. The same checklist for both nephrologist and patient might improve the communication.

In many hospitals, patients are informed about the increased risks of HPV related genital abnormalities before the transplantation. In this period, patients may experience anxiety or distress because of their health issues. This information is briefly repeated when the patients are discharged from the hospital after the transplantation. RTRs as well as nephrologists mention that this might be the wrong moment for this information. They both suggest that when the renal function is stable and the patient is referred back to their own referring nephrologist, would be a good moment for education on the post transplantation period, including reasons for gynaecological screening.

In the Netherlands, the participation rate of the general population in cervical cancer screening programme is not at its optimum. Women who do not attend cervical screening within two reminders, are called ‘non-responders’. Research showed that this group of women has a higher incidence of cervical (pre)malignancies compared to ‘responders’. To reach these ‘non-responders’, a cervico-vaginal self-sampling method to detect HPV may be an alternative. Self-sampling is highly accepted and even preferred over physician taken sample in 95% of cases by women and the sensitivity of high risk HPV testing on self-sampling material equals sampling materials obtained by physicians (42). The implementation of self-sampling in the Netherlands showed a response rate of approximately 30% in the non-responders of the population-based screening (43, 44). Self-sampling will therefore be implemented to be available to the non-responder group of the new Dutch national screening program. Self-sampling was preferred over physician taken sample because it was easy to use with simple instructions and less painful, which was confirmed by the female RTRs in our focus groups. The nephrologists mentioned all of the above arguments as well and pointed out that self-sampling is easy to implement in the annual check up of female RTRs.
CONCLUSION

Based on the findings of this study, we suggest every female RTR should receive a small checklist one year after transplantation (or when referred back to their own nephrologist), with the examinations they need to undergo at different time intervals. Furthermore, HPV self-sampling in the annual check-up by the nephrologist (with referral to the gynaecologist in case of HPV-positivity) would obviate a lot of barriers and meets the main facilitators. Implementing these changes might improve the participation rate of female RTRs in gynaecological screening.
REFERENCE LIST


32. Gottvall M, Larsson M, Hoglund AT, Tyden T. High HPV vaccine acceptance despite low awareness among


IMPROVEMENT OF GYNAECOLOGICAL SCREENING OF FEMALE RENAL TRANSPLANT RECIPIENTS BY SELF-SAMPLING FOR HPV DETECTION

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Luuk B. Hilbrands,
Kim A. Meeuwis,
Muriël C. van Bergen – Verkuyten,
Brigitte F. Slangen,
Michelle M. van Rossum,
Janette Rahamat-Langendoen,
Leon F. Massuger,
Joanne A. de Hullu,
Willem J. Melchers

ABSTRACT

Objectives: Female renal transplant recipients (RTRs) have increased risk for developing human papillomavirus (HPV)-related (pre)malignancies of the lower genital tract. Annual cervical screening is advised for RTRs, but the participation rate is low. The aim of this study is to investigate whether HPV self-sampling is suitable for gynaecological screening of RTRs to increase participation rate.

Methods: A large cohort of 253 RTRs was investigated for the prevalence of HPV. All participants received a device for a cervico-vaginal self-sample. Questionnaires were sent to assess the experience with this device. High-risk (hrHPV) presence was determined with the SPF<sub>10</sub>-LiPA<sub>25</sub> system and GP5+/6+ PCR. HrHPV positive patients underwent gynaecologic examination.

Results: More than 90% of the patients rated their experience with the self-sample device as good to excellent, and 77% preferred self-sampling over a physician taken sample. Approximately 35 of 217 women tested hrHPV positive with SPF<sub>10</sub>-LiPA<sub>25</sub>, and 22 tested positive with the GP5+/6+ PCR. Eleven hrHPV-positive patients had clinically relevant gynecological abnormalities, and they all tested positive with GP5+/6+ PCR.

Conclusions: Self-sampling is clinically applicable in a gynaecological screening and is preferred by female RTRs. Therefore, self-sampling could be implemented with the aim to increase the participation rate of female RTRs in yearly gynaecological screening.
INTRODUCTION

Female renal transplant recipients (RTRs) have a markedly increased risk of developing human papillomavirus (HPV)-related (pre)malignancies of the female lower genital tract (1-5). They have a 14-fold increased risk of cervical cancer and up to 50-fold increased risk of vulvar cancer because of the lifelong use of immunosuppressive drugs. Human papillomavirus plays a key role in the oncogenesis of these malignancies in RTRs. This is in contrast with the general population in which HPV, although causing nearly 100% of the cervical cancers, causes only 20% of the vulvar (pre) malignancies (6).

Because of the higher risk of (pre)malignant cervical lesions and the need for early detection, because of the limited treatment options due to the kidney transplant in the pelvis, guidelines and publications advise annual cervical screening for female RTRs (7-9). However, evidence on the optimal frequency of cervical screening is scarce. In daily practice, we found the average screening interval in female RTRs of 5 years (10, 11). A possible explanation for this long-interval screening in RTRs could be that the female RTRs and/or the nephrologists focus more on the preservation of renal function than on long-term complications. Other possible reasons for non-attending screening could be embarrassment and fear of the gynaecological examination or lack of time to schedule an appointment.

The participation rate of women in the Dutch well-organized national screening program, which is currently based on cervical cytology, is only approximately 65% (12). Women not participating after invitation (“nonparticipants”) have a higher risk of developing cervical (pre)malignancies (13, 14).

High-risk (hr)HPV tests have a higher sensitivity in detecting cervical intraepithelial neoplasia (CIN) 2/3 than cervical screening by PAP smear (~90% versus ~60%) and screening based on hrHPV tests affords better protection against cervical cancer (15-17). Therefore, it was decided to introduce the hrHPV test as primary screening tool in the general population in The Netherlands in 2017 (18).

Human papillomavirus testing can be adequately and reliably performed on a cervicovaginal sample taken by women themselves (self-sampling) (19, 20). Self-sampling is highly accepted by women and the sensitivity of hrHPV testing on self-sampling material equals that obtained with sampling by physicians (21). The implementation of self-sampling in The Netherlands showed a response rate of approximately 30% in the nonparticipants of the population-based screening (22, 23). Self-sampling will therefore be available to the nonparticipant group of the Dutch national screening program. Because the female RTRs who do not participate in yearly gynaecological screening can be considered as “nonparticipant”, self-sampling might be a promising option to increase the yearly screening rate in female RTRs.

The aim of this study is to investigate whether HPV self-sampling is suitable to implement in gynaecological screening of RTRs and can ultimately increase the participation rate.
METHODS

Female RTRs were invited to participate in a study to investigate the prevalence of HPV in female RTRs. 24 Female RTRs who fulfilled the following criteria were invited to participate: (1) renal transplantation at the Radboud University Medical Center (Radboudumc), Nijmegen, or the Maastricht University Medical Center (MUMC), Maastricht, The Netherlands, in the period 1968-2008; (2) a functioning donor kidney as of February 2012; (3) living in The Netherlands; and (4) older than 18 years. Of the 626 invited participants, 253 RTRs (40%) gave their written informed consent for this study.

All 253 participants from the cohort study were asked to self-collect a cervicovaginal sample in the privacy of their own home with the dry Evalyn Brush® system (Rovers Medical Devices B.V., Oss, The Netherlands), which was proven to provide similar results as a physician-taken sample for hrHPV detection (20, 25, 26). Patients received the collection device and written instructions with illustrations and returned the package comprising a leak proof seal bag, absorption sheet, and a plastic return envelope complying with the packing instruction for the sending of biological substances (category B) per mail. All samples were stored dry and certified the unique study code. Each recipient received a questionnaire, which assessed the experience of the RTRs regarding self-sampling with the dry Evalyn Brush® system. The questionnaire also contained items to assess socio-demographic characteristics, medical data, and sexual behaviour. Questionnaires could be filled out using a digital (secured) system or via a paper version. All questionnaires were provided and saved with a unique study code.

The self-sampling device was resuspended in 1·5 ml ThinPrep medium at the laboratory of the Department of Medical Microbiology at the Radboudumc. The vials were vortexed for 3x15s, stored overnight at 4°C, and again vortexed for 2x15s. From each resuspended dry specimen, 200 μl was used for DNA extraction with the MagNaPure 96 (Roche Molecular Diagnostics). The purified DNA was eluated in 50μl TE-buffer. For detection and genotyping of HPV, broad spectrum HPV amplification was performed using the short-PCR-fragment assay (SPF$^{10}$-LiPA$^{25}$; Labo Bio-medical Products B.V., Rijswijk, The Netherlands). This assay amplifies a small fragment of 65-bp from the L1 open reading frame and allows detection of a broad range of HPV genotypes and has a high analytical sensitivity. The SPF$^{10}$-LiPA$^{25}$ system will detect a relatively high amount of HPV not associated with genital (pre)malignancies. On the other hand, the GP5+/6+ PCR enzyme immunoassay (EIA) is less sensitive, but is more specific in detecting (pre)malignancies. Patients who tested hrHPV positive with the SPF$^{10}$-LiPA$^{25}$ system were invited for gynaecological examination. Gynaecological examination comprised an extensive inspection of the genital area and a smear of the cervix or vaginal vault in case of hysterectomy in the past. In case of any abnormal finding during this visit, patients were referred for additional tests (i.e., vulvar biopsy, colposcopy) and treatment according national guidelines.

To assess the clinical applicability of HPV testing on self-sampling material in the gynaecological screening of female RTRs, any samples that were hrHPV positive with the highly sensitive SPF10-
LiPA25 were retested with a clinically validated HPV test, the GP5+/6+ PCR EIA (EIA HPV GP HR kit, Diassay, Voorburg, The Netherlands). Women with SPF10-LiPA25 HPV-negative samples were not retested with the GP5+/6+ PCR. As a quality control for the presence of DNA and absence of PCR inhibitors in the isolated material, a PCR for β-globin was performed. The HPV results of the GP5+/6+ PCR EIA were related to the cytological and/or histological outcome of the gynaecological examination.

