



**HAL**  
open science

## ”Primary Squamous Cell Carcinoma of the Vagina: HPV Detection, p16INK4A Overexpression and Clinicopathological Correlations”

Victoria Fuste, Marta del Pino, Assumpta Perez, Angel Garcia, Aureli Torne,  
Jaume Pahisa, Jaume Ordi

### ► To cite this version:

Victoria Fuste, Marta del Pino, Assumpta Perez, Angel Garcia, Aureli Torne, et al.. ”Primary Squamous Cell Carcinoma of the Vagina: HPV Detection, p16INK4A Overexpression and Clinicopathological Correlations”. *Histopathology*, 2010, 57 (6), pp.907. 10.1111/j.1365-2559.2010.03727.x . hal-00601179

**HAL Id: hal-00601179**

**<https://hal.science/hal-00601179>**

Submitted on 17 Jun 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Histopathology



## “Primary Squamous Cell Carcinoma of the Vagina: HPV Detection, p16INK4A Overexpression and Clinicopathological Correlations”

Journal:	<i>Histopathology</i>
Manuscript ID:	HISTOP-10-09-0603.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	25-Feb-2010
Complete List of Authors:	Fuste, Victoria; Hospital Clinic, Pathology del Pino, Marta; Hospital Clinic, Obstetrics and Gynaecology Perez, Assumpta; Hospitals Vall d' Hebron, Pathology Garcia, Angel; Hospitals Vall d' Hebron, Obstetrics and Gynaecology Torne, Aureli; Hospital Clinic, Obstetrics and Gynaecology Pahisa, Jaume; Hospital Clinic, Obstetrics and Gynaecology Ordi, Jaume; Hospital Clinic, Pathology
Keywords:	Vagina, Squamous cell carcinoma, p53, p16, Human papilloma virus



**“Primary Squamous Cell Carcinoma of the Vagina: HPV  
Detection, p16<sup>INK4A</sup> Overexpression and Clinicopathological  
Correlations”**

Victòria Fusté,<sup>1</sup> Marta del Pino,<sup>2</sup> Assumpta Pérez,<sup>3</sup> Angel Garcia,<sup>4</sup> Aureli Torne,<sup>2</sup> Jaume Pahisa<sup>2</sup> & Jaume Ordi<sup>1</sup>

<sup>1</sup> *Department of Pathology,* <sup>2</sup> *Department of Obstetrics and Gynaecology,*  
*Hospital Clínic-IDIBAPS, University of Barcelona, Barcelona, Spain;*

<sup>3</sup> *Department of Pathology,* <sup>4</sup> *Department of Obstetrics and Gynaecology,*  
*Hospitals Vall d’Hebron, Barcelona*

Address for correspondence:

Jaume Ordi M.D., PhD. Department of Pathology, Hospital Clínic, C/ Villarroel  
170,  
08036-Barcelona, Spain.

Tel +34 93 227 5450; Fax: +34 93 227 5717; e-mail: [jordi@clinic.ub.es](mailto:jordi@clinic.ub.es)

**RUNNING TITLE:** HPV in vaginal carcinoma

**Key Words:** Vagina —Squamous cell carcinoma—p53—p16—human  
papilloma virus—HPV

**Abbreviations:** HPV, Human papillomavirus; SCCVa, squamous cell  
carcinoma of the vagina; VaIN, vaginal intraepithelial neoplasia; CIN cervical  
intraepithelial neoplasia; VIN vulvar intraepithelial neoplasia

## ABSTRACT

**Aim:** To determine the role of HPV in the pathogenesis of primary squamous cell carcinoma of the vagina (SCCVa), and to evaluate its clinico-pathological significance.

**Methods:** All cases of SCCVa diagnosed over a 15 year period from two hospitals in Barcelona, Spain (n=32) were retrieved. Patients with a history of carcinoma of the cervix diagnosed less than 5 years before were excluded. HPV was detected and typed by PCR using the SPF10 primers. Immunohistochemistry for p16 and p53 was performed.

**Results:** HPV was detected in 25 cases (78.1%). HPV16 was the most prevalent type. Patients with HPV-positive tumours were frequently associated with a history of carcinoma or intraepithelial neoplasia of the cervix or vulva diagnosed more than 5 years before (56% vs. 0%; p=0.01). HPV-positive tumours were more frequently of non-keratinizing, basaloid or warty type than HPV-negative neoplasms (84% vs. 14.3%; p<0.001), and almost constantly showed diffuse positive staining for p16<sup>INK4a</sup> (96%, vs. 14.3%; p<0.001). The sensitivity and specificity of p16 to identify HPV-positive tumours were 96% and 85.7% respectively.

**Conclusions:** A high number of SCCVs are related to HPV infection and may be identified by immunostaining for p16. HPV-positive tumours tend to affect women with history of cervical neoplasm.

## Introduction

Primary carcinoma of the vagina is a rare disease accounting for about 1-2% of all gynaecologic malignances.<sup>1-4</sup> Invasive squamous cell carcinoma (SCCVa) is the most frequent tumour representing 65-95% of all cases.<sup>3, 5-10</sup> Despite all therapeutic advances, the overall 5-year survival rate is still poor.<sup>1, 3, 11</sup>

Human papillomavirus (HPV) has been clearly implicated in the pathogenesis of malignant tumours of the female lower genital tract. HPV has been detected in almost 100% of cervical carcinomas.<sup>12-15</sup> In vulvar carcinoma HPV has been identified in 20-35% of all cases, being detected in 75-100% of basaloid and warty carcinomas and in less than 20% of keratinizing squamous cell carcinomas.<sup>16-20</sup> Because of the low frequency of SCCVa, few series have been published focusing on this tumour, and consequently there is scant information on the implication of HPV infection with discrepant results in the published series. There is also limited information on whether there are pathological characteristics associated to HPV-positive tumours and the clinical consequences of HPV infection. Moreover, it has not been determined whether p16<sup>INK4A</sup> immunostaining, which in several locations such as the vulva or the head and neck region<sup>18, 21</sup> has been shown to be a reliable marker for HPV-associated tumours can also be a useful marker of HPV infection in vaginal neoplasms.

Finally, it has been shown that about 30% of patients with SCCVa have a history of prior dysplasia or carcinoma of the uterine cervix.<sup>3, 5, 18, 21-24</sup> A 5 year free period is generally considered the distinction between a recurrent cervical carcinoma and a new primary vaginal carcinoma, as 95% of the recurrences of

cervical carcinoma occur within five years.<sup>25, 26</sup> Nevertheless, there is a general concern about this threshold and several authors have expanded that period to 10 years<sup>6</sup> or have even excluded all patients with any previous history of cervical pathology.<sup>13</sup> Thus, it is not clear whether patients with previous cervical lesions diagnosed more than 5 years before should or should not be considered as primary SCCVa.

Our study was aimed to evaluate the role of HPV in the pathogenesis of SCCVa with its possible clinico-pathological implications, to analyze the usefulness of p16<sup>INK4A</sup> as a marker for HPV-associated SCCVa, determining whether it may improve the results of pure histological classification or not, and to evaluate the clinical impact on previous history of cervical or vulvar intraepithelial or invasive neoplasm.

## Materials and methods

### CASE SELECTION

All cases of invasive squamous cell carcinoma of the vagina diagnosed in a period of 15 years (1994-2008) were retrieved from the surgical pathology files of two tertiary institutions in Barcelona, Spain, the Hospital Clinic and the Hospital Vall d'Hebron. Thus, 32 cases were included in the study.

