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Reliability of sentinel node assay in vulvar cancer: The first Croatian validation trial

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ABSTRACT

Objective. To evaluate the reliability of sentinel node assay in early stage vulvar cancer patients by using preoperative lymphoscintigraphy.

Methods. Technetium-99m colloid albumin was injected intradermally around the tumor for lymphoscintigraphic mapping and intraoperative hand-held gamma probe detection of sentinel nodes. For all patients, sentinel node biopsy was followed by inguinofemoral lymphadenectomy, regardless of the sentinel lymph node status.

Results. From December 2008 until May 2011, 25 consecutive patients with T1 or T2 stage of vulvar squamous cell cancer were enrolled. The median age of patients was 69 years (range, 48–79). The detection of sentinel lymph node was successful in all 25 patients. A total of 36 sentinel lymph nodes were harvested and metastatic carcinoma was identified in 12 sentinel nodes from 8 patients. There was 1 patient with metastatic non-sentinel lymph node despite the negative sentinel node. Two patients with negative sentinel nodes proven by routine histopathological examination were positive by immunohistochemical staining. The sensitivity, specificity and negative predictive value of sentinel node assay with immunohistochemistry included were 89%, 100%, and 94%, respectively.

Conclusions. Lymphoscintigraphy and sentinel lymph node biopsy under gamma-detecting probe guidance proved to be an easy and reliable method for the detection of sentinel node in early vulvar cancer. Immunohistochemical analysis improves the sensitivity for the detection of regional micrometastases. The sentinel node assay is highly accurate in predicting the status of the remaining inguinofemoral lymph nodes. Our results indicate that patients best suited to SLN assay have had a simple punch biopsy to confirm the diagnosis rather than a previous tumor excision. This technique represents a true advance in the selection of patients for less radical surgery.

Introduction

Vulvar cancer accounts for 5% of all female genital malignant neoplasms and about 1% of all malignancies in women [1,2]. The incidence in Croatia is 3.4 per 100,000 female population, which is approximately 80 newly diagnosed cancers per year [3]. Although vulvar cancer was previously almost exclusively seen in women between 65 and 75 years of age [4,5], recent studies have shown that 20% of these cancers now occur in women younger than 50 years [6–8]. An increasing incidence of a tumor precursor (vulvar intraepithelial neoplasia) and invasive vulvar cancer has been noted over the last few decades [7–9], what is explained by the extending life span of the female population [10] and the increasing prevalence of oncogenic types of human papillomavirus infections [6,11].

About 90% of all primary vulvar malignancies are squamous cell carcinomas (SCCs) and the remaining 10% comprise a variety of tumors ranging from malignant melanoma to adenocarcinoma of the Bartholin's gland, extramammary Paget's disease, basal cell carcinoma, sarcomas and lymphoma [12]. The dissemination pattern of SCC of the vulva is predominantly lymphogenic, while spread by a direct extension may occur, although less frequent. Hematogenous spread is extremely rare, especially in the absence of lymph node metastases [1].

Standard treatment for an early stage vulvar cancer is a partial or total vulvectomy with inguinofemoral lymph node dissection (ILND) [10,13]. However, a significant treatment-related morbidity has been observed concomitantly. Early and late complications after ILND were reported in more than 70% of patients. Main complications are wound breakdown and/or infection of the groin, lymphocyst formation and chronic leg lymphedema [14,15].

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The most significant predictor of both the failure to survive and the recurrence is the inguinofemoral lymph node (ILN) involvement [4,16]. The likelihood of regional metastasis is related to the depth, size and histological grade of the primary tumor [10,13,17].

The sentinel lymph node (SLN) is defined as the first node of a regional lymphatic basin that receives lymphatic flow from the tumor, thus representing an elective site of lymph node metastasis. Histologically negative SLN should guarantee the absence of disease in the remaining regional lymph nodes [18,19]. A validation study of SLN biopsy should establish standard guidelines for deciding on the extent of lymphadenectomy in vulvar cancer but still allow staging of a tumor.

Material and methods

Study population

From December 2007 to May 2011, a prospective clinical trial for the evaluation of SLN assay in patients with an early vulvar SCC was performed at the Zagreb University Hospital Center. Twenty-five consecutive patients with T1 or T2 stage [20] of vulvar SCC with a depth of invasion more than 1 mm and clinically no suspicious inguinofemoral lymph nodes were enrolled in the study. A tumor located within 2 cm from the midline of the vulva was considered a midline lesion. Only patients with previously untreated vulvar SCC, where a radical excision or a complete vulvectomy sufficed to resects the primary lesion, were eligible. Multifocality was not an exclusion criterion. The Ethics Committee of the Zagreb University Hospital Center gave the approval for this study. All patients signed an informed consent form.

