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Sentinel lymph node biopsy in vulvar cancer: a multicenter evaluation of procedure's feasibility for Israeli patients

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Summary

Purpose: To evaluate the accuracy, safety, and outcome of sentinel lymph node biopsy (SLNB) in early-stage vulvar cancer and determine the applicability of this procedure for selected patients in Israel. *Materials and Methods:* Forty-five patients with T1 squamous cell carcinoma (SCC) of the vulva who underwent surgery with SLNB between 2002-2011 were included. SLN was detected using both radioactive tracer and blue dye. All resected nodes underwent pathological examination. The accuracy, recurrence rates, and complications of the procedure were analyzed. *Results:* There was a significant correlation between radioactive reading intensity and SLN detection in frozen section ($p < 0.0003$, $p < 0.0001$). A weaker correlation existed with use of blue dye ($p = 0.04$, $p = 0.09$). For metastatic LNs, the detection rates of both agents were similar. The false negative for metastatic SLN detection in frozen section was 12.5%, while the false positive was 2%. The rate of inguinal recurrence without local recurrence was 4.4%. For patients with unifocal vulvar disease and a negative sentinel node the seven-year survival rate was 94%. *Conclusion:* SLNB is an effective and safe procedure for Israeli patients with early-stage SCC of the vulva. Recurrence rates and disease-free survival are similar to the reported literature and morbidity is low compared to radical inguinal LNs resection.

Key words: Vulva; Carcinoma; Sentinel lymph node biopsy.

Introduction

Vulvar cancer is a rare malignancy. According to the latest out of only two epidemiological studies conducted in Israel [1, 2], squamous cell carcinoma (SCC) constitutes 70% of vulvar malignancies in Jewish Israeli women with a mean annual incidence of 0.07−7.2 per 100,000 in different age groups [2]. Lymphatic spread, the strongest predicting factor for survival [3], may occur early and 12% of T1 tumors will eventually spread to the inguinal LNs [4]. Patients with negative LNs have a five-year survival rate of 90% as apposed to 50% for patients with positive nodes [5]. Groin dissections are associated with early postoperative morbidity including wound infection and breakdown in 20−40% of cases, as well as with late postoperative morbidity, including chronic leg edema, functional impairment and chronic skin infections. In an effort to reduce postoperative morbidity, the use of sentinel lymph node biopsy (SLNB) has been proposed for early-stage patients. The SLN is defined as the first LN in the lymphatic pathway that drains the primary tumor [6], therefore tumor cells within it can predict the state of following LNs in the pelvis. Negative SLN obviates complete groin dissection [6]. The SLN can be detected using either a blue dye or radioactive agent, both injected around the tumor [7] and overall detection rates can be maximized by using both [8, 9]. In their meta-analysis, Covens *et al.* reported an 87% per groin detection rate using radiocolloid tracer combined with blue dye, with a 6.4% false negative rate. Recurrence rates were 2.8% and 1.4% for SLNB versus inguinofemoral LNs dissection, respectively, and SLNB was recommended for patients meeting the clinical criteria, provided that the logistic and human requirements are met [10].

Groin dissection was an integral part of the surgical treatment for vulvar cancer in Israel until recently. Here, the authors present the first summary of a series of patients who underwent SLNB in their country, in order to evaluate the learning curve of this procedure. Considering the low volumes in which this operation is performed by individual gynecologists in Israel, it is cardinal to ensure that it is a suitable alternative for groin dissection in candidate patients before it can become the working routine. Using both blue dye and radioactive agents the authors evaluate the accuracy, safety, and outcome of SLNB, thereby determining

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Table 1. — *General demographic characteristics of the study population.*

	n = 45
Age (years, mean ± SD)	70 ± 13.4
Gestation (n, mean ± SD)	4.2 ± 5
Parturition (n, mean ± SD)	2.76 ± 2.08
Age of menopause (years, mean ± SD)	46.8 ± 13.2

the eligibility of this procedure for selected patients in Israel.

Materials and Methods

This retrospective multicenter study included three gynecologic units in Israel engaged in surgical treatment of vulvar cancer, including SLN detection.

Data was collected for all patients with SCC of vulva operated between 2002 -2011. Included lesions were all Stage T1, smaller than four cm, with stromal invasion deeper than one mm and without clinical suspicion of Inguinal LN involvement.

