Validation and application of the sentinel lymph node concept in malignant vulvar tumours

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Abstract. *Purpose:* Inguinal lymphadenectomy, unilateral or bilateral, is widely used in cases of vulvar squamous cell carcinoma and melanoma but has a high morbidity. Sentinel lymph node (SLN) biopsy may be used in the management of these patients. The aims of this study were firstly to determine the reliability of SLN biopsy in predicting regional lymph node status and secondly to apply this technique in the routine clinical setting.
Methods: We prospectively studied 70 women with vulvar malignancies. The first 50 cases were of squamous vulvar cancer and were used to validate the SLN technique in this clinical setting (validation group). Once a satisfactory success rate had been achieved in the validation group, the SLN technique was applied to a further 20 patients with vulvar malignancies, i.e. squamous cell carcinoma (n=12) and melanomas (n=8) (application group). Dynamic and static images were acquired after the injection of 74–148 MBq of a colloidal albumin, and continued until SLN identification. Fifteen minutes before surgery, blue dye injection was administered in a similar manner to the radiocolloid. After incision, a hand-held gamma probe was used to find the SLN. In the validation group, dissection of the SLN was always followed by lymphadenectomy. In the application group, this procedure was only performed if the SLN was positive for metastases. For pathological staging, samples were evaluated using haematoxylin and eosin and immunohistochemistry.
Results: In the validation group, lymphoscintigraphy allowed SLN detection in 49/50 patients (98%). Blue dye detected the SLN in 40/50 patients (80%). In 16 patients (33%), the SLN showed metastases in the pathology study. All 33 patients with negative SLN had regional lymph nodes negative for metastases (negative predictive value 100%). In the application group, lymphoscintigraphy showed drainage to an SLN in 19 out of 20 patients (95%) and blue dye demonstrated a stained SLN in 17/20 patients (85%). Seven of the 19 SLN-identified nodes (37%) were positive for metastases.
Conclusion: SLN identification permits the accurate pathological study of regional nodes and could reduce the high morbidity of current surgical treatment in vulvar tumour patients if the technique were to be adopted on a routine clinical basis.

Keywords: Vulvar cancer – Lymphoscintigraphy – Blue dye – Gamma probe – Sentinel node – Malignant melanoma

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Introduction

Vulvar cancer is responsible for 3–4% of female malignancies, and is particularly prevalent in older women, with a mean presentation age of about 70 years. Although only accounting for 0.3% of all female cancer deaths, the general increase in life expectancy has raised awareness of carcinoma of the vulva among gynaecological malignancies [1]. About 90% of vulvar cancers are squamous, with melanoma, Paget's disease, adenocarcinoma, basal cell carcinoma and sarcoma being less frequent. The most common localisation of vulvar carcinoma is in the labia

majora, but the labia minora, clitoris or perineum may also be primary sites [2].

The status of the regional lymph nodes is one of the most important prognostic indicators in vulvar cancer patients. The risk of lymphatic metastases in squamous vulvar cancer ranges from 20% to 90% depending on the tumour stage (5–30% in tumours of FIGO stage Ib) [3]. Classically, unilateral or bilateral inguinofemoral lymphadenectomy is performed together with radical vulvectomy. However, while such surgery permits accurate prognostic evaluation, it is accompanied by high morbidity in both the short and the long term (leg oedema, wound breakdown or lymph cysts are possible) [4]. Moreover, this treatment has been shown to be excessive and unnecessary in at least 70% of stage Ib patients. It is currently accepted that these patients need a more conservative treatment. In this context it is important to develop new techniques which can accurately determine the nodal status, thus reducing morbidity [5].

First introduced by Morton et al. in 1992, the concept of the SLN, the node in a lymphatic area that reflects the tumour status of the lymphatic basin, has been validated in patients with melanoma and breast cancer [6–8]. Two techniques have been suggested for identifying the SLN in the vulva, namely vital dye (isosulfan blue) [9, 10] and lymphoscintigraphy followed by gamma probe-guided surgery [11, 12].

Nowadays, SLN biopsy has an established role in malignant melanoma and breast cancer patients and important modifications in staging classifications have been made since its introduction. However, the literature contains a relatively low number of reports and cases on the use of this technique in vulvar malignancies. The hypothesis of this study was that SLN biopsy accurately predicts the tumoural status of the lymphatic drainage basin in vulvar malignancies, so allowing its inclusion on a routine clinical basis. Therefore, the aim of the study was to clarify the feasibility of lymphoscintigraphy, combined with hand-held gamma probe and vital dye, in detecting the SLN in vulvar cancer patients and to evaluate its reliability in predicting the regional lymph node status.

