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Multivariate analysis of prognostic factors in primary squamous cell vulvar cancer: The role of perineural invasion in recurrence and survival

Federico Ferrari [a, *], Sara Forte [b], Laura Ardighieri [c], Emma Bonetti [b], Barbera Fernando [d], Enrico Sartori [b], Franco Odicino [b]

[a] Department of Obstetrics and Gynecology, Spedali Civili of Brescia, Piazzale Spedali Civili 1, Brescia, 25126, Italy
[b] Department of Obstetrics and Gynecology, University of Brescia, Italy
[c] Department of Pathology, Spedali Civili of Brescia, Italy
[d] Department of Radiotherapy, Spedali Civili of Brescia, Italy

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ABSTRACT

Objective: to assess the prognostic role in recurrence and survival of perineural invasion (PNI) in vulvar squamous cell cancer (VSCC).

Methods: Patients underwent primary surgery for VSCC from January 2009 to December 2016 served as the study cohort. We collected demographic, clinical, pathological characteristics and follow-up data, and we compared them among PNI-negative versus -positivepatients. We calculated disease-free survival (DFS) and overall survival (OS) using Kaplan-Meier and univariate log-rank test. We conducted a multivariate analysis with cox-proportional hazard models for DFS and OS, including age, tumor size, depth of invasion, free tumor margin <8 mm, high-grade histology, lymph vascular space invasion, PNI, extracapsular lymph nodal disease, lymph nodal ratio >0.2 and FIGO Stage 2009 (Early I-II versus Advanced III-IV).

Results: We found 74 patients with a PNI prevalence of 31.1%. The 5-year DFS was favourable for PNI-negative patients (72% versus 18%; $p = 0.00$). The 5-year OS was 75% versus 35% in favor of PNI-negative patients ($p = 0.00$). The subgroup analysis conducted among stage confirmed a decreased DFS and OS in PNI-positive patients. Multivariate analysis showed that PNI (HR 2.74; CI95% 1.10–7.13; $p = 0.03$) and extracapsular lymph nodal disease (HR 13.54; CI95% 2.87–64.07; $p = 0.01$) are independent prognostic factors for earlier recurrence. OS was significantly reduced in case of PNI (HR 4.93; CI95% 1.33–18.35; $p = 0.01$) and extracapsular lymph nodal disease (HR 10.63; CI95% 1.65–68.57; $p = 0.01$).

Conclusions: PNI is an independent prognostic factor for aggressive behavior and unfavorable course in VSCC and should be considered in adjuvant treatment planning.

Introduction

Vulvar cancer is the fourth most common gynecological malignancy, and it represents up to 6% of gynecologic cancers [1]. Vulvar squamous cell cancer (VSCC) is the most frequent histology, and surgery is the mainstay of treatment. Known risk factors for vulvar cancer include advanced-age, smoking, the presence of lichen sclerosus, immunodepression, history of vulval intraepithelial neoplasia, HPV-related lesion and cervical cancer [2].

The tailoring of adjuvant treatment is based on surgical pathological findings, and current evidence could not uniquely identify aggressive vulvar cancer. Many prognostic factors were identified and most relevant were included in the last version of the FIGO staging system, such as tumor size and extracapsular nodal involvement [2]. However, other factors, such as depth of invasion of the stroma or reduced length of free surgical margins are weaker prognostic factors. Perineural invasion (PNI) is a manifestation of cutaneous squamous cell carcinomas, it carries a much higher risk for local and distant recurrence and may require a more aggressive

* Corresponding author.
E-mail address: f.ferrari.obgyn@gmail.com (F. Ferrari).

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treatment [3]. Outcomes of primary squamous cancers of other anatomical districts are adversely affected by the presence of PNI, such as in patients with squamous cell cancer of the upper aerodigestive tract, resulting in increased locoregional recurrences and decreased survival [4].

The negative prognostic role of PNI in VSCC is not supported yet by robust evidence [5,6]; in fact, it's still not routinely considered during the planning of adjuvant treatment. The aim of this study is to demonstrate the prognostic value of PNI in recurrence and survival outcomes and hence to encourage the consideration of this rare feature in clinical practice.