The treatment protocol included resection of the primary tumor combined with SLN identification by both a radioactive marker and blue dye. A day before surgery, 0.2 to 0.6 ml of 60-millibecquerel (mBq) 99mTc were injected into the skin around the tumor, followed by lymphoscintigraphy and pencil marking of the SLN approximated location on the skin. At the day of surgery, under general anesthesia, two ml of blue dye were injected into the marked location. Following intraoperative identification of the SLN using a gamma-detecting probe, all significantly radioactive and blue LNs were dissected and probe examination was repeated. Unless the radioactive reading decreased to background levels, the dissection continued in search for additional nodes. SLNs were analyzed by frozen sections. When the SLN was negative, no further treatment was performed. In case of metastatic SLN, extensive inguinal LNs dissection was carried out, either in the primary surgery (positive SLN in the frozen section) or in a repeated surgery (when the gland was found positive only with Hematoxylin and Eosin (H&E) or immunohistochemistry (IHC) staining of the final specimen). In cases of more than one positive node or extranodal metastases, complementary radiotherapy treatment (50 Gy) to the groin/pelvis was performed.

SLNs were cut in the middle in order to preserve tissue for cytogenetic testing. Then, four sections were sampled from one half of the node for routine H&E stainings. IHC was only performed on sentinel nodes that were negative in routine examination.

All statistical analyzes were performed using SPSS software, version 11. Differences in demographic characteristics of patients were analyzed using chi square test (χ^2). For continuous variables, *t*-test was performed. Survival was calculated using the Kaplan-Meier curve. Statistical differences were considered significant when $P < 0.05$. Surveillance period for survival rates included the time between the primary surgery and the date of last examination or death.

Table 2. — *Clinical and intra- and postoperative characteristics of the study population.*

	Number of patients (%)
Symptom	
Pruritus	19 (42.2)
Pain	15 (33.3)
Palpable mass	11 (24.4)
Sign	
Ulcer	22 (48.9)
Palpable mass	23 (51.1)
Grade in biopsy	
Well differentiated SCC	30 (66.7)
Moderately differentiated SCC	12 (26.7)
Poorly differentiated SCC	3 (6.7)
Location	
Clitoris	9 (20.5)
Rt. labia	18 (40.9)
Lt. labia	2 (4.5)
Fourchette	15 (34.1)
Palpable size	
≤ 2 cm	27 (60)
> 2 cm	18 (40)
Type of surgical procedure	
Wide local excision (patients, %)	6 (13.3)
Radical vulvectomy (patients, %)	39 (86.7)
Histology	
Tumor volume (cm^2, ± SD)	4 ± 4.6
Stromal Invasion (cm, ± SD)	2.9 ± 4.2 mm
Tumor diameter (cm, ± SD)	2.3 ± 1.4 cm
Free margins (patients, %)	41 (91.1)
Stage	
Ia (patients, %)	19 (42.2)
Ib (patients, %)	18 (40)
II (patients, %)	
IIIa (patients, %)	5 (11.1)
IIIb (patients, %)	3 (6.7)
LVSI (patients, %)	2 (4.4)

SCC – squamous cell carcinoma. Rt. – right. Lt – left. LVSI – lymphovascular space invasion.

Results

Between 2002-2011, 45 patients with vulvar cancer underwent vulvectomy with SLNB in three large medical centers in Israel. Their mean age was 70 years. All three medical centers met the criteria. General demographic characteristics are presented in Table 1.

Clinical characteristics and intra- and postoperative characteristics are summarized in Table 2. In the majority of cases, intraoperative assessment revealed Stage Ia or Ib disease (37 patients (82.2%)), free surgical margins [41 patients (91.1%)], and no LVSI (43 patients (95.6%)).

SLN detection was performed utilizing injection of both Patent Blue and radiocolloid. For radioactive readout, reading intensity was divided to weak, medium or strong. Table 3 shows a significant correlation between 99mTc

Table 3. — *Radioactive intensity or Methylene blue coloration and SLN detection.*

	Frozen section – Lt. groin				Frozen section – Rt. groin			
	SLN not detected (n,%)	Benign SLN detected (n,%)	Metastatic SLN detected (n,%)	*p*-value	SLN not detected (n,%)	Benign SLN detected (n,%)	Metastatic SLN detected (n,%)	*p*-value
Radioactive intensity				< 0.0003				< 0.0001
No uptake	2 (100)		1 (50)		1(100)			
Weak		1 (4)				1 (4.2)		
Medium		7 (28)	1 (50)			4 (16.7)	2 (40)	
Strong		17 (68)				19 (79.2)	3 (60)	
Blue coloration				0.04				0.09
No coloration	2 (100)	5 (19.2)	1 (33.3)		1(100)	4 (15.4)	1 (16.7)	
Coloration		21 (80.8)	2 (66.7)			22 (84.6)	5 (83.3)	

Rt. – right. Lt. – left. SLN – sentinel lymph node.