All tissues had been routinely fixed in 4% buffered neutral formalin and embedded in paraffin. In all cases, both haematoxylin and eosin (H&E) glass slides and paraffin blocks were available for review. In 17 patients (53.1%) a colpectomy or a wide resection was performed. In 15 patients (46.9%) only a small biopsy was available. The clinical charts were reviewed in all cases. The medical records were abstracted to obtain information on patient's age, clinical

symptoms, location, other previous or concurrent neoplasms or premalignant lesions, stage, treatment and clinical outcome. Patients with a previous history of carcinoma of the uterine cervix diagnosed less than 5 years before were excluded from the study.<sup>25, 26</sup>

## HISTOLOGICAL EVALUATION

Representative blocks of all cases were chosen. Review of H&E stained slides of the tumour specimens was performed independently by two pathologists who did not know the results of HPV testing or the tumour staging and clinical outcome. Tumours were classified and graded according to the World Health Organization (WHO) classification. When differences between the two independent evaluations were detected, a new evaluation was conducted and a consensus diagnosis was reached.

## DNA EXTRACTION

In all cases a representative block including both the invasive carcinoma as well as the associated vaginal intraepithelial neoplasia (VaIN) lesion was selected. 10 µm thick sections of formalin-fixed, paraffin embedded tissues were cut using the sandwich technique, which implies that the first and last sections are stained with H&E and used to confirm the adequacy of the sample, whereas the central sections are used for DNA extraction. The microtome blade was replaced after each case; a blank paraffin block was cut prior to cutting a new sample. A vacuum cleaner was used to avoid cross-contamination between paraffin blocks. The tissue sections were digested with proteinase K in a volume of 250 µl at 56° C overnight. Proteinase K was heat inactivated at 95° C for 10 minutes. A 1/10 dilution of the sample was used for PCR (10 µl).

## HPV DETECTION AND TYPING

A broad-spectrum HPV DNA amplification was performed using the short PCR fragment (SPF10) primer set (Innogenetics Diagnostica, Spain) as previously described.<sup>18</sup> The SPF10 primers amplify a 65-bp fragment from the L1 region of the HPV genome. PCRs were performed in 50  $\mu$ L reaction following standard conditions. For HPV amplification, a 9 minute denaturation step at 94°C was followed by 40 cycles of amplification using 1.5 I.U. DNA polymerase (Amplitaq Gold DNA polymerase, Applied Biosystems) with a thermocycler (PTC-200, Peltier Thermal Cycler, MJ Research, Waltham, MA). Each cycle included a denaturation step at 94°C for 30 seconds, a primer annealing step at 52°C for 45 seconds, and a chain elongation step at 72°C for 45 seconds. The final elongation step was prolonged by 5 minutes. The PCR products were subjected to electrophoresis in a 3% agarose gel and the 65-bp product was visualized with ethidium bromide staining. Additional confirmation of the presence of amplified HPV-specific sequences was performed using HPV DNA enzyme immunoassay, a microliter plate-based hybridization assay. All samples were then incubated with the INNO-LIPA HPV research assay (15). Twenty-five individual HPV genotypes (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70 and 74) can be identified simultaneously in a single assay. In this assay 10  $\mu$ L of denatured HPV PCR product was hybridized to the genotype-specific probes immobilized as parallel lines on a nitrocellulose strip. After the washing step, the products of hybridization were detected by a colour reaction with alkaline-phosphatase-streptavidin conjugate and substrate (5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium), which resulted



in a purple precipitate. The results of the hybridization were assessed visually by comparing to the standard grid.

To confirm DNA amplification a second PCR was conducted with primers  $\beta$ -globin-PCO3 and  $\beta$ -globin-PCO5. In each series of cases we included a positive control (squamous cell carcinoma of the cervix known to harbour HPV16), as well as a negative control (distilled water).

#### IMMUNOHISTOCHEMISTRY

Immunohistochemical studies were done with the automated immunohistochemical system Autostainer Link 48® (Dako Co, Carpinteria, CA, USA). p16<sup>INK4a</sup> was detected using the CINtec histology kit (clone E6H4, mtm Laboratories, Heidelberg, Germany), following the manufacturer's protocol. In each series a negative control consisting of a normal cervix, as well as a positive control consisting of a CIN3, were included.

The evaluation of the p16<sup>INK4A</sup> immunostained slides was performed on 3 different staining patterns: negative, focal, or diffuse staining. Negative staining was defined as non-immunoreactive; focal staining was defined as non continuous staining of isolated cells or small cell clusters. Diffuse staining was defined as a continuous staining of cells of the basal and parabasal layers, independently of the positive or negative staining in the centre of the tumour nests. p16<sup>INK4A</sup> expression was nuclear, as well as cytoplasmic. Only cases with diffuse staining were considered as positive for analytical purposes.<sup>23</sup>

p53 was detected with the monoclonal antibody DO-7 (Dako). In each series a positive control consisting of an endometrial serous papillary carcinoma for p53 was included. p53 immunostaining was scored as negative when <1%

of cells were positive, (+) when less than 5% were positive, (++) when less than 25% of cells were positive and (+++) when more than 25% of positive cells were observed. <sup>18</sup>

## STATISTICAL ANALYSIS

Data was analyzed with the program SPSS (Version 15.0, SPSS Inc, Chicago, IL, USA). Chi-square analysis or Fisher's exact test were used for comparisons between qualitative variables and Student's t test and ANOVA were applied for quantitative variables according to application conditions.

## Results

### HPV DETECTION AND CLINICAL CORRELATIONS

Table 1 lists the age, location, previous VaIN, previous or concurrent other associated neoplasms, as well as the results of HPV DNA detection for each individual tumour. Of all tumours, 25 out of 32 (78.1%) were HPV positive, whereas only 7 SCCVa were HPV-negative. All HPVs detected were of high risk types and HPV16 was the type most frequently identified (18 out of 25 [72.0%] HPV-positive cases).

Patients with HPV-positive tumours were significantly younger than patients with HPV-negative neoplasms ( $62.6 \pm 13.8$  vs.  $74.0 \pm 8.5$ ;  $p=0.049$ ). All tumours arising in women under 60 years (7 women) were positive for HPV. A previous history of invasive carcinoma (7 patients) or high-grade intraepithelial neoplasia of the uterine cervix (CIN2-3, 6 cases) or vulva (basaloid VIN3, 1 patient) was identified in 56.0% (14/25) of women with HPV-positive tumours and none of HPV-negative tumours ( $p=0.010$ ). In all the cases the cervical or

vulvar neoplasm had been diagnosed more than 5 years before the diagnosis of SCCVa and no evidence of relapse of the cervical tumour or premalignant lesion had been detected during a period of at least 5 years. No differences in mean age were observed between women with history of previous cervical or vulvar neoplasm and women with HPV-positive tumours and no previous history of neoplasm ( $61.7 \pm 15.1$  vs.  $63.8 \pm 12.8$ ;  $p=0.715$ ). Nineteen patients (59.4%) presented because abnormal vaginal bleeding, in 11 women (34.4%) the tumour was detected because of abnormal Pap test and in two a vaginal mass was detected in a routine gynaecological examination. The upper third of the vagina was exclusively involved in 8/25 (32.0%) HPV-positive and 0/7 (0%) HPV-negative tumours ( $p=0.149$ ).

#### HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISTICS

The histological and immunohistochemical characteristics of HPV-positive and negative tumours are shown in table 2. Overall, 15 SSCVa were non-keratinizing, 10 were keratinizing, 4 were basaloid and 3 were of warty type. 21 out of 25 HPV-positive tumours (84.0%) were of non-keratinizing, basaloid or warty types, whereas only 4/25 (16.0%) tumours were of keratinizing type. In contrast, 6 out of 7 HPV-negative tumours (85.7%) were of keratinizing type ( $p<0.001$ ). There was no association between HPV-type and any histological type of tumour.