Material and methods

We conducted a retrospective analysis of women who underwent upfront surgery for VSCC at the Department of Obstetrics and Gynecology of Spedali Civili of Brescia (Italy), a tertiary university hospital in northeast Italy, from January 2009 to December 2016 using a surgical departmental database after Institutional Review Board approval (Number 3413). We excluded patients treated with neoadjuvant chemotherapy and those with incomplete data or lost to follow-up. The period of study began when systematic evaluation of PNI was introduced in the pathology report and it promptly finished ensuring a minimum significant clinical follow-up of two years. We collected demographic and clinical data such as age, BMI, smoking status and presence of lichen sclerosus. We analyzed the patterns of treatment considering vulvar surgery (simple versus radical surgery), lymph nodal staging (unilateral or bilateral systematic inguino-femoral lymph node dissection; sentinel mapping was not performed yet in our practice during the study period [7]) and adjuvant treatment (external-beam beam radiotherapy directed to vulvar and/or inguinofemoral, external, iliac nodal regions with or without concomitant chemotherapy). Vulvar-directed radiotherapy was indicated based on classical primary tumor risk factors [8]. Isolated presence of PNI with negative lymph nodal status did not represent an indication for tumor-directed radiotherapy in our practice.

We collected surgical-pathological outcomes, such as tumor histology, size, cancer multifocality and histology grade, presence of lymphovascular space invasion (LVSI), depth of infiltration expressed in millimeters (DOI), presence of PNI and length of free pathological margin expressed in millimeters. We classified free pathological margin as “close margin” if less than 8 mm from the lesion, otherwise as “far margin”; in case of margin involved by tumor or high-grade dysplastic lesion, patients were submitted to further surgical excision and hence re-classified accordingly. Original hematoxylin and eosin histology stain slides of tumor resections of each patient were collected and PNI was identified throughout the whole area of tumor sections, serially under 100x magnification and then confirmed under 200x magnification. PNI was defined as positive when infiltration was identified in any layer of the nerve sheath or in case of tumor involvement of more than one-third of the circumference of the nerve; in doubtful cases, a dual immunohistochemical (IHC) staining with S-100 and AE1/3 was used (Fig. 1). We identified the lymph nodal status (negative or positive for cancer), the presence of extracapsular disease and number of positive lymph nodes over total lymph nodes removed, known as lymph nodal ratio (LNR) and FIGO Stage 2009. We collected relevant clinical events, such as the first recurrence and cause-specific deaths and we calculated respectively the disease-free survival (DFS) and overall survival (OS) in months. DFS was measured from the date of the end of treatment to the date of recurrence or death; OS was measured from the date of the end of treatment until the date of last contact or death. Patients without recurrence or death were censored at their last contact date. Local and lymph nodal recurrences were proven by biopsy, while distant recurrences were assessed using multimodal imaging after exclusion of other primitive cancers.

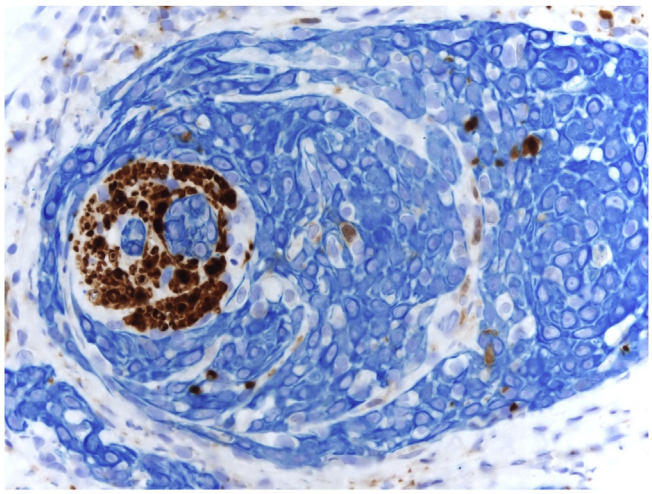

Fig. 1. Perineural invasion (PNI) in vulvar specimen. Dual immunohistochemical staining with S-100 (brown) and AE1/3 (blue) showing PNI in pathological specimen.