RESULTS

Of the 253 patients who signed the informed consent form, 217 (86%) returned the sample, and 212 (84%) patients filled out the questionnaire. 157 patients returned the sample and filled out the questionnaire completely. These 157 patients who completed questionnaires were used to assess the opinion of the RTRs on the self-collected cervicovaginal sample with the dry Evalyn Brush® system. The included patients had a median age of 56 years (range 23-79) and the interval after their first transplantation was 10 years (range 3-37). One hundred thirty-five (86%) of 157 patients used the self-sampling device without any problems. Problems that did occur include pain with insertion and unpleasant feeling when turning the brush or blood on the device. From the total group, 144 (92%) patients rated their experience with and convenience of using the self-sampling device as good to excellent. The instructions for using the brush were considered good to excellent by 153 (97%) of the 157 female RTRs. The feelings that patients experienced during self-sampling are summarized in Figure 1. Overall, self-sampling did not raise feelings of shame and was not referred to as scary or unpleasant. Most patients were confident that they performed the self-sampling correctly, and 68% rated the sampling as not difficult at all. Cervical screening using the self-sampling method was preferred above sampling by a physician by 119 (76%) of the 157 female RTRs, and 3 female RTRs had no preference for either.

Most patients used a calcineurine inhibitor (tacrolimus/cyclosporine A), in most cases with corticosteroids and in some cases with a proliferation inhibitor (azathioprine/mycophenolate mofetil). Time on immunosuppressive medication and the type of immunosuppressive treatment were not related to HPV prevalence (24).

Of the 217 completed self-samples, 35 samples (16%; 95% confidence interval [CI], 11%-21%) were positive for hrHPV by the sensitive SPF10 test. These RTRs were invited to the outpatient clinic of the Department of Obstetrics and Gynaecology for gynaecological examination. Of these 35 samples, 20 (57%; 95% CI, 41%-73%) samples tested hrHPV positive with the clinically validated GP5+/6+ PCR, and the overall hrHPV positivity was 9% (95% CI, 5%-13%) with GP5+/6+ PCR. The 20 samples that were hrHPV positive with the GP5+/6+ PCR included all 11 samples of patients with clinically relevant cervical/vaginal and/or vulvar abnormalities (Table 1). In the remaining 9 cases that were hrHPV positive with the GP5+/6+ PCR, there were no abnormal cytology results. Two of the 11 patients had HPV-related vulvar abnormalities, consisting of usual vulvar intraepithelial neoplasia (uVIN) and condylomata
Figure 1. Feelings of 157 female RTRs experienced during self-sampling.

Table 1. Details of clinically relevant genital abnormalities found in female RTRs, who tested hrHPV positive on self-sampling material.

<table>
<thead>
<tr>
<th>N=35</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No relevant abnormalities</strong></td>
<td>24</td>
</tr>
<tr>
<td><strong>Clinically relevant abnormalities</strong></td>
<td>11</td>
</tr>
<tr>
<td>Cervical</td>
<td></td>
</tr>
<tr>
<td>- Moderate dysplasia</td>
<td>5</td>
</tr>
<tr>
<td>- Severe dysplasia</td>
<td>2</td>
</tr>
<tr>
<td>Vulvar/vaginal</td>
<td></td>
</tr>
<tr>
<td>- Condylomata</td>
<td>1</td>
</tr>
<tr>
<td>- uVIN 2-3</td>
<td>1</td>
</tr>
<tr>
<td>Combined cervical/vaginal and vulvar</td>
<td></td>
</tr>
<tr>
<td>- Moderate vaginal dysplasia and uVIN 3</td>
<td>1</td>
</tr>
<tr>
<td>- Moderate dysplasia cervix and condylomata</td>
<td>1</td>
</tr>
</tbody>
</table>

*U*VIN indicates usual vulvar intraepithelial neoplasia, + 1 patient also had moderate dysplasia of the cervix.*
DISCUSSION

Our study showed that 75% of the included female RTRs preferred self-sampling to a physician taken sample. Furthermore, hrHPV testing on self-sampling material with a clinically validated HPV test seems a reliable tool in detecting clinically relevant gynaecological abnormalities in female RTRs.

The question whether HPV testing on self-sampling material is equivalent to HPV testing on clinician-collected samples was addressed in a recent meta-analysis by Arbyn et al. (21). It was concluded that PCR with the GP5+/6+ primers have a similar sensitivity and specificity (91-95% and 51-54% respectively) on self-samples versus clinician-taken samples for detecting cervical abnormalities. Our study confirms the clinical applicability of the PCR on self-sampling material in female RTRs, which is an important finding, even in a small study population. Implementation of this self-sampling strategy offers the potential to increase the participation rate in the yearly gynaecological screening of female RTRs. Yearly HPV self-sampling as a routine at the outpatient clinic nephrology with referral to the gynaecologist in case of an HPV positive test could comprise the gynaecological screening of female RTRs.

Interestingly, a secondary finding in our study was that also vulvar abnormalities may be detected by self-sampling using HPV PCR, even in the absence of cervical/vaginal abnormalities. Although there were only 2 patients with vulvar abnormalities in this small population, it is of the utmost importance that vulvar abnormalities in female RTRs are detected in an early stage, as the vulvar (pre)malignancies tend to develop more rapidly in female RTRs (27). In the general population, a majority of vulvar squamous cell carcinomas (SCCs) consists of non HPV-related SCCs in a background of lichen sclerosus and/or differentiated VIN (28). However, in women treated with immunosuppressive drugs, almost 100% of SCCs are HPV-related vulvar (pre)malignancies and/or usual VIN (29). We did not find any publications on the use of cervicovaginal self-sampling in relation to the presence of vulvar (pre)malignancies. The RTRs that were hrHPV negative did not undergo gynaecological examination, so the prevalence of vulvar abnormalities in this group is not entirely clear. Our findings emphasize the importance of inspection of the total genital area in hrHPV-positive female RTRs.

The participation rate of female RTRs in annual cervical screening is low, despite their increased risk of developing genital abnormalities. The reasons for not participating in screening are not entirely clear. Most likely, the main focus of the female RTRs is on the preservation of the renal transplant and less on the prevention of long-term complications. Another explanation of the low participation rate may be the age of the RTR population. In the general population, age has an influence on cervical screening uptake, and the uptake is worse in women over 55 years (30). Participants in our study had a median age of 56 years. The influence of educational level was also investigated, but yielded no significant effect on screening participation (30). A study among RTRs in Sydney showed that awareness of increased cancer risk was present, but patients were primarily focused on skin cancer (31). It also may be hypothesized that patients do not receive or do not recall information on
the need for gynaecological screening. More insight in the reasons for non-attending might help us to improve the gynaecological screening in female RTRs.

**STRENGTHS AND LIMITATIONS**

This study is the first to assess the experiences of female RTRs with an HPV self-sampling device. Furthermore, we compared the results of the analytical sensitive SPF10-LiPA25 test with the clinically sensitive GP5+/6+ PCR and related the outcome with clinically relevant gynaecological abnormalities, which is of great importance for possible implementation of HPV testing in the gynaecological screening. A limitation of the study is the composition of the study population. There could be population bias because the female RTRs who participated already have a positive opinion on HPV self-sampling at study entry. We have no information on the opinion of the female RTRs who did not participate. Most RTRs are focused on the preservation of the renal transplant and already need to go to the hospital regularly. Furthermore, the self-sampling technique is new and not yet introduced in the general population, and nephrologists are not yet informed about HPV self-sampling. This could be explanations for the participation rate of 40%.

**CONCLUSIONS**

In conclusion, HPV testing on cervicovaginal self-sampling material could be a reliable tool for gynaecological screening in female RTRs. The experiences of the female RTRs in this study with the self-sampling device were quite favourable. Human papillomavirus self-sampling could be implemented in the follow up of female RTRs, mainly to ensure and improve the yearly gynaecological screening in these women.

In future research, it would be interesting to assess the barriers and facilitators in gynaecological screening of female RTRs. The results might present suggestions for further improvement of gynaecological screening.
REFERENCE LIST


GENERAL DISCUSSION
GENERAL DISCUSSION

In this thesis, different studies on vulvar- and cervical (pre)malignancies in either the general population and renal transplant recipients were described. This chapter will focus on two important issues in more extent; tumour localization in vulvar squamous cell carcinoma (SCC) and screening of HPV-related genital (pre)malignancies in renal transplant recipients (RTRs). These topics are highly relevant because of their influence on (future) clinical practice.

1. VULVAR CANCER: ROLE OF TUMOUR LOCALIZATION?

CLITORAL TUMOUR: WORST PROGNOSIS

In Chapter 2 we have shown that vulvar SCC with clitoral involvement has a worse prognosis compared to other localizations on the vulva. The localization itself appeared not to be an independent risk factor, but several tumour characteristics that were in itself independent risk factors were more often present in tumours with clitoral involvement. The characteristics larger tumour diameter, deeper tumour invasion, more lymphovascular space invasion (LVSI), positive surgical margins, and the presence of lymph node metastases were independently related to prognosis.

Patients’ and doctors’ delay in the diagnostic process may be an explanation for the larger and deeper invading tumours around the clitoris. Patients may fear the implications of the lesion to the clitoral function which could lead to reluctance to visit a doctor combined with uncertainty, fear and shame. Diagnostic delay by doctors in clitoral SCC might be enhanced by the hesitation to biopsy the lesion at the outpatient clinic, because of fear damaging the clitoris.

Another hypothesis for the increased depth of invasion and more LVSI in clitoral SCC could be that clitoral tissue is more prone for tumour invasion due to higher blood circulation and a large number of (lymph) vessels and nerves. This might stimulate faster and deeper tumour growth. In general, the prognosis of vulvar SCC is mainly based on the presence and number of inguinofemoral lymph node metastases which are representative for unfavourable histopathological factors such as larger and deeper invading tumours (1).

The shorter distance from tumour to the inguinofemoral lymph nodes may also play a role in higher rate of lymph node metastases compared to tumours that are located further from the groins. Metastases to the groins develop via embolization; one could imagine that a larger distance to the groins may lead to a longer interval for groin nodes to become positive. Furthermore, tumours with clitoral involvement had more often positive or surgical margins <8mm. These factors might increase the local recurrence rate, however it is still a debate whether close surgical margins truly influence recurrence rates (2).