All but one HPV-positive tumours were strongly positive, showing diffuse basal staining (score +++ ) for p16<sup>INK4a</sup> regardless of their histological type. The staining for p16<sup>INK4a</sup> was intense and clear both in the nuclei and in the

cytoplasm and no background was observed in negative cases or in negative areas of positive cases. Figure 1 illustrates the histological as well as the p16<sup>INK4a</sup> staining of four HPV-positive tumours with non-keratinizing, basaloid and keratinizing features. p53 was positive in 3 out of 25 HPV-positive tumours. One of them was negative for p16<sup>INK4a</sup>. In this case a HPV52 was repeatedly identified. In the HPV-negative group only one case showed intense and diffuse positivity for p16<sup>INK4a</sup> (score +++). This positive staining coexisted with a strong and staining for p53. Figure 2 illustrates the histological as well as the p16<sup>INK4a</sup> staining of two HPV-negative tumours with keratinizing and non-keratinizing. Table 3 shows the sensitivity, specificity, and predictive values for the diagnosis of HPV-positive carcinoma of the vagina according to the following diagnostic approaches: diffuse positivity for p16<sup>INK4a</sup>, negative staining for p53 and non-keratinizing, basaloid or warty histology.

#### TREATMENT AND FOLLOW-UP DATA

Table 4 shows the treatment received by each patient, and the follow-up results. Twelve out of 23 women (47.8%) with HPV-positive tumours and adequate follow-up and none out of 5 (0%) women with HPV-negative tumours were alive without disease (p=0.052). Five out of 25 (24.0%) women with HPV-positive tumours and 2/5 women (40.0%) with HPV-negative tumours died of disease (p=0.587). The median survival was 113.9 months for women with HPV-positive and 19.7 months for women with HPV-negative tumours respectively (p=0.152). In the HPV-positive group of patients no differences were observed in terms of survival between patients with a previous history of cervical carcinoma (0 out of 7 died of disease), high grade premalignant cervical

or vulvar neoplasia (3 out of 7, 37.5% died of disease), or no previous history (3 out of 11, 27.3% died of disease) ( $p=0.200$ ).

## Discussion

Our results confirm previous evidence showing that, similarly to vulvar invasive squamous cell carcinoma, invasive SCCVa develops following two different etiopathogenic pathways: one group is related to HPV infection, whereas the second group of tumours arises via independent-HPV pathways. However, in contrast with the 20-35% of tumours positive for HPV in the vulva<sup>18</sup>,<sup>27, 28</sup> an overwhelming number of SCCVa (over 75% of the invasive SCCVa in the present series) are HPV-positive. The high percentage of HPV-positive tumours observed in our series is similar to the 81% observed in a recent series published by Ferreira et al.<sup>13</sup> but is clearly higher than the percentages reported in older series. Although these variations might in part reflect different prevalence of HPV infection in different areas, they are likely caused mainly by the higher sensitivity of the HPV detection techniques used in these recent reports. In this regard, in our study, like in the report by Ferreira et al, the SPF10 primers have been used to detect HPV. These primers, which amplify a small region of 65 bp, are particularly useful for studies based on formalin-fixed, paraffin embedded tissue, and have been shown to be more sensitive than other PCRs in this type of material.<sup>18</sup>

In our study, HPV16 was identified in 72% of HPV-positive SCCVa. This percentage is higher than the percentage of HPV16 reported in the uterine cervix.<sup>14</sup> This high percentage is however, similar to that observed in other reports focused on SCCVa.<sup>13</sup> These high percentages of HPV16 involvement

observed in the vagina are as well similar to those observed in the vulva.<sup>18</sup> Moreover, percentages even higher than those (around 90%) have been reported in most studies focusing on head and neck tumours.<sup>21, 29-31</sup> Thus, our data indicate that, as in all regions and particularly in sites other than the uterine cervix, HPV16 is by far the most frequently involved type and that currently developed vaccines may prevent the large majority SCCVa associated with HPV infection.

As it has been shown in the vulva, women with HPV-positive SCCVa were significantly younger than patients with HPV-negative tumours. Nevertheless, although it has been suggested that SCCVa associated to HPV could be similar to cervical cancers in terms of risk factors and prognostic,<sup>3, 5, 22-24</sup> there are important differences between both neoplasm, particularly in age of presentation. SCCVa and cervical cancer occur in different age groups: while cervical carcinomas affect women younger than 60 years, SCCVa, is mainly a disease of elderly postmenopausal women, as showed in previous studies.<sup>3, 7-9, 22, 32</sup> On our study, only 7 tumours, all of them positive for HPV (21.9% of all SCCVa; 28% of HPV-positive tumours) developed in women under 60 years.

In agreement with previous reports<sup>3, 5, 22-24</sup> our results show that a high percentage of women with SCCVa have a history of prior dysplasia or invasive carcinoma of the uterine cervix. Following general consensus we have used in this report a 5 year free period between any previous cervical or vulvar neoplasm or relapse and the diagnosis of the SCCVa as the limit to distinguish a recurrent cervical carcinoma and a new primary vaginal carcinoma. Nevertheless, there is some concern about this threshold and several authors have expanded that period to 10 years<sup>6</sup> or have even excluded all patients with

any previous history of cervical pathology.<sup>13</sup> In our study no significant differences were observed in age, and more importantly in terms of survival, between patients with SCCVa-HPV positive and previous history of cervical or vulvar neoplasia, and those with SCCVa-HPV positive and no previous history of cervical or vulvar disease. Thus, our findings suggest that this 5 year free period is an adequate threshold to distinguish a recurrent cervical carcinoma and a new primary vaginal carcinoma.

Our series confirms previous data showing that most HPV-positive SCCVa are of non-keratinizing, basaloid or warty types, whereas most HPV-negative tumours are commonly of keratinizing type.<sup>13</sup> However, as observed in the vulva,<sup>18</sup> some overlap exists between the histological types and the association with HPV. Thus, 16% of all HPV-positive SCCVa were of keratinizing type, whereas 14.3% of the HPV-negative SCCVa showed non-keratinizing features.

The present study confirms previous data from vulva,<sup>18, 33</sup> penis,<sup>34</sup> head and neck region,<sup>21, 29, 31, 35</sup> and anal canal<sup>36</sup> showing that, as observed in the uterine cervix,<sup>37-39</sup> an intense and diffuse expression of p16<sup>INK4A</sup> in a SCCVa indicates a high-risk HPV-associated process, this being irrespective of the histological type. An intense and diffuse p16<sup>INK4A</sup> expression was observed in 96% HPV-positive SCCVa and only in one (14.3%) non-HPV associated SCCVa. In our series, the sensitivity and specificity of p16<sup>INK4a</sup> immunostaining to detect HPV-associated carcinomas (96% and 87.5% respectively) were higher than the sensitivity and specificity of histological classification and those of p53 negative staining. Thus, our results clearly indicate that p16<sup>INK4A</sup> immunostaining can be a useful tool to properly classify SCCVa into HPV-

positive or HPV-negative and can be considered as almost equivalent to HPV detection.

Finally no significant differences in terms of clinical stage, local recurrence or survival were observed between HPV-positive and HPV-negative tumours. However, the results are limited in this respect due to the small number of HPV-negative tumours observed in our series, and should be confirmed in larger studies.