All variables were compared among patients with and without PNI. Univariate analysis using Pearson chi-squared and Mann–Whitney U tests were conducted. Kaplan-Meier curves and log-rank test were used to assess differences in DFS and OS using PNI as the stratifying factor; further sub-groups analyses were then performed according to stage (Early I-II versus Advanced III-IV). We conducted multivariate analyses with cox-proportional hazard models using a stepwise forward selection method for DFS and OS, including age, tumor size, depth of invasion, close margin, high-grade histology, LVSI, PNI, the presence of extracapsular lymph nodal disease, LNR >0.2 and stage. Lymph nodal status was excluded from the model since its direct dependence with the stage. Differences were considered statistically significant at a p-value < 0.05. All statistical tests were performed using IBM SPSS for Windows, Version 23.

Results

A total of 95 Caucasian women affected by primary VSCC were surgically treated in our department during the study period. Seventy-four patients fulfilled inclusion criteria and the demographic, clinical and pathologic characteristics are listed in Table 1. After pathologic review, PNI was identified in 23 patients for a prevalence rate of 31.1% (CI95% 21–43) and IHC was used in 10 cases (43%). No differences were noted in age, BMI, smoking status and presence of lichen sclerosus. PNI was significantly found more frequently among patients with stage III-IV compared with lower stages (65.2% versus 34.8%; $p = 0.00$). Positive lymph nodes were more frequent in patients with PNI (65.2% versus 15.7%; $p = 0.01$), as well as the prevalence of a LNR > 0.2 (26% versus 2%; $p = 0.01$). Extracapsular lymph nodal disease was seen exclusively in patients with PNI (prevalence rate of 30.4%). Lymph nodal staging was performed more frequently in patients with PNI (95.6% versus 65%; $p = 0.01$), whereas unilateral lymph nodal staging was performed in the minority of patients. Patients with PNI were submitted more frequently to adjuvant treatment (47% versus 20%; $p = 0.06$) as reported in Table 2. Thirty-nine recurrences were identified for a recurrence rate of 52.7% (CI95% 41–64) with a higher rate of recurrences in patients with PNI (82.6% versus 39.2%; $p = 0.00$). Lymph nodal recurrences were 43.5% versus 5.9%, respectively in

Table 1
Demographic, clinical and surgical-pathological characteristics of the study chort.

Variable	All patients n = 74	PNI Negative n = 51	PNI Positive n = 23	p
Age				
Mean (range)	73.5 (31–92)	75 (31–92)	73 (44–90)	0.57[a]
Median (IQR)	75.5 (17)	77 (14)	75 (22)	
BMI				
Mean (range)	25.2 (17–50)	24 (17–50)	26 (19–41)	0.24[a]
Median (IQR)	24 (5)	24 (6)	24.5 (8)	
Smoking status, n (%)				
Never	58 (78.4%)	40 (78.4%)	18 (78.3%)	0.45[b]
Current	8 (10.8%)	6 (11.8%)	2 (8.7%)	
Past	6 (8.1%)	4 (7.8%)	2 (8.7%)	
Unknown	2 (2.7%)	1 (2%)	1 (4.3)	
Lichen sclerosus, n (%)	15 (20.3%)	9 (17.6%)	6 (26%)	0.59[b]
Multifocal, n (%)	11 (14.9%)	7 (13.7%)	4 (17.4%)	0.46[b]
HG Histology, n (%)	11 (14.9%)	5 (9.8%)	6 (26%)	0.74[b]
Stage (FIGO 2009), n (%)				
Early I-II	51 (69%)	43 (84.3%)	8 (34.8%)	0.00[b]
Advanced III-IV	23 (31%)	8 (15.7%)	15 (65.2%)	
Tumor Size				
Mean (range)	32.7 (6–80)	30.3 (6–70)	38 (15–80)	0.10[a]
Median (IQR)	30 (21)	25 (20)	32 (40)	
LVSI, n (%)	24 (32.4%)	10 (19.6%)	14 (60.9%)	0.01[b]
DOI ≥ 1 mm, n (%)	68 (91.9%)	45 (88.2%)	23 (100%)	0.22[b]
Surgical margin, n (%)				
Positive	9 (12.2%)	7 (13.7%)	2 (8.7%)	0.17[b]
<8 mm (Close margin)	43 (58.1%)	26 (51%)	17 (73.9%)	
≥8 mm (Far margin)	22 (29.7%)	18 (35.3%)	4 (17.4%)	
Positive node(s), n (%)	23 (31.1%)	8 (15.7%)	15 (65.2%)	0.01[b]
Extracapsular nodal disease, n (%)	7 (9.45%)	0 (0%)	7 (30.4%)	0.01[b]
LNR > 0.2, n (%)	7 (12,2%)	1 (2%)	6 (26%)	0.01[b]

BMI: Body Mass Index; HG: High Grade; FIGO: International Federation of Obstetrics and Gynecology; LVSI: Lymph Vascular Space Invasion; DOI: Depth Of Invasion; LNR: Lymph Nodal Ratio.
[a] Mann-Whitney test.
[b] Pearson chi-squared.

