Chapter

HPV Infection and Vulvar Cancer

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Abstract

Although the strong association between human papilloma virus (HPV) and cervical cancer has been widely demonstrated, it seems that uterine cervix cancer is not the only gynecologic malignancy induced by this pathogenic agent. It has been shown that HPV infection plays a central role in the development of vulvar cancer too, HPV 16 and 18 being the most frequently reported genotypes that might induce this kind of lesions. This aspect presents a particular importation, patients diagnosed with HPV-related vulvar cancer reporting a more favorable trend in regard with the long-term outcome. The current chapter aims to describe the pathogenesis as well as the therapeutic options and the long-term outcomes of patients in which association between HPV and vulvar cancer can be assessed.

Keywords: HPV, infection, squamous cell carcinoma, vulvar cancer, preneoplastic disease

1. Introduction

Discovering human papilloma virus (HPV) represented a crucial step in understanding and preventing the apparition of cervical cancer in women worldwide, the most frequently incriminated carcinogenic subtypes including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 [1]. However, it has been demonstrated that HPV infection plays a central role in the development of other malignancies such as vulvar, vaginal or anal cancer in women and anal or penile cancer in men [2].

2. The role of HPV in the development of premalignant vulvar lesions

Due to the fact that the incidence of vulvar squamous cell carcinoma has reported a continuous increase in the last decades, attention was focused on determining the pathogenesis of this lesion as well as on improving the therapeutic options [3, 4].

The main premalignant vulvar lesion consists of vulvar intraepithelial neoplasia (VIN), with an increasing incidence in the last decades; moreover, it seems that the age at diagnosis of this pathological feature has been consistently dropping in the last period of time, especially due to the relative increase of HPV infections [5, 6]. However, it seems that there are two different pathways leading to the apparition of this premalignant lesion; the first one is mainly related to type 16 HPV infection, while the second one is rather related to the presence of a nonneoplastic chronic inflammatory condition, lichen sclerosus [7, 8].

Squamous cell carcinomas represent more than 90% of all vulvar cancer and are associated with several histopathological subtypes such as keratinized, basaloid warty or verrucous lesions; moreover, it seems that basaloid and warty lesions are more commonly seen in younger women, being usually associated with HPV DNA positivity. Contrarily, keratinized lesions that usually develop from chronic dermatoses such as lichen sclerosus are not associated with HPV infection and develop in older patients [9]. In cases associated with lichen sclerosus, the premalignant disease is usually referred as differentiated VIN (dVIN) [10]. A third category has been also proposed, comprising the VIN lesions, which could not be classified in either of the two above-mentioned classes. This category is referred as VIN, unclassified type [11].

In order to study the clinicopathological characteristics of lichen-related vulvar carcinomas, Regauer et al. conducted a study on 38 patients diagnosed with this pathology [12]. Among these cases, 32 patients presented solitary lesions, while the remaining 6 patients presented multifocal lesions, all cases being HPV and p16 negative. As for the stage of disease, inguinal metastases were present in 42% of cases at the time of presentation. When it comes to the performed therapy, radical surgery (consisting of radical resection with negative margins) was performed in 36 cases, while the remaining 2 cases were submitted to radiochemotherapy. However, 14 of the 36 surgically treated patients developed recurrent disease on the residual mucosa, 68% of them being diagnosed within the first year. Moreover, 14 of the 38 patients died of the disease. In this way, the study came to demonstrate the strong association between the presence of lichen, the absence of HPV and a relatively poorer outcome of this class of patients [12].

In HPV-related lesions, it seems that the immune system of the host encounters a failure in order to produce an effective response to HPV; the longer time the HPV infection persists, the longer certain oncoproteins such as E6 or E7 will interfere with the cyclic cellular mechanisms, inducing cellular escape from the apoptotic process, and therefore malignant transformation [13, 14].

As for the VIN grading system, the first classification was proposed in 1986 by the International Society for the Study of Vulvo-Vaginal Diseases and included the division of VIN in three grades [15]; two decades, later the same organization decided to change the grading system. Lesions that had been previously considered as VIN 1 have been regarded since that moment as warts or HPV infections, while VIN 2 and 3 have been generally referred as VIN [7, 15].

More recently, in order to provide a more specific classification, the Lower Anogenital Squamous Terminology Committee graded all the HPV-related tumors of the anogenital tract into two categories depending on the degree of differentiation: the first category, LSIL (low-grade squamous intraepithelial lesions) refers to lesions presenting a lower grade of pathological transformation, while the second type of lesions HSIL (high-grade squamous intraepithelial lesions) refers to lesions with a higher grade of pathological transformation [16].