In summary, our study confirms that SCCVa occur in two different groups of patients. HPV-positive SCCVa, which account for over 75% of all SCCVa, arise in younger women, and are frequently associated with previous HPV-positive tumours or premalignant lesions of the uterine cervix, or vulva. Histologically, HPV-positive SCCVa are frequently of non-keratinizing, basaloid or warty type, and overexpress p16<sup>INK4a</sup>. In contrast, HPV-negative tumours, arise in elderly women, are histologically of keratinizing type and do not overexpress p16<sup>INK4a</sup>. Our study indicates that p16<sup>INK4a</sup> is a reliable marker for HPV-positive SCCVa and improves the results of pure histological classification.

### **Acknowledgements**

The authors are grateful to Ms. Rosana Millan and Ms. Ingrid Victoria for their technical assistance in the preparation of histological sections and Ms. Montserrat Tortosa and Ms. Elena Gonzalvo for their help with the immunohistochemical studies. We thank Mr. Ray Stanvius for the English revision of the manuscript.



**Table 1.** Cases of primary squamous cell carcinoma of the vagina with age, tumour location, other associated neoplasms and HPV type

Case	Age	Tumour location	Previous VaIN	Associated neoplasm	HPV type
1	53	Lower	Unknown	No	16
2	47	Upper	Unknown	CIN3	16
3	49	Upper	Yes	Carcinoma cervix	16
4	52	Upper	Unknown	CIN3	16
5	65	Unknown	Yes	Carcinoma cervix, VIN3	16
6	72	Lower	Yes	No	16
7	66	Upper	No	Carcinoma cervix	16
8	79	Lower	Yes	CIN3, Melanoma	16
9	86	Lower	Yes	VIN3	16
10	72	Middle	Unknown	No	16
11	65	All	Yes	No	16
12	78	Lower	Yes	Carcinoma cervix	16
13	45	Upper	Yes	Carcinoma cervix	16
14	38	Upper	Yes	Carcinoma cervix	16
15	72	Middle and lower	No	CIN3	16
16	61	Middle and lower	No	No	16
17	69	All	No	No	16
18	63	All	Unknown	CIN3	16
19	69	All	No	Carcinosarcoma endometrium	35
20	47	Upper	Yes	No	51
21	44	Middle	No	No	52
22	89	Middle	Unknown	No	52
23	75	All	Unknown	Carcinoma cervix	58
24	61	Upper	Yes	No	59
25	49	All	Yes	CIN2	68
26	71	All	No	No	Negative
27	82	Middle and lower	No	No	Negative
28	83	Middle	Unknown	No	Negative
29	75	Upper and middle	Unknown	No	Negative
30	60	All	Unknown	No	Negative
31	67	Lower	Unknown	No	Negative
32	80	Middle and lower	No	No	Negative

**Table 2.** Histological and immunohistochemical characteristics of human papillomavirus-positive and -negative primary squamous cell carcinomas of the vagina

	High-risk human papillomavirus		p value
	Positive (n=25)	Negative (n=7)	
<b>Histologic type</b>			0.006
Keratinizing	4 (16.0 %)	6 (85.7 %)	
Non-keratinizing	14 (56.0%)	1 (14.3%)	
Basaloid	4 (16.0 %)	0 (0 %)	
Warty	3 (12.0 %)	0 (0 %)	
<b>Immunohistochemistry</b>			
p16 <sup>INK4A</sup> positivity	24 (96.0 %)	1 (14.3 %)	<0.001
p53 positivity	3 (12.0 %)	4 (64.5 %)	0.025

**Table 3.** Sensitivity, specificity, positive and negative predictive values for the diagnosis of human papillomavirus-associated carcinoma based on diffuse positivity for p16<sup>INK4A</sup>, negative staining for p53 and basaloid or warty histology.

	<b>p16<sup>INK4A</sup> diffuse</b>	<b>p53 (-)</b>	<b>Non-keratinizing/ basaloid/warty histology</b>
<b>Sensitivity</b>	96.0%	88.0%	95.5%
<b>Specificity</b>	85.7%	57.1%	60.0%
<b>Positive predictive value</b>	96.0%	88.0%	84.0%
<b>Negative predictive value</b>	85.7%	57.1%	85.7%

**p16<sup>INK4A</sup> diffuse:** Continuous staining of cells of the basal and parabasal layers

**p53 (-):** Less than 25% of positive cells (score 0, + or ++)

**Table 4.** Primary squamous cell carcinoma of the vagina with treatment, relapse, and follow-up

Case	Stage	Treatment	Relapse	Months follow-up	Follow-up
1	II	Radiotherapy	No	159.5	AWOD
2	I	Radiotherapy	Pelvis	33.2	DOD
3	Unknown	Radiotherapy + Surgery <sup>a</sup>	Vagina	15.6	AWOD
4	IV	Radiotherapy + Surgery <sup>c, d</sup>	Pelvic	15.6	DOD
5	Unknown	Surgery <sup>e</sup>	Vagina	148.3	AWD
6	II	Radiotherapy	Vagina	9.4	AWOD
7	I	Radiotherapy + Surgery <sup>a</sup>	No	134.2	AWOD
8	II	Radiotherapy + Surgery <sup>a</sup>	Vagina	136.4	AWD
9	I	Radiotherapy	Pelvis	24.3	AWOD
10	III	Radiotherapy	No	106.0	AWOD
11	I	Radiotherapy + Surgery <sup>e</sup>	Pelvic and abdominal	104.0	AWOD
12	II	Surgery <sup>a</sup>	No	2.2	AWOD
13	I	Radiotherapy + Surgery <sup>a</sup>	No	42.1	AWOD
14	I	Radiotherapy + Surgery <sup>a</sup>	Pelvis	83.7	AWOD
15	II	Radiotherapy + Chemotherapy	No	26.1	AWOD
16	IV	Chemotherapy	No	40.1	AWD
17	III	Palliative	Progression	31.2	DOD
18	IV	Surgery <sup>a</sup>	Pelvis and abdomen	57.4	AWD
19	IV	Radiotherapy	Progression	9.0	AWD

20	II	Surgery <sup>a, b, c</sup>	No	12.4	AWOD
21	II	Chemotherapy + Radiotherapy + Surgery <sup>b, c, e</sup>	Vagina	12.8	AWD
22	II	Radiotherapy	Vagina	1.5	DOD
23	III	Chemotherapy + Radiotherapy	No	0.9	AWD
24	II	Surgery <sup>a, c</sup>	Vagina	3.2	DOD
25	III	Radiotherapy + Chemotherapy	No	10.5	DOD
26	III	Surgery <sup>a, b</sup>	Pelvis	11.4	AWD
27	III	Radiotherapy	Progression	7.3	AWD
28	II	Radiotherapy	No	36.2	AWD
29	IV	Surgery <sup>a</sup>	No	15.7	DOCT*
30	II	Radiotherapy + Chemotherapy	No	4.9	DOCT**
31	II	Radiotherapy	Vagina + lymph nodes	12.8	DOD
32	III	Palliative	Progression	0.5	DOD

<sup>a</sup>: Partial colectomy; <sup>b</sup>: total hysterectomy with bilateral salpingoophorectomy; <sup>c</sup>: Pelvic lymphadenectomy; <sup>d</sup>: Posterior exenteration; <sup>e</sup>: Total colectomy; AWOD: Alive without disease; AWD: alive with disease; DOD: Dead of disease; DOC: Dead of complications of treatment; \* dead of surgical complications; \*\*: dead of sepsis

**LEGEND OF FIGURES**

**Figure 1.** Human papillomavirus-positive squamous cell carcinomas of the vagina. A: Basaloid; B: warty, C: keratinizing types, H&E staining. Inset in B koilocytotic change. Diffuse immunohistochemical expression of p16<sup>INK4A</sup> (A',B',C').

**Figure 2.** Human papillomavirus-negative vulvar squamous cell carcinomas. A, B, C: keratinizing type. A: H&E, B: negative staining for p16<sup>INK4A</sup>; C: negative staining for p53; D, E, F: non-keratinizing type. D: H&E, E: negative staining for p16<sup>INK4A</sup>; F: positive staining for p53.