Table 2
Pattern of treatment of the study cohort.

Treatment modalities	All patients n = 74	PNI Negative n = 51	PNI Positive n = 23	p
Radical surgery, n (%)	56 (75.6%)	37 (73%)	19 (82.6%)	0.58[a]
Second surgery for margin, n (%)	6 (8.1%)	4 (7.8%)	2 (8.6%)	0.73[a]
Lymph node staging, n (%)	55 (74.3%)	33 (65%)	22 (95.6%)	0.01[a]
Unilateral staging, n (%)	11 (14.9%)	6 (11.5%)	5 (21.7%)	0.46[a]
Submitted to Adjuvant treatment, n (%)	21 (28.4%)	10 (20%)	11 (47%)	0.06[a]

[a] Pearson chi-squared.

positive and negative PNI patients (p = 0.00). No differences in the rates of local, distant or multiple recurrences were noted (Table 3). Overall, the median follow up in our study cohort was 45 months (range 3–115). The median time to relapse was 27 (CI95% 20.5–31.4) and 7.6 months (CI95% 3.74–13.4) respectively for PNI-negative and -positive patients (p = 0.00), in fact, DFS at 5-years was decreased in PNI-positive patients (72% versus 18%; p = 0.00). OS at 3- and 5-years was respectively 92% and 75% for PNI-negative patients, and 41% and 35% for PNI-positive patients (p = 0.00); patients showing PNI had a median estimate survival of 30 months (CI95% 12.6–48). Subgroup analysis confirmed the role of PNI in early-stage patients with a greater DFS (recurrence rate of 76% versus 25% at 5-years with p = 0.05) and a longer OS (survival rate of 92% versus 60% at 5-years with p = 0.01) both in favor of PNI-negative patients. Also in advanced-stage, PNI-negative patients had both longer DFS (recurrence rate of 50% versus 13% at 5-years, with p = 0.03) and OS (survival rate of 75% versus 22% at 5-years with p = 0.02); the median estimate survival was 15 months (CI95% 9.9–19.7) in presence of PNI. All Kaplan-Meier curves are available as supplementary material. Multivariate analysis with

Table 3
Pattern of recurrences of the study cohort (Patients are repeated if they have more than a recurrence type).

Type of recurrence	All patients n = 74	PNI Negative n = 51	PNI Positive n = 23	p
Any type (total), n (%)	39 (52.7%)[a]	20 (39.2%)	19 (82.6%)	0.00[b]
Local, n (%)	28 (37.8%)	18 (35.3%)	10 (43.5%)	0.33[b]
Lymph nodal, n (%)	13 (17.6%)	3 (5.9%)	10 (43.5%)	0.00[b]
Distant, n (%)	3 (4.1%)	1 (2%)	2 (8.7%)	0.22[b]
Multiple, n (%)	3 (4.1%)	1 (2%)	2 (8.7%)	0.22[b]

[a] Absolute count of patients who recurred.
[b] Pearson chi-squared.

Table 4
Multivariate cox-proportional hazard models using a stepwise forward selection method for disease free survival (DFS) and overall survival (OS). The statistically significant factors are highlighted in bold.

