

Original Research

Utility of Intraoperative Frozen Section of the Inguinofemoral Sentinel Lymph Node in Vulvar Cancer: A Retrospective Cohort

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Abstract

Objective: Sentinel lymph node dissection (SLND) spares most patients diagnosed with clinically early-stage vulvar cancer from undergoing complete inguinofemoral lymphadenectomy (IFLND). We sought to evaluate the intraoperative examination of frozen sentinel lymph node (SLN) sections to assess the need for IFLND. **Methods:** We identified patients with vulvar cancer treated at a tertiary referral center between January 2006 and December 2019 who either underwent SLND or met the eligibility criteria to receive SLND. All patients were restaged according to the International Federation of Gynecology and Obstetrics (FIGO) 2009 guidelines. The records of each patient were reviewed for disease characteristics, follow-up status, patient demographics, SLN eligibility, and surgical and pathologic variables. **Results:** Of 142 eligible patients, 76 underwent SLND (53.5%) for a total of 118 groins assessed. We found no statistically significant differences in characteristics between the cohorts that received or lacked SLN examination. The SLN was detected in 90.8% (95% Confidence Interval (CI): 81.9%–96.2%) of patients. SLNs of 52 patients were sent for frozen section, and the results were used to direct further surgical intervention. The results of the frozen section pathology and the final pathology report exhibited a high degree of correlation per patient (100%) and per groin (98.7%). The 1 incorrect groin was negative on frozen but positive on final pathology and therefore no patients received an incorrect complete groin dissection. We observed no statistically significant differences in recurrence-free survival or overall survival rates between those patients who received or did not receive SLND. **Conclusions:** The use of intraoperative frozen section is appropriate for assessing node status at time of initial surgery. The use of this method decreases the incidence of reoperation by identifying those patients who warrant immediate IFLND.

Keywords: vulvar; sentinel; lymph nodes; frozen section; surgery

1. Introduction

Vulvar carcinomas account for 6% of gynecologic cancers. Nearly 6500 new cases of vulvar carcinoma are diagnosed in the U.S. annually [1]. Lymph node status is the single most important prognostic factor for patients with vulvar carcinoma [2,3]. The 5-year overall survival (OS) rate of patients without nodal involvement is 91% versus 52% for patients with nodal involvement [4]. In clinically early-stage disease where the tumor size is less than four cm in diameter, approximately one third of patients will have involvement of the lymph nodes [5,6].

Determination of lymph node status is typically accomplished via bilateral or unilateral inguinofemoral lymphadenectomy (IFLND). IFLND is associated with high rates of wound complications including lymphedema (28%), lymphocyst formation (40%), infection (39%), and wound breakdown (17%) [6–9]. Therefore, the selection of patients eligible for unilateral and/or sentinel lymph node dissection (SLND) instead of IFLND represents an important step in reducing the morbidity associated with complete bilateral IFLND [6,10–12]. SLND is an accurate means of assessing lymph node status in vulvar cancer [13]. SLND

is associated with decreased morbidity and rates of detection of lymph node metastases that are equivalent to those of complete bilateral IFLND without an increased risk of groin recurrence or compromising disease specific survival. Isolated groin recurrences following a negative SLND are less than 3% [14,15].

Patients with a sentinel lymph node (SLN) that is enlarged >2 mm (positive for metastasis) or that possesses extracapsular extension will require a complete IFLND with subsequent radiation-based therapy. Its importance for patient prognosis was demonstrated in GROINSS-V-II study [16]. In 162 patients with macrometastases (>2 mm) and <4 cm primary tumors, radiation treatment in the absence of IFLND resulted in a 2-year recurrence rate of 25.0% versus 8.2% in the presence of IFLND ($p = 0.012$) [16]. Unfortunately, complete IFLND may require a second operation if its need is only discovered subsequent to the first surgery. The aim of this study is to examine the utility of examining SLN frozen sections during the initial surgery to identify positive SLNs in patients who can be subjected to complete IFLND immediately, at the time of initial surgery.



2. Materials and Methods

2.1 Inclusion and Exclusion Criteria

We performed a retrospective chart review of all patients treated for vulvar cancer at the West Cancer Center and Research Institute, Division of Gynecologic Oncology in Memphis, TN from January 2006 to December 2019. The project was initiated after exempt review and approval of the protocol under U.S. regulation 45CFR46.101(b)(4) by the University of Tennessee Health Science Center (UTHSC) Institutional Review Board (IRB). All STROBE guidelines were followed.