Anatomic localization and histopathological characteristics of a tumour cannot be changed. More direct lymphatic drainage from the clitoris does not totally explain the higher rate of positive lymph
nodes, since Iversen et al. (3) assessed lymph drainage from different vulvar areas and found no difference in uptake of $^{99m}$Tc-colloid between different injection sites (including clitoris) (3). However, the particles of $^{99m}$Tc-colloid are extremely small and may pass through the lymphatic system more easily than tumour cells. In vulvar SCC the perineum is further from to the groin nodes than the clitoris and tumour cells may cluster together and may not reach the groin nodes in contrast to the tumour cells from the primary vulvar SCC located on the clitoris. The only factors that may be influenced are either the patient/doctors delay and the treatment. Earlier detection of tumour might minimize tumour invasion. This could be established by better education of general practitioners and gynaecologists. Patient education is also very important, especially to diminish the feelings of shame concerning vulvar complaints. Prognosis of vulvar SCC is worse in case of more local recurrences. The question rises whether primary treatment can be improved to decrease the number of local recurrences resulting in a better prognosis. There is no clear definition of local vulvar SCC recurrence in the current literature. There is no consensus on the minimum or maximum time span between the primary tumour and the recurrence. Some authors define local recurrence as the new appearance of a tumour after therapy with radical intent and a disease-free period of at least 6 months (4). Most real local recurrences occur within 2 years after primary treatment, so recurrences after 2–3 years might be considered de novo tumours based on the adjacent premalignancy (5, 6). The incidence of isolated local recurrences is 20–23% (4, 7, 8). More than 50% of all recurrences are local. So, how can we minimize local recurrences in vulvar SCC with clitoral involvement and try to improve survival in these patients? Is more radical surgery necessary? Are all the local recurrences diagnosed until now true recurrences or de novo tumours? The width of the tumour-free margin is one of the most clinically important and controversial topics in treatment of vulvar SCC. Although it is obvious that a tumour positive margin is associated with an increased local recurrence rate, the association between the width of the tumour-free margin and local recurrence rate is less clear (6, 9-13). Studies yielded varying results with regard to the tumour-free margin and the risk of local recurrence (6, 9, 10, 12, 13). A recent study by Nooij et al. (2) showed no clear difference in the risk of local recurrence in the <8 versus ≥8 mm group and showed positive surgical margins the only independent risk factor for local recurrences. Based on these study results more extensive surgery does not seem to be the solution in the prevention of local recurrences. Does this also mean that re-excision in case of a surgical margin <8 mm is not necessary based on these results? Apparently, only positive margins give a proven higher risk of local recurrence. More research on this topic is certainly needed but adapting this conclusion would lead to a substantial decrease in the number of repeat surgical procedures significantly decreasing patient morbidity. It seems that more information on the localization of the primary tumour could give more information on the approach of an alleged recurrence. Nowadays the time span between primary treatment and the occurrence of a new tumour is leading in the determination of recurrence or de
novo tumour. Because documentation of the exact location of the primary tumour is often absent, the distance to the primary site to define recurrence is difficult to assess. In ongoing and future prospective studies, introduction of digital cameras may be helpful in reporting the exact location of the primary tumour. Digital documentation of the localization of the primary tumour will provide better demarcation of the tumour site and be used in defining recurrence. Recurrent tumours located >1cm from the primary tumour site could be considered de novo also when the tumour develops within 2 years after primary treatment. The underlying premalignancy (lichen sclerosus (LS), differentiated vulvar intraepithelial neoplasia or high-grade squamous intraepithelial lesion (HSIL) of the vulva) is probably more important for developing local recurrences (de novo?) than the tumour free margins but good clinical studies are lacking. Currently, the standard treatment of vulvar SCC is local excision and sentinel lymph node (SLN) procedure of the groins (14). In case of a local recurrence the treatment is re-excision and inguinofemoral lymphadenectomy with all the associated morbidity; the indication for lymphadenectomy results in losing the advantages of only a SLN procedure in case of a negative SLN. Prevention of local recurrences is of utmost importance for either mortality and morbidity in patients with vulvar SCC. Besides the use of digital cameras, genetic profiling of primary vulvar SCCs and the local recurrences may be helpful in distinguishing between real local recurrences and de novo vulvar SCC. Furthermore, in case of HPV-related vulvar SCC, HPV genotyping could also help in defining between recurrence and de novo tumour. When another HPV genotype is found in the recurrent SCC it is more likely a de novo tumour. These tools should be part of future studies on the role of tumour free margins and the underlying premalignancy with respect to local recurrences and prognosis.

**PERINEUM: BEST PROGNOSIS**

In contrast to the bad prognosis for patients with vulvar SCC located on the clitoris, patients with vulvar SCC on the perineum have a better prognosis. We showed in Chapter 3 that HPV-related vulvar SCCs are more often located on the perineum. It seems that HPV-associated vulvar SCC has a better prognosis. Another explanation for the favourable prognosis could be the early detection of vulvar SCC on the perineum, as a tumour on the perineum could give more and earlier discomfort for the patient. This might result in an earlier presentation to the general practitioner. Furthermore, the perineum is a relative safe place to take a biopsy. All these factors might contribute to more early stage vulvar SCC on the perineum.

Patients with vulvar SCC on the perineum have a relatively good prognosis. Chapter 3 showed that HPV-relation has a positive influence on the prognosis. The question rises whether the localization or the HPV relation are explanations for the better prognosis? Nowadays, the HPV status of vulvar SCC is not assessed in the diagnostic process because lack of consequences for the treatment.

Vulvar cancer is primarily surgically treated with wide local excision and SLN procedure of the groin nodes independent of the oncogenesis (HPV-related or LS-related). What is known about the role of HPV in other malignancies? Cancer of the cervix and vagina are almost always HPV-
related and treatment consists of surgery and/or radiotherapy dependent on the stage of disease. Head- and neck tumours comprise tumours of the mouth, oropharynx, hypopharynx, and larynx. The oropharyngeal SCCs are partly HPV-related and treated with (chemo)radiation therapy. The last decade, head- and neck tumours (especially oropharyngeal tumours) have been extensively reviewed since a rise in incidence has been observed in the past years. The increasing incidence correlates with the increase of HPV infections associated with alterations in sexual behaviour with a trend toward increased number of sexual partners and increased number of orogenital partners (15). HPV-related head- and neck tumours differ from the traditional tumours in the region and are associated with improved response to therapy and longer life-span. The HPV-positive tumours are more sensitive for radiotherapy than the HPV-negative tumours, probably based on intact p53 protein (16). Despite the numerous differences in HPV-related and non HPV-related head- and neck tumours, currently there is no specific algorithm to treat HPV-related head- and neck tumours. However, it has been suggested to minimize the intensity of the radiotherapy treatment due to the better response to radiotherapy. In head- and neck cancer hypoxia seems to play an important role. Hypoxia is the result of an imbalance between oxygen delivery and oxygen consumption, and hypoxic areas are a common feature in solid tumours. Hypoxia activates various signalling pathways that enhance the survival of tumour cells (e.g., HIF-1 and AKT) and is, therefore, an inherent negative factor for outcome. Hypoxia induces stabilization of the transcription factor HIF-1alpha (HIF-1α), associated with (chemo)radiotherapy resistance in oropharyngeal SCC. HIF-1α protein overexpression is associated with worse overall survival in oropharyngeal SCC, especially in the HPV-positive tumours (17).

Radiotherapy in vulvar cancer is used adjuvant in case of positive groin nodes, in case of close/positive surgical margins, and in combination with chemotherapy in locally advanced vulvar SCC. In line with HPV-related head- and neck tumours, HPV-related vulvar SCC may benefit from treatment with radiotherapy. We need to investigate the effect of radiotherapy in vulvar SCC further. Recently, the worldwide multicenter study (Groningen International Study on Sentinel nodes in Vulvar cancer (GROINSS-V) II) has been closed awaiting for final results; the effect of only radiotherapy after positive sentinel lymph nodes in patients with early stage vulvar SCC is the main aim of the study. Furthermore, a study on chemoradiation in vulvar SCC is ongoing in patients with primary or recurrent vulvar SCC with locally advanced disease not curable with surgery unless extensive reconstructive surgery. We suggest to investigate the role of HPV status of all primary and recurrent vulvar SCCs of these two studies in relation to the efficacy of radiotherapy between non HPV-related and HPV-related vulvar SCC. Assessing the role of hypoxia in vulvar SCC could give more insight in the difference in prognosis between HPV-related and non HPV-related vulvar SCC. This may ultimately contribute to better personalized cancer care.

Furthermore, is surgery of inguinofemoral lymph nodes always necessary in case of a small vulvar SCC located on the perineum? What do we know about small anal tumours, which are often located close to the perineum? In other words, are there similarities between these two tumours? It might be argued that the lymph drainage from the perineum differs from the other tumour localizations
of the vulva and is more comparable to anal cancer? There are no studies on this topic.

Anal cancer can be divided into anal canal cancer and anal margin cancer, where anal margin cancer is often SCC of the skin around the anus and represents one-fourth to one-third of all SCC of the anus. Tumours are HPV-related in 70-80% of the cases. The incidence of metastatic lymph nodes is related to tumour size with 0% in tumours less than 2 cm, 23% of tumours 2 to 5 cm and 67% of tumours greater than 5 cm (18). Tumours that are limited to the anal margin are treated similar to cutaneous SCC elsewhere on the body with wide local excision (WLE). Only for patients with larger tumours, nodal involvement, or invasion of the sphincter muscle, treatment with chemoradiation is the appropriate treatment. In these cases inguinalfemoral nodes should always be part of the irradiation field (19). As in vulvar SCC, inguinalfemoral groin node status is an important prognostic factor in anal cancer (20). Small anal margin SCC is treated like cutaneous SCC, so what is the reason to treat all vulvar SCC with >1 mm invasion depth with WLE and SLN procedure? In vulvar cancer untreated positive groin nodes/groin recurrences decreases the prognosis significantly. Since the perineum is so close to the anal margin it could be hypothesized that lymph drainage is comparable. Before we can change the surgical treatment of perineal SCC, we should assess the percentage of groin node metastases in all perineal SCCs < 2 cm, between 2-5 cm, and larger than 5 cm. In vulvar SCC invasion depth of the tumour plays an important role in treatment decision, so this tumour characteristic should definitely be measured next to tumour diameter. Based on these results, small perineal vulvar SCCs could possibly be treated with only WLE like cutaneous SCC and anal margin SCC and diminish disease burden and treatment morbidity.

