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Original Research

Clinical characteristics and correlates of vulvar high-grade squamous intraepithelial lesion involving the clitoris

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Summary

Purpose: Vulvar high-grade squamous intraepithelial lesion (VHSIL) involving the clitoris is a rare and ill-defined condition. The authors evaluated the patient characteristics associated with clitoral *vs*. non-clitoral VHSIL and the clinical correlates of the disease. *Materials and Methods:* Data from 216 consecutive patients seen at an Italian tertiary referral centre (1981-2014) were analysed using the chi-square test and multiple logistic regression models. *Results:* Clitoral VHSIL was detected in 41 (19%) patients. HIV infection, associated cervical and vaginal intraepithelial neoplasia, and multifocality were significantly ($p < 0.05$) associated with clitoral VHSIL. In multivariate analysis, multifocality retained a strong effect [odds ratio (OR), 17.5; 95% confidence interval (CI), 6.9-44.7]. Clitoral VHSIL was a weak risk factor for patient loss to follow-up in univariate (24.4% *vs*. 13.1%; $p = 0.072$) and multivariate analysis (OR, 2.13; 95% CI, 0.92-4.92). The 60-month persistence/recurrence-free survival was non-significantly lower for patients with clitoral VHSIL. *Conclusion:* Multifocality was independently associated with clitoral VHSIL and this, in turn, with patient loss to follow-up.

Key words: Clitoris; Vulvar neoplasms; Vulvar high-grade squamous intraepithelial lesion; Vulvoscopy.

Introduction

Despite the worldwide increase in the incidence of vulvar tumours [1-4], vulvar high-grade squamous intraepithelial lesion (VHSIL) remains an uncommon and poorly characterized condition. The disease has insidious onset, varied and non-specific appearance, and unpredictable behavior [5, 6]. In addition, the pathogenesis of symptoms and presentations is virtually unknown, because the clinical features of VHSIL have been insufficiently studied and reported.

There is increasing circumstantial evidence that a subset of VHSILs, those involving the clitoris (hereafter referred to as clitoral VHSILs), are particularly worthy of epidemiological and clinical attention. On the one hand, it has been reported that the ongoing increasing trend in VHSIL incidence is mainly accounted for by the portion of vulva that is situated between the urethra and the clitoris [7-9]. On the other hand, it has been demonstrated that clitoral VHSIL is independently associated with an increased risk of detection of a clinically unrecognized invasive vulvar carcinoma (IVC) at vulvoscopy-guided biopsy [10].

The importance of these findings depends on the fact that invasive vulvar carcinoma involving the clitoris has unfavourable pathologic and clinical characteristics, that is, larger tumor size, deeper stromal invasion, more frequent spread to lymph nodes, and reduced survival [11-13]. This may be caused by the rich blood circulation of the area, which could stimulate faster and deeper tumor growth. An alternative explanation may be a physician's delay in diagnosis, caused by hesitation to perform biopsies in order to avoid injury to the region [13]. From a clinical point of view, an additional problem is that the treatment of advanced-stage lesions involving the midline structures poses specific challenges [14].

These facts, coupled with the notion that VHSIL has a potential to progress to IVC [15], have provided us with the rationale for a systematic evaluation of the determinants and clinical correlates of clitoral involvement in VHSIL. A better characterization of the disease would permit an earlier recognition, with prompt assessment and diagnosis and rapid initiation of treatment. This, in turn, would prevent the unrecognized progression to a potentially life-threatening invasive lesion.

Materials and Methods

Both this study and a previous one [10] are part of a broader ongoing clinical investigation into the presentation, diagnosis, treatment, and outcomes of vulvar low-grade SIL (VLSIL), VHSIL, differentiated-type vulvar intraepithelial neoplasia (dVIN), and IVC. This project is taking place at a tertiary referral

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Table 1. — *Significant univariate determinants of the involvement of clitoris in vulvar high-grade squamous intraepithelial lesion (total number of patients, 216).*

Determinant	Patients			Multivariate analysis*		
	Total no.	No. (%) with involvement of clitoris**	*p* value	OR	95% CI	*p* value
HIV infection						
No	202	35 (17.3)		Variable removed		
Yes	14	6 (42.9)	0.018			>0.10
Associated CIN or VaIN						
No	160	25 (15.6)				
Yes	56	16 (28.6)	0.033	Variable removed		>0.10
Multifocality						
No	136	6 (4.4)		1.00	(referent)	
Yes	76	34 (44.7)	0.000	17.5	(6.9-44.7)	0.000