Materials and methods

Patients

From June 1998 to July 2005 we prospectively studied 70 women (mean age 71 years, range 41–95) with vulvar tumours, previously diagnosed by gynaecological exploration and biopsy. Sixty-two of these patients presented squamous carcinoma and the remaining eight had a malignant melanoma.

Validation group

The first 50 patients with squamous carcinoma (mean age 75 years, range 41–95) formed the validation group. This meant that the SLN technique was used to assess the reliability of the technique in this particular malignancy. The inclusion criterion was the need to perform a lymphadenectomy in patients who had been proposed for a curative surgical procedure.

The number of patients was selected so as to form a robust group for this particular malignancy. Our primary end-points were to have both a visualisation rate in lymphoscintigraphy and a surgical identification rate higher than 90%, with a false negative rate of less than 5%.

Application group

After evaluating the validation group results, the next 20 patients (mean age 68 years, range 40–88), comprising 12 with squamous carcinoma and eight with malignant melanoma, were scheduled initially to undergo only the SLN technique. Then, depending on their SLN pathological result, a lymphadenectomy was or was not performed.

This study was approved by the institutional ethics committee and all patients gave their written consent.

Preoperative lymphoscintigraphy

One day prior to surgery, all patients underwent lymphoscintigraphy following the perilesional and intradermal administration of between two and four doses, each of 37 MBq (with an overall volume of 0.1 ml), of colloidal albumin labelled with ^{99m}Tc (Lymphoscint/Nanocoll, Nycomed Amersham-Sorin, Saluggia, Italy). No anaesthetic agent was placed in the syringes; however, all patients received a topical anaesthetic in the vulvar area in order to avoid a painful injection. All injection procedures were performed by the nuclear medicine physician. Scintigraphic images were obtained in a large field of view gamma camera fitted with a low-energy, high-resolution collimator. Dynamic images of 30 s each, with a matrix size of 128×128, were acquired for a total of 10 min (Fig. 1). Following this, planar images (anterior and lateral) of 5 min, with a matrix size of 256×256, were acquired at 30 min and 2 h after injection. Delayed images were obtained where necessary. A thin rectangular phantom with 37 MBq of ^{99m}Tc was used as a flood source to visualise the body contour. A lead shield was also used to hide the area of injection, so that the lymphatic drainage was clearer (Fig. 2).

SLNs were defined as each node with a direct lymphatic channel from the tumour site. In cases where the channel was not visualised, the first lymph node that appeared on scintigraphy was assumed to be the SLN. In this context, lateral views may be helpful to differentiate between superficial and deep nodes, especially if multiple nodes are visualised (Fig. 3). Once localised, the location of the SLN was marked on the skin with indelible ink.

Surgery

Fifteen minutes before surgical incision, 1 ml of blue dye was intradermally administered, by the nuclear medicine physician, in the same way as the radiocolloid injection. Isosulfan blue (Lymphazurin, Ben Venue Labs, Bedford, OH, USA) and methylene blue (Lab. Dr. Carreras, Barcelona, Spain) were used. In our institutions the nuclear medicine physician goes to the surgical room and handles the gamma probe, aiding the surgeons in localising the SLN.

A 2- to 3-cm incision was made at the previously marked location in order to reach the SLN. A hand-held gamma probe (Navigator, USSC, Norwalk, CT, USA) guided the surgeon to the SLN. A 10:1 ratio between the SLN and the background tissue activity was