Covariate	DFS			OS		
	HR	95% CI	p	HR	95% CI	p
Age	1.00	0.97–1.03	0.92	1.02	0.97–1.07	0.41
Tumor Size	1.00	0.97–1.04	0.67	1.01	0.97–1.05	0.64
DOI	0.91	0.83–1.02	0.67	0.95	0.83–1.10	0.49
Close margin	1.69	0.60–4.75	0.32	4.43	0.78–25.37	0.10
HG Histology	2.63	0.87–7.94	0.08	0.93	0.21–4.16	0.92
LVSI	1.13	0.41–3.13	0.82	0.55	0.14–2.23	0.41
PNI	**2.74**	**1.10–7.13**	**0.03**	**4.93**	**1.33–18.35**	**0.01**
Extracapsular ND	**13.54**	**2.87–64.07**	**0.01**	**10.63**	**1.65–68.57**	**0.01**
LNR > 0.2	1.10	0.34–3.58	0.10	0.56	0.09–3.29	0.52
FIGO 2009 Stage	1.56	0.65–3.76	0.32	3.50	0.89–13.68	0.07

HR: Hazard Ratio; CI: Confidence Interval; DOI: Depth Of Invasion; HG: High Grade; LVSI: Lymph Vascular Space Invasion; PNI: Perineural Invasion; ND: Nodal Disease; LNR: Lymph Nodal Ratio; FIGO: International Federation of Obstetrics and Gynecology.

cox-proportional hazard models (Table 4) showed a significant statistically decreased DFS in presence of PNI (HR 2.74; CI95% 1.10–7.13; $p = 0.03$) and in case of extracapsular lymph nodal disease (HR 13.54; CI95% 2.87–64.07; $p = 0.01$), while high-grade histology approached statistical significativity. OS was significantly reduced in positive PNI patients (HR 4.93; CI95% 1.33–18.35; $p = 0.01$) and in case of extracapsular lymph nodal disease (HR 10.63; CI95% 1.65–68.57; $p = 0.01$).

Discussion

In our study, the presence of PNI in VSCC is associated with lymph nodal metastasis at diagnosis, a three-fold increased risk of earlier recurrence and a five-fold risk of death. At 5-years of follow-up, only 35% of patients with PNI were cancer-free.

Data in the literature regarding the role of PNI in vulvar cancer is lacking [9], although our findings confirm the most recent growing body of evidence [6]. Papers reviewing the presence of PNI are few and scanty, in fact, in literature, the prevalence rate of this pathological feature in VSCC ranges from 7.6% to 52% [5,6]. In our study, there are consecutive cases of VSCC and systematic evaluation of PNI brought to a prevalence rate of 31.1%, which is a not negligible quote of patients. The first study reporting PNI in vulvar cancer was a small retrospective study of 22 patients affected by early-stage disease [8], where authors concluded that PNI was strongly associated with lymph nodal metastasis at diagnosis, although it was seen only in two patients. A further study found a PNI prevalence of 21.4% in a wider case series of 71 patients [10] and in this study, it was statistically significantly associated with survival at univariate analysis, but not confirmed in multivariate analysis. The highest prevalence rate was seen in a retrospective review [11] of 103 cases where 52% of the specimens showed PNI; of interest, 69% of patients who recurred were positive for PNI at diagnosis; in fact, it resulted as an independent predictor of recurrence at multivariate analysis. In this study, no significant association with lymph nodal involvement was confirmed, albeit it suggests that PNI could be associated with advanced disease. In a previous retrospective study, regarding prognostic factors in vulvar cancer, including patients treated with primary surgery, PNI approached statistical significativity with an increased risk of recurrence after adjustment for the stage [12]. A recent study by Salcedo et al. found a PNI prevalence rate of 7.7% in a single institution cohort of more than four-hundred patients and confirmed the role of this factor as an independent predictor of recurrence in multivariate analysis; moreover, PNI was associated with positive lymph nodes, advanced disease and LVSI. During years the role of PNI in vulvar cancer shifted from an unreported histologic feature and apparently not related to recurrence [12] to a role of known prognostic factor and correlated with positive lymph nodes [13]; further, it was stated to be an independent indicator of risk for recurrence in vulvar cancer, independently of the DOI, lymph nodal involvement, LVSI and stage [11]. Interestingly, in the Brigham and Women's Hospital tumor staging system [14] for cutaneous squamous cell carcinomas, a multivariate analysis confirmed the role of PNI, tumor diameter ≥ 2 cm, poorly differentiated histology and tumor invasion beyond fat as predictors of worse outcomes; furthermore, this analysis confirmed, for the aforementioned factors, their role in identifying a high-risk subset of patients with an increased risk of metastasis and death. Recently a new classification system by the Harvard University, the Brigham Women's Vulvar Tumor Classification, identified five risk factors that were independent predictors of poor outcomes, and among these PNI was not included [15]; authors, however, stated as a limitation the study design, that precluded the evaluation of very rare prognostic factors such as PNI, LVSI and immunosuppression. Moreover, detection of PNI in the surgical specimens is a challenge for the pathologist in view of processing artifacts or inadequate specimen assessment, and this could lead to an increased false-negative rate. Among classical pathological risk factors, in our study we found that in case of PNI, the median tumor size was almost ten millimeters bigger and the rates of close margins were higher; however, these findings are not significantly different from PNI-negative patients, and hence we could not draw any conclusion regarding their prognostic role. An interesting explorative hypothesis could link the presence of PNI to a bigger tumor and hence a closer free margin, that could amplify or partially explain the prognostic role of PNI. Unfortunately, our results are not sufficient to support these findings.