Sentinel lymph node mapping technique

Lymphoscintigraphy was performed to establish the vulvar lymphatic drainage and to define the location of SLN. Technetium-99m (Tc-99m) colloid albumin was used for lymphatic mapping [21,22]. The procedure was as follows: three hours before an elective surgery, the patients were transported to the nuclear medicine unit where they received a peritumoral injection of 15–20 MBq Tc-99m colloid albumin (SentiScint, Medi-Radiopharma Ltd, Hungary; with more than 80% of particles between 100 and 600 nm in size).

Generally, 5 MBq of Tc-99m colloids per injection in a volume of 0.1 ml were administered intradermally with a 25-gauge needle at four sites around the primary tumor or the residual biopsy scar at the junction with healthy skin. Dynamic scintigraphy was commenced immediately using a large field scintillation camera (Siemens Diacam, Medical Imaging Concepts, INC). Static planar images were taken afterwards in anterior and lateral positions. Each planar acquisition lasted 5 min and was obtained every 20–30 min until the sentinel node was visualized. The scintigraphic hot spot *in vivo* was detected using a hand-held gamma detecting probe (GDP) (neo2000, Neoprobe Corporation, Dublin, UK) and was marked on the skin [23]. No adverse effects were noted.

Surgery procedure

Three hours after administration of the tracer, the patients were carried into the operation room. Foremost, the hand-held GDP was used before making the skin incision in order to identify the area of the greatest activity to confirm the location of SLN. A small 2–4 cm incision was made over the hot spot and surgical extirpation of SLN was performed. When the first SNL was identified and removed, the groin region was rescanned. If radioactivity at a level greater than 10% of the first excised SNL was detected, the dissection was continued to find an additional SLN. The removed SLNs were measured *ex vivo* in order to ensure that the correct nodes were removed. In all patients, SLN biopsy was followed by a complete ILND, regardless of the SLN status. Finally, vulvectomy or radical local excision with 2 cm clinical free margins was performed. The operations were all performed by the same surgeon (JZ).

Conventional histopathology and immunohistochemical analysis

The SLN was excised together with a rim of the surrounding tissue and sent as a separate specimen for histological examination. The lymph node was cut into 3 mm thick slices, completely processed, and paraffin embedded and one hematoxylin–eosin (HE) slide of 3 μm thickness was made at first instance [24]. If no metastases were found, immunohistochemical (IHC) staining was performed [25,26]. A pan-Cytokeratin (CK AE1/3) antibody (Dako, Denmark) was used. All non-SLNs were examined with a standard HE technique as described. In cases where SLNs were negative by using cytokeratin stain, the status of non-SLNs was also confirmed by IHC. In all cases, a tissue section of the primary vulvar cancer was immunostained to confirm tumor cell positivity.

Statistical analysis

Statistical analysis was performed using Statistica 9 software (STATSOFT Inc, Tulsa, OK). Categorical data describing patient and tumor characteristics are expressed as numbers and frequencies. The data were presented as medians (range) and Mann–Whitney *U*-test was used to evaluate differences between the groups. A significance level of 0.05 was chosen.

Results

A study of 25 patients, who underwent SLN biopsy followed by inguinofemoral lymphadenectomy and radical excisions of vulvar tumor, is detailed in Table 1. Patients ranged in age from 48 to 79 years with a median age of 69 years. Primary tumors were T1 (28%) or T2 (72%) stage of SCCs ranging from 1.2 to 7.5 cm in diameter (median 3.2 cm). The median value of depth of tumor invasion was 5.0 mm (range, 1.1–14.0).

A total of 50 groin dissections were performed, with a total number of the removed lymph nodes being 443 (Table 2). The median number of the dissected lymph nodes was 17 (range, 8–32), and 9 (range, 4–20) per patient and per groin, respectively. In the first 10 patients, the median number of the removed nodes was 13 (range, 8–18) and in the remaining 15 it was 19 (range, 10–32), ($P<0.05$).

At least one SLN was identified in all patients (100% detection rate). A total of 36 SLNs was isolated with the median number of 1 node per patients (range, 1–3). Furthermore, in 9 of 25 patients

Table 1
Summary of patients (N = 25) and tumor characteristics.

Characteristics	Value (%)	Range
Age (years, median)	69	48–79
Primary tumor position (%)		
Midline[a]	14 (56)	
Lateral[b]	11 (44)	
FIGO clinical stage (%)		
1B	7 (28)	
II	18 (72)	
Primary tumor, diameter (median, cm)	3.2	1.2–7.5
Depth of invasion (median, mm)	5.0	1.1–14.0
1.1–3.0 mm	4	
3.1–5.0 mm	9	
>5.0 mm	12	
Grade of differentiation		
Well	10	
Moderately	9	
Poorly	6	

[a] Defined as <2 cm from midline.
[b] Defined as ≥2 cm from midline.