When it comes to the HPV-related VIN development, it seems that HPV DNA integration into the host cell genome plays a crucial role [17, 18]. An interesting study conducted on this theme was published by Peter Hillemanns and Xiuli Wang in Gynecologic Oncology in 2006 [19]. The study included 30 patients diagnosed with VIN at the University of Munich-Grosshaderm, Germany; among the 30 patients, HPV DNA was detected in 25 women, the main identified subtypes including HPV 16 and HPV-18. The presence of HPV-16 or HPV-18 DNA was reported in eight cases, all of them being diagnosed with multicentric lesions of VIN, one of them also associating areas of vulvar carcinoma. Therefore, the authors concluded that the integration of HPV-DNA in the hosts presents a central role when it comes to the progression of the vulvar lesions to advanced or multifocal VIN lesions or even vulvar carcinomas [19].

3. Epidemiology of HPV-related vulvar neoplasms

A recent study regarding the epidemiology of HPV-related vulvar neoplasms originates from the National Cancer Institute, Bethesda MD, United States of America, and was conducted by Brinton et al. [20]. The study was conducted on 201,469 women, 370 of them being diagnosed with vulvar neoplasms including 198 cases with grade 3 vulvar intraepithelial neoplasms. The mean age at the time of conducting the study was 61.8 years; among these cases, most patients were white, married, with a high level of education and parous. Moreover, a significant number of cases had been previously submitted to hysterectomy or reported a chronic usage of oral contraceptives or menopausal hormones, while more than one quarter of patients were obese. After a mean follow-up interval of 13.8 years, 170 cases developed vulvar cancer (the mean age at diagnosis being 71 years), while 198 cases developed grade 3 vulvar intraepithelial neoplasia (at a mean age of 67.5 years). Demographic risk factors included nonwhite women, as well as the marital status (divorced or separated women reporting a significantly higher risk of development of the disease); however, the educational status did not influence the risk of the development of any kind of vulvar lesions. As for the parity status, the risk of vulvar lesions was significantly decreased among women who had delivered. Moreover, patients with a previous history of hysterectomy reported a trend to a higher risk of vulvar neoplasm development, although this fact was not statistically significant. However, this difference was not found among cases submitted to a prior oophorectomy. When it comes to the relationship between the body mass index and the risk of developing vulvar lesions, obese patients (defined through a body mass index higher than 30 kg/m^2) had a significantly higher risk of developing invasive vulvar cancer; however, this relationship could not be established for patients with vulvar intraepithelial neoplasms. As for other lifestyle factors, diabetes or alcohol consumption did not influence the risk of vulvar neoplasm development, but smoking constituted in a significant risk factor. In the meantime, administration of hormonal therapies or oral contraceptives was only associated with the risk of development of intraepithelial lesions and not with the apparition of vulvar invasive cancer. Moreover, smoking was a risk factor especially among patients with HPV-related diseases [20].

4. The influence of HPV infection on the overall prognostic in patients diagnosed with vulvar squamous cell carcinoma

Although vulvar squamous cell carcinoma is not a common malignancy, its estimated incidence ranging between 3 and 5% of all gynecological malignancies, it seems that HPV infection plays a central role in its development; therefore, it is estimated that up to 70% of cases diagnosed with this malignancy present in fact HPV-related lesions [21, 22]. However, the rate of correlation between HPV infection and vulvar squamous cell carcinoma widely varies between different studies depending on the detection method and the included tumoral histopathological subtypes [23, 24].

Due to the fact that other related HPV infection malignancies (such as head or neck tumors) are associated with a significantly better outcome when compared to HPV-negative lesions, attention was focused on determining whether a similar relationship could be established between this type of infection and the overall survival in vulvar cancer patients. The improved outcome of patients diagnosed with HPV-related head or neck tumors seems to be explained especially through a better response to radiochemotherapy [23].

However, scarce data have been reported so far. An interesting study conducted on this theme was published by Alonso et al. in Gynecologic Oncology Journal in 2011 [23]. The study included 98 patients diagnosed with vulvar squamous cell carcinoma between 1995 and 2009 in which the authors studied the presence of HPV DNA; among these cases, 19 patients were diagnosed with HPV-associated infection, HPV-16 being the most prevalently detected subtype. Therefore, HPV-16 subtype was reported in 14 cases, one of these cases presenting in the meantime HPV 56 co-infection; HPV-33 was found in two patients, whereas HPV-31, 51 and 52 were each reported in 1 patient. When it comes to the clinical characteristics for the two subgroups, patients presenting with HPV-related tumors were significantly younger when compared to those in whom the presence of the infection could not be demonstrated (68 years versus 78 years, $p = 0.005$). As for the other clinical factors that had been studied (such as FIGO stage at diagnostic, the median dimension of the tumor, association of ulceration, invasion depth and lymph node metastases) as well as for the type of performed therapeutic strategy (resection or radiochemotherapy), there was no significant difference between patients with HPV-related tumors when compared to those in whom HPV infection had not been observed [23].

