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The association of pre-treatment HPV subtypes with recurrence of VIN

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ABSTRACT

Objective: To assess whether pre-treatment HPV types are associated with recurrence of high-grade vulvar intraepithelial neoplasia (VIN2+).
Study design: Data of consecutive patients with pretreatment HPV DNA test undergoing treatment for VIN2+ were retrospectively collected. Risk factors promoting the risk of VIN2+ persistence and recurrence were analyzed using Kaplan-Meier and Cox hazard proportional models.
Results: 64 patients had pretreatment vulvar-vaginal HPV DNA test. Two were excluded due to the presence of synchronous vulvar cancer, thus leaving 62 patients for the final analysis. HPV16, HPV18, HPV31 and HPV33 were the most common HPV genotype detected, occurring in 15 (24.2%), 4 (6.5%), 8 (12.9%) and 5 (8.0%) patients, respectively. HPV was not detected in 19 (30.6%) patients. During a mean (SD) follow up of 56.7 (±26.7) months, 10 (16.1%) patients had VIN2+ persistence/recurrence. Mean (SD) lesion-free interval was 51.7 (±31.4) months. Via multivariate analysis, pretreatment infection from HPV31 (HR:46.7(95%CI:4.21,518.4); p = 0.02) and HPV33 (HR:77.0(95%CI:6.73,881.9); p < 0.001) correlated with an increased risk of VIN2+ persistence/recurrence. Additionally, we observed that patients undergoing surgical excision followed by LASER ablation experienced a trend towards lower recurrence rate than patients undergoing other surgical or medical treatments (HR:0.20(95%CI:0.03,1.09); p = 0.05). Two (3.2%) patients developed progression to vulvar cancer.
Conclusions: Owing to the inherent biases of the retrospective study design and the small sample size, our data have to be corroborated by larger and prospective studies. HPV31 and HPV33 have a potential role in predicting VIN2+ persistence/recurrence. These findings will be paramount, owing to the implementation of new immunization programs.

Introduction

Vulvar intraepithelial neoplasia (VIN) is a precancerous condition of the vulva, often associated with high-risk human papillomavirus (HPV) genotypes [1]. Owing to the high virulence and raising diffusion of HPV infection, VIN incidence is estimated to dramatically increase, especially in young women [2,3]. In 1995, Monk et all suggested the association between HPV infection and the occurrence of vulvar cancer in young women [4].

There is still no consensus on the optimal management of VIN. Growing data suggest that irrespective to treatment modalities, high-grade, usual type VIN (VIN2+) is associated with low rates of complete eradication and consequent high rates of recurrence and progression (about 30% and 3–9%, respectively) [5]. Historically, positive margins after excision and multifocal disease are considered risk factor for developing recurrent disease [5–7]. Additionally, smoking history is associated with recurrence [5].

Evidence learned from cervical precancerous conditions suggests that different subtypes of HPV might correlate with different risk of persistence/recurrence and progression [8,9]. However, data regarding subtypes of HPV involved in VIN2+ and their significance are scant. Recently, a systematic review on the prevalence of HPV in genital precancerous and cancerous conditions, suggested that HPV16, 18, 31, 33, 45 and 52 are the most common HPV genotypes involved in the pathogenesis of

Abbreviations: VIN, vulvar intraepithelial neoplasia; HPV, human papillomavirus; CO_2, carbon dioxide; IRB, Institutional Review Board; 5-FU, 5-flurouracil.

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VIN2+ and vulvar cancer [10]. In particular HPV16 was detected in about 70% and 50% of VIN2+ and vulvar cancer, respectively [10,11].

In the present paper, we sought to assess the prevalence and significance of different HPV infections among patients affected by VIN2+, in order to investigate a possible association between pretreatment HPV infection and risk of VIN2+ persistence/recurrence. Additionally, as a secondary endpoint we aimed at describing the risk of developing recurrence among VIN2+ patients, and their risk of progression to invasive cancer.