## References

1. Beller U, Maisonneuve P, Benedet JL, *et al.* Carcinoma of the vagina. *Int. J. Gynaecol. Obstet.* 2003 Oct;**83 Suppl 1**:27-39.
2. Chyle V, Zagars GK, Wheeler JA, Wharton JT, Delclos L. Definitive radiotherapy for carcinoma of the vagina: outcome and prognostic factors. *Int. J. Radiat. Oncol. Biol. Phys.* 1996 Jul 15;**35**(5):891-905.
3. Creasman WT, Phillips JL, Menck HR. The National Cancer Data Base report on cancer of the vagina. *Cancer* 1998 Sep 1;**83**(5):1033-1040.
4. Hellman K, Hellstrom AC, Silfversward C, *et al.* Cancer of the vagina: Laminin-5gamma2 chain expression and prognosis. *Int. J. Gynecol. Cancer* 2000 Sep;**10**(5):391-396.
5. Hellman K, Alaiya AA, Schedvins K, Steinberg W, Hellstrom AC, Auer G. Protein expression patterns in primary carcinoma of the vagina. *Br. J. Cancer* 2004 Jul 19;**91**(2):319-326.
6. Hellman K, Lundell M, Silfversward C, Nilsson B, Hellstrom AC, Frankendal B. Clinical and histopathologic factors related to prognosis in primary squamous cell carcinoma of the vagina. *Int. J. Gynecol. Cancer* 2006 May;**16**(3):1201-1211.
7. Lindeque BG. The role of surgery in the management of carcinoma of the vagina. *Baillieres Clin. Obstet. Gynaecol.* 1987 Jun;**1**(2):319-329.

8. Pingley S, Shrivastava SK, Sarin R, *et al.* Primary carcinoma of the vagina: Tata Memorial Hospital experience. *Int. J. Radiat. Oncol. Biol. Phys.* 2000 Jan 1;**46**(1):101-108.
9. Ptackova B. Primary carcinoma of the vagina. *Neoplasma* 1968;**15**(3):309-322.
10. Stock RG, Chen AS, Seski J. A 30-year experience in the management of primary carcinoma of the vagina: analysis of prognostic factors and treatment modalities. *Gynecol. Oncol.* 1995 Jan;**56**(1):45-52.
11. Beller U, Benedet JL, Creasman WT, *et al.* Carcinoma of the vagina. FIGO 6th Annual Report on the Results of Treatment in Gynecological Cancer. *Int. J. Gynaecol. Obstet.* 2006 Nov;**95 Suppl 1**:S29-S42.
12. Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. *J. Clin. Pathol.* 2002 Apr;**55**(4):244-265.
13. Ferreira M, Crespo M, Martins L, Felix A. HPV DNA detection and genotyping in 21 cases of primary invasive squamous cell carcinoma of the vagina. *Mod. Pathol.* 2008 Aug;**21**(8):968-972.
14. Munoz N, Bosch FX, de SS, *et al.* Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N. Engl. J. Med.* 2003 Feb 6;**348**(6):518-527.
15. Walboomers JM, Jacobs MV, Manos MM, *et al.* Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J. Pathol.* 1999 Sep;**189**(1):12-19.



16. Al-Ghamdi A, Freedman D, Miller D, *et al.* Vulvar squamous cell carcinoma in young women: a clinicopathologic study of 21 cases. *Gynecol. Oncol.* 2002 Jan;**84**(1):94-101.
17. Madeleine MM, Daling JR, Carter JJ, *et al.* Cofactors with human papillomavirus in a population-based study of vulvar cancer. *J. Natl. Cancer Inst.* 1997 Oct 15;**89**(20):1516-1523.
18. Santos M, Landolfi S, Olivella A, *et al.* p16 overexpression identifies HPV-positive vulvar squamous cell carcinomas. *Am. J. Surg. Pathol.* 2006 Nov;**30**(11):1347-1356.
19. Schiffman M, Kjaer SK. Chapter 2: Natural history of anogenital human papillomavirus infection and neoplasia. *J. Natl. Cancer Inst. Monogr* 2003;(31):14-19.
20. Trimble CL, Hildesheim A, Brinton LA, Shah KV, Kurman RJ. Heterogeneous etiology of squamous carcinoma of the vulva. *Obstet. Gynecol.* 1996 Jan;**87**(1):59-64.
21. Alos L, Moyano S, Nadal A, *et al.* Human papillomaviruses are identified in a subgroup of sinonasal squamous cell carcinomas with favorable outcome. *Cancer* 2009 Jun 15;**115**(12):2701-2709.
22. Perez CA, Grigsby PW, Garipagaoglu M, Mutch DG, Lockett MA. Factors affecting long-term outcome of irradiation in carcinoma of the vagina. *Int. J. Radiat. Oncol. Biol. Phys.* 1999 Apr 1;**44**(1):37-45.
23. Leminen A, Forss M, Lehtovirta P. Therapeutic and prognostic considerations in primary carcinoma of the vagina. *Acta Obstet. Gynecol. Scand.* 1995 May;**74**(5):379-383.

24. Murad TM, Durant JR, Maddox WA, Dowling EA. The pathologic behavior of primary vaginal carcinoma and its relationship to cervical cancer. *Cancer* 1975 Mar;**35**(3):787-794.
25. Hellman K, Silfversward C, Nilsson B, Hellstrom AC, Frankendal B, Pettersson F. Primary carcinoma of the vagina: factors influencing the age at diagnosis. The Radiumhemmet series 1956-96. *Int. J. Gynecol. Cancer* 2004 May;**14**(3):491-501.
26. Tabata T, Takeshima N, Nishida H, Hirai Y, Hasumi K. Treatment failure in vaginal cancer. *Gynecol. Oncol.* 2002 Feb;**84**(2):309-314.
27. Kurman RJ, Toki T, Schiffman MH. Basaloid and warty carcinomas of the vulva. Distinctive types of squamous cell carcinoma frequently associated with human papillomaviruses. *Am. J. Surg. Pathol.* 1993 Feb;**17**(2):133-145.
28. Toki T, Kurman RJ, Park JS, Kessis T, Daniel RW, Shah KV. Probable nonpapillomavirus etiology of squamous cell carcinoma of the vulva in older women: a clinicopathologic study using in situ hybridization and polymerase chain reaction. *Int. J. Gynecol. Pathol.* 1991;**10**(2):107-125.
29. Gillison ML, Koch WM, Capone RB, *et al.* Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J. Natl. Cancer Inst.* 2000 May 3;**92**(9):709-720.
30. Herrero R, Castellsague X, Pawlita M, *et al.* Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. *J. Natl. Cancer Inst.* 2003 Dec 3;**95**(23):1772-1783.