CIN: cervical intraepithelial neoplasia, VaIN: vaginal intraepithelial neoplasia, HIV: human immunodeficiency virus, OR: odds ratio, CI: confidence interval.
** From a multiple logistic regression model with backward stepwise selection of variables. Patient age, year of diagnosis, body mass index, and associated dermatoses were removed from the model as nonsignificantly (p >0.10) contributing to its likelihood.*
*** After a sensitivity analysis, two patients with missing data were classified as free of involvement of clitoris.*

Table 2. — *Association between the involvement of clitoris in vulvar high-grade squamous intraepithelial lesion and the risk of patients being lost to follow-up (total number of patients, 216).*

Involvement of clitoris	Patients			Univariate odds ratio**	95% CI	*p* value
	Total no.	No. (%) lost to follow-up*	*p* value			
No***	175	23 (13.1)			(referent)	
Yes	41	10 (24.4)	0.072	2.13	(0.92-4.92)	0.076

*CI: confidence interval. * Follow-up was conducted every three-four months for the first two years post-treatment, and every six months thereafter in the absence of recurrent disease. All patients were treated at least four months before follow-up data were obtained. Loss to follow-up was defined as no visit after treatment.*
*** From a multiple logistic regression model. No covariates were entered because all of the other factors studied (patient age, year of diagnosis, body mass index, HIV infection, associated CIN or VaIN, associated dermatoses, lesion size, multifocality, lesion appearance, type of treatment, and surgical margin involvement) were not significantly (p > 0.05) associated with patient loss to follow-up. *** After a sensitivity analysis, two patients with missing data were classified as free of involvement of clitoris.*

centre for vulvar disease in north-western Italy, that was created in 1981. The procedures for referral and admittance have remained unchanged since then. Patients are seen on an outpatient basis.

The diagnostic and therapeutic protocols for the management of study patients are described in the authors' previous article [10] and elsewhere [16-18]. In brief, the examination of the vulva was performed by the naked eye. A magnifying lens or a colposcope was non-systematically used to better evaluate the margins of lesions. Routine application of acetic acid was not used. The location, focality, size, color, surface, thickness, and margination of the lesion were assessed [19]. After administering local anesthesia, a cold knife biopsy of the worst-looking area was taken. Once the histological diagnosis of VHSIL was established, patients were submitted to excisional treatment. Biopsy and surgical specimens were processed according to a standard institutional procedure [16, 17]. Follow-up was conducted every three to four months for the first two years post-treatment, and every six months thereafter in the absence of recurrent disease. All the diagnostic and treatment procedures were performed by selected staff.

The clinical and pathological records of patients histologically diagnosed with VLSIL, VHSIL, and dVIN between 1981 and 2014 were reviewed by trained personnel. A structured set of criteria was used. For patients with multiple independent diagnoses, the index lesion was identified. Prior to the adoption of the current terminology [20], the classification of vulvar intraepithelial lesions was modified twice during the study period [21, 22]. As a consequence, the classification of specimens was made using additional information from the descriptive diagnostic reports. Clitoral VHSIL was defined as a disease involving the clitoris with or without spread to other parts of the vulva.

The case series included 302 patients. After exclusion of patients who were diagnosed with VLSIL (n=52) and dVIN (n=21), who refused treatment (n=7), and who received imiquimod as primary treatment (n=5), there were 216 eligible patients. Their median age was 50 (range, 19-88) years. Their clinical characteristics at presentation are reported in details in the authors' previous article [10].

The study was designed to determine (1) whether the patient characteristics (patient age, year of diagnosis, body mass index, HIV infection, associated CIN or vaginal intraepithelial neoplasia (VaIN), associated dermatoses, and multifocality, as independent variables) influence the involvement of clitoris in VHSIL (yes *vs*. no, as a dependent variable), and (2) whether the involvement of clitoris (as an independent variable) influences the clinical characteristics of the disease (symptoms, size in mm, clinical appearance, color, type of treatment, surgical margin involvement, compliance to follow-up, and persistence/ recurrence-free survival, as dependent variables). Multifocality was treated as a patient characteristic because it was expected that a multifocal spread, as an independent factor, increases the likelihood of involvement of clitoris. Compliance to follow-up – a patient characteristic – was included in the second part of analysis in order to confirm or refute the hypothesis that a risk factor for invasive carcinoma detection was also a risk factor for discontinuation of clinical surveillance.