2. HPV IN RENAL TRANSPLANT RECIPIENTS: HOW TO OPTIMIZE SCREENING AND VACCINATION

In this thesis we demonstrated an increased cervical HPV prevalence in patients before and after renal transplantation (Chapter 5). All these patients use immunosuppressants: drugs that lower the body's ability to reject a transplanted organ. We suggested that the start of immunosuppressants after renal transplantation results in re-activation of latent HPV infection and not by acquiring new infections. Remarkably, there was also a higher cervical HPV prevalence found in female patients with end stage renal disease before transplantation compared to women in the general population in the same age group. A likely explanation is that (some) patients with end stage renal disease are already immunocompromised based on either their underlying kidney disease and/or immunosuppressive medication. Due to short follow-up time, the persistence of the hrHPV infections after transplantation could not be established, nor could an increase in incidence of genital (pre)malignancies be observed. However, the results explain the increased risk of HPV-related cervical- and vulvar (pre)malignancies in women after renal transplantation.
SCREEnING
Knowing that HPV infections may contribute to the increased incidence of HPV-related (pre)malignancies in RTRs, the question arises how cervical screening could help to reduce the incidence of HPV-related genital malignancies.

International guidelines suggest cervical screening after renal transplantation (21-23) to be performed annually. However, the level of evidence for this high frequency of screening is low. Patients with HIV are screened annually and twice in the first year after diagnosis (24), but they differ from transplant patients with respect to the degree of immunosuppression and the risk of acquiring genital HPV infections. Does the literature provide us with information on cervical screening in immunocompromised patients, other than HIV patients? Patients with systemic inflammatory disease, such as inflammatory bowel disease (IBD), rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), who are also treated with immunosuppressive drugs, have an increased risk of cervical dysplasia as well (25-30). Of these patient groups, especially patients with SLE are at higher risk. A meta-analysis by Zard et al. (31) showed an increased risk of high grade cervical squamous intraepithelial lesions for patients with SLE with a cumulative odds ratio of 8.6. There is currently no international consensus on the frequency of screening in these patient groups.

Even in the general population there is no international consensus on frequency and target age group in cervical screening (Table 1) (32).

<table>
<thead>
<tr>
<th>Country</th>
<th>Target range (in years)</th>
<th>Screening interval in years</th>
<th>Smears per women lifelong</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belgium</td>
<td>25-64</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>Denmark</td>
<td>23-59</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>Finland</td>
<td>30-60</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>France</td>
<td>25-65</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>Germany</td>
<td>≥ 20</td>
<td>1</td>
<td>50+</td>
</tr>
<tr>
<td>Italy</td>
<td>25-64</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>Netherlands</td>
<td>30-60</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Sweden</td>
<td>20-59</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>UK (England)</td>
<td>20-65</td>
<td>3 or 5</td>
<td>16-10</td>
</tr>
<tr>
<td>Australia</td>
<td>18-70</td>
<td>2</td>
<td>43</td>
</tr>
<tr>
<td>USA</td>
<td>21-65</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>Canada</td>
<td>30-69</td>
<td>3</td>
<td>14</td>
</tr>
</tbody>
</table>
During recent years, the Dutch well-organized screening programme invited women between 30-60 years every 5 years and screening was based on cytological examination of PAP smears. The choices for this target range and screening interval were based on cost-effectiveness analyses. High-risk (hr) HPV tests have a higher sensitivity in detecting CIN 2/3 than cervical screening by PAP smear (~90% versus ~60%) and screening based on hrHPV tests affords better protection against cervical cancer (32-34). Therefore, it was decided to introduce the hrHPV test as primary screening tool in the general population in the Netherlands from 2017 (35). This means that the primary triage is an hrHPV test; only in case of positive hrHPV test, cytologic examination of the smear will be performed. The changes in the Dutch screening programme are displayed in Table 2.

**Table 2. Changes in the Dutch screening programme**

<table>
<thead>
<tr>
<th></th>
<th>Old screening programme</th>
<th>New screening programme (start 2017)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invitation by</td>
<td>General practitioner/screening organization</td>
<td>screening organization</td>
</tr>
<tr>
<td>Screening test</td>
<td>Cytology</td>
<td>hrHPV-test</td>
</tr>
<tr>
<td>Target range</td>
<td>30 – 60 years</td>
<td>30 – 60 years</td>
</tr>
<tr>
<td>Age at invitation (years)</td>
<td>30, 35, 40, 45, 50, 55, 60</td>
<td>30, 35, 40, 50, 60 (45, 55, 65 in case hrHPV positive in previous round)</td>
</tr>
<tr>
<td>Number of screening rounds</td>
<td>7</td>
<td>min. 5 - max. 8</td>
</tr>
</tbody>
</table>

Like for the cytological screening, it is challenging to give an evidence based advise on the optimal strategy for screening of female RTRs with the hrHPV-test. The Dutch guideline cervical intraepithelial neoplasia (CIN), which includes screening based on hrHPV testing, does not recommend more frequent screening in female RTRs, but is this justified? In our view, the following aspects of screening should be considered in more detail based on the results presented in this thesis: pretransplantation screening, the target age range of screening, and the optimal screening interval.

**PRETRANSPLANTATION SCREENING**

Meeuwis et al. (36) showed that there are two peaks in the incidence of genital (pre)malignancies after transplantation: either shortly (<three years) or >8-10 years after transplantation. It is likely that the first peak is explained by abnormalities already present at the time of transplantation since a large number of the female RTRS in this study never participated in cervical screening. Fairley et al. (37) showed an overall HPV prevalence of 20% in dialysis patients. Next to a higher HPV prevalence, it has been shown that dialysis patients have an increased risk of cancer, including cervical- and vulvovaginal cancer (38). In our study, 10% of the female renal transplant candidates
had an abnormal cervical smear which in half of the cases was a HSIL, and two patients newly developed HSIL within two years after transplantation. In addition, the risk of HPV reactivation due to immunosuppressive therapy will be highest during the first year after transplantation because of more intense immunosuppression. We therefore think a recent screening test (participation in national screening programme is sufficient) should be hrHPV negative or hrHPV positive with normal cytology before patients can be listed for a cadaveric donor transplantation, or be planned for a living donor transplantation.

**AGE RANGE OF SCREENING**

In the national programme screening starts at the age of 30 years and continues until the age of 60 years. In the general population cervical cytological abnormalities are common at young age, but regress in almost all cases. Therefore and to prevent overtreatment, screening is not indicated before the age of 30 years in the general population. However, in female RTRs <30 years of age the chance of progression of cytological abnormalities is higher and the rate of spontaneous regression of CIN lesions is lower than in the general population (39). Therefore, we suggest to start the screening of female RTRs at 25 years of age, provided that women are sexually active. We chose 25 years, because in the general population the incidence rates of cervical cancer start around the early twenties. Below the age of 25 years, cervical cancer is hardly diagnosed. The national screening programme screens females until the age of 60 years. Our studies did not provide information on the behaviour of HPV infections or the development of genital (pre)malignancies in female RTRs >60 years of age. Furthermore, no other studies investigated this specific group of patients in extent. These advises only comprise screening, but in case of complaints (abnormal vaginal bleeding) gynaecological examination should be performed despite the age of the woman.

**FREQUENCY OF SCREENING**

It is known that female RTRs have an increased risk of developing HPV-related malignancies (increased standardized incidence ratios between 1.0-2.5 for cervical cancer and 7.3-23.9 for vulvar/vaginal cancer), but we do not know how long it takes for a (pre)malignancy to develop in female RTRs. The data in Chapter 5 of this thesis show that latent HPV infections are re-activated after transplantation, but do not provide information on the persistence of these infections nor on the time course of developing genital (pre)malignancies.

To properly advise on the frequency of screening, more research into these issues needs to be done. A first step would be to extend the follow up of the study population from Chapter 5. We could assess HPV status, genotype, and the presence of cytological abnormalities 5 years after renal transplantation (RT). This would give us information on the persistence of the HPV infection and if persistence is established, the effect on developing genital (pre)malignancies. Nevertheless, due to the fact that female RTRs are lifelong treated with immunosuppressive drugs we should consider a more aggressive course of (pre) malignant HPV-induced malignancies. Moreover, the mere presence of a renal graft in the pelvic region may hamper adequate treatment of different stages of cervical cancer: full pelvic lymphadenectomy is technically impossible while radiotherapy of the pelvis will result in dysfunction of the transplanted kidney. Based on these considerations we
propose to limit the maximum time interval between two screening tests to 5 years, which deviates from the national programme for women above 40 years of age (Table 2).

Taken together, there are no solid data to support annual cervical screening of female RTRs. On the other hand, the screening programme for the general population does not take into account some issues that are specific for this population. Until we have the results of our follow up studies, we would like to give the following recommendations concerning the cervical screening in female RTRs:

- All female RTRs should be strongly advised to take part in the national screening programme.
  Any abnormal test result should be evaluated according to the guidelines in this programme;
- A screening test should be performed before transplantation in all women between 30 and 60 years of age according to national screening programme;
- The starting age of screening after transplantation should be lowered from 30 to 25 years in case of sexual activity;
- The interval between screenings test should be maximized to 5 years (no 10 year intervals in female RTRs in case of hrHPV negativity and normal cytology).

The Dutch society for Organ Transplantation and the Dutch society for Obstetrics and Gynaecology should cooperate on potential amendments to the Dutch CIN guideline for organ transplant recipients. Ideally, a national consensus should be reached on gynaecological screening in all immunocomprised patients.

IMPLEMENTATION OF SCREENING – PATIENTS AS PARTNERS

Meeuwis et al. (40) showed that the actual screening frequency in female RTRs was 0.2 cervical smears per year, while 30% never got a cervical smear after RT at all. A major step forward would be to reach the female RTRs that do not participate in screening at all (35% of all invited women), because we know that 50% of all women with cervical cancer (in the whole Dutch population) never participated in screening (41). An HPV self-test, which provided an increase in participation of 10% in the general population and is now offered to non-responders in the national screening programme, might strongly improve the participation rate of non-participating female RTRs. Furthermore, screening participation can be increased by educating the female RTRs on the risks and symptoms of genital (pre)malignancies. Renal transplant patients receive a lot of information at once when they are discharged after their renal transplant surgery. The female RTRs in the focus groups of Chapter 6 suggested to repeat the information on gynaecological screening approximately 6-12 months after transplantation and to develop a checklist with all the necessary examinations during follow-up after transplantation. Female RTRs stated that the repetition of the information is more important than the manner of information supply. The following patient factors are known to be associated with a lower participation rate in cervical screening programmes: higher age, associated morbidity, lower income, and long-term care residence (42). Efforts to increase the participation rate should therefore be targeted on patients with these characteristics. The general practitioner should be notified that participation to the screening programme is mandatory in these patients and an extra reminder might be necessary. Several different decision aids, for example paper-
based information booklets, audio books, videodiscs, CD-ROM programmes, and internet sites, are developed to enable patients to make an informed decision about diagnostic and therapeutic procedures. A successful decision-making procedure will enhance patient autonomy (43, 44). Optimizing the contents and timing of education on the risks of HPV-related genital abnormalities could increase the participation in gynecological screening. In the upcoming years, the development of an app or roadmap with all the recommended post-transplantation check-ups could enhance patient autonomy and participation rate.