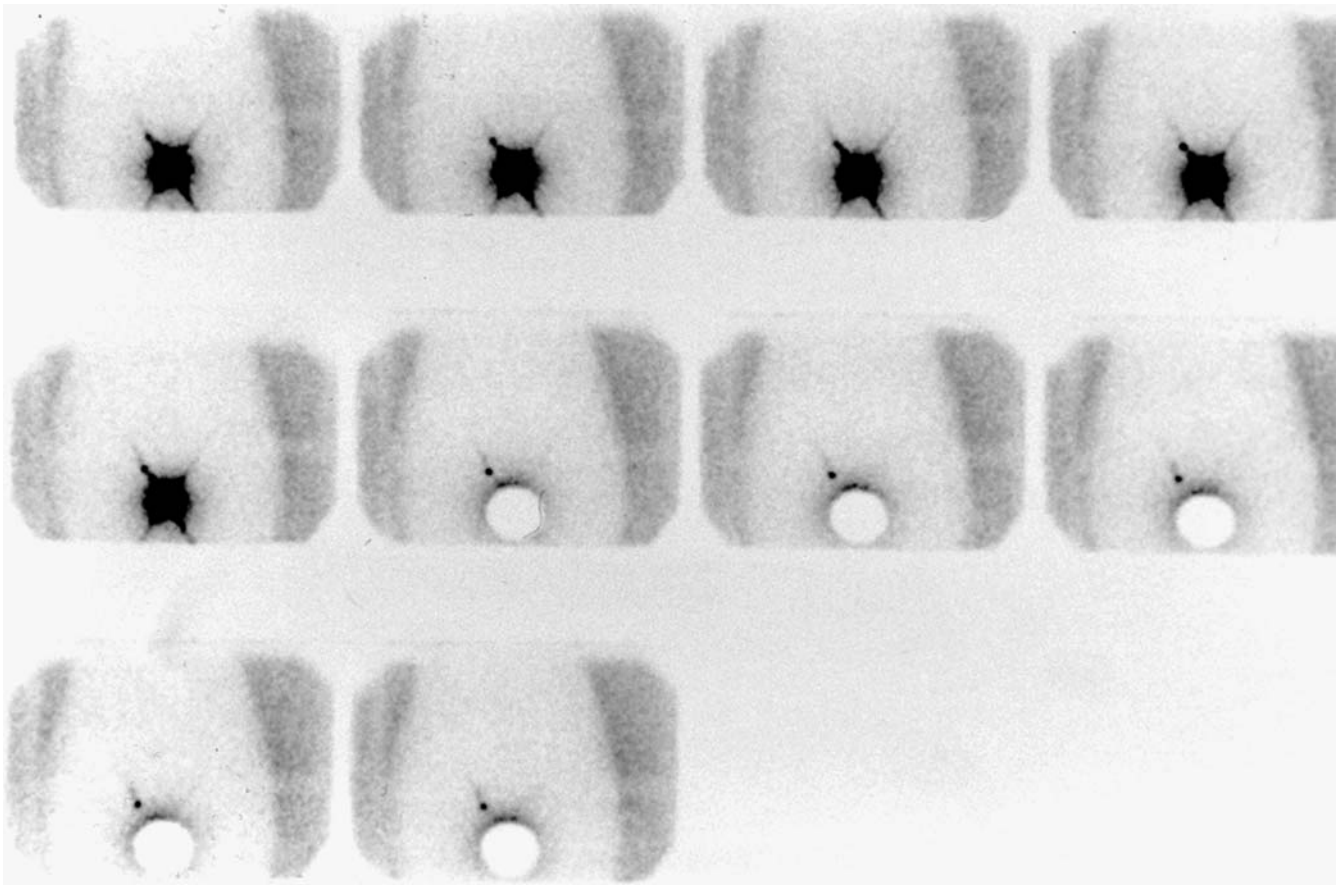

Fig. 1. Dynamic images (1 image/30 s) in the anterior view, showing the regional lymphatic drainage to the right groin. The SLN was localised in the right groin. The body contour was delimited by a rectangular thin phantom

considered significant, as was blue colouration of the SLN or the lymph channel leading to the SLN. After SLN identification, a probe search in the surgical field was done to confirm that there was no other significant activity. The node or nodes harvested were isolated and labelled as SLNs for pathological analysis. Following the SLN procedure a complete inguinofemoral lymphadenectomy was performed in the first 50 patients with squamous cancer, representing our learning curve in this malignancy.

In the application group, the same surgery was performed but lymphadenectomy was only done if the SLN was positive on pathological assessment.