The most relevant studies that focused on the correlation between HPV status and clinicopathological findings of patients with vulvar cancer are summarized in **Table 1**.

When it comes to the long-term outcomes, both disease-free and overall survival were significantly influenced by the FIGO stage at diagnosis, while the association of HPV infection showed no significant influence. Moreover, no significant difference was reported between the association of radiotherapy, HPV infection and overall survival; however, cases in which radiotherapy was associated reported a higher morbidity rate. In univariate analysis, the most important factors associating with the risk of disease progression and mortality were represented by the age over 78 years, FIGO stages III–IV, tumor size larger than 20 mm, ulceration, invasion depth and the presence of lymph node metastases; however, in multivariate

Name, year	No of cases (total)	HPV-related cases	Non–HPV-related cases	Factors significantly associated with HPV infection
Hinten, 2018 [25]	318	55	263	Patients' age, smoking status, immune status, history of lichen sclerosus, diameter of the tumor, lympho-vascular space invasion, FIGO stage, risk of recurrence
Yap, 2018 [26]	40	14	26	Lower risk of recurrence *- No correlation between HPV status and age, TNM classification or type of treatment, disease-free survival interval, overall survival*
Brinton, 2017 [20]	370	—	—	Smoking, age, obesity
Rasmussen, 2018 [27]	1638	541	1097	Higher overall survival rate in HPV-related lesions
Monk, 1995 [28]	55	33	22	Patients' age, smoking status, histopathological subtype *- No correlation between HPV status and FIGO stage, grade of the tumor, type of therapy*

Table 1.
Correlation between HPV status and clinicopathological findings in patients with vulvar cancer.

analysis, only the association of lymph node metastases was still significantly associated with the mortality risk. When it comes to the influence of HPV infection, a significant association could not be seen even after adjusting for age. These data come to suggest that a supplementary mechanism might be involved in HPV-related vulvar neoplasms when compared to head and neck HPV-related neoplasms [23].

However, these results were not sustained by more recent studies conducted on the theme of the prognostic significance of HPV infection in patients diagnosed with vulvar squamous cell carcinoma and submitted to radiotherapy. In the article published by Lee in the same journal in 2016, contradictory results were found [29]. The study included 57 patients diagnosed with this pathological entity between 1985 and 2011 in Brigham and Women's Hospital and Dana Farber Cancer Institute who were treated with postoperative radiotherapy with radical intent or as part of the salvage setting. In all cases the presence of the following genotypes was studied: 6, 11, 16, 18, 26, 31, 33, 35, 40, 45, 51, 52, 56 and 59; similar to Alonso's study, HPV-16 genotype was the most commonly encountered subtype. When it comes to the long-term outcomes, patients with p16-positive tumors reported a significantly better five-year progression-free and overall survival rates. Moreover, in univariate analysis, older age at diagnosis as well as higher FIGO stage and development of recurrent disease were associated with increased risk of progressive disease and mortality-related disease; however, association of chemotherapy did not significantly impact on the overall survival. When a multivariate analysis was performed, the presence of p16 staining was associated with higher progression-free survival rates as well as with lower rates of recurrence [29]. The reported results of this study were similar to those regarding head and neck HPV-induced malignancies, the presence of HPV infection being associated with a better response to radiotherapy.

A recent study that was conducted by Hinten et al. that will be published in 2018 in Gynecologic Oncology Journal demonstrated that in fact HPV-positive and negative vulvar cancer represent in fact two different pathologic entities with different localization and different prognosis. The study was conducted between March 1988 and January 2015 and included 318 patients. Among these cases, HPV-related disease was reported in 55 cases, while the remaining 263 had non–HPV-related vulvar neoplastic lesions [25]. The authors demonstrated that HPV-related lesions were more often localized on the perineum when compared to non-HPV lesions. When it comes to the long-term outcomes, the authors demonstrated that patients with HPV-induced lesions reported a better outcome in terms of both disease-free survival and overall survival when compared to non-HPV lesions; therefore, the 5-year and total disease-free survival were 76 versus 46%, and 28 versus 13% in HPV-related lesions versus non–HPV-related lesions. In the meantime, the 5-year and total overall survival rates were 85 versus 57% in HPV-related lesions, and only 16% in non-HPV lesions. Another important prognostic factor that significantly influenced survival was the site of the lesions; therefore, even among patients with HPV-related lesions, cases presenting with perineal development reported a significantly better prognosis when compared to nonperineal HPV-induced lesions; the difference remained significant in terms of both disease-free and overall survival. Moreover, among patients presenting with perineal vulvar cancer, HPV-induced malignancies reported a more favorable outcome when compared to non–HPV-induced lesions. In the meantime, disease-free survival was also significantly influenced by FIGO stage and diameter of the tumor, while the overall survival was significantly influenced by age at primary treatment, stage at diagnosis, tumor diameter and relapse as well as by the perineal localization of the lesions. Moreover, the 10-year survival rate was significantly influenced by age at the time of initial treatment, FIGO stage at diagnosis, tumoral diameter, p16 expression and perineal localization of the lesions. In terms of histopathological characteristics,