Methods

A large institutional database of more than 13,000 women who had HPV DNA test between 1998 and 2015 was retrospectively reviewed, searching records of women affected by VIN2+. All patients gave consent for the use of personal information for health research. The Institutional Review Board (IRB) of National Cancer Institute – Milan approved this study.

Endpoints measure was the risk of VIN2+ persistence/recurrence. Demographic details, data about HPV genotype(s) detected as well as data on treatment and follow-up were searched in order to identify predictors for VIN2+ recurrence.

VIN2+ diagnosis was defined as the presence of moderate (VIN2) or severe (VIN3) vulvar intraepithelial dysplasia. Full thickness carcinoma in situ was classified as VIN3.

Vulvo-vaginal specimens were tested for HPV with the Clinical Array Technology (CLART; Genomica, Madrid, Spain) HPV 2 assay, which combines highly specific and sensitive PCR with the technology of low-density arrays. Viral DNA was extracted from specimens obtained with a brush or cotton swab. Each sample was resuspended in a tube containing 3 mL of 0.9% Sodium Chloride Solution and stored at −20 °C until it was processed. The method is based on PCR amplification of a 450-bp fragment within the highly conserved L1 region of the viral genome followed by hybridization with specific probes for each HPV type. It allows detection of minimal quantities of viral DNA of the most clinically relevant HPV types. The whole procedure was performed in two physically separated areas: the pre-PCR area, where samples were prepared and DNA was extracted, and the post-PCR area, where products were amplified and then visualized. Strict procedures were developed to avoid specimen contamination, such as always keeping physically separated the material from the two areas, changing gloves frequently, keeping working areas clean and using pipette tips containing filters. For each HPV test, a pair of primers permitting the amplification of a fragment of the human cystic fibrosis transmembrane conductance regulator (CFTR) gene was used as a genomic DNA control: this was essential for confirming a negative result, since it indicated the presence of DNA from the patient even if HPV was not found. Also, a pair of primers for the amplification of a modified plasmid was used as a PCR control: this was essential to distinguish between an inhibited amplification reaction and a sample that contained no DNA.

All patients were evaluated volvoscopically and colposcopically on an outpatient basis [12]. All examinations and vulvo-vaginal samples (HPV DNA test and vulvar biopsies) were performed by a dedicated team of gynecologic oncologists. Treatments included use of topical agents (e.g., flurouracil (5-FU), Imiquimod), diatermocoagulation and surgical excision (with cold knife or carbon dioxide (CO2) LASER) followed by LASER ablation of the margins. Treatment using 5-FU and Imiquimod required 2–6 weeks of therapy. LASER surgery allows simultaneous photothermal ablation and coagulation. However, during the study period standard treatment consisted in excision of the lesion (either performed with cold knife or LASER) followed by LASER ablation. No patients had ablative surgery without excision. Generally, medical treatments were delivered to patients who refused surgical treatment.

When different grade of VIN severity were observed, patients were classified with the highest grade of VIN. After treatment follow up schedule consisted in clinical visit and colposcopy every four months for the first year than every six months. Histological assessment was not routinely performed but on clinical suspicion. Persistence was defined as the presence of a VIN2+ lesion at the first follow up visit; VIN2+ recurrence was defined as the presence of biopsy-proven diagnosis of VIN2+ after one negative follow up (anytime). Patients with recurrent VIN1 were not included in this analysis.

Statistical analysis

Data are summarized using basic descriptive statistics. Odds ratio (OR) and 95% confidence intervals (CI) were reported when appropriate. Risk of VIN2+ persistence/recurrence and the risk of developing vulvar cancer were evaluated using Kaplan-Meier and Cox proportional hazard models. Hazard ratio (HR) and 95% confidence intervals (CI) were calculated. Univariate and multivariate models were performed when appropriate. All covariates with a p value less than 0.10, based on univariable model were included in the multivariable model. Length of follow-up was considered from end of treatment and date of last follow-up. All p values were two-sided. P values < 0.05 were statistically significant. Statistical analyses were performed using GraphPad Prism version 6.0 for Mac (GraphPad Software, San Diego CA) and IBM-Microsoft SPSS (SPSS Statistics. International Business Machines Corporation IBM. 2013. Armonk, USA) version 20.0 for Mac.