Patients who either received or were eligible for SLND met the inclusion criteria for the study. Patients were excluded if they had advanced and/or metastatic disease or were not eligible for SLND. Surgeon preference dictated lymph node approach. Eligibility for SLND on patients receiving IFLND was determined by preoperative exam and imaging. Eligibility for SLND required a diagnosis of vulvar cancer, the absence of clinically or radiologically suspicious lymph nodes, a depth of tumor invasion greater than one mm, tumor size less than four cm when measured edge-to-edge on the longest axis, and no prior history of treatment for vulvar carcinoma, including chemotherapy, pelvic or inguinal radiotherapy, and prior vulvar surgery (aside from excisional biopsy). All patients were restaged to International Federation of Gynaecology and Obstetrics (FIGO) 2009 staging guidelines [17].

2.2 Sentinel Node Acquisition

Radiocolloid was utilized for the detection of the SLN. One to six hours preoperatively, 0.5 to 1.0 mL of Technetium-99 microsulfur colloid was injected intradermally in each quadrant around the tumor to identify afferent lymphatic channels. Intraoperatively, a handheld gamma counter in a sterile sleeve was passed transcutaneously over the tissue to identify lymph nodes that emit the tracer. A radioactive lymph node was considered sentinel if its radioactivity was at least 2 times greater than background levels. Following its excision, the SLN was removed from the surgical field, and the gamma counter was used to confirm it was radioactive, with threshold levels considered to be those at least 10 times higher than the background level. The surgical site was re-examined with the gamma counter to identify any remaining radioactive nodes. Some surgeons preferred to include intradermal blue dye (isosulfane or methylene blue) with the Technetium-99 tracer in select patients. This was performed following induction of anesthesia and sterile preparation and draping. Incisions were made shortly after dye injection. Lymph nodes were labeled as sentinel nodes with “blue” and/or “radioactive” qualifiers and sent for pathology with specification of superficial inguinal vs femoral.

2.3 Pathology

The decision to request intraoperative frozen sections was made by the surgeon according to their preference. Identified SLNs were sent to the pathology laboratory for preparation of frozen sections. Briefly, the SLN was frozen in a cryostat and cut into 4 μm -thick slices perpendicular to the longest axis at intervals of 2 to 3 mm. Four to six serial frozen sections were subjected to hematoxylin and eosin (H&E) staining and evaluated according to standard protocol by a board-certified pathologist with expertise in gynecologic malignancies.

Thereafter, any remaining lymph node tissue was fixed in buffered 4% formaldehyde (pH 7.2) and embedded in paraffin. Ultra-staging was performed on all SLNs, regardless of frozen pathologic results. In cases of SLNs that were assessed to be negative, the tissue was examined after immunohistochemical staining for cytokeratin 19 (CK19). Surgical findings were correlated with the final pathology report.

2.4 Statistical Analyses

Patient demographic and disease characteristics were compared between SLN surgical groups using Chi-square and *t*-test analyses. Survival endpoints, including time to recurrence- or relapse-free survival (RFS) and overall survival (OS), were estimated as described by Kaplan and Meier. RFS was defined as the time from surgery until recurrence was detected by examination or imaging. Those patients who did not experience recurrence were scored as censored at the date of their last follow up visit. OS was calculated as the time from surgery until death from any cause. Surviving patients were scored as censored at the date of last follow up. All analyses were performed using Statistical Package for the Social Sciences Release 27 software (SPSS 27, IBM, Armonk, NY). All reported *p* values and 95% confidence intervals (CI) are two-sided and unadjusted for multiple comparisons.