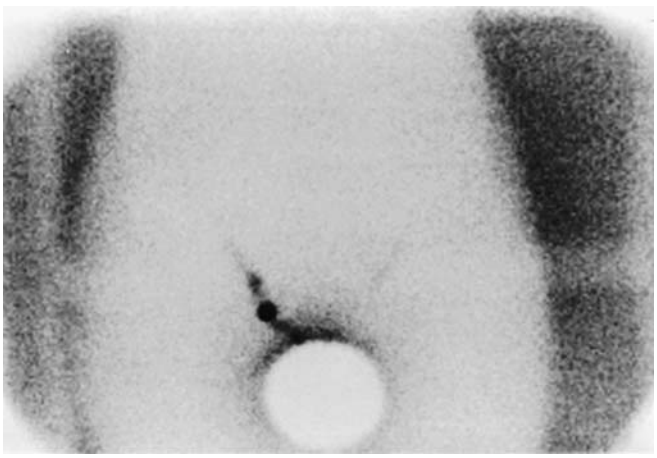

Fig. 2. Static image in the anterior view showing an SLN localised in the right groin. A secondary lymph node is also visible above the SLN. A circular lead shield was used to minimise the lesion activity

Pathological study

During the validation phase, the pathological study of SLN and lymphadenectomy nodes was performed in delayed fashion. Once collected, each SLN was fixed in 10% neutral buffered formalin. Two-millimetre-thick sections following the shortest diameter of the SLN were performed, and the complete SLN was submitted for histological analysis. The tissue was embedded in paraffin wax using standard techniques. Sections with a thickness of 4 μm were stained with haematoxylin and eosin (H&E) and examined under a light microscope. If these first sections were negative, two new sections were performed at an interval of 400 μm. These sections were stained, one with H&E and the other using immunohistochemistry techniques for wide-spectrum cytokeratin (Cytokeratin AE, Dako Co, Carpinteria, CA, USA) for squamous cancer and HMB-45 antibody (Biogenex-Menarini, San Ramon, CA, USA) and S-100 protein (Dako Co, Denmark) for melanoma. The inguinal lymphadenectomy specimens were macroscopically dissected to isolate all the lymph nodes, which were cut and routinely processed and stained with H&E.

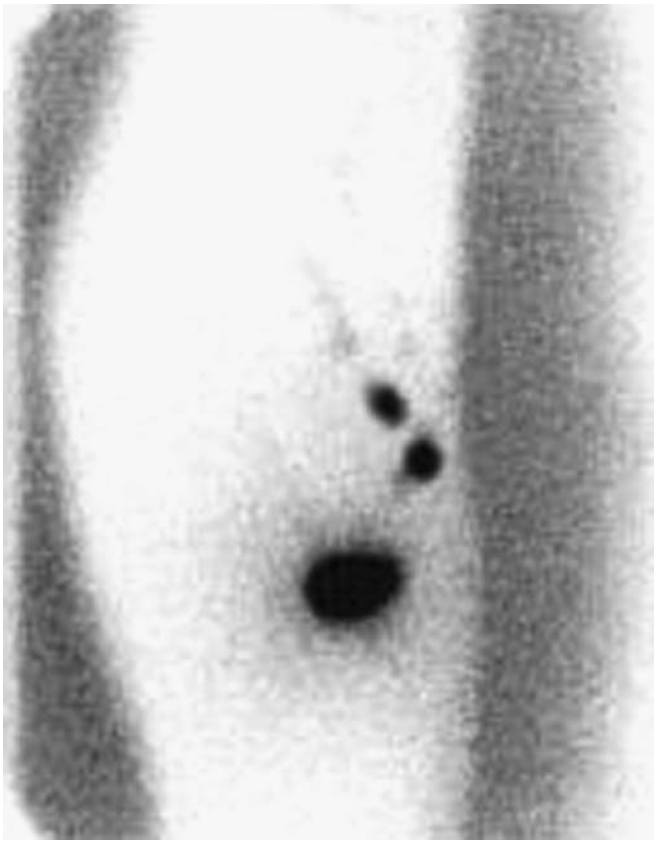

Fig. 3. Static image in the right lateral view showing superficial and deep nodes

In the application group, intraoperative evaluation of SLNs was performed in order to achieve an earlier result and thus avoid the need for delayed lymphadenectomy if this assessment showed metastases. This evaluation was performed by imprint cytology and H&E staining.

Results

Validation group

In this group, 43 out of 50 patients presented lesions that were clinically limited to the vulva (23 stage Ib and 20 stage II) (Table 1). They underwent an inguinal lymphadenectomy with separate incisions followed by radical wide excision or radical modified vulvectomy, depending on the characteristics of the lesion. One patient with Ib stage vulvar cancer also presented a synchronous stage Ib carcinoma in the uterine cervix. In the remaining seven patients the cancer extended beyond the anatomical limits of the vulva (stage III). One patient at stage III, owing to extension to the urethral meatus, had undergone a previous local excision of the tumour.