VACCINATION

Trial results showed high efficacy in the general population of the quadrivalent (HPV genotypes 16, 18, 6, and 11) and bivalent (HPV genotypes 16 and 18) prophylactic HPV vaccines (>90%) against the targeted HPV infection, CIN and vulvar HSIL lesions, and genital warts (in case of quadrivalent vaccine) (45). Since 2015, a third, nonavalent HPV vaccine has been developed to protect against an additional five oncogenic types (HPV genotypes 31, 33, 45, 52, and 58) and showed an overall vaccine efficacy of 96.7% in women aged 16-29 years in the general population (46). This vaccine also gives more protection against other anogenital HPV-related (pre)malignancies such as vaginal-, vulvar-, and anal (pre)malignancies. To date, there are only a few studies conducted with the quadrivalent HPV vaccine in immunocompromised patients. Nelson et al. (47) showed a good response to the quadrivalent HPV vaccine in a group of girls and young women (9-21 years of age) with chronic kidney disease. Similar responses were observed in adults on dialysis and with late-stage chronic kidney disease (48). No serious vaccine-related adverse events were observed. However, Nelson et al. (47) also showed a less robust response to the vaccine among those girls and women with a kidney transplant with only about 50-75% of patients achieving seropositivity. On the contrary, two other studies on HPV vaccination in immunocompromised adolescents (after solid organ transplantation, stem cell transplantation, and auto-immune disorders) showed an adequate immune response to the quadrivalent HPV vaccine with seroconversion to all four HPV genotypes (49, 50). In adult transplant recipients, however, suboptimal immunogenicity of the quadrivalent HPV vaccine was observed (51). Vaccinating female patients with end-stage renal disease (ESRD) before they undergo transplantation might diminish the prevalence of hrHPV infections and prevent development of genital (pre)malignancies after transplantation. Meeuwis et al. (52) as well as Chapter 5 of this thesis, showed a broad spectrum of HPV genotypes in female RTRs, which would not be covered by the existing quadrivalent HPV vaccine. Consequently, patients with ESRD should be vaccinated with the nonavalent vaccine to optimize the coverage. There is a need to investigate the efficacy of the nonavalent vaccine in female patients with ESRD and in female RTRs. The design of such a study should include serological and cervical monitoring of HPV infection before and after RT. Moreover the influence of various immunosuppressive drugs on the efficacy of vaccination should be addressed. Introduction of HPV vaccination in female patients with ESRD does not imply that cervical screening becomes unnecessary. These women need to be screened, as do the women in the general population.
3. **FINAL CONSIDERATIONS**

A new entity in vulvar SCC could be diagnosed based on localization of the tumour and HPV-relationship. Further research should bring us more insight in the influence of these two features of the tumours on the current treatment guidelines in vulvar SCC. In the management of HPV-related genital (pre)malignancies in RTRs the main focus should be on encouraging patients to participate in the national cervical screening programme. Further research on persistence of HPV infections, incidence of genital (pre)malignancies, and the role of vaccination in female RTRs and other immunocomprised patients should be conducted to develop a well designed screening programme for all immunocomprised patients.


SUMMARY / SAMENVATTING
SUMMARY

CHAPTER 1
Most common lower genital (pre)malignancies arise from the cervix and the vulva. The oncogenesis of cervical (pre)malignancies is extensively researched and an infection with high-risk Human Papillomavirus (hrHPV) is an essential step in the development of cervical cancer. Vulvar cancer arises from two different pathways: a non HPV-related (70%) and an HPV-related pathway (30%). In vulvar cancer the exact role of HPV in the oncogenesis is unclear. Patients who use immunosuppressants have an increased risk for HPV infections. The largest immunocomprised patient groups are HIV-infected patients and organ transplant recipients. The renal transplant recipients (RTRs) are the subject of this thesis. Studies have shown that RTRs have an increased risk of cervical- and vulvar (pre)malignancies due to an increased prevalence of HPV.

To be able to get more insight in the oncogenesis of vulvar cancer in the general population and to explore the behaviour of HPV and the screening possibilities in female RTRs, the following aims are composed in this thesis:

1. To assess the influence of tumour localization in vulvar cancer on the prognosis (Chapter 2).
2. To define the predilection site and prognosis of HPV-related vulvar cancer on the vulva compared to non HPV-related vulvar cancer (Chapter 3).
3. To establish the HPV prevalence before and after renal transplantation in females with an end-stage renal disease (Chapter 5).
4. To determine the barriers and facilitators for annual gynaecological screening in female RTRs from patients’ and professionals’ perspective (Chapter 6).
5. To assess the experience of female RTRs with and the clinical applicability of HPV self-sampling in gynaecological screening (Chapter 7).

CHAPTER 2
The overall 5-year survival of vulvar squamous cell carcinoma (SCC) is 70%. The aim of this study was to investigate if clitoral involvement of the tumour led to a worse prognosis. We performed a retrospective study with all consecutive patients with primary vulvar SCC treated with surgery. In total 347 patients were included in the analysis: 72 patients had a tumour with clitoral involvement and 275 patients had a tumour without clitoral involvement. We found that patients with a clitoral SCC more often had larger and deeper invaded tumours, lymphovascular space involvement (LVSI), positive surgical margins and a higher percentage of positive lymph nodes. The 5- and 10- year DSS rates of the clitoral involvement group were lower than in the non clitoral involvement group. We could not confirm clitoral involvement as an independent risk factor, however, the clinical- and histopathological characteristics of the tumours with clitoral involvement were independent risk factors for a worse prognosis. It is important to recognize and quickly refer patients with vulvar tumours with clitoral involvement to prevent diagnostic delay. The next chapter describes the role of tumour localization of vulvar SCC in more extent.
CHAPTER 3
There are two etiologic pathways for vulvar SCC. The first occurs most frequently and presents mainly in elderly women, often in a background of lichen sclerosus and/or differentiated vulvar intraepithelial neoplasia (dVIN). The second is related to hrHPV infection with its precursor lesion high grade squamous intraepithelial lesion (HSIL) of the vulva, formerly usual vulvar intraepithelial neoplasia. Although a clear relation is established, the precise role of HPV in the oncogenesis of vulvar SCC remains unknown. Our hypothesis is that micro-trauma on the perineum facilitate access of HPV to the cell as a first step in the oncogenesis of HPV-related vulvar SCC. The aim of this study was to assess the predilection site and survival of HPV-related vulvar SCCs compared to non HPV-related vulvar SCCs. All consecutive patients with primary vulvar SCC between March 1988 and January 2015 were analyzed. All available histological specimen were tested on HPV with the SPF10-LiPA25 system assay and p16INK4a staining was performed using CINtec® histology kit. Vulvar SCCS were considered HPV-related in case of either >25% p16INK4a expression and HPV positive or >25% p16INK4a expression, HPV negative, and HSIL next to the tumour. The tumour localization, disease specific survival (DSS), disease free survival (DFS) and overall survival (OS) of patients with HPV-related and non HPV-related vulvar SCC were compared. In total 318 patients were included: 55 (17%) patients had an HPV-related vulvar SCC with 93% hrHPV positive tumours (Group 1) and 263 (83%) patients had a non HPV-related vulvar SCC with 92% hrHPV negative tumours (Group 2). The tumours in Group 1 were significantly more often located at the perineum compared to Group 2, respectively 30% compared to 14%. Furthermore, there was significantly better DSS, DFS and OS in the HPV-related vulvar SCC patients. All patients with tumours with perineal involvement had better survival compared to the patients with tumours without perineal involvement. Comparing the HPV-related with perineal involvement (N = 20) to the non HPV-related vulvar SCCs with perineal involvement (N = 35) showed significantly better survival rates for the HPV-related vulvar SCCs. HPV-related vulvar SCCs are more frequently located at the perineum and have a favourable prognosis compared to non HPV-related vulvar SCCs. Both localization of the tumour and the HPV-related pathway could explain the favourable prognosis. HPV-related vulvar malignancies seem to be a separate entity within vulvar SCC.

CHAPTER 4
Chapter 4 is a review about HPV-related (pre)malignancies of the female anogenital tract in RTRs. Renal transplantation and the risk for developing malignancies in general are discussed. Furthermore, HPV, cervical-, vulvar-, and anal (pre)malignancies in general and specified in female RTRs are extensively described.

CHAPTER 5
We designed a prospective study to assess the HPV prevalence before and after renal transplantation. Female patients with end-stage renal disease (ESRD), who were judged whether they were appropriate candidates for renal transplantation (RT), were invited to participate in this study. In total 123 patients participated in the study. All patients underwent gynaecological examination, including cervical/vaginal cytology and HPV test, at first contact, after 1- and 2 years.
The patient was asked to self collect a cervico-vaginal sample with a collection device at the first visit and at home every three months. Furthermore, patients filled out questionnaires on relations/sexual behaviour at start of the study and with every self-sample moment. HPV detection was performed by a sensitive test, the SPF10-LiPA25 system, optimally assessing genital prevalence and a clinically validated test, COBAS 4800, assessing the relevant infections at that time point. In total 65 patients were transplanted. hrHPV prevalence assessed with the sensitive test significantly increased from 19% before to 31% after RT. Using the clinically validated test, hrHPV prevalence increased from 10% before to 14% after. Eight patients (12.3%) had cytological abnormalities: five high-grade lesions. No relevant changes in sexual behaviour were reported. Thirty-three patients, not undergoing RT, showed a hrHPV prevalence of 21% at study entry and 27% after 12 months with the sensitive test. With the clinically validated test this value remained stable at 16%. In this cohort of patients with end-stage renal disease, the hrHPV prevalence was higher than the age-specific hrHPV prevalence in the general population, before as well as after the transplantation. The increase in hrHPV prevalence after transplantation, without any change in sexual behaviour, suggests activation of latent HPV infections during immunosuppression. The activation of latent HPV infections could contribute to the increased risk of HPV-related cervical- and vulvar (pre)malignancies in female RTRs.