31. El-Mofty SK, Lu DW. Prevalence of human papillomavirus type 16 DNA in squamous cell carcinoma of the palatine tonsil, and not the oral cavity, in young patients: a distinct clinicopathologic and molecular disease entity. *Am. J. Surg. Pathol.* 2003 Nov;**27**(11):1463-1470.
32. Tjalma WA, Monaghan JM, de Barros LA, Naik R, Nordin AJ, Weyler JJ. The role of surgery in invasive squamous carcinoma of the vagina. *Gynecol. Oncol.* 2001 Jun;**81**(3):360-365.
33. Santos M, Montagut C, Mellado B, *et al.* Immunohistochemical staining for p16 and p53 in premalignant and malignant epithelial lesions of the vulva. *Int. J. Gynecol. Pathol.* 2004 Jul;**23**(3):206-214.
34. Rubin MA, Kleter B, Zhou M, *et al.* Detection and typing of human papillomavirus DNA in penile carcinoma: evidence for multiple independent pathways of penile carcinogenesis. *Am. J. Pathol.* 2001 Oct;**159**(4):1211-1218.
35. Klussmann JP, Gultekin E, Weissenborn SJ, *et al.* Expression of p16 protein identifies a distinct entity of tonsillar carcinomas associated with human papillomavirus. *Am. J. Pathol.* 2003 Mar;**162**(3):747-753.
36. Lu DW, El-Mofty SK, Wang HL. Expression of p16, Rb, and p53 proteins in squamous cell carcinomas of the anorectal region harboring human papillomavirus DNA. *Mod. Pathol.* 2003 Jul;**16**(7):692-699.
37. Wang HL, Lu DW. Detection of human papillomavirus DNA and expression of p16, Rb, and p53 proteins in small cell carcinomas of the uterine cervix. *Am. J. Surg. Pathol.* 2004 Jul;**28**(7):901-908.

38. Klaes R, Benner A, Friedrich T, *et al.* p16INK4a immunohistochemistry improves interobserver agreement in the diagnosis of cervical intraepithelial neoplasia. *Am. J. Surg. Pathol.* 2002 Nov;**26**(11):1389-1399.
39. Negri G, Egarter-Vigl E, Kasal A, Romano F, Haitel A, Mian C. p16INK4a is a useful marker for the diagnosis of adenocarcinoma of the cervix uteri and its precursors: an immunohistochemical study with immunocytochemical correlations. *Am. J. Surg. Pathol.* 2003 Feb;**27**(2):187-193.

**Table 1.** Cases of primary squamous cell carcinoma of the vagina with age, tumour location, other associated neoplasms and HPV type

Case	Age	Tumour location	Previous VaIN	Associated neoplasm	HPV type
1	53	Lower	Unknown	No	16
2	47	Upper	Unknown	CIN3	16
3	49	Upper	Yes	Carcinoma cervix	16
4	52	Upper	Unknown	CIN3	16
5	65	Unknown	Yes	Carcinoma cervix, VIN3	16
6	72	Lower	Yes	No	16
7	66	Upper	No	Carcinoma cervix	16
8	79	Lower	Yes	CIN3, Melanoma	16
9	86	Lower	Yes	VIN3	16
10	72	Middle	Unknown	No	16
11	65	All	Yes	No	16
12	78	Lower	Yes	Carcinoma cervix	16
13	45	Upper	Yes	Carcinoma cervix	16
14	38	Upper	Yes	Carcinoma cervix	16
15	72	Middle and lower	No	CIN3	16
16	61	Middle and lower	No	No	16
17	69	All	No	No	16
18	63	All	Unknown	CIN3	16
19	69	All	No	Carcinosarcoma endometrium	35
20	47	Upper	Yes	No	51
21	44	Middle	No	No	52
22	89	Middle	Unknown	No	52
23	75	All	Unknown	Carcinoma cervix	58
24	61	Upper	Yes	No	59
25	49	All	Yes	CIN2	68
26	71	All	No	No	Negative
27	82	Middle and lower	No	No	Negative
28	83	Middle	Unknown	No	Negative
29	75	Upper and middle	Unknown	No	Negative
30	60	All	Unknown	No	Negative
31	67	Lower	Unknown	No	Negative
32	80	Middle and lower	No	No	Negative

**Table 2.** Histological and immunohistochemical characteristics of human papillomavirus-positive and -negative primary squamous cell carcinomas of the vagina

	High-risk human papillomavirus		p value
	Positive (n=25)	Negative (n=7)	
<b>Histologic type</b>			0.006
Keratinizing	4 (16.0 %)	6 (85.7 %)	
Non-keratinizing	14 (56.0%)	1 (14.3%)	
Basaloid	4 (16.0 %)	0 (0 %)	
Warty	3 (12.0 %)	0 (0 %)	
<b>Immunohistochemistry</b>			
p16 <sup>INK4A</sup> positivity	24 (96.0 %)	1 (14.3 %)	<0.001
p53 positivity	3 (12.0 %)	4 (64.5 %)	0.025

**Table 3.** Sensitivity, specificity, positive and negative predictive values for the diagnosis of human papillomavirus-associated carcinoma based on diffuse positivity for p16<sup>INK4A</sup>, negative staining for p53 and basaloid or warty histology.

	<b>p16<sup>INK4A</sup> diffuse</b>	<b>p53 (-)</b>	<b>Non-keratinizing/ basaloid/warty histology</b>
<b>Sensitivity</b>	96.0%	88.0%	95.5%
<b>Specificity</b>	85.7%	57.1%	60.0%
<b>Positive predictive value</b>	96.0%	88.0%	84.0%
<b>Negative predictive value</b>	85.7%	57.1%	85.7%