non–HPV-related lesions presented a larger diameter and were associated with a deeper invasion, more frequent metastases at the level of the lymphatic nodes and, therefore, a more frequent association of adjuvant radiotherapy. This different outcome could be explained by a more aggressive biological behavior of non–HPV-related lesions as well as by the older age at diagnosis, elderly patients feeling most often ashamed to address to the gynecologist for such lesions [25]. All these data enabled the authors to conclude that most probably HPV and non–HPV-related lesions are in fact two different entities with different pathogenesis and different outcomes. Another possible explanation is related to the p53 status, non-HPV lesions being most commonly associated with a higher level of p53, and, in consequence, with a more aggressive biology of the tumor [30].

Another recent study that focused on determining the prognostic significance of human papilloma virus and p16 expression in patients with vulvar squamous cell carcinoma submitted to radiotherapy was conducted by Yap et al. and has been recently published in Clinical Oncology Journal [26].

5. Factors influencing relapse in patients with premalignant or malignant diseases

Starting from the observation that patients with similar stages of disease who receive similar treatment strategies had a very different evolution, the researchers tried to identify the potential factors that influenced this evolution.

5.1 The influence of DNMT expression in development of recurrent vulvar cancer

DNMT (DNA methyltransferases), the enzyme that dictates and maintains DNA methylation patterns through the genome, seems to have significant differences in terms of expression in patients with vulvar carcinomas. Among the general name of DNMT, there are in fact three enzymes with various influences on the methylation process. DNMT1 is directly involved in the methylation process in normal cells; however, it seems that it plays a certain role in tumorigenesis too. DNMT3A and DNMT3B represent two other enzymes that present low expression levels in adult cells; however, these molecules seem to be overexpressed in several epithelial tumors [31, 32]. Moreover, their expression is associated with poor prognosis in patients with such epithelial tumors. A recent study conducted on this theme was published in Gynecologic Oncology Journal in 2016 and included patients treated for vulvar squamous cell carcinomas at the Pan Birmingham Gynecological Cancer Center between 2001 and 2008 [33]. The authors demonstrated the overexpression of DNMT1 in 83% of cases and of DNMT3A in 44% of cases, while the overexpression of DNMT3B was present in 42% of patients. After determining these parameters, the authors studied their influence on the risk of recurrence. DNMT3A was associated with a 4.5 fold increased risk of developing recurrent vulvar cancer; in multivariate analysis, the overexpression of this enzyme was also significantly correlated with a higher risk of local recurrence. Moreover, the authors tested the patients with overexpression of DNMT3A for CDKN2A, an indicator of HPV-induced dysplasia, and demonstrated that among patients with negative staining for CDKN2A, the overexpression of DNMT3A was significantly higher. Similar to DNMT3A, a higher level of DNMT3B was significantly associated with the risk of recurrence; however, the levels of this enzyme could not be correlated with CDKN2A expression. As for DNMT1 levels, there was no significant

correlation between this parameter and the risk of vulvar cancer recurrence; similar to DNMT3B, no significant correlation could be found between DNMT1 levels and CDKN2A expression [33].

5.2 The influence of pretreatment subtype of HPV on the risk of relapse in patients with VIN

Another interesting topic in regard to the influence of HPV on the overall prognostic in patients with premalignant or malignant lesions is related to the effect of various viral subtypes on the long-term outcomes of these patients. A recent study conducted in Milan by Bogani et al. and published in 2017 in the European Journal of Obstetrics and Gynecology and Reproductive Biology included 64 patients diagnosed with high-grade VIN [34]. Among these cases, 41 patients had a previous history of HPV infection, the most commonly incriminated subtypes being HPV 16, 18, 31 and 33. As for the performed procedures, most often it consisted of LASER ablation, excision or diathermocoagulation. After a mean follow-up of 56.7 months, 10 patients were diagnosed with VIN2+ persistence or relapse, the mean disease-free survival being 51.7 months; the authors demonstrated that a pretreatment infection with HPV 31 or HPV 33 subtype was associated with an increased risk of developing recurrent or persistent disease. Moreover, patients submitted to surgical excision followed by LASER ablation experienced a lower rate of relapse when compared with other types of therapies. These facts were explained through two mechanisms: the first one is related to the fact that HPV16, as well as HPV 31 and 33, was associated with multifocal lesions, while multifocal lesions usually associate with a higher risk of persistent/recurrent disease; the second mechanism is probably related to the fact that HPV 31- and HPV33-induced lesions usually associate with a more rapid pattern of growth [34].