PNI is linked to increased mortality, recurrence and overall worse outcomes in many other types of malignant tumors. The most prominent example derives from squamous cell head and neck cancers; actually, PNI and LVSI have been extensively associated with the risk of local recurrences and lymph node metastasis in advanced-stage of oral squamous cell cancer; a multivariate analysis confirmed the role of PNI as an independent predictor for OS and DFS, in fact, in oral carcinoma surgical specimens, it has a significant impact on survival outcomes for patients with advanced-stage submitted to radical surgery and adjuvant treatment. Based on these outcomes, systematic evaluation of PNI in head and neck cancer was recommended. Another type of squamous cell cancer recently investigated for the role of PNI is cervical cancer, where patients affected by early-stage PNI positive squamous cervical cancer exhibited shorter DFS and OS; in fact, patients with PNI were more likely to exhibit adverse histopathological features, such as greater tumor size, increased DOI, parametrial invasion, presence of LVSI and lymph nodal metastases at diagnosis, which are factors that have an important role in tailoring adjuvant therapy. A previous meta-analysis demonstrated that cervical cancer with PNI was associated with a poor OS rate and suggested that it could be an independent prognostic factor for cervical cancer and the decision to deliver adjuvant therapy after surgery [16].

Evidence about the prognostic role of PNI is available also in non-squamous cell cancers. A recent study [17] highlights the role of PNI in predicting survival in patients with primary operable colorectal cancer; authors, in fact, concluded that it is a poor independent prognostic factor, and they claimed it could complement staging for stage II-III colorectal cancer. Also in operated pancreatic cancer, PNI is correlated with a poor prognosis and is associated with positive lymph nodes, pancreatitis and elevated CA19.9 serum levels [18]. In prostate cancer [19], PNI predicts lethal

outcome independently from other clinical factors and its presence is supposed to create a microenvironment that promotes cancer aggressiveness.

The role of nerve's invasion in cancer progression and lymph nodal metastasis is relatively unknown, and one of the hypotheses comprises that nerves could be a reservoir for cancer cells that let them spread to the draining regional lymph nodes. In the future, working on animal models could let us understand the pathogenesis of PNI-related metastasis and gene profiling would help in risk stratification regardless of cancer type [20].

Our study has strengths and limitations. Among strengths, our cohort is one of the largest case series of consecutive VSCC with documented PNI, and this is the first study that includes PNI and all the known prognostic factors for VSCC in a multivariate analysis for recurrence and survival. Furthermore, at our institution, we have high-volume surgeons and dedicated pathologist and radiotherapist for gynecological oncology. Finally, all patients were upfront staged according to FIGO 2009, and hence we have reduced any bias regarding a heterogeneous pattern of adjuvant treatment. Among limitations, our study was performed in a single center and therefore, results require external validation to support widespread changes in practice. Since the retrospective nature of the study, it may not be exempt from selection bias, since we excluded twenty-one patients from our analysis due to incomplete data.

Conclusions

Our results suggest that PNI is an independent prognostic factor for both earlier lymph nodal recurrence and death in VSCC. A growing body of evidence supports our findings, and hence thorough attention should be given to this pathological feature. In fact, we think that PNI should be a potential point of discussion in the future development of the FIGO staging system for its role as a poor prognostic risk factor. For instance, a vulvar cancer biopsy reporting the presence of PNI should address physicians to suspect lymph nodal involvement. Prospective multicenter studies are needed to identify PNI in VSCC and its impact on the choice of lymph nodal staging and need for adjuvant treatment.