3. Results

From January 2009 to December 2019, 142 patients diagnosed with early vulvar cancer met the inclusion criteria for this study. Demographics of patients undergoing SLND compared to IFLND without SLN are shown in Table 1. SLND was performed with Technetium-99 in 76 patients (nine of whom also received intradermal methylene blue dye). A total of 118 groins were evaluated, 34 of which were ipsilateral and 42 bilateral. A SLN was detected in 69 of 76 patients (90.8%; 95% CI: 81.9%–96.2%). The remaining seven patients ultimately underwent a complete dissection, with one having positive nodes on final pathology. Frozen sections were performed in 52/69 of the patients where SLN was identified, nine of whom were identified as having a positive SLN (17.3%, 95% CI: 8.2%–30.3%). Eight of the nine patients subsequently underwent an IFLND after identification of the frozen section as posi-

Table 1. Patient demographic and clinical characteristics by SLN technique.

Item	No SLN technique	SLN technique	Overall	<i>p</i> value
	n = 66	n = 76	(n = 142)	
Mean age, years (\pm SD)	60.4 (\pm 12.1)	63.6 (\pm 13.4)	62.1 (\pm 12.9)	0.140
Race, n, (%)				
White	49 (74.2)	62 (81.6)	111 (78.2)	0.289
Black	15 (22.7)	14 (18.4)	29 (20.4)	0.529
Other	2 (3.0)	0 (0.0)	2 (1.4)	0.126
Mean BMI, kg/m ² (\pm SD)	29.3 (\pm 7.2)	31.6 (\pm 8.0)	30.6 (\pm 7.7)	0.077
Median tumor size ^a , mm (range)	20 (2–40)	20 (1.7–90)	20 (1.7–90)	0.903
Median depth of invasion ^b , mm (range)	5.5 (1–25)	5 (0.7–24)	5 (0.7–25)	0.631
Tumor histology, n (%)				
Squamous cell	58 (87.9)	63 (82.9)	121 (85.2)	0.407
Melanoma	3 (4.5)	5 (6.6)	8 (5.6)	0.603
Other	5 (7.6)	8 (10.5)	13 (9.2)	0.542
Final Stage, n (%)				
IA/IB	53 (80.3)	52 (68.4)	105 (73.9)	0.107
II/IIIA/IIIB/IIIC	13 (19.7)	24 (31.6)	37 (26.1)	
Area of Involvement, n (%)				
Lateral	32 (48.5)	41 (53.9)	73 (51.4)	0.516
Midline	34 (51.5)	35 (46.1)	69 (48.6)	
Median nodes identified (n) (range)	5.5 (0–23)	4 (1–24)	4 (0–24)	0.020
Final node status, n (%)				
Positive	12 (18.2)	15 (19.7)	27 (19.0)	0.810
Negative	52 (78.8)	61 (80.3)	113 (79.6)	0.826
Not obtained	2 (3.0)	0 (0)	2 (1.4)	0.126
Complications				
No	45 (68.2)	67 (88.2)	112 (78.9)	0.004
Yes	21 (31.8)	9 (11.8)	30 (21.1)	

^aNot available for two patients; ^bNot available for three patients; *p* values in bold text were statistically significant (*p* < 0.05). SD, standard deviation.

tive (seven ipsilateral, one bilateral). Because this is a retrospective study, we were unable to identify intraoperative factors that warranted delaying the IFLND in the remaining patient. By patient, intraoperative frozen sections exhibited 100% correspondence with final pathology.

A SLN was detected in 104 of 118 groins evaluated (88.1%; 95% CI: 80.9%–93.4%) and, of these, frozen sections were performed in 78 (26 bilateral, 26 ipsilateral). Of the 25 patients with midline lesions who underwent frozen section, 19 were negative in both groins bilaterally, three were negative on one side and positive on the other, and one was positive in both groins bilaterally. Two patients (one positive and one negative on frozen section) received unilateral groin evaluation despite having a midline lesion, although both exhibited final pathology that corresponded with the results of the frozen section.

Of the 27 patients with lateral lesions who underwent frozen section, 22 were negative on the ipsilateral side. Bilateral evaluation was performed in three patients, one of whom was negative bilaterally, while the other two patients

were positive in the ipsilateral groin. Final pathology reports were consistent with the frozen pathology in 37/37 left groins and 40/41 right groins, resulting in a 98.7% correspondence (Table 2). The single instance of inconsistent pathology involved a midline lesion where bilateral SLND was performed. The frozen section was positive in the left groin and negative in the right. However, the final pathology report revealed both SLNs to be positive. No patients incorrectly underwent a complete IFLND due to a false positive frozen section of the SLNs.