When it comes to the influence of HPV infection on the risk of recurrence of VIN, a recent study published by Satmary et al. in Gynecologic Oncology Journal in 2018 comes to demonstrate a significant relationship between these two entities [35]. The study included 784 patients with histopathological diagnostic of vulvar intraepithelial neoplasia which were treated with curative intent; however, 26,3% of cases developed recurrent intraepithelial neoplasia while 2,2% of these cases developed vulvar cancer. Among these cases, 25.9% of patients were 40 years of age or less, 23.9% were aged between 41 and 50 years, 24.6% were aged between 51 and 60 years, while the remaining 25.6% of patients were aged over 60 years. As for the immunity status, immunosuppression was reported in 189 cases and was caused by immune suppressant therapies (such as prednisone or methylprednisolone) in all but two patients (who were known to have HIV infection). When it comes to the performed therapy, it consisted of local excision in 54.8% of cases, laser therapy in 19.3% of cases and topical medication as single therapy or in association with excision or with laser in the remaining patients; in 17% of cases, data regarding therapy were not reported. However, cases in which the initial therapeutic option was not known were excluded from further study regarding recurrent disease. Among the 650 patients who benefited from any kind of treatment recurrence occurred in 171 cases, after a median disease-free survival interval of 16.9 months, while the median follow-up period was 89 months. Moreover, it seems that 75% of cases recurred within 43.1 months. When analyzed according to the age at the time of diagnosis, recurrence rate was significantly higher among patients over the age of 50 ($p = 0.0031$) when compared to younger patients. In univariate analysis, a significant association was also found between the risk of recurrence and the immunity system of the

patient, association of cervical intraepithelial neoplasia and increased BMI; in multivariate analysis, only age over 50 years, immunity status and association of cervical intraepithelial neoplasia were significantly associated with the risk of recurrence. When it comes to the influence of the type of treatment, in multivariate analysis, a trend toward a higher rate of recurrence was reported in cases submitted to nonexcisional therapies. As for the cases in which progression to vulvar cancer was encountered, the median time to progression to malignancy was 36.2 months. When studying only the patients who developed recurrences, the authors demonstrated that the relapse was significantly associated with increased age (patients over 50 years of age reporting a higher risk of recurrence), immunosuppression, positive resection margins and adjacent areas of lichen sclerosus or HPV infection [35].

5.3 The influence of TP53 gene on the risk of recurrence of vulvar cancer

Another factor that seems to influence the evolution of patients with vulvar cancer is represented by TP53 expression. Moreover, it has been widely demonstrated that antitumor agents activating the TP53 tumor suppression gene can be safely used as adjuvant therapy for cases exhibiting this gene. When it comes to the association between HPV-related infection and TP53 expression, in cases diagnosed with head and neck malignancies, disruptive TP53 mutations were exclusively seen in HPV-negative tumors; moreover, these lesions were associated with poorer outcomes when compared with HPV-positive lesions [36].

6. The potential preventive role of HPV vaccination against HPV-induced vulvar cancer

In order to diminish the risk of development of HPV-related malignancies, the quadrivalent and the two-valent HPV vaccines were approved to be used in both males and females in the European Union since 2006 and 2007, respectively [37, 38]. The main viral subtypes that are controlled by these two vaccines are HPV-16 and HPV-18, while the quadrivalent vaccine also contains proteins derived from HPV6 and 11 [37]. Studies have demonstrated that using HPV vaccines in HPV-naïve persons protects against both benign and malignant conditions such as condylomas, perineal and anal neoplasia in men as well as cervical cancer in women [38]. Therefore, routine HPV vaccination has been recommended in Europe in 12-year-old girls since 2009, in order to decrease the risk of development of such pathologies [39]. Due to the fact that there is a strong relationship between HPV infection and certain cases of vulvar cancer, a decreased incidence of this pathology is to be expected in the next decades, once the HPV vaccination has been widely implemented [40].