Results

Overall, 206 patients had treatment for VIN2+ at National Cancer Institute between 1998 and 2015. Among those, 64 (31%) patients had pretreatment vulvar-vaginal HPV DNA test two patients were excluded due to the presence of an invasive vulvar cancer diagnosed at the time of surgical excision, thus leaving 62 (30%) patients for the final analysis. No patients who had HPV testing were excluded since they have no data The flowchart displaying the study design is shown in Fig. 1. Patients' mean (SD) age was 45.6 (16.4) years. History of previous HPV infection(s) was reported in 41 (66.1%) patients. HPV16, HPV18, HPV31 and HPV33 were the most common HPV genotype detected, occurring in 15 (24.2%), 4 (6.5%), 8 (12.9%) and 5 (8.0%) patients, respectively. HPV was negative in 19 (30.6%) patients. Treatments included topical agents, excision followed by LASER ablation, excision plus diathermocoagulation in 7 (11.3%), 57 (87%) and 1 (1.7%) patients, respectively. Disease involved margins were observed in eight (14.5%) patients.

During a mean (SD) follow up of 56.7 (±26.7) months, 10 (16.1%) patients had VIN2+ persistence/recurrence. Mean (SD) lesion-free interval was 51.7 (±31.4) months. Twenty six patients were tested for presence of HPV after treatment: 12 (46.1%) patients had HPV persistence after treatment; while, 14 (53.9%) had not. Via multivariate analysis, pretreatment infection from HPV31 (HR: 46.7 (95%CI: 4.21, 518.4); $p = 0.02$) and HPV33 (HR: 77.0 (95%CI: 6.73, 881.9); $p < 0.001$) were associated with an increased risk of developing VIN2+ persistence/recurrence. Additionally, we observed that patients undergoing surgical excision (either with LASER or cold knife) followed by LASER ablation experienced a trend towards lower recurrence rate than patients undergoing other surgical or medical treatments (HR 0.20 (95%CI: 0.03, 1.09); $p = 0.05$). Fig. 2 shows recurrence-free interval curves according to different HPV genotypes.

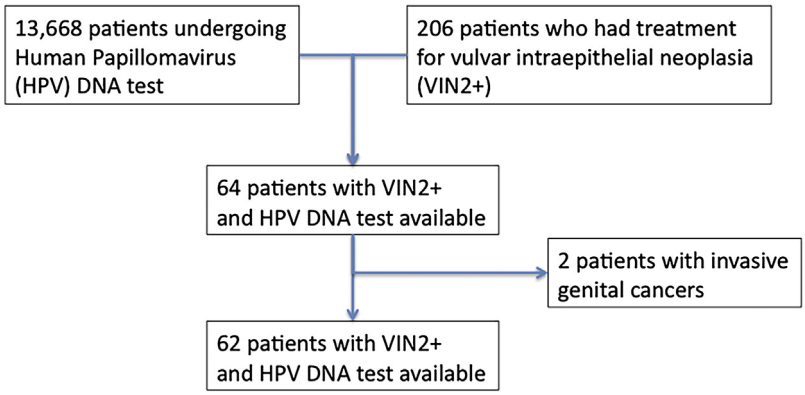

Fig. 1. Study design.

Among the patients who had recurrent disease, four (40%; 6.4% of the whole patients cohort) had a secondary recurrence. Recurrence-free interval curves are shown in Fig. 3. Two (3.2%) patients developed progression to invasive genital cancers (Fig. 3C).