In terms of safety, 2.6% of patients in the SLND group experienced a superficial wound infection compared to 13.6% in the IFLND group (Table 3). Thirty-one patients had recurred during the follow up period. One (1.3%) patient in the SLND group recurred in the groin, compared with two (3.0%) patients who recurred in the IFLND group, although this result was not statistically significant (*p* = 0.478). The patient in the SLND group who recurred exhibited one positive node after initial surgery, whereas the two patients in the IFLND group who recurred had negative

Table 2. Characteristics of groins with SLN method performed, by final node status.

Item	Final node positive	Final node negative	Overall
	n = 17	n = 87	n = 104 (%)
Frozen pathology positive	10	0	10 (9.6)
Frozen pathology negative	1	67	68 (65.4)
Frozen section not performed	6	20	26 (25.0)

Table 3. Complications, as sorted by nodal approach.

Item	No SLN technique	SLN technique	Overall	<i>p</i> value
	n = 66 (%)	n = 76 (%)	n = 142 (%)	
Wound dehiscence	6 (9.1)	4 (5.3)	10 (7.0)	0.373
Superficial infection	9 (13.6)	2 (2.6)	11 (7.7)	0.014
Lymphedema	6 (9.1)	3 (3.9)	9 (6.3)	0.211
Deep vein thrombosis	1 (1.5)	1 (1.3)	2 (1.4)	0.920
Lymphocele	3 (4.5)	5 (6.6)	8 (5.6)	0.603

Several patients experienced more than one complication; *p* values in bold text were statistically significant ($p < 0.05$).

nodes on final pathology. All three of these patients exhibited midline lesions. Thirty-eight patients expired during the follow up period, 22 of whom experienced disease recurrence. The five-year RFS was 75.4% (95% CI; 69.3%–81.5%) in the SLN group and 72.6% (95% CI; 65.7%–79.5%) in the non-SLN group, a difference that was not statistically significant ($p = 0.943$) (Fig. 1). The 5-year OS was 74.4% (95% CI; 68.4%–80.4%) in the SLN group and 70.9% (95% CI; 63.6%–78.2%) in the non-SLN group, a difference that was also not statistically significant ($p = 0.504$) (Fig. 2).

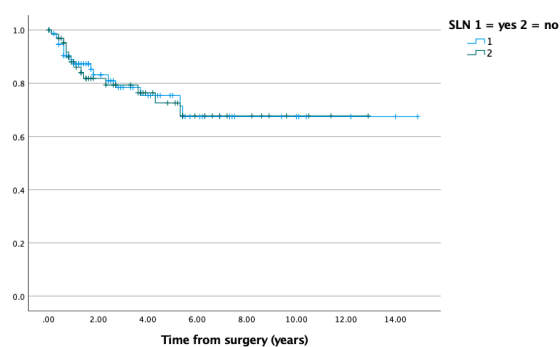


Fig. 1. Recurrence Free Survival (RFS). 31 patients were found to have recurred during follow up.

4. Discussion

This retrospective study sought to investigate the utility of intraoperative frozen sections at the time of SLND in early-stage vulvar cancer. We identified a high correspondence rate between intraoperative frozen section pathology and final pathology in SLNs. Intraoperative frozen section

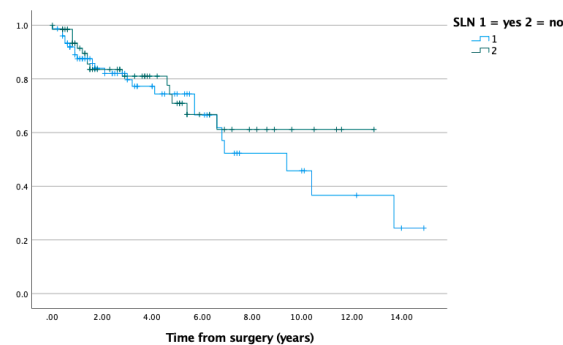


Fig. 2. Overall Survival (OS). 38 patients died during the follow up period and 22 experienced prior recurrence.

had a 100% positive predictive value and 98.5% (95% CI: 91.2%–99.8%) negative predictive value when compared to final pathology. Thus, no patients was incorrectly subjected to a complete IFLND subsequent to a false positive result. In addition, we found that there was no statistically significant difference in OS and RFS between those who were subjected to SLN and those who were not.