In order to maximize the protective effect of vaccination against HPV-related diseases, a nine-valent second generation of HPV vaccine was proposed; this nine-valent HPV vaccine is expected to offer protection against the seven high-risk HPV subtypes (HPV 16/18/31/33/45/52/58) as well as against low-risk subtypes such as HPV 6/11. In this way, it is expected to provide a significant degree of protection against the main nine subtypes of HPV, which are responsible for up to 90% of all genital warts. The nine-valent vaccine proved to be effective in order to prevent 97% of all high-grade premalignant lesions of cervix, vulva and vagina. In the study conducted by Hartwig et al. and published in 2015, which included all HPV-related malignancies reported in Europe in the year 2013, the authors demonstrated the efficacy of the second-generation HPV vaccine [2].

7. Future perspectives

Once the benefits of vaccination in terms of prevention of HPV-related malignancies are widely demonstrated, another problem is reported, the one of the vaccine's costs. It seems that, especially in the developing countries, where the incidence of HPV-related malignancies is higher, the accessibility to HPV vaccines is lower due to its price. Therefore, one of the future perspectives in regard to HPV-related diseases is lowering the price of the vaccine and increasing in this way the general accessibility to these products. Moreover, if we take into consideration the fact that a significant number of women self-refer to the gynecologist when neoplastic disease is already present, it seems that an important future perspective should refer to the development of a vaccine that could also have a therapeutic role [41].

8. Conclusion

HPV infection seems to play a central role in developing premalignant or malignant vulvar lesions. Patients diagnosed with HPV-related lesions tend to have a younger age at diagnosis, especially due to the association with the presence of this virus. However, HPV-induced vulvar neoplastic lesions seem to have a better outcome in terms of both disease-free survival and overall survival when compared with non–HPV-related lesions. When it comes to the prevention of these lesions, it seems that anti-HPV vaccination might play a role; however, more studies are still needed in order to clearly state this aspect.

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References

[1] IARC. Monographs on the Evaluation on Carcinogenic Risks to Humans. A Review of Human Carcinogens. Part B: Biological Agents. Vol. 100. Lyon: International Agency for Research on Cancer; 2012

[2] Hartwig S, Baldauf JJ, Dominiak-Felden G, Simondon F, Alemany L, de Sanjosé S, Castellsagué X. Estimation of the epidemiologic alburden of HPV-related anogenital cancers, precancerous lesions, and genital warts in women and men in Europe: Potential additional benefit of a nine-valent second generation HPV vaccine compared to first generation HPV vaccines. Papillomavirus Research. 2015;**1**:90-100

[3] Judson PL, Habermann EB, Baxter NN, Durham SB, Virnig BA. Trends in the incidence of invasive and in situ vulvar carcinoma. Obstetrics and Gynecology. 2006;**107**:1018-1022

[4] Hampl M, Deckers-Figiel S, Hampl JA, Rein D, Bender HG. New aspects of vulvar cancer: Changes in localization and age of onset. Gynecologic Oncology. 2008;**109**:340-345

[5] Iversen T, Tretli S. Intraepithelial and invasive squamous cell neoplasia of the vulva: Trends in incidence, recurrence, and survival rate in Norway. Obstetrics and Gynecology. 1998;**91**:969-972

[6] Gupta J, Pilotti S, Rilke F, Shah K. Association of human papillomavirus type 16 with neoplastic lesions of the vulva and other genital sites by in situ hybridization. The American Journal of Pathology. 1987;**127**:206-215

[7] Sideri M, Jones RW, Wilkinson EJ, Preti M, Heller DS, Scurry J, Haefner H, Neill S. Squamous vulvar intraepithelial neoplasia: 2004 modified terminology, ISSVD Vulvar Oncology Subcommittee. The Journal of Reproductive Medicine. 2005;**50**:807-810

[8] van de Nieuwenhof HP, Bulten J, Hollema H, Dommerholt RG, Massuger LF, van der Zee AG, de Hullu JA, van Kempen LC. Differentiated vulvar intraepithelial neoplasia is often found in lesions, previously diagnosed as lichen sclerosus, which have progressed to vulvar squamous cell carcinoma. Modern Pathology. 2011;**24**:297-305

[9] de Sanjose S, Bruni L, Alemany L. HPV in genital cancers (at the exception of cervical cancer) and anal cancers. Presse Médicale. 2014;**43**:e423-e428

[10] Preti M, Igidbashian S, Costa S, Cristoforoni P, Mariani L, Origoni M, Sandri MT, Boveri S, Spolti N, Spinaci L, Sanvito F, Preti EP, Falasca A, Radici G, Micheletti L. VIN usual type—From the past to the future. Ecancermedicalscience. 2015;**9**:531

[11] Preti M, Scurry J, Marchitelli CE, Micheletti L. Vulvar intraepithelial neoplasia. Best Practice & Research. Clinical Obstetrics & Gynaecology. 2014;**28**:1051-1062

[12] Regauer S, Reich O, Eberz B. Vulvar cancers in women with vulvar lichen planus: A clinicopathological study. Journal of the American Academy of Dermatology. 2014;**71**:698-707