**p16<sup>INK4A</sup> diffuse:** Continuous staining of cells of the basal and parabasal layers

**p53 (-):** Less than 25% of positive cells (score 0, + or ++)

For Peer Review

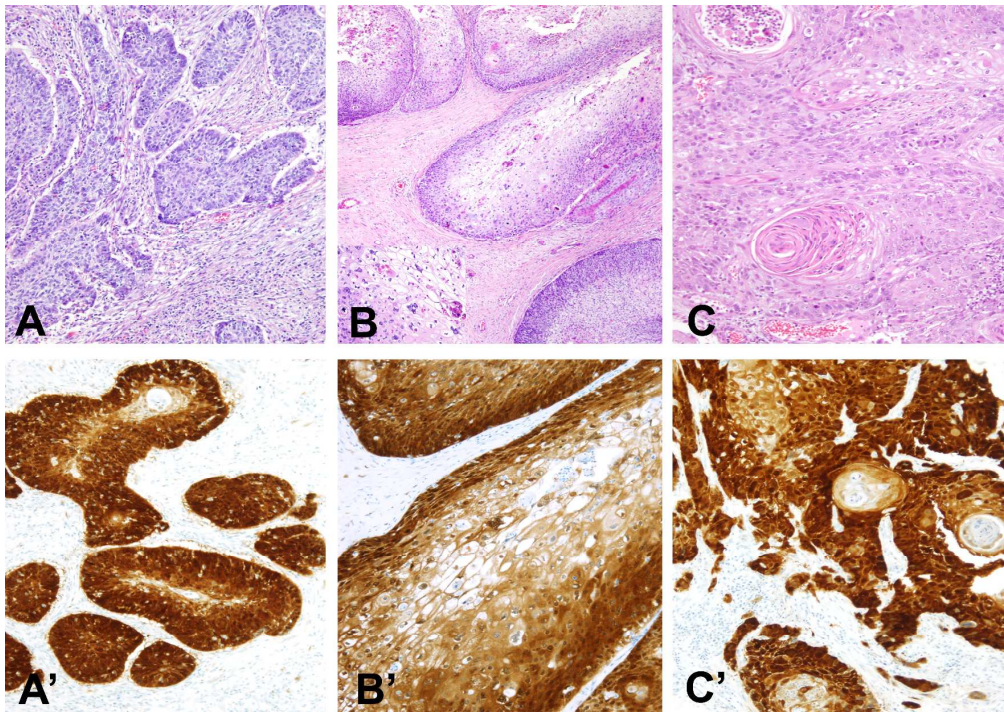


**Table 4.** Primary squamous cell carcinoma of the vagina with treatment, relapse, and follow-up

Case	Stage	Treatment	Relapse	Months follow-up	Follow-up
1	II	Radiotherapy	No	159.5	AWOD
2	I	Radiotherapy	Pelvis	33.2	DOD
3	Unknown	Radiotherapy + Surgery <sup>a</sup>	Vagina	15.6	AWOD
4	IV	Radiotherapy + Surgery <sup>c, d</sup>	Pelvic	15.6	DOD
5	Unknown	Surgery <sup>e</sup>	Vagina	148.3	AWD
6	II	Radiotherapy	Vagina	9.4	AWOD
7	I	Radiotherapy + Surgery <sup>a</sup>	No	134.2	AWOD
8	II	Radiotherapy + Surgery <sup>a</sup>	Vagina	136.4	AWD
9	I	Radiotherapy	Pelvis	24.3	AWOD
10	III	Radiotherapy	No	106.0	AWOD
11	I	Radiotherapy + Surgery <sup>e</sup>	Pelvic and abdominal	104.0	AWOD
12	II	Surgery <sup>a</sup>	No	2.2	AWOD
13	I	Radiotherapy + Surgery <sup>a</sup>	No	42.1	AWOD
14	I	Radiotherapy + Surgery <sup>a</sup>	Pelvis	83.7	AWOD
15	II	Radiotherapy + Chemotherapy	No	26.1	AWOD
16	IV	Chemotherapy	No	40.1	AWD
17	III	Palliative	Progression	31.2	DOD
18	IV	Surgery <sup>a</sup>	Pelvis and abdomen	57.4	AWD
19	IV	Radiotherapy	Progression	9.0	AWD

20	II	Surgery <sup>a, b, c</sup>	No	12.4	AWOD
21	II	Chemotherapy + Radiotherapy + Surgery <sup>b, c, e</sup>	Vagina	12.8	AWD
22	II	Radiotherapy	Vagina	1.5	DOD
23	III	Chemotherapy + Radiotherapy	No	0.9	AWD
24	II	Surgery <sup>a, c</sup>	Vagina	3.2	DOD
25	III	Radiotherapy + Chemotherapy	No	10.5	DOD
26	III	Surgery <sup>a, b</sup>	Pelvis	11.4	AWD
27	III	Radiotherapy	Progression	7.3	AWD
28	II	Radiotherapy	No	36.2	AWD
29	IV	Surgery <sup>a</sup>	No	15.7	DOCT*
30	II	Radiotherapy + Chemotherapy	No	4.9	DOCT**
31	II	Radiotherapy	Vagina + lymph nodes	12.8	DOD
32	III	Palliative	Progression	0.5	DOD

<sup>a</sup>: Partial colpectomy; <sup>b</sup>: total hysterectomy with bilateral salpingoophorectomy; <sup>c</sup>: Pelvic lymphadenectomy; <sup>d</sup>: Posterior exenteration; <sup>e</sup>: Total colpectomy; AWOD: Alive without disease; AWD: alive with disease; DOD: Dead of disease; DOC: Dead of complications of treatment; \* dead of surgical complications; \*\*: dead of sepsis



297x209mm (300 x 300 DPI)

Review

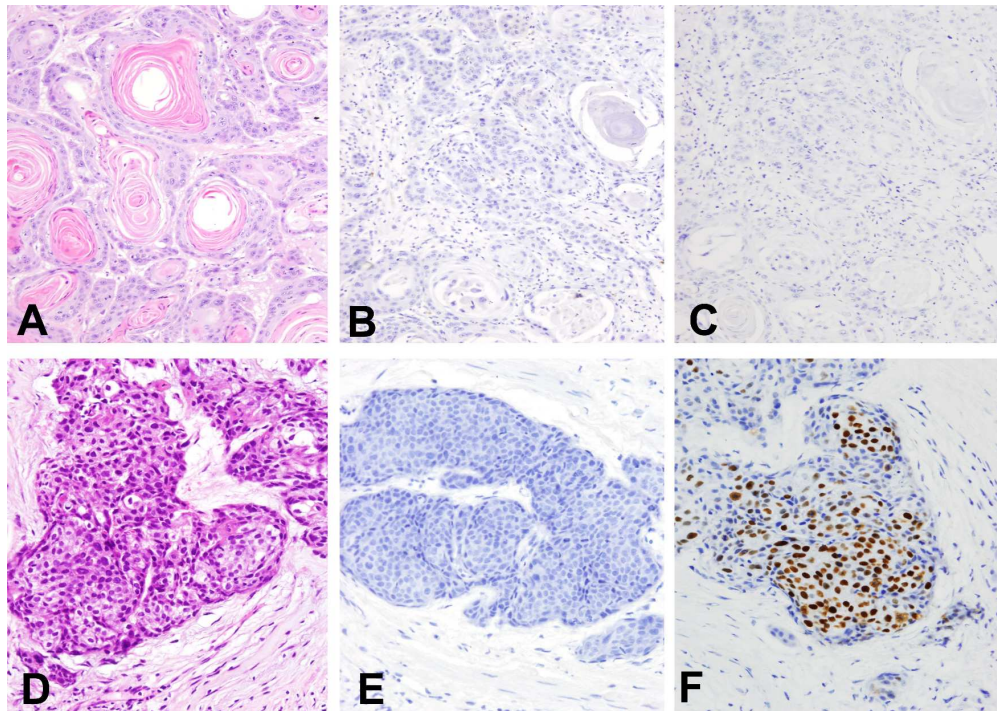


Figure 1. Human papillomavirus-positive squamous cell carcinomas of the vagina. A: Basaloid; B: warty, C: keratinizing types, H&E staining. Inset in B koilocytotic change. Diffuse immunohistochemical expression of p16INK4A (A',B',C'). 297x209mm (300 x 300 DPI)

**LEGEND OF FIGURES**

**Figure 1.** Human papillomavirus-positive squamous cell carcinomas of the vagina. A: Basaloid; B: warty, C: keratinizing types, H&E staining. Inset in B koilocytotic change. Diffuse immunohistochemical expression of p16<sup>INK4A</sup> (A',B',C').

**Figure 2.** Human papillomavirus-negative vulvar squamous cell carcinomas. A, B, C: keratinizing type. A: H&E, B: negative staining for p16<sup>INK4A</sup>; C: negative staining for p53; D, E, F: non-keratinizing type. D: H&E, E: negative staining for p16<sup>INK4A</sup>; F: positive staining for p53.

## References

1. Beller U, Maisonneuve P, Benedet JL, *et al.* Carcinoma of the vagina. *Int. J. Gynaecol. Obstet.* 2003 Oct;**83 Suppl 1**:27-39.
2. Chyle V, Zagars GK, Wheeler JA, Wharton JT, Delclos L. Definitive radiotherapy for carcinoma of the vagina: outcome and prognostic factors. *Int. J. Radiat. Oncol. Biol. Phys.* 1996 Jul 15;**35**(5):891-905.
3. Creasman WT, Phillips JL, Menck HR. The National Cancer Data Base report on cancer of the vagina. *Cancer* 1998 Sep 1;**83**(5):1033-1040.
4. Hellman K, Hellstrom AC, Silfversward C, *et al.* Cancer of the vagina: Laminin-5gamma2 chain expression and prognosis. *Int. J. Gynecol. Cancer* 2000 Sep;**10**(5):391-396.
5. Hellman K, Alaiya AA, Schedvins K, Steinberg W, Hellstrom AC, Auer G. Protein expression patterns in primary carcinoma of the vagina. *Br. J. Cancer* 2004 Jul 19;**91**(2):319-326.
6. Hellman K, Lundell M, Silfversward C, Nilsson B, Hellstrom AC, Frankendal B. Clinical and histopathologic factors related to prognosis in primary squamous cell carcinoma of the vagina. *Int. J. Gynecol. Cancer* 2006 May;**16**(3):1201-1211.
7. Lindeque BG. The role of surgery in the management of carcinoma of the vagina. *Baillieres Clin. Obstet. Gynaecol.* 1987 Jun;**1**(2):319-329.
8. Pingley S, Shrivastava SK, Sarin R, *et al.* Primary carcinoma of the vagina: Tata Memorial Hospital experience. *Int. J. Radiat. Oncol. Biol. Phys.* 2000 Jan 1;**46**(1):101-108.