The single most important prognostic factor for vulvar cancer is the status of the lymph nodes (nodal status) [2,3]. There is increasing interest in utilizing pre-operative imaging as a means of assessing nodal status [18,19]. However, surgical pathology remains the gold standard method to assess nodal status. The most common method used for intraoperative evaluation of pathologic specimens is the frozen tissue section, although studies on its use in vulvar cancer have been limited. A recent meta-analysis described the use of intraoperative frozen section in 326 breast cancer patients undergoing SLN assessment [20]. The authors found a 100% positive predictive value and 83.2% negative

predictive value. Brunner *et al.* [21] evaluated 44 patients with vulvar cancer undergoing SLND similarly found 100% positive predictive value for examination of intraoperative frozen tissue sections. Three patients received false negative results and all 3 were diagnosed with micrometastasis that was identified on the pathology final report.

The SLN detection rate of 90.8% and isolated groin recurrence rate of 1.3% in this study are consistent with the established literature [6,14,22,23]. Similar detection rates and recurrence profiles across studies attests to the safety of SLND in a variety of settings, including academic medical centers and community hospitals. We found no statistically significant difference in OS and RFS between those patients subjected to SLND and those who were not.

This study is limited by the lack of standardization of SLN approach and selection. Technetium-99-labeled sulfur colloid was used alone in 76 patients or in combination with blue dye in nine patients as the surgeon preferred; none of the patients examined was subjected to lymphoscintigraphy. Though blue dye was not routinely used, its addition may not have improved the detection rate. In a large series of melanoma cases, the addition of radiocolloid increased the detection rate from 87% to 99% and only one blue SLN was identified that was not radioactive [24]. Other studies also indicate that the improvement in detection rate can be attributed mostly to the use of the radiocolloid rather than the combination of dye and radiocolloid [25]. Lymphoscintigraphy might have been of use in patients with ambiguous laterality, although it is unlikely to have improved the detection rate [26]. In this study, 17 patients with a total of 26 sentinel lymph nodes failed to receive frozen section examination for no reason that was documented. Unfortunately, since this study was retrospective in nature, we are unable to determine the circumstances that warranted exclusion of frozen section examination. Since this study is retrospective, it is possible that some patients may have experienced complications that were not fully described in the patients' medical records. Strengths include the confirmation of SLN status with immunohistochemical assessment, ultra-staging, and uniform use of radiocolloid. Immunohistochemical and ultra-staging improved chances of detecting microscopic disease that can be easily missed using standard sampling protocols, resulting in increased regional nodal failure from undertreatment [27].

Given the excellent positive predictive value for vulvar carcinoma in this study and for other solid tumors in the literature, the examination of intraoperative frozen SLN sections should be considered in the management of suspected early-stage vulvar cancer. We found that no patients were incorrectly subjected to more aggressive surgery such as complete IFLND inguofemoral lymphadenectomy due to a false positive SLN. By correctly identifying patients who should undergo further surgery at the time of their initial SLN procedure, patients may be spared a second procedure, thereby reducing cost, potential morbidity and mor-

tality of a second operative procedure, and potential delay in starting essential chemotherapy or radiotherapy. Integration of intraoperative frozen section analysis into the methodology of future inguofemoral lymph node studies will further establish the utility of this approach.

5. Conclusions

In conclusion, our findings support the utility of intraoperative frozen section analysis to assess the status of the lymph nodes during initial surgery, with 100% positive predictive value and 98.5% negative predictive value.

Author Contributions

NZ—Data curation, Formal analysis, Writing—Review & Editing; JG—Data curation, Writing—Review & Editing; BW—Writing—Review & Editing; CC—Data curation, Writing—Original Draft; MR—Conceptualization, Methodology, Writing—Review & Editing; AE—Conceptualization, Methodology, Supervision, Writing—Original Draft/Review & Editing.

Ethics Approval and Consent to Participate

The IRB determined exempt review under 45CFR46.101(b)(4). All STROBE guidelines were followed. Consent was waived as this was a retrospective review and no patient identifiers are present.

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Conflict of Interest

AE reports consulting fees from AstraZeneca and Eisai during the conduct of the study. The other authors declare no conflict of interest.

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