The SLN was visualised by lymphoscintigraphy in 49/50 patients (98%). In 40 out of 49 cases (82%), dynamic images showed a lymph channel leading to the SLN (Fig. 1). Lymphatic drainage was unilateral in 30 of the 49 patients (61%) and bilateral in the remaining 19 patients who had successful lymphoscintigraphy (39%) (Fig. 4). The SLN was not localised by lymphoscintigraphy in a patient with stage III squamous cancer who had previously undergone a complete local excision and had been injected with radiotracer around the scar.

During surgery the SLN was identified with blue dye in 40 out of 50 patients (80%) and with radiotracer in 49 out of 50 patients (98%). The overall results are listed in Table 1. In all cases the SLN was identified below Camper's fascia and in most cases superior to the inguinal fold. Ninety-four SLNs were harvested in these patients (mean of 1.9 SLNs per patient). In 19 patients (39%) only one SLN was harvested, in 18 patients (37%) two SLNs, in ten patients (20%) three SLNs, in one patient (2%) four SLNs and in one patient (2%) five SLNs (Fig. 4).

Five hundred and twenty-eight non-SLNs were removed in 49 out of the 50 patients in this group (mean 10.7 nodes per patient; range 0–30). Metastases were found in 17 SLNs from 16 patients (33%). Five out of 17 metastatic SLNs presented micrometastases. These patients presented stage III (n=4), stage II (n=8) or stage Ib (n=4) lesions. Ten metastatic nodes were observed among non-SLNs from lymphadenectomy specimens. All of these came from patients with at least one positive SLN. One of these positive non-SLNs, confirmed by histological study, was observed in a patient who presented with macroscopically suspicious lymphadenopathy in the right groin, while the remaining seven nodes extracted from this groin were negative. Although lymphoscintigraphy showed bilateral drainage, the right SLN was not sought, owing to the palpable adenopathy. In the left groin of this patient, a positive SLN and four other negative nodes were found. Importantly, in all patients with a negative SLN, the remaining lymph nodes were also negative (negative predictive value 100%).

Patients were followed up over a mean period of 24 months. Only one patient, with synchronous vulvar and cervical cancer, presented a pelvic recurrence of cervical cancer in the 13th month and died in the 20th month. Four patients developed a local recurrence in the scar area (successfully resolved by performing a local resection) and are alive without recurrence.

Application group

In this group, 12 out of 20 patients presented lesions clinically limited to the vulva (seven stage Ib and five stage II). The eight remaining patients all presented malignant melanoma, with a Breslow thickness ranging from 1.2 to 7 mm. None of these patients had any palpable lymph nodes under clinical exploration. In the patients who had melanoma of the vulva, SLN localisation was part of their attendance protocol as the SLN procedure had been validated in our institutions for melanoma and breast cancer.

The SLN was visualised by lymphoscintigraphy in 19 out of 20 patients (95%). In 16 of these 19 cases, dynamic images showed a lymph channel towards the SLN (84%).

Table 1. Clinical, scintigraphic, surgical and pathological data of the patients included in the validation group