Table 4. — *Tumor location and radioactive intensity or Methylene Blue coloration in SLN.*

	Clitoris (n, %)	Lt. Labia (n, %)	Rt. Labia (n, %)	Forshette (n, %)	*p*-value
Radioactive intensity					
Lt. groin					0.636
No uptake		3 (17.6)	2 (33.3)		
Weak		1 (5.9)			
Medium	3 (50)	3 (17.6)	2 (33.3)		
Strong	3 (50)	10 (58.8)	2 (33.3)	2 (100)	
Rt. groin					0.624
No uptake	1 (16.7)	2 (22.2)	1 (6.7)		
Weak		1 (11.1)			
Medium	2 (33.3)	1 (11.1)	2 (13.3)	1 (50)	
Strong	3 (50)	5 (55.6)	12 (80)	1 (50)	
Methylene Blue coloration					
Lt. groin					0.33
No coloration	1 (12.5)	6 (35.3)	3 (50)		
Coloration	7 (87.5)	11 (64.7)	3 (50)	2 (100)	
Rt. groin					0.2
No coloration		4 (40)	3 (20)		
Coloration	7 (100)	6 (60)	12 (80)	2 (100)	

Rt. – right. Lt. – left.

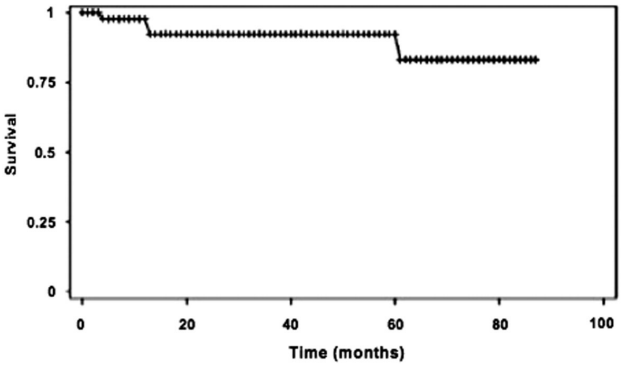

Figure 1. — Postoperative survival curve. Cox survival analysis of the study cohort presenting the fraction of surviving patients *vs*. months post-operation.

reading intensity and SLN detection in frozen section with $p < 0.0003$, $p < 0.0001$ for left and right groins, respectively. A weaker correlation exists between Patent Blue uptake and SLN detection in frozen section with $p = 0.04$, $p = 0.09$ for left and right groins, respectively. A total of seven patients had metastatic LNs detected in the final pathological examination. One of them underwent pre-operative Patent blue injection only. All other six metastatic LN were detected by both methods, except for one patient with Stage 3b tumor located at the left labia for whom the metastatic LN was undetected by both methods and underwent groin dissection at the time of the primary surgery. Seven patients were diagnosed with metastatic LN in frozen section, of which one with Stage 1b in the final pathology. She had a left-sided tumor, underwent Patent blue injection only, and blue colored LNs were detected bilaterally. The frozen section of the left SLN indicated metastatic involvement but her final pathology was negative. Hence, in the present cohort, the false negative for metastatic SLN detection in frozen section was 12.5% while the false positive was 2%.

Table 4 shows that there is no correlation between tumor location and SLN location, as detected by radioactive labeling, $p = 0.624$ and $p = 0.636$ for left and right groin, respectively. The same is true for Patent Blue coloration with $p = 0.33$ and $p = 0.2$ for left and right groins, respectively. Radioactive uptake by the SLN can be increased bilaterally with any tumor location.

Six women had recurrence 6 to 17 months from the primary surgery, all in Stage 1b. Four of them (8%) had local recurrence. In all six cases, the final pathology matched the results of the frozen section. Three women had groin recurrence 12-14 months, one of which could be attributed to local recurrence.

Two patients (4.4%) developed an anaphylactic allergic reaction to Patent Blue. Six patients (13%) had surgical

wound infection, two of them with metastatic lymph nodes. Four patients (8.8%) had postoperative fever in the absence of wound infection. Three patients developed lymphedema. All three had a metastatic inguinal LN and underwent extensive inguinal dissection.