CHAPTER 6
Several national and international guidelines recommend annual cervical screening in female RTRs. Gynaecological examination should definitely also include inspection of the vulvar area, but no recommendations are specified. The participation rate in screening is low in female RTRs. The reasons for not participating in screening are unclear. In this chapter the barriers and facilitators for gynaecological screening were assessed both from a female RTRs’ as a nephrologists’ perspective. We conducted focus group interviews and divided the mentioned barriers and facilitators according to four influencing factors: patient, professional, organisation, and finance. Both nephrologists and female RTRs mentioned similar barriers: uncomfortable and less reliable examination by a general practitioner (GP) compared to examination by a gynaecologist, limited knowledge of professionals, and limited information supply to patients on increased risk for developing HPV-related genital (pre)malignancies. However, female RTRs focused more on unpleasantness of the examination itself. Total agreement between female RTRs and nephrologists was found on the facilitators: a reminder for annual gynaecological screening, a checklist for all the examinations the female RTRs should undergo in which timeframe, integration of gynaecological examination in annual check-up, HPV self-sampling and information supply at the right moment. We recommended that every female RTR should receive a small checklist one year after transplantation. Furthermore, HPV self-sampling in the annual check-up by the nephrologist (with referral to the gynaecologist in case of HPV positivity) would obviate a lot of barriers and meets the main facilitators. Implementing these changes might improve the participation rate of female RTRs in gynaecological screening.
CHAPTER 7
In this chapter we explore the role of HPV self-sampling in gynaecological screening. HPV self-sampling has shown to reach non-participants in cervical screening in the general population. However, no research has been done to assess the clinical applicability of HPV testing on self-sampling material in female RTRs. The aim of this study is to investigate whether HPV self-sampling is suitable for gynaecological screening of RTRs. A large cohort of 253 RTRs was investigated for the prevalence of HPV. All participants received a device for a cervico-vaginal self-sample. Furthermore, questionnaires were sent to assess the experience with this device. High risk (hr)HPV presence was determined with the SPF$_{10}$-LiPA$_{25}$ system and when patients tested hrHPV positive, gynaecological examination was performed. The hrHPV positive samples were retested with the GP5+/6+ PCR to assess the clinical applicability of HPV self-sampling in female RTRs. More than 90% of the patients rated their experience with self-sampling device as good to excellent and 77% preferred self-sampling over a physician taken sample. Thirty-five of 217 women (16%) tested hrHPV positive with SPF$_{10}$-LiPA$_{25}$, and 22 tested positive with the GP5+/6+ PCR. Eleven of the 35 hrHPV-positive patients (31%) had clinically relevant gynecological abnormalities, and they all tested positive with GP5+/6+ PCR. Eleven hrHPV positive patients had clinically relevant gynaecological abnormalities and they all tested positive with GP5+/6+ PCR. Self-sampling could be applicable in gynaecological screening and is preferred by female RTRs. Therefore, self-sampling could be implemented with the aim to increase the participation rate of female RTRs in yearly gynaecological screening.
SAMENVATTING

HOOFDSTUK 1
De meest voorkomende genitale (pre)maligniteiten bij vrouwen ontstaan in het gebied van de cervix en de vulva. De oncogenese van cervicale (pre)maligniteiten is al uitgebreid onderzocht: een infectie met het hoog risico Humaan Papillomavirus (hrHPV) is een essentiële stap in de ontwikkeling tot een cervix carcinoom. Bij het plaveiselcel carcinoom (PCC) van de vulva zijn twee verschillende ontstaanswijzen beschreven: een niet HPV-gerelateerde (70%) en een HPV-gerelateerde (30%) ontstaanswijze, waarbij deze laatste gelijkenissen vertoont met het ontstaan van cervixcarcinoom. Patiënten die immuunsuppressiva gebruiken hebben een verhoogd risico op het krijgen van een HPV infectie. Patiënten met HIV en ontvangers van een orgaantransplantaat zijn de grootste groep immuungecompromiteerde patiënten. Verschillende studies tonen aan dat niertransplantatiepatiënten een groter risico hebben op het ontwikkelen van cervicale – en vulvaire (pre)maligniteiten als gevolg van een verhoogde prevalentie van HPV infecties. Het huidige proefschrift gaat met name over de vrouwelijke niertransplantatiepatiënten omdat deze patiëntengroep steeds frequenter met HPV-gerelateerde problematiek gezien wordt op de polikliniek Gynaecologie. Om meer inzicht te krijgen in de oncogenese van het vulvacarcinoom in de algemene populatie en om het biologisch gedrag van HPV en screeningmogelijkheden bij niertransplantatiepatiënten te onderzoeken, zijn de volgende onderzoeksvragen geformuleerd:

1. Wat is de invloed van tumorlokalisatie op de prognose van het vulvacarcinoom? (Hoofdstuk 2)
2. Wat is de voorkeurslokalisatie en prognose van het HPV gerelateerde vulvacarcinoom vergeleken met het niet HPV gerelateerde vulvacarcinoom? (Hoofdstuk 3)
3. Hoe verhoudt de HPV prevalentie zich voor en na niertransplantatie bij vrouwen met eind stadium nierziekte? (Hoofdstuk 5)
4. Welke factoren beïnvloeden de deelname aan gynaecologische screening van vrouwelijke niertransplantatiepatiënten vanuit het perspectief van patiënten en professionals? (Hoofdstuk 6)
5. Wat is de ervaring van vrouwelijke niertransplantatiepatiënten met en de klinische toepasbaarheid van de HPV zelftest bij gynaecologische screening? (Hoofdstuk 7)

HOOFDSTUK 2
De 5-jaarsoverleving van het PCC van de vulva is 70%. Vanuit de kliniek bestond het gevoel dat de tumoren in het clitorisgebied een slechtere prognose hebben. Het doel van de studie in Hoofdstuk 2 was om te onderzoeken of de lokalisatie van een tumor bij de clitoris inderdaad leidt tot een slechtere prognose. We hebben de data van alle chirurgisch behandelde patiënten met een primair PCC van de vulva retrospectief geanalyseerd. In totaal hebben we 347 patiënten geïncludeerd in de analyse: 72 patiënten hadden een clitoris tumor en 275 patiënten hadden een tumor zonder betrokkenheid van de clitoris. De resultaten lieten zien dat de clitoris tumoren vaak groter en dieper geïnvasive waren, lymfavatinvasie lieten zien, positieve snijranden en een hoger percentage positieve lymfklieren hadden. De 5- en 10-jaars overleving van de patiënten met clitoris tumoren was slechter dan in de groep zonder clitoris betrokkenheid. We konden de lokalisatie bij/op de
clitoris niet als een onafhankelijke risicofactor bevestigen, maar de klinische en histopathologische eigenschappen van deze tumoren waren wel onafhankelijke risicofactoren voor een slechtere prognose. Het is dus belangrijk om patiënten met een vulvacarcinoom met clitorisbetrokkenheid te herkennen en snel te verwijzen om vertraging in het diagnostische proces te voorkomen. In het volgende hoofdstuk komt de rol van tumor lokalisatie van het vulvacarcinoom uitgebreider aan bod.

**HOOFDSTUK 3**

Er zijn twee ontstaanswijzen beschreven voor het PCC van de vulva. De eerste komt het meest voor en voornamelijk bij oudere vrouwen tegen een achtergrond van lichen sclerosus en / of gedifferentieerde vulaire intraepitheliale neoplasie (dVIN). De tweede ontstaanswijze is gerelateerd aan een hoog risico (hr) HPV infectie met hoog-graadige squameuze intraepitheliale neoplasie (HSIL) van de vulva, voorheen usual VIN, als premaligne afwijking. Het is duidelijk dat HPV een rol speelt in de oncogenese van het vulvacarcinoom, maar de precieze rol blijft onduidelijk. Onze hypothese is dat microtraumata op het perineum de toegang van HPV tot de cel faciliteren en fungeren als eerste stap in de oncogenese van het HPV-gerealiseerde vulvacarcinoom. Het doel van deze studie was om de voorkeurslokalisatie van de HPV-gerealiseerde tumor te bepalen in vergelijking met de niet HPV-gerealiseerde tumoren. Daarnaast werd de prognose vergeleken tussen patiënten met een HPV-gerealiseerd en een niet HPV-gerealiseerd vulvacarcinoom. Alle patiënten, die chirurgisch behandeld werden voor een primair PCC van de vulva in de periode maart 1988 en januari 2015 werden geanalyseerd. Al het beschikbare histologische materiaal werd getest op HPV, zowel hoog als laag risico typen, met de SPF10-LiPA25-systeem assay en er werd een p16INK4a-kleuring uitgevoerd met behulp van CINtec® histologie kit. Vulaire tumoren werden beschouwd als HPV-gerelateerd in het geval van >25% p16INK4a expressie en hrHPV positiviteit of in het geval van >25% p16INK4a expressie, afwezigheid van hrHPV en HSIL naast de tumor. De tumor lokalisatie bij en de prognose van patiënten met HPV-gerealiseerde en niet HPV-gerealiseerde PCC van de vulva werden vergeleken. Er werden in totaal 318 patiënten geanalyseerd: 55 (17%) patiënten hadden een HPV-gerealiseerd vulvacarcinoom (Groep 1) en 263 (83%) patiënten hadden een niet HPV-gerealiseerd vulvacarcinoom (Groep 2). De tumoren in Groep 1 waren significant vaker op het perineum gelokaliseerd in vergelijking met Groep 2, respectievelijk 30% ten opzichte van 14%. Bovendien hadden patiënten met een HPV-gerealiseerd vulvacarcinoom een aanzienlijk betere overleving. Alle patiënten met tumoren op het perineum hadden een betere overleving ten opzichte van de patiënten met tumoren zonder perineale betrokkenheid. Het vergelijken van de HPV-gerealiseerde tumoren met perineale betrokkenheid (N = 20) met de niet HPV-gerealiseerde tumoren met perineale betrokkenheid (N = 35) toonde significant betere overlevingscijfers in de HPV-gerealiseerde groep. HPV-gerealiseerde PCC van de vulva komen vaker voor op het perineum en hebben een gunstigere prognose in vergelijking met niet HPV-gerealiseerde PCC van de vulva. Zowel de lokalisatie van de tumor als de HPV-gerealiseerde ontstaanswijze kan de gunstige prognose verklaren. HPV-gerealiseerde vulva tumoren lijken een aparte entiteit te zijn binnen het vulvacarcinoom. Verder onderzoek zal moeten uitwijzen of er plaats is voor alternatieve behandelingsopties voor HPV-gerealiseerde vulvacarcinomen.
HOOFDSTUK 4
Hoofdstuk 4 is een review welke een duidelijk overzicht van HPV-gerelateerde (pre)maligniteiten van het anogenitale gebied bij vrouwelijke niertransplantatiepatiënten weergeeft. In dit hoofdstuk worden niertransplantatie en het risico op het ontwikkelen van maligniteiten besproken. Daarnaast komen HPV-gerelateerde cervicale-, vulvaire- en anale (pre)maligniteiten in de normale populatie en bij vrouwelijke niertransplantatiepatiënten uitgebreid aan bod.