Case	Age (yrs)	Lesion location	Stage	Lymphatic drainage	Blue dye staining	Radiotracer uptake	SLN pathological study
1	72	Right labium majus	Ib	Right groin	Yes	Yes	1 (−) R
2	77	Clitoris	II	Bilateral	Yes	Yes	1 (−) L
3	91	Right labium majus	II	Right groin	Yes	Yes	1 (+) R
4	58	Right labium minus	Ib	Right groin	Yes	Yes	1 (−) R
5	80	Left labium majus	III	Left groin	Yes	Yes	1 (+) L
6	41	Left labium majus	Ib	Left groin	Yes	Yes	3 (−) L
7	70	Left labium majus	Ib	Bilateral	Yes	Yes	1 (−) R and 2 (−) L
8	89	Right labium majus	Ib	Right groin	No	Yes	1 (−) R
9	67	Periurethral	III	Not found	No	No	Not found
10	71	Right labium majus	Ib	Right groin	No	Yes	2 (−) R
11	82	Left labium majus	II	Bilateral	No	Yes	1 (−) R and 2 (−) L
12	86	Left labium majus	III	Left groin	Yes	Yes	1 (−) L
13	81	Right labium majus	II	Right groin	Yes	Yes	1 (−) R
14	86	Left labium majus	III	Left groin	Yes	Yes	2 (−) L
15	74	Left labium and perineum	Ib	Left groin	Yes	Yes	2 (−) R and 1 (−) L
16	77	Right labium minus	II	Bilateral	Yes (right)	Yes	2 (−) R and 1 (−) L
17	70	Left labium majus	Ib	Left groin	Yes	Yes	1 (−) L
18	49	Clitoris	II	Bilateral	Yes	Yes	1 (−) R and 1 (+) L
19	74	Left labium minus	Ib	Left groin	Yes	Yes	1 (−) L
20	69	Right labium majus/clitoris	III	Bilateral	Yes	Yes	1 (+) R and 1 (−) L
21	95	Right labium majus	II	Right groin	Yes	Yes	2 (−) R
22	65	Left labium majus	Ib	Left groin	Yes	Yes	2 (−) L
23	86	Right labium majus	II	Right groin	Yes	Yes	2 (−) R
24	69	Right labium majus	Ib	Right groin	No	Yes	1 (−) R
25	75	Clitoris/periclitoris	III	Bilateral	No	Yes	2 (−) R and 1 (+) L
26	74	Left labium majus	III	Left groin	Yes	Yes	2 (+) L
27	79	Both labia minora	II	Bilateral	Yes	Yes	1 (+) R and 2 (−) L
28	80	Clitoris/periclitoris	II	Bilateral	Yes (right)	Yes	1 (−) R and 1 (−) L
29	50	Vulva and anus	Ib	Left groin	No	Yes	1 (−) L
30	92	Clitoris	II	Left groin	No	Yes	1 (+) and 1 (−) L
31	69	Left labium minus/clitoris	Ib	Left groin	No	Yes	1 (+) L
32	87	Clitoris/periclitoris	Ib	Bilateral	Yes	Yes	2 (−) R and 3 (−) L
33	76	Both right labia	Ib	Bilateral	Yes	Yes	3 (−) R and 1 (−) L
34	81	Right labium majus	II	Right groin	Yes	Yes	1 (+) and 1 (−) R
35	75	Right labium minus	II	Bilateral	Yes	Yes	1 (+) L
36	88	Left labium minus/clitoris	II	Bilateral	Yes	Yes	1 (−) R and 1 (−) L
37	75	Right labium majus	II	Right groin	Yes	Yes	1 (+) and 1 (−) R
38	77	Left labium minus	Ib	Left groin	Yes	Yes	1 (+) and 1 (−) L
39	76	Right labium minus	II	Bilateral	Yes	Yes	1 (+) R
40	70	Right labium majus	Ib	Right groin	Yes	Yes	1 (−) R
41	82	Right labium majus	II	Right groin	Yes	Yes	1 (+) R
42	83	Right labium minus	Ib	Bilateral	Yes	Yes	1 (−) R and 1 (−) L
43	75	Right labium minus	Ib	Bilateral	No	Yes	2 (−) R and 1 (−) L
44	69	Left labium minus	II	Bilateral	Yes	Yes	1 (−) R and 2 (−) L
45	76	Left labium minus	Ib	Left groin	Yes	Yes	1 (+) L
46	71	Left labium minus	Ib	Left groin	Yes	Yes	2 (−) L
47	82	Right labium minus	Ib	Right groin	Yes	Yes	3 (−) R
48	76	Right labium majus	II	Right groin	Yes	Yes	2 (−) R
49	66	Right labium minus	Ib	Bilateral	Yes	Yes	1 (−) R
50	79	Clitoris	II	Bilateral	Yes	Yes	1(−) R and 1 (−) L

R right groin, *L* left groin, *(−)* negative for metastasis, *(+)* positive for metastasis

Lymphatic drainage was unilateral in 11 out of 19 patients (58%) and bilateral in the remaining eight patients with successful lymphoscintigraphy (42%). The SLN was not localised by lymphoscintigraphy in one patient with superficial spreading malignant melanoma with a Breslow thickness of 3.9 mm.