Comment

The present paper investigated the prevalence and prognostic significance of pretreatment HPV infection among patients with VIN2+, thus reporting a number of noteworthy findings. First, pretreatment positivity for high-risk HPV might correlate with an increased risk of persistent/recurrent disease. Second, pretreatment infection from HPV31 and HPV33 is associated with an increased risk of VIN recurrence. Third, local excision (performed either with cold knife or LASER) followed by LASER ablation of surgical margins is associated with a lower risk of recurrence.

Evidence from cervical precancerous conditions suggested that HPV subtypes involved in pre-cancerogenic processes have an important role determining patients' prognosis [8,9]. Similarly, HPV persistence after treatment is the main prognostic factor influencing the risk of cervical dysplasia recurrence. However, there are still no data regarding the prognostic impact of different HPV subtypes in VIN2+. The present paper for the first time in

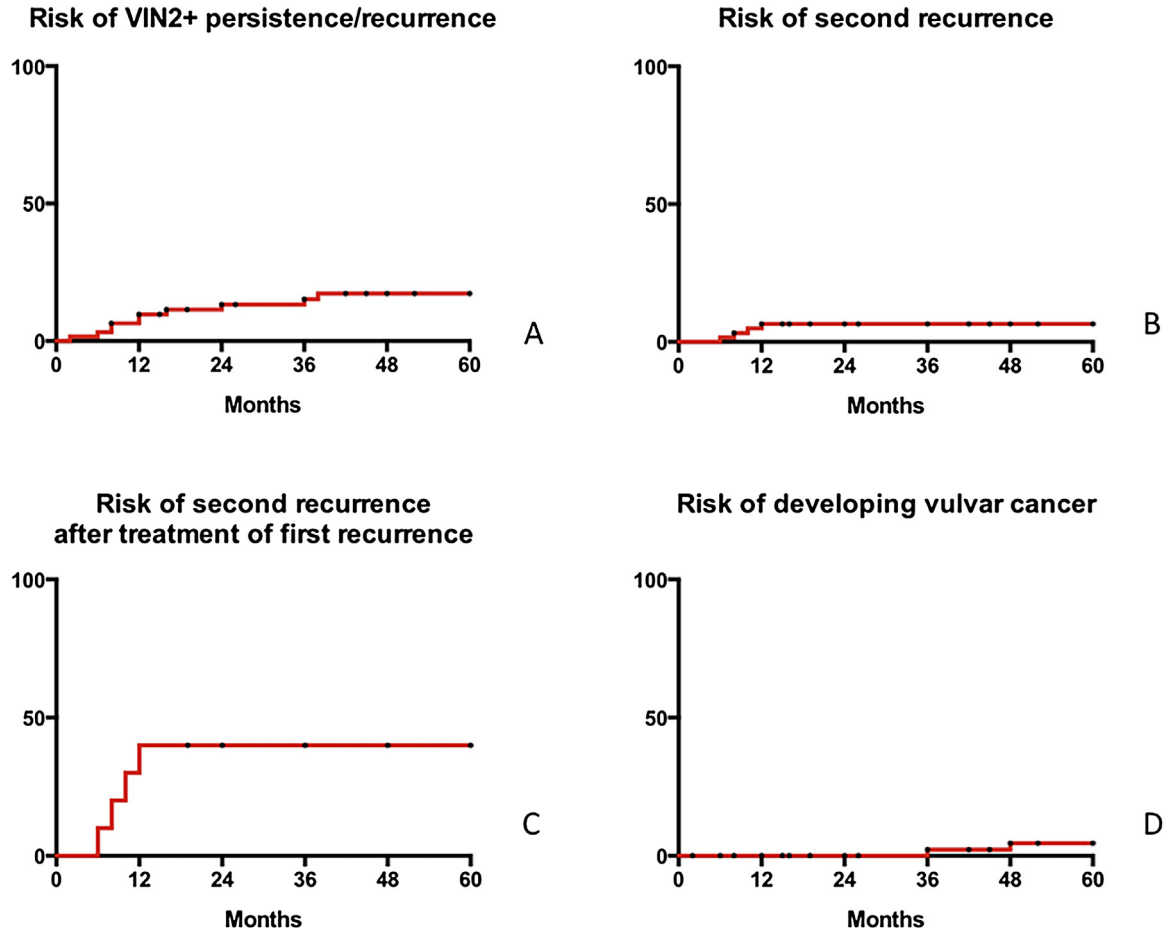

Fig. 2. Role of HPV infection in predict VIN2+ recurrence.
(A) Pretreatment HPV-16 infection; (B) Pretreatment HPV-18 infection; (C) Pretreatment HPV-31 infection; (D) Pretreatment HPV-33 infection.

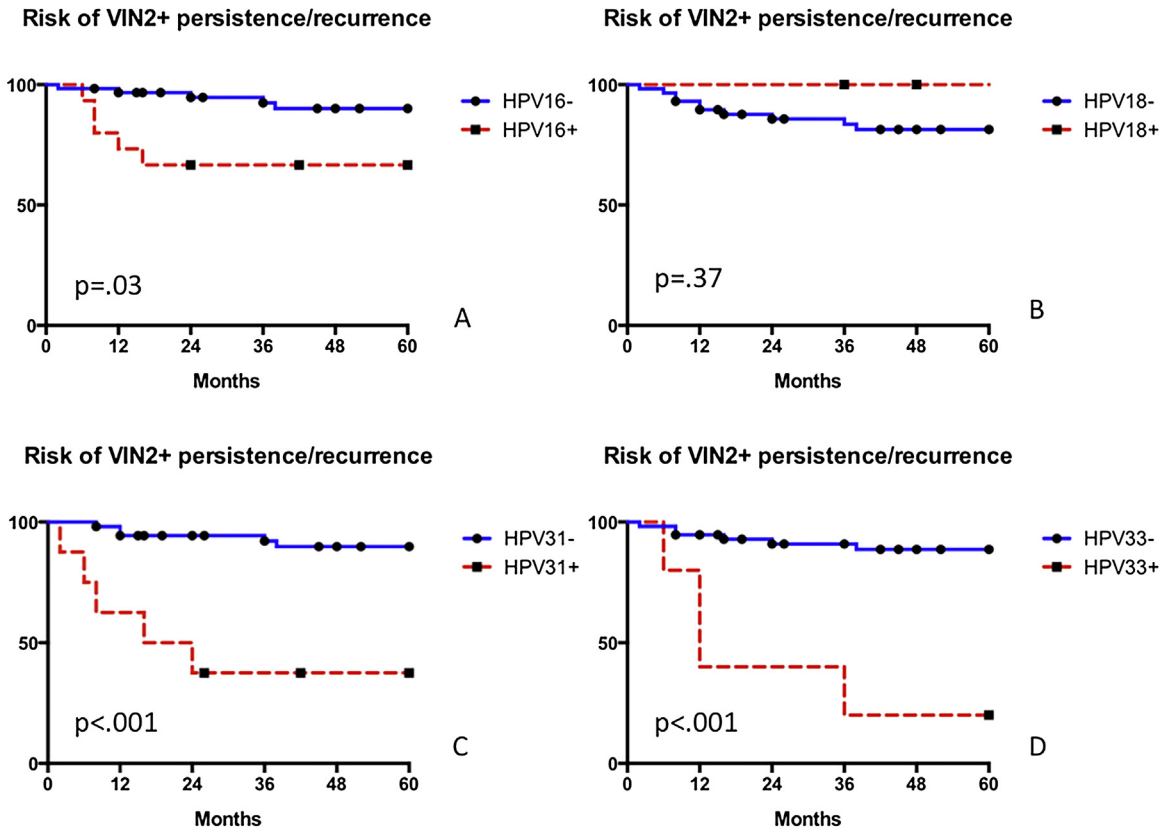

Fig. 3. Risk of VIN2+ recurrence and progression.
(A) First persistence/recurrence; (B) Second persistence/recurrence; (C) Vulvar cancer.