[13] van der Avoort I, Shirango H, Hoevenaars BM, Grefte JM, de Hullu JA, de Wilde PC, Bulten J, Melchers WJ, Massuger LF. Vulvar squamous cell carcinoma is a multifactorial disease following two separate and independent pathways. International Journal of Gynecological Pathology. 2006;**25**:22-29

[14] Hoevenaars BM, van der Avoort I, de Wilde PC, Massuger LF, Melchers WJ, de Hullu JA, Bulten J. A panel of p16(INK4A), MIB1 and p53 proteins can distinguish between the 2 pathways leading to vulvar squamous cell

carcinoma. International Journal of Cancer. 2008;**123**:2767-2773

[15] Wilkinson EJ, Kneale BL, Lynch FW. Report of the ISSVD Terminology Committee: VIN. The Journal of Reproductive Medicine. 1986;**31**:973-974

[16] Darragh TM, Colgan TJ, Cox JT, Heller DS, Henry MR, Luff RD, McCalmont T, Nayar R, Palefsky JM, Stoler MH, Wilkinson EJ, Zaino RJ, Wilbur DC. The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions: Background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. Archives of Pathology and Laboratory Medicine. 2012;**136**:1266-1297

[17] van Beurden M, ten Kate FJ, Smits HL, Berkhout RJ, de Craen AJ, van der Vange N, Lammes FB, ter Schegget J. Multifocal vulvar intraepithelial neoplasia grade III and multicentric lower genital tract neoplasia is associated with transcriptionally active human papillomavirus. Cancer. 1995;**75**:2879-2884

[18] Remmink AJ, Walboomers JM, Helmerhorst TJ, Voorhorst FJ, Rozendaal L, Risse EK, Meijer CJ, Kenemans P. The presence of persistent high-risk HPV genotypes in dysplastic cervical lesions is associated with progressive disease: Natural history up to 36 months. International Journal of Cancer. 1995;**61**:306-311

[19] Hillemanns P, Wang X. Integration of HPV-16 and HPV-18 DNA in vulvar intraepithelial neoplasia. Gynecologic Oncology. 2006;**100**:276-282

[20] Brinton LA, Thistle JE, Liao LM, Trabert B. Epidemiology of vulvar neoplasia in the NIH-AARP Study. Gynecologic Oncology. 2017;**145**:298-304

[21] Pinto AP, Schlecht NF, Pintos J, Kaiano J, Franco EL, Crum CP, Villa LL. Prognostic significance of lymph node variables and human papillomavirus DNA in invasive vulvar carcinoma. Gynecologic Oncology. 2004;**92**:856-865

[22] Sutton BC, Allen RA, Moore WE, Dunn ST. Distribution of human papillomavirus genotypes in invasive squamous carcinoma of the vulva. Modern Pathology. 2008;**21**:345-354

[23] Alonso I, Fuste V, del Pino M, Castillo P, Torne A, Fuste P, Rios J, Pahisa J, Balasch J, Ordi J. Does human papillomavirus infection imply a different prognosis in vulvar squamous cell carcinoma? Gynecologic Oncology. 2011;**122**:509-514

[24] Pinto AP, Signorello LB, Crum CP, Harlow BL, Abrao F, Villa LL. Squamous cell carcinoma of the vulva in Brazil: Prognostic importance of host and viral variables. Gynecologic Oncology. 1999;**74**:61-67

[25] Hinten F, Molijn A, Eckhardt L, Massuger LFAG, Quint W, Bult P, Bulten J, Melchers WJG, de Hullu JA. Vulvar cancer: Two pathways with different localization and prognosis. Gynecologic Oncology. 2018;**149**:310-317

[26] Yap ML, Allo G, Cuartero J, Pintilie M, Kamel-Reid S, Murphy J, Mackay H, Clarke B, Fyles A, Milosevic M. Prognostic significance of human papilloma virus and p16 expression in patients with vulvar squamous cell carcinoma who received radiotherapy. Clinical Oncology (Royal College of Radiologists (Great Britain)). 2018;**30**:254-261

[27] Rasmussen CL, Sand FL, Hoffmann FM, Kaae AK, Kjaer SK. Does HPV status influence survival after vulvar cancer? International Journal of Cancer. 2018;**142**:1158-1165

[28] Monk BJ, Burger RA, Lin F, Parham G, Vasilev SA, Wilczynski SP. Prognostic significance of human papillomavirus DNA in vulvar carcinoma. Obstetrics and Gynecology. 1995;**85**:709-715