HOOFDSTUK 5
Om de HPV prevalentie vóór en na niertransplantatie te bepalen, hebben we een prospectieve studie opgezet die in Hoofdstuk 5 worden weergegeven. Vrouwelijke patiënten met eindstadium nierziekte, die op de polikliniek Nefrologie onderzocht werden op geschiktheid voor een niertransplantatie, werden uitgenodigd om deel te nemen aan onze studie. In totaal zijn er 123 patiënten geïncludeerd in de studie. Alle patiënten ondergingen gynaecologisch onderzoek, inclusief cervicale / vaginale cytologie en een HPV test, die 1 en 2 jaar na het eerste contact werden herhaald. De patiënt werd gevraagd om zowel bij het eerste bezoek als thuis om de drie maanden, een cervico-vaginale uitstrijkje af te nemen met een zelftest. Bij de start van de studie en bij elk zelfafname moment hebben patiënten vragenlijsten over hun relaties / sekseueel gedrag ingevuld. De aanwezigheid van HPV werd bepaald door enerzijds een gevoelige test, het SPF10-liPA 25 -systeem, waarbij hele kleine hoeveelheden van zowel laag risico als hoog risico HPV typen ontdekt konden worden en anderzijds een klinisch gevalideerde test, COBAS 4800, waarbij alleen de klinisch relevante hrHPV infecties op dat moment werden beoordeeld. In totaal ondergingen 65 patiënten een niertransplantatie. De hrHPV prevalentie bepaald met de gevoelige test was aanzienlijk gestegen van 19% vóór tot 31% na de niertransplantatie. De hrHPV prevalentie bepaald met de klinisch gevalideerde test was gestegen van 10% naar 14%. Acht patiënten (12,3%) hadden cytologische afwijkingen waarvan vijf hoog-gradige afwijkingen. Er werden geen relevante veranderingen in seksueel gedrag gemeld. Drieëndertig patiënten, die geen niertransplantatie ondergingen, hadden een hrHPV prevalentie van 21% bij de start van de studie en van 27% na 12 maanden (gemeten met de gevoelige test). Met de klinisch gevalideerde test bleef deze waarde stabiel op 16%. Opvallend is dat in dit cohort van patiënten met eindstadium nierziekte de prevalentie van de hrHPV infecties hoger was dan de leeftijdsspecifieke hrHPV prevalentie in de algemene populatie, zowel vóór als ook na de transplantatie. De stijging van de hrHPV prevalentie na transplantatie, zonder enige verandering in seksueel gedrag, suggereert reactivatie van latente hrHPV infecties tijdens gebruik van immunosuppressieve medicatie. De activering van latente hrHPV infecties kan bijdragen aan het verhoogde risico op HPV-gerelateerde cervicale- en vulvaire (pre)maligniteiten bij vrouwelijke niertransplantatiepatiënten.

HOOFDSTUK 6
Vanwege het verhoogde risico op het ontwikkelen van HPV-gerelateerde genitale (pre) maligniteiten adviseren verschillende nationale en internationale richtlijnen vrouwelijke niertransplantatiepatiënten om jaarlijks cervicale screening te laten verrichten. Het percentage vrouwelijke niertransplantatiepatiënten dat deelneemt aan de screening is relatief laag; slechts
70% van de vrouwen laat screening uitvoeren na niertransplantatie. De redenen om niet aan deze screening deel te nemen zijn onduidelijk. In Hoofdstuk 6 werden de factoren die de deelname aan gynaecologische screening beïnvloeden vanuit het perspectief van zowel vrouwelijke niertransplantatiepatiënten als nefrologen belicht. We organiseerden focusgroep interviews en deelden de factoren van invloed in vier categorieën: patiëntgebonden, professional, organisatie en financieel. Zowel nefrologen als vrouwelijke niertransplantatiepatiënten noemden dezelfde factoren die de deelname aan screening negatief beïnvloeden: het gynaecologisch onderzoek wordt pijnlijker ervaren wanneer uitgevoerd door een huisarts en minder betrouwbaar beschouwd dan wanneer het uitgevoerd wordt door een gynaecoloog, de beperkte kennis van professionals over en beperkte informatievoorziening aan patiënten met een verhoogd risico op het ontwikkelen van HPV-gerelateerde (pre)maligniteiten. Echter, vrouwelijke niertransplantatiepatiënten gaven aan dat het ongemak van het onderzoek zelf de grootste belemmerende factor is. De vrouwelijke niertransplantatiepatiënten en nefrologen waren het eens wat betreft de bevorderende factoren voor deelname aan screening: een jaarlijkse oproep, een checklist voor alle onderzoeken die de vrouwelijke niertransplantatiepatiënten zouden moeten ondergaan, integratie van gynaecologisch onderzoek bij de jaarlijkse controle van de nefroloog, het gebruik van de HPV zelftest en het geven van informatie over gynaecologische screening op het juiste moment na de niertransplantatie. Op basis van deze uitkomsten adviseerden wij het meegeven van een kleine checklist aan iedere vrouwelijke niertransplantatiepatiënt een jaar na de transplantatie. Het gebruik van de HPV zelftest bij de jaarlijkse controle door de nefroloog (met verwijzing naar de gynaecoloog bij hrHPV positiviteit) zou veel belemmering wegnemen en zelfs kunnen stimuleren om deel te nemen aan de gynaecologische screening. Het toepassen van deze twee veranderingen zou in onze ogen de deelname van vrouwelijke niertransplantatiepatiënten aan gynaecologische screening substantieel kunnen verbeteren.

HOOFDSTUK 7
In dit hoofdstuk wordt de rol van de HPV zelftest bij gynaecologische screening onderzocht. In de algemene populatie heeft het aanbieden van de HPV zelftest gezorgd voor een stijging in deelname aan het bevolkingsonderzoek baarmoederhalskanker en is aangetoond dat de HPV test op zelfafname materiaal even betrouwbaar is als op materiaal afgenomen door een arts. Er is echter nog geen onderzoek gedaan naar de klinische toepasbaarheid van het testen van HPV op zelfafname materiaal bij vrouwelijke niertransplantatiepatiënten. Het doel van deze studie was om te testen of de HPV zelftest geschikt is voor gynaecologische screening van niertransplantatiepatiënten. Een eerder onderzoek uitgevoerd door onze onderzoeksgroep heeft de prevalentie van HPV van een cohort van 253 niertransplantatiepatiënten bepaald. Alle deelnemers ontvingen een brush voor het afnemen van cervico-vaginaal materiaal en een vragenlijst waarin gevraagd werd naar de ervaringen met deze HPV zelftest. Het zelfafname materiaal werd getest op de aanwezigheid van hrHPV met behulp van het SPF 10-LiPA 25-systeem, een zeer gevoelige assay. De patiënten ondergingen een gynaecologisch onderzoek wanneer zij positief testten voor HPV. Er werden 35 van de 217 patiënten (16%), die zelfafname materiaal hadden ingestuurd, hrHPV positief bevonden met het SPF 10-LiPA 25-systeem. Elf hrHPV positieve patiënten (31%) hadden een gynaecologische afwijking.
Wij hebben het HPV positieve materiaal opnieuw getest met de klinisch gevalidateerde GP5+/6+ PCR om de klinische toepasbaarheid van de HPV zelftest bij vrouwelijke niertransplantatiepatiënten te beoordelen. De resultaten van de vragenlijsten lieten zien dat meer dan 90% van de patiënten de zelftest als goed tot uitstekend beoordeelden en 77% gaf zelfs de voorkeur aan de HPV zelftest in plaats van een uitstrijkje afgenomen door een arts. Alle 11 hrHPV positieve patiënten met een afwijking werden ook hrHPV positief bevonden met de GP5+/6+ PCR. Wij concludeerden hieruit dat de HPV zelftest betrouwbaar is en de voorkeur heeft van de vrouwelijke niertransplantatiepatiënten bij gynaecologische screening. De HPV zelftest zou geïmplementeerd kunnen worden om de deelname van vrouwelijke niertransplantatiepatiënten aan de gynaecologische screening te verhogen.
APPENDIX

CURRICULUM VITAE
PUBLICATIELIJST
DANKWOORD
CURRICULUM VITAE

PUBLICATIELIJST


DANKWOORD

Het is klaar en wat is dat een heerlijk gevoel. Ik was inmiddels ook wel een ‘kantooruin fossiel’ dus het werd tijd. In dit dankwoord, vaak het meest gelezen onderdeel van het proefschrift, wil ik heel graag een aantal mensen bedanken.

Beste Leon, echt ongelooflijk hoe jij altijd met nieuwe en interessante invalshoeken aankwam tijdens ons overleg. Je hebt de gave om ons onderzoekers te inspireren om verder te kijken dan onze neus lang is. Bedankt voor je fijne begeleiding!

Beste Luuk, jouw kennis over alles wat met niertransplantatie te maken heeft, was onmisbaar bij het tot stand komen van dit proefschrift. Jouw kritische blik heeft er voor gezorgd dat de artikelen tot een hoger niveau getild werden. Bedankt voor het kijkje in de keuken van de niertransplantatie, jouw interesse in onze projecten en vlotte correcties van de artikelen.