The duration of postoperative follow-up ranged from three to 87 months and four patients passed away during that time period. Two cases were disease-related; one patient passed away three months post-op following pulmonary metastasis and another patient passed away 12 month post-op with local recurrence. Two patients passed away from unrelated reasons; the first had local recurrence 12 months post-op but died of cardiac disease 60 months after surgery. The second died of lung disease free of tumor. Using Kaplan-Meir survival analysis, the average survival period was 56.125 ± 2.48 months. Cox survival analysis presented in Figure 1 depicts the postoperative survival rate *vs*. time in months.

Discussion

Local or wide radical excision with superficial and deep inguinal LN dissection is still considered the standard surgical procedure for SCC of vulva in many centers. Short- and long-term postoperative complications include wound infection, wound breakdown, chronic leg edema, functional impairment, and chronic skin infections. In an effort to reduce these morbidities, SLNB is gradually becoming the more prevalent therapeutic approach provided that a suitable setting exists. Levenback *et al.* [11] found that the detection rates of SLN by blue dye alone ranged from 60−90%. In this study the authors found that in 17 out of 68 groins, a SLN was not detected using this method, setting the detection rate at 75%. Moore *et al.* [12] found that the detection rates of SLN using an agent labeled with sulfur-bound radioactive technetium 99 are approximately 100%. In the present study the authors showed a 91% detection rate using this method. In one out of the four cases in which a SLN was not detected, groin dissection revealed a stiff, fully metastatic LN occupied entirely by tumor cells, which could have prevented lymphatic drainage and absorption of radioactive agents or blue dye from the tumor bed.

SLN detection success rates can be maximized by dual labeling, with both blue dye and isotopes [8, 9]. The present authors found a significant correlation between radioactive readings and inguinal SLN detection, $p < 0.0003$ and $p < 0.0001$ for left and right groin, respectively. A weaker correlation was demonstrated between Patent Blue coloration and SLN detection, in both sides. Hence, SLN detection by a radioactive marker is more sensitive. In four out of 26 (15%) cases involving the left groin, a non-colored SLN was discovered by technetium. The same was demonstrated for cases involving the right groin - five out of 29 (17%). In cases of metastatic LNs, the sensitivity of both labeling methods was identical. Being that blue dye injection is performed as part of the surgery, requires less operator experience and costs less, future studies in a larger cohort should be performed in order to answer whether blue dye only can be used for SLNB.

For midline tumors, SLN detection rates in both sides are very high compared to laterally positioned tumors in which SLN detection rates are high [13]. Contrary to the literature, in the present cohort the authors could not find a correlation between tumor location and SLN location as detected by radioactive labeling of the right and left groin, $p = 0.624$ and $p = 0.636$, respectively. Likewise, there was no correlation between tumor location and SLN location detected by Blue coloring. In light of these finding, bilateral radioactive examination for SLN location should be carried out regardless of tumor lateralization.

Homesley *et al.* [5] reported metastatic spread to the inguinal LNs in 10−15% of patients with T1 or T2 tumors, meaning that 85−90% of patients may undergo unnecessary inguinal dissection with all the associated short- and long-term morbidity. In the present cohort only three women developed lymphedema. It is noteworthy that all three had a metastatic inguinal SLN, leading to extensive inguinal dissection. Six women (13%) had surgical wound infection, none of them leading to wound breakdown. Additionally, two patients (4.4%) developed an anaphylactic allergic reaction to Patent Blue. Surgery was completed as scheduled in both cases, but proper evaluation and planning are warranted.

In this multicenter retrospective study, three out of 37 women diagnosed with benign SLN had inguinal recurrence (8.1%). Thus, the rate of inguinal recurrence without local recurrence is only 4.4%, similar to the reported literature: 5% in patients with T1 and T2 undergoing complete groin dissection [14]. Patients with palpable metastases or a history of groin operation resulting in lymph tract destruction are not candidates for this procedure.

For patients with unifocal vulvar disease and a negative sentinel node, the seven-year survival rate was 94%. Combined with the low recurrence rates for patients with benign SLN, this procedure is a safe alternative for radical LN dissection in selected cases in Israel.

Conclusion

The present study demonstrates the effectiveness and safety of SLNB in early-stage SCC of the vulva performed in Israel, with no change in recurrence rates or disease-free survival compared to the method used so far. In addition, this method allows prevention of short- and long-term morbidity caused by radical inguinal LNs resection. Due to the scarcity of this disease, in order to optimize the surgical performance of this rare procedure in small countries like Israel, the establishment of a single, nation-wide referral center should be considered.

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