[29] Lee LJ, Howitt B, Catalano P, Tanaka C, Murphy R, Cimbak N, DeMaria R, Bu P, Crum C, Horowitz N, Matulonis U, Viswanathan AN. Prognostic importance of human papillomavirus (HPV) and p16 positivity in squamous cell carcinoma of the vulva treated with radiotherapy. Gynecologic Oncology. 2016;**142**:293-298

[30] Hay CM, Lachance JA, Lucas FL, Smith KA, Jones MA. Biomarkers p16, human papillomavirus and p53 predict recurrence and survival in early stage squamous cell carcinoma of the vulva. Journal of Lower Genital Tract Disease. 2016;**20**:252-256

[31] Li M, Wang Y, Song Y, Bu R, Yin B, Fei X, Guo Q, Wu B. Expression profiling and clinicopathological significance of DNA methyltransferase 1, 3A and 3B in sporadic human renal cell carcinoma. International Journal of Clinical and Experimental Pathology. 2014;**7**:7597-7609

[32] Zhang JJ, Zhu Y, Zhu Y, Wu JL, Liang WB, Zhu R, Xu ZK, Du Q, Miao Y. Association of increased DNA methyltransferase expression with carcinogenesis and poor prognosis in pancreatic ductal adenocarcinoma. Clinical and Translational Oncology. 2012;**14**:116-124

[33] Leonard S, Pereira M, Fox R, Gordon N, Yap J, Kehoe S, Luesley D, Woodman C, Ganesan R. Over-expression of DNMT3A predicts the risk of recurrent vulvar squamous cell carcinomas. Gynecologic Oncology. 2016;**143**:414-420

[34] Bogani G, Martinelli F, Ditto A, Signorelli M, Taverna F, Lombardo C, Chiappa V, Leone Roberti MU, Recalcati D, Scaffa C, Perotto S, Sabatucci I, Indini A, Lorusso D, Raspagliesi F. The association of pre-treatment HPV subtypes with recurrence of VIN. European Journal of Obstetrics, Gynecology, and Reproductive Biology. 2017;**211**:37-41

[35] Satmary W, Holschneider CH, Brunette LL, Natarajan S. Vulvar intraepithelial neoplasia: Risk factors for recurrence. Gynecologic Oncology. 2018;**148**:126-131

[36] Westra WH, Taube JM, Poeta ML, Begum S, Sidransky D, Koch WM. Inverse relationship between human papillomavirus-16 infection and disruptive p53 gene mutations in squamous cell carcinoma of the head and neck. Clinical Cancer Research. 2008;**14**:366-369

[37] de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, Tous S, Felix A, Bravo LE, Shin HR, Vallejos CS, de Ruiz PA, Lima MA, Guimera N, Clavero O, Alejo M, Llombart-Bosch A, Cheng-Yang C, Tatti SA, Kasamatsu E, Iljazovic E, Odida M, Prado R, Seoud M, Grce M, Usubutun A, Jain A, Suarez GA, Lombardi LE, Banjo A, Menendez C, Domingo EJ, Velasco J, Nessa A, Chichareon SC, Qiao YL, Lerma E, Garland SM, Sasagawa T, Ferrera A, Hammouda D, Mariani L, Pelayo A, Steiner I, Oliva E, Meijer CJ, Al Jassar WF, Cruz E, Wright TC, Puras A, Llave CL, Tzardi M, Agorastos T, Garcia-Barriola V, Clavel C, Ordi J, Andujar M, Castellsague X, Sanchez GI, Nowakowski AM, Bornstein J, Munoz N, Bosch FX. Human papillomavirus genotype attribution in invasive cervical cancer: A retrospective cross-sectional worldwide study. Lancet Oncoloy. 2010;**11**:1048-1056

[38] Garland SM, Hernandez-Avila M, Wheeler CM, Perez G, Harper DM, Leodolter S, Tang GW, Ferris DG, Steben M, Bryan J, Taddeo FJ, Railkar R, Esser MT, Sings HL,

Nelson M, Boslego J, Sattler C, Barr E, Koutsky LA. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. New England Journal of Medicine. 2007;**356**:1928-1943

[39] Lynge E, Rygaard C, Baillet MV, Dugue PA, Sander BB, Bonde J, Rebolj M. Cervical cancer screening at crossroads. APMIS. 2014;**122**:667-673

[40] Skorstengaard M, Thamsborg LH, Lynge E. Burden of HPV-caused cancers in Denmark and the potential effect of HPV-vaccination. Vaccine. 2017;**35**:5939-5945

[41] Bolhassani A. Future prospects in HPV prevention and treatment. In: Azam Bolhassani. HPV Infections: Diagnosis, Prevention, and Treatment. Bentham Science Publishers; 2018. p. 220-226. DOI: 10.2174/97816810861701180101