9. Ptackova B. Primary carcinoma of the vagina. *Neoplasma* 1968;**15**(3):309-322.
10. Stock RG, Chen AS, Seski J. A 30-year experience in the management of primary carcinoma of the vagina: analysis of prognostic factors and treatment modalities. *Gynecol. Oncol.* 1995 Jan;**56**(1):45-52.
11. Beller U, Benedet JL, Creasman WT, *et al.* Carcinoma of the vagina. FIGO 6th Annual Report on the Results of Treatment in Gynecological Cancer. *Int. J. Gynaecol. Obstet.* 2006 Nov;**95 Suppl 1**:S29-S42.
12. Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. *J. Clin. Pathol.* 2002 Apr;**55**(4):244-265.
13. Ferreira M, Crespo M, Martins L, Felix A. HPV DNA detection and genotyping in 21 cases of primary invasive squamous cell carcinoma of the vagina. *Mod. Pathol.* 2008 Aug;**21**(8):968-972.
14. Munoz N, Bosch FX, de SS, *et al.* Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N. Engl. J. Med.* 2003 Feb 6;**348**(6):518-527.
15. Walboomers JM, Jacobs MV, Manos MM, *et al.* Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J. Pathol.* 1999 Sep;**189**(1):12-19.
16. Al-Ghamdi A, Freedman D, Miller D, *et al.* Vulvar squamous cell carcinoma in young women: a clinicopathologic study of 21 cases. *Gynecol. Oncol.* 2002 Jan;**84**(1):94-101.

17. Madeleine MM, Daling JR, Carter JJ, *et al.* Cofactors with human papillomavirus in a population-based study of vulvar cancer. *J. Natl. Cancer Inst.* 1997 Oct 15;**89**(20):1516-1523.
18. Santos M, Landolfi S, Olivella A, *et al.* p16 overexpression identifies HPV-positive vulvar squamous cell carcinomas. *Am. J. Surg. Pathol.* 2006 Nov;**30**(11):1347-1356.
19. Schiffman M, Kjaer SK. Chapter 2: Natural history of anogenital human papillomavirus infection and neoplasia. *J. Natl. Cancer Inst. Monogr* 2003;(31):14-19.
20. Trimble CL, Hildesheim A, Brinton LA, Shah KV, Kurman RJ. Heterogeneous etiology of squamous carcinoma of the vulva. *Obstet. Gynecol.* 1996 Jan;**87**(1):59-64.
21. Alos L, Moyano S, Nadal A, *et al.* Human papillomaviruses are identified in a subgroup of sinonasal squamous cell carcinomas with favorable outcome. *Cancer* 2009 Jun 15;**115**(12):2701-2709.
22. Perez CA, Grigsby PW, Garipagaoglu M, Mutch DG, Lockett MA. Factors affecting long-term outcome of irradiation in carcinoma of the vagina. *Int. J. Radiat. Oncol. Biol. Phys.* 1999 Apr 1;**44**(1):37-45.
23. Leminen A, Forss M, Lehtovirta P. Therapeutic and prognostic considerations in primary carcinoma of the vagina. *Acta Obstet. Gynecol. Scand.* 1995 May;**74**(5):379-383.
24. Murad TM, Durant JR, Maddox WA, Dowling EA. The pathologic behavior of primary vaginal carcinoma and its relationship to cervical cancer. *Cancer* 1975 Mar;**35**(3):787-794.



25. Hellman K, Silfversward C, Nilsson B, Hellstrom AC, Frankendal B, Pettersson F. Primary carcinoma of the vagina: factors influencing the age at diagnosis. The Radiumhemmet series 1956-96. *Int. J. Gynecol. Cancer* 2004 May;**14**(3):491-501.
26. Tabata T, Takeshima N, Nishida H, Hirai Y, Hasumi K. Treatment failure in vaginal cancer. *Gynecol. Oncol.* 2002 Feb;**84**(2):309-314.
27. Kurman RJ, Toki T, Schiffman MH. Basaloid and warty carcinomas of the vulva. Distinctive types of squamous cell carcinoma frequently associated with human papillomaviruses. *Am. J. Surg. Pathol.* 1993 Feb;**17**(2):133-145.
28. Toki T, Kurman RJ, Park JS, Kessis T, Daniel RW, Shah KV. Probable nonpapillomavirus etiology of squamous cell carcinoma of the vulva in older women: a clinicopathologic study using in situ hybridization and polymerase chain reaction. *Int. J. Gynecol. Pathol.* 1991;**10**(2):107-125.
29. Gillison ML, Koch WM, Capone RB, *et al.* Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J. Natl. Cancer Inst.* 2000 May 3;**92**(9):709-720.
30. Herrero R, Castellsague X, Pawlita M, *et al.* Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. *J. Natl. Cancer Inst.* 2003 Dec 3;**95**(23):1772-1783.
31. El-Mofty SK, Lu DW. Prevalence of human papillomavirus type 16 DNA in squamous cell carcinoma of the palatine tonsil, and not the oral cavity, in young patients: a distinct clinicopathologic and molecular disease entity. *Am. J. Surg. Pathol.* 2003 Nov;**27**(11):1463-1470.

32. Tjalma WA, Monaghan JM, de Barros LA, Naik R, Nordin AJ, Weyler JJ. The role of surgery in invasive squamous carcinoma of the vagina. *Gynecol. Oncol.* 2001 Jun;**81**(3):360-365.
33. Santos M, Montagut C, Mellado B, *et al.* Immunohistochemical staining for p16 and p53 in premalignant and malignant epithelial lesions of the vulva. *Int. J. Gynecol. Pathol.* 2004 Jul;**23**(3):206-214.
34. Rubin MA, Kleter B, Zhou M, *et al.* Detection and typing of human papillomavirus DNA in penile carcinoma: evidence for multiple independent pathways of penile carcinogenesis. *Am. J. Pathol.* 2001 Oct;**159**(4):1211-1218.
35. Klussmann JP, Gultekin E, Weissenborn SJ, *et al.* Expression of p16 protein identifies a distinct entity of tonsillar carcinomas associated with human papillomavirus. *Am. J. Pathol.* 2003 Mar;**162**(3):747-753.
36. Lu DW, El-Mofty SK, Wang HL. Expression of p16, Rb, and p53 proteins in squamous cell carcinomas of the anorectal region harboring human papillomavirus DNA. *Mod. Pathol.* 2003 Jul;**16**(7):692-699.
37. Wang HL, Lu DW. Detection of human papillomavirus DNA and expression of p16, Rb, and p53 proteins in small cell carcinomas of the uterine cervix. *Am. J. Surg. Pathol.* 2004 Jul;**28**(7):901-908.
38. Klaes R, Benner A, Friedrich T, *et al.* p16INK4a immunohistochemistry improves interobserver agreement in the diagnosis of cervical intraepithelial neoplasia. *Am. J. Surg. Pathol.* 2002 Nov;**26**(11):1389-1399.

39. Negri G, Egarter-Vigl E, Kasal A, Romano F, Haitel A, Mian C. p16INK4a is a useful marker for the diagnosis of adenocarcinoma of the cervix uteri and its precursors: an immunohistochemical study with immunocytochemical correlations. *Am. J. Surg. Pathol.* 2003 Feb;**27**(2):187-193.

For Peer Review