Patient loss to follow-up was defined as no visit after treatment. Disease persistence/recurrence was defined as the histological diagnosis of VHSIL or invasive vulvar carcinoma during follow-up. Differences in proportions were tested for significance with the chi-square test for heterogeneity and trend and the Fisher exact test. Differences in distributions were tested with the non-

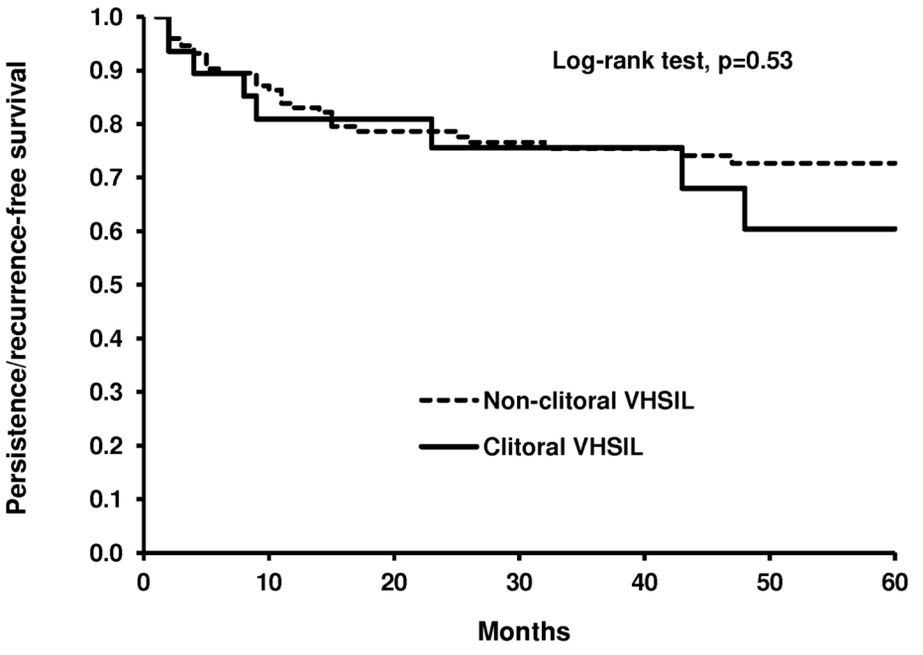

Figure 1. — Kaplan-Meier curve of persistence/recurrence-free survival of patients with vulvar high-grade squamous intraepithelial lesion (VHSIL) by involvement of clitoris (no, n=175; yes, n=41). Persistence/ recurrence-free survival at 60 months is 0.60 *vs.* 0.73. The log-rank test is two-sided.

parametric Mann-Whitney test. Statistical significance was set at $p = 0.05$. A p value > 0.05 and < 0.10 was considered to indicate a borderline level of significance. Significant univariate explanatory factors were further examined in multiple logistic regression models with backward stepwise selection of variables. The level of significance for removal of variables was set at $p = 0.10$.

The analysis of persistence/ recurrence-free survival was truncated at 60 months because very few patients were followed beyond this time interval. Recurrence-free survival curves were generated using the Kaplan-Meier method and compared by the two-sided log-rank test. Statistical significance was set at $p = 0.05$.

Results

Clitoral VHSIL was detected in 41 (19%) patients. Their distribution by year of diagnosis was similar to that of patients with non-clitoral disease (median year, 2005 *vs.* 2003). The former were younger (median age, 44 years *vs.* 52 years), but not significantly so. Body mass index and the detection of associated dermatoses (lichen planus, lichen sclerosus, squamous hypertrophy, other) had no significant effects on clitoral involvement.

As shown in Table 1, HIV infection, associated CIN and VaIN, and multifocality were significant determinants of clitoral involvement in univariate analysis. Multiple logistic regression analysis showed that associated CIN and VaIN and HIV infection, after adjustment for multifocality, did not retain a significant association with clitoral involvement. Multifocality was confirmed to be a strong independent determinant.

Clitoral involvement in VHSIL had no significant effects on the proportion of patients reporting subjective symptoms, the proportion of lesions ≥ 20 mm in size, and the distribution of lesions by clinical appearance (macule, nodule, papule, plaque, ulcer) and color (reddish, whitish, and hyperpigmentation).