Beste Willem, bedankt voor je altijd snelle en kritische commentaar op de verschillende stukken. Deze commentaren waren altijd verpakt in korte, maar krachtige e-mails. Ik heb ook erg genoten van onze overlegmomenten, die altijd begonnen met de verschillende blessures die we weer hadden opgelopen met hockey en tennis (en dan beweren ze dat sporten gezond is).

Zonder de deelname van de patiënten aan de verschillende studies, was er nu geen proefschrift geweest. Bij dezen wil ik graag alle vrouwen bedanken voor hun deelname.

Beste Andries, aan het begin van mijn promotietraject was jij het aanspreekpunt over alles wat met transplantatie te maken had. Ik heb erg fijn met je samengewerkt. Bedankt voor jouw laagdrempeligheid en interesse in het promotietraject en in mij persoonlijk.

Beste Michelle van Rossum, ook jij hebt aan de start van mijn promotietraject gestaan. De affiniteit met de dermatologie werd gaandeweg minder waardoor onze samenwerking afliep. Bedankt voor je hulp en belangstelling voor de voortgang gedurende mijn promotietraject.
Beste Ruud, jouw deur stond altijd open voor vragen over het onderzoek. Ik heb veel gehad aan jouw kennis over HPV en cervicale afwijkingen. Helaas heb ik je maar heel even als opleider meege- maakt, maar we komen elkaar vast nog tegen. Succes in Eindhoven.

Beste Joanna, jij bent iemand die statistiek op een begrijpelijke manier kan uitleggen en betrekken op een specifiek onderwerp. Ondanks je drukke agenda was er altijd tijd voor overleg. Bedankt voor je hulp bij de CMO aanvraag en bij alle projecten die daaruit voort vloeiden.

Lieve Annemiek, jij hebt mij geïntroduceerd in de wereld van de niertransplantatie. Er komt heel wat bij kijken voordat iemand een transplantatie kan ondergaan. Wat betreft het voorbereiden van patiënten op hun transplantatie, ben jij de spin in het web. Ik heb bewondering voor je inzet en heb erg fijn met je samengewerkt. Zonder jouw hulp bij het plannen van de studiepatiënten was het niet gelukt. Bedankt ook voor de gezelligheid!

Beste Muriël, wat heb je een werk verzet om alle HPV testen voor de studie uit te voeren. De uitslagen waren altijd vlot beschikbaar en bij onduidelijkheden nam je ruim de tijd voor uitleg. Heel erg bedankt voor de fijne samenwerking.

Beste Hans en Peter, in de laatste periode van mijn promotietraject hebben we behoorlijk wat coupes bekeken. Hans, ondanks dat de stapel met plankjes steeds hoger werd, vonden we de tijd om samen naar de coupes te kijken. Natuurlijk hoorde daar ook interessante casusbesprekingen bij. Peter, we hebben in een paar weken tijd een hoop werk verzet. Het was niet gelukt zonder jouw hulp. Allebei heel erg bedankt! De coupes moesten gesneden en gekleurd worden; medewerkers van de afdeling Pathologie bedankt voor de ondersteuning met blokjes opzoeken in het archief, opvragen bij andere ziekenhuizen en tijdens het snijden. Meike heel erg bedankt voor de gezelligheid.

Lieve (oud)tuinbewoners, wat hebben we een leuke tijd gehad met z’n allen. Ik heb het geluk gehad dat ik deel uitmaakte van twee generaties tuinbewoners. Heerlijk om alle overwinningen en tegenslagen te kunnen delen met jullie! Daarnaast waren de congressen (met Melbourne als hoogtepunt, toch Sophieke?!), weekendjes weg, etentjes, wintersport, kerstlunches en promotiefeestjes met de bijbehorende presentaties iets om naar uit te kijken. Ik ben blij dat velen van jullie collega’s zijn of worden in de toekomst. Jullie zijn stuk voor stuk toppers!

Er zijn een paar mensen die ik bij naam en toenaam wil bedanken. Allereerst de ‘vulvameisjes’. Lieve Hedwig, je was mijn begeleidster tijdens mijn wetenschappelijke stage en later collega in het JBZ. Jouw efficiëntie is jaloersmakend en je lach aanstekelijk. Ik heb veel van je geleerd. Hopelijk mogen we in de toekomst weer samenwerken. Lieve Loes, wat hebben we het gezellig gehad in de tuin, maar zeker op congressen in Parijs, Milaan en Amsterdam. Helaas geen collega’s binnen de gynaecologie, maar ik heb er geen twijfel over dat je een fantastische huisarts wordt. Lieve Kim, wat hebben we toch een mooie onderzoekslijn opgezet met de vrouwelijke niertransplantatiepatiënten. Erg bedankt voor al je hulp bij het opzetten van mijn prospectieve studie. Succes met je opleiding tot Dermatoloog en we zien elkaar zeker nog! En dan de ‘nieuwe’ generatie ‘vulvameisjes’ Michelle
van der Linden en Anne-Floor. Jullie zijn allebei waardige opvolgers (hihi) en ik wens jullie veel succes met jullie onderzoek. Ik hoorde niet helemaal bij de HPV club, maar ik heb wel veel hulp gehad van Remko, Pleun en Renee. Remko ‘de professor’, erg fijn om af en toe met je te sparren over het onderzoek. Daarnaast ga ik denk ik nooit iemand anders een frikadel XXL zien eten. Pleuntje, jouw organisatorische talent is bewonderenswaardig en jouw humor verfrissend. Succes met het afronden van jouw proefschrift. Lieve Renee, je was mijn reddende engel aan het einde van mijn onderzoekstijd. Zonder jou zat ik denk ik nu nog te pipetteren en was de afdeling Pathologie zeker overstroomd (oeps).

Financiële ondersteuning is onmisbaar voor het doen van onderzoek. De Nierstichting heeft ons project gesteund en mede daardoor is dit proefschrift tot stand gekomen.

Secretariaat Verloskunde&Gynaecologie, wat zijn jullie een gezellige club en een mooi visitekaartje voor de afdeling. Bedankt voor al jullie hulp.

Medewerkers polikliniek Verloskunde&Gynaecologie, ik voelde met meeteen op mijn gemak op de polikliniek. Eerst alleen met mijn studiepatiënten, maar later ook als arts op de Vulvapoli. Bedankt voor jullie ondersteuning, verwerken van de foto’s, maken van afspraken en het kletsen tussen de afspraken door.

Polikliniek Nefrologie, post Geel, ik was toch een beetje een vreemde eend in de bijt met mijn grijze ‘steriele bak’ op de vrijdagmiddag. Bedankt voor de gastvrijheid en behulpzaamheid.

Alle gynaecologisch oncologen, bedankt voor jullie input tijdens de onco-avonden en voor de gezelligheid tijdens de verschillende congressen.

Studenten en arts-assistenten, bedankt voor jullie hulp bij de verschillende projecten en voor het draaiende houden van de studies tijdens mijn zwangerschapsverlof.

Beste Lenno, ik wist niet dat je met de trein kon reizen met een glas bubbels in plaats van op een stapelbed. Bedankt voor je begeleiding op de vulvapoli. Ik heb erg veel van je geleerd.

Beste gynaecologen, arts-assistenten en verloskundigen uit het Jeroen Bosch Ziekenhuis, ik ben in een warm bad terecht gekomen in Den Bosch. Bedankt voor de fijne samenwerking en op naar nog een paar mooie jaren.

Lieve Floor, lieve buuf, wie had gedacht dat een vergadering van de VVE zo leuk kon zijn. We zijn he-las geen echte buufoes meer, maar zo voelt het wel. Bedankt voor de vele wandelingen in de mooie bossen in Zeist, theetjes en pannenkoeken bij theehuis Rhijnauwen. Je bent een schat.

Lieve Linde, ik vind het nog altijd jammer dat we elkaar pas ontdekt hebben tijdens het co-weekend in Rotterdam. We kunnen namelijk heerlijk samen praten, lachen en genieten van lekker eten en
drinken. Ik heb genoten van onze weekenden weg naar o.a. Haarlem, dagjes sauna, en gezellige etentjes. Dit moeten we er zeker inhouden. Je bent een lieve vriendin en ik ben blij dat jij mijn paranimf bent.

Lieve Karin, garden mattie van het eerste uur, haha. Jij bent een van die personen waarbij je aan een woord/blik genoeg hebt. Vanaf het begin in de kantoortuin hebben we erg veel lol gehad; de ‘fout’ uurtjes, onderzoekersweekenden en promotiefeestjes (echte C&W 2.0). Nu zijn we samen in opleiding en volgen er meer mooie feestjes. Fantastisch dat jij naast mij staat als paranimf (op hoge hakken natuurlijk).

Lieve Harrie en Ilse, dat ik van de ‘koude’ kant ben heb ik bij jullie nooit gemerkt. Al vanaf het begin hebben jullie mij opgenomen in de familie. Bedankt voor het oppassen op de woensdagen, zodat ik wat meer tijd had voor het onderzoek.

Lieve papa en mama, de basis van wie ik ben is door jullie gelegd. Al vroeg hebben jullie mij geleerd om verantwoordelijkheid te nemen en zelfstandig te zijn. Daarnaast was en is er nog steeds, altijd tijd voor een kopje koffie, potje tennissen en een knuffel. We zien elkaar regelmatig nu we weer in Nijmegen wonen en dat vind ik fijn. Yfke, en straks Aukje ook, genieten altijd volop van de dagen bij opa Bas en oma Liene. Ik hou van jullie!

Lieve Lotte, mijn lieve kleine grote zus. Ondanks dat je niets met geneeskunde hebt en al helemaal niet met ‘rare’ dingen aan de schaamlippen, ging je met me mee naar Lissabon voor het HPV congres en heb je zelfs gekeken naar mijn presentatie (en de presentaties na mij met hele ‘vieze’ plaatjes). Ik ben trots op wat je allemaal bereikt hebt en dat je mijn zus bent.

Ik hou van jou lieve Paul, mijn man, mijn lief en mijn mix partner. Je bent een fantastische vader voor onze dochters en zonder jou was het me niet gelukt. Ik ben gelukkig met jou.