Literature investigated this issue. In fact, genital sampling for detection of HPV is very uncommon [10]. For instance, using a large institutional computerized database including more than 13,000 women undergoing genital sampling for the detection of HPV, we extracted only 62 (>0.5%) patients affected by VIN2+.

Few researches investigated how different factors influence the risk of VIN recurrence [5–7]. Wallbillich et al., reviewed data of more than 300 patients affected by VIN2+ [5], reporting that about 30% of patients developed recurrent VIN2+. Smoking history, lesion size and positive margins were the most important prognostic covariates. Additionally, they reported that LASER ablation is associated with a higher risk of recurrent disease in comparison with excision and topical treatment (i.e., imiquimod) [5]. However, they reported that recurrence is more likely in cases with disease involved margins compared to disease free margins (31.5% vs. 10.9%) [5]. These data are corroborated by others [6,7,13]. Jones et al., reported that patients with disease involved margins experience a 3.3 higher risk of recurrent disease in comparison with patients with disease free margins [6]. Similarly, van Seters et al., and Modesitt et al., observed that the risk of developing local recurrence was decreased of 30% in case of lesion-free margins [7,13]. In our series, we observed a recurrence rate of 16.1%; the use of excision (either performed using cold knife or CO2 LASER) followed by margins ablation might explain this finding.

However, to date no specific guidelines have rated the effectiveness of various treatments for the management of VIN2+. Although Wallbillich et al., suggested that local imiquimod is superior to local excision and LASER ablation, these data are not confirmed by other studies [5]. In fact, previous investigation and the Cochrane review reported similar outcomes after various surgical treatments [13–15]. Hillemanns and Wang studied the prevalence and integration of high-risk HPV DNA in VIN1+ [11]. They studied 30 patients with histologically proved VIN, thus observing that eight patients out of ten harbored high-risk HPV DNA, with HPV16 being the most predominant HPV subtype [11]. This finding is in agreement with our data, that reported that HPV16 is the most prevalent HPV type detected (about 35% of patients with HPV-related VIN2+). However, for the first time in the Literature we observed that HPV31 and HPV33 associated with a high recurrence rate in comparison to other HPV subtypes. Although this finding represents the main strength of our investigation, the absence of the similar results in previous studies by other Authors limits the generalization of our results. In fact, we stress that our results should be considered as preliminary and have to be confirmed by other prospective studies.

Two points regarding interpretation of our results deserve to be addressed. First, high-risk HPV subtypes (like HPV 16, HPV31 and HPV33) are related to the presence of multifocal lesions. Mulifocalitity is a well-known risk factor for genital dysplasia persistence/recurrence [5–7]. Second, HPV31 and HPV 33 are probably related to more rapidly growing lesions in comparison with HPV16 [11,16,17]. Considering these two points, we might speculate that patients with infection from HPV31 and HPV33 seem to have more frequently multifocal lesions with a high progression rate.

The inherent weaknesses of the present study included its being a retrospective single centre study design and the small sample size. In addition it must be considered the long-time study period in witch the cases were collected. Moreover, from a pure statistical point of view the relative low prevalence of HPV31 and HPV33 in our population leads to wide CI in our analyses, thus limiting the statistical power of our data.

As aforementioned, our innovative findings represent the most important strength of the present investigation. Additionally, our is the larger study reporting data on the prevalence of HPV infection among patients with VIN2+.

In conclusion, the present study investigated the prevalence and prognostic significance of pretreatment HPV infection among patients affected by VIN2+. We observed that pretreatment infections from HPV31 and HPV33 are the main prognostic factors influencing the risk of local recurrence. Although the fact that HPV31 and HPV33 are related to multifocal and rapidly growing VIN2+ might explain this linkage, further investigations are warranted in order to assess and better clarify this association.

Conflicts of interest

The authors declare no conflicts of interest. No funding sources supported this investigation.

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