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Competing interests

Nothing to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejso.2019.07.029.

References

[1] Alkatout I, Schubert M, Garbrecht N, et al. Vulvar cancer: epidemiology, clinical presentation, and management options. Int J Womens Health 2015;20:305–13.
[2] Hacker NF, Barlow EL. Staging for vulvar cancer. Best Pract Res Clin Obstet Gynaecol 2015;29:802–11.
[3] Karia PS, Morgan FC, Ruiz ES, Schmults CD. Clinical and incidental perineural invasion of cutaneous squamous cell carcinoma. JAMA Dermatol 2017;153:781–8.
[4] Chatzistefanou I, Lubek J, Markou K, Ord RA. The role of perineural invasion in treatment decisions for oral cancer patients: a review of the literature. J Cranio-Maxillofacial Surg 2017;45:821–5.
[5] Baiocchi G, Mantoan H, de Brot L, et al. How important is the pathological margin distance in vulvar cancer? Eur J Surg Oncol 2015;41:1653–8.
[6] Salcedo MP, Sood AK, Dos Reis R, et al. Perineural invasion (PNI) in vulvar carcinoma: a review of 421 cases. Gynecol Oncol 2018;152:101–5.
[7] Scavone J, Damast S, Dorigo O, et al. NCCN guidelines version 2.2019 vulvar cancer (squamous cell carcinoma). 2018. https://www.nccn.org/professionals/physician_gls/pdf/vulvar.pdf. [Accessed 13 July 2019].
[8] Rowley KC, Gallion HH, Donaldson ES, et al. Prognostic factors in early vulvar cancer. Gynecol Oncol 1988;31:43–9.
[9] Nicoletto MO, Parenti A, Del Bianco P, et al. Vulvar cancer: prognostic factors. Anticancer Res 2010;30:2311–7.
[10] Lerma E, Matias-Guiu X, Lee SJ, Prat J. Squamous cell carcinoma of the vulva: study of ploidy, Hpv, p53, and prb. Int J Gynecol Pathol 1999;18:191–7.
[11] Holthoff ER, Jeffus SK, Gehlot A, et al. Perineural invasion is an independent pathologic indicator of recurrence in vulvar squamous cell carcinoma. Am J Surg Pathol 2015;39:1070–4.
[12] Preti M, Ronco G, Ghiringhello B, Micheletti L. Recurrent squamous cell carcinoma of the vulva: clinicopathologic determinants identifying low risk patients. Cancer 2000;88:1869–76.
[13] Rowley KC, Gallion HH, Donaldson ES, et al. Prognostic factors in early vulvar cancer. Gynecol Oncol 1988;31:43–9.
[14] Karia PS, Jambusaria-Pahlajani A, Harrington DP, et al. Evaluation of American joint committee on cancer, international union against cancer, and Brigham and Women's hospital tumor staging for cutaneous squamous cell carcinoma. J Clin Oncol 2014;32:327–34.
[15] Le ST, Karia PS, Vollenhoven BJ, Besaw RJ, Feltmate CM, Schmults CD. Evaluation of AJCC and an alternative tumor classification system for primary vulvar squamous cell carcinoma. J Natl Compr Cancer Netw 2018;16:42–9.
[16] Cui L, Shi Y, Zhang GN. Perineural invasion as a prognostic factor for cervical cancer: a systematic review and meta-analysis. Arch Gynecol Obstet 2015;292:13–9.
[17] Zhou Y, Wang H, Gong H, Cao M, Zhang G, Wang Y. Clinical significance of perineural invasion in stages II and III colorectal cancer. Pathol - Res Pract 2015;211:839–44.
[18] Yang Y-H, Liu J-B, Gui Y, Lei L-L, Zhang S-J. Relationship between autophagy and perineural invasion, clinicopathological features, and prognosis in pancreatic cancer. World J Gastroenterol 2017;23:7232–41.
[19] Zareba P, Flavin R, Isikbay M, et al. Transdisciplinary prostate cancer partnership (ToPCaP), perineural invasion and risk of lethal prostate cancer. Cancer Epidemiol Biomark Prev 2017;26:719–26.
[20] Liebig C, Ayala G, Wilks JA, Berger DH, Albo D. Perineural invasion in cancer. Cancer 2009;115:3379–91.