Clitoral VHSIL was more often treated with partial vulvectomy (*vs.* excisional biopsy) than non-clitoral VHSIL (36.6% *vs.* 19.4%; $p = 0.018$). After simultaneous adjustment for patient age, lesion size, multifocality, and clinical appearance, all of which were independent risk factors for partial vulvectomy, the association was no longer significant.

Clitoral VHSIL was more often associated with surgical margin involvement (79.4% *vs.* 61.8%; $p = 0.055$), but not significantly so after adjustment for year of diagnosis, associated CIN or VaIN, and multifocality. All of these factors were independently associated with surgical margin status.

Patients with clitoral VHSIL were more likely to be lost to follow-up (Table 2). Clitoral involvement was the only risk factor for loss to follow-up, although at a borderline level of significance. As shown in Figure 1, the lower 60 month persistence/recurrence-free survival for patients with clitoral VHSIL did not reach the level of statistical significance.

Discussion

In the first part of the present study, the authors identified important clinical determinants of clitoral VHSIL. The disease was independently more frequent among patients with multifocal lesions. The effect of HIV infection and associ-

ated CIN or VaIN was not confirmed by multivariate analysis, which suggests that their univariate association with the involvement of clitoris is attributable to their strong association with multifocality. Although without a causal role, however, the concomitance of HIV infection and CIN or VaIN remains a clinical problem, because the presence of CIN or VaIN increases the risk of relapse of VHSIL [23] and HIV-positive women are more likely to develop invasive vulvar carcinoma and vulvar, vaginal, and perianal intraepithelial neoplasia [24, 25]. The concomitance of HIV infection, multiple HPV-related diseases, and clitoral VHSIL has also behavioral and psychosexual implications [26]. Behavioral change strategies in addition to pre- and post-treatment counseling might be helpful.

Although the role of multifocality was expected, the finding is nevertheless important. Multifocality has often been associated with the risk of recurrence of VHSIL [27, 28]. This association needs to be further confirmed, and it is also necessary to assess whether the frequent clitoral involvement in multifocal diseases explains, at least in part, the risk increase.

In the second part of analysis, the authors assessed which clinical characteristics of VHSIL the involvement of clitoris determines. From this perspective, too, the present findings were worthy of note. First, it is of clinical interest that clitoral VHSIL was associated with an increased risk of surgical margin involvement, although this was actually due to the confounding effect of multifocality and other factors. Second, patients with clitoral VHSIL were more likely to be lost at follow-up and, interestingly, the involvement of clitoris was the only demonstrable risk factor for this. Studies on the quality of life of patients with VHSIL have focused on the impact of treatment on the psychosexual sphere and the sexual well-being, with many reporting an increased risk for dysfunctions [29]. The present authors suggest that the clitoral disease may also be associated with acute psychological distress and depressive reaction. The increased risk of follow-up failure must be viewed in the light that clitoral involvement in VHSIL is related to multifocality, a determinant of the risk of recurrence, and is a risk factor for clinically unrecognized invasive carcinoma [10]. Given these two associations, a poor patient's compliance to follow-up may have harmful consequences. Counseling and information are crucial in the management of patients with clitoral VHSIL [30], especially if associated with HIV infection and other HPV-related lesions.

The higher rate of patients lost to follow-up and the relatively short duration of clinical surveillance for most patients suggest that the negative results of persistence/recurrence-free survival analysis be considered with caution. Although to a non-significant extent, the Kaplan-Meier curves in Figure 1 separate after approximately four years since treatment. More long-term follow-up research is needed to document whether patients treated for clitoral VHSIL have an increased risk of disease relapse.

The present authors have already discussed the methodological issues involved in this study, including the long duration of patient accrual, the limited statistical power, and the possibility of a selection bias [10]. In particular, the limited size of the case series suggests caution when considering some negative results of analysis. The possibility that results are affected by a selection bias seems less likely because this case series differs only in some patient characteristics from previously reported case series [5, 31].

This study complements and extends the authors' previous work [10]. Overall, they conclude that patients presenting with multifocal VHSIL should undergo an accurate search for the involvement of clitoris. If this is detected, a timely, correct, and more liberal use of vulvar biopsy is indicated in order to rule out the presence of stromal invasion. Pre- and post-treatment counseling on follow-up steps is highly recommended.

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