During surgery the SLN was identified with blue dye in 17 out of 20 patients (85%) and with radiotracer in 19 out

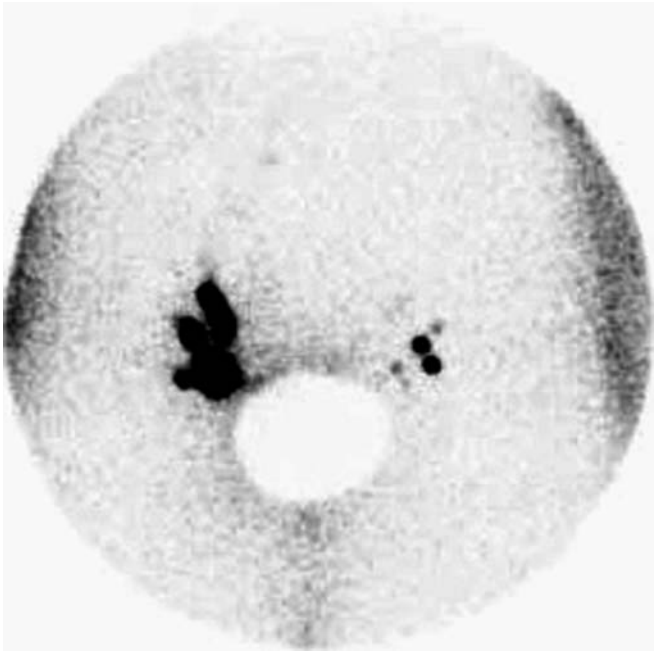

Fig. 4. Anterior view static image showing very high uptake in the right groin and two SLNs in the left groin. Although there may have been SLN overestimation in the right groin, during surgery three SLNs were excised (corresponding to the areas of highest uptake). Overall, five SLNs were excised in this patient

of 20 patients (95%). The overall results are listed in Table 2.

As in the validation group, in all cases the SLN was identified below Camper's fascia and superior to the inguinal fold. Thirty-eight SLNs were harvested in these patients (mean of 2 SLNs per patient). In six patients (32%) only one SLN was harvested, in nine patients (47%) two SLNs, in three patients (16%) three SLNs and in one patient (5%) five SLNs. The patient with negative visualisation of SLN on lymphoscintigraphy did not show radiotracer or blue dye uptake during surgery and bilateral lymphadenectomy was performed. Three metastatic nodes were found (one in the right groin and two in the left) among the ten nodes harvested.

Seven patients presented metastases in their SLNs (four of the squamous cancer patients and three of the malignant melanoma patients), i.e. 37% of the group. It is worth noting that five of these patients had micrometastases, one had macrometastases and one presented isolated tumour cells. Lymphadenectomy was performed in all but one of these patients because the Gynaecological Oncology Committee decided to use radiation therapy to treat the patient with isolated tumour cells. In the six patients who had a lymphadenectomy, 84 non-SLNs were harvested, only one of which showed metastatic involvement.

One of the malignant melanoma patients (Breslow thickness of 5 mm) who presented with metastatic involvement of the SLN showed metastatic dissemination in the 12th month following diagnosis and died in the 14th month. None of the SLN-negative patients showed recurrence during the follow-up period.

Discussion

Lymphatic mapping of vulvar cancer by using radiotracers and blue dye opens up a new approach in the management of these patients. The SLN concept, validated in melanoma and breast cancer, affords the possibility of avoiding unnecessary lymphadenectomies. In vulvar cancer, pelvic or deep groin node involvement is rare if superficial groin nodes are normal. Iversen and Aas found that when the inguinal nodes are free of metastases, the pelvic nodes are never involved and thus, pelvic lymphadenectomy is unnecessary [13].

The SLN technique provides an opportunity to clearly identify the nodes most likely to harbour metastases. In our series we always found the SLN above the inguinal fold and in some cases level with the inguinal ligament or at the entrance to the inguinal duct. The first publication relating to SLN identification in vulvar cancer related to research carried out by Levenback et al. in 1994 [9]. These investigators identified SLNs intraoperatively in a series of nine patients using blue dye. Later, this group published its experience with blue dye (21 and 52 patients) [14, 15], reporting SLN identification success rates of 86% and 88%. The introduction of radiotracers allowed improved localisation of the SLN (as well as better reliability), and the localisation rate rose to 98% in our study. The use of radiotracers enhances the localisation of the SLN and gives better results than when the blue dye technique alone is used. Therefore, a combination of the two methods is the best approach.

As in malignant melanoma, the SLN was identified soon after injection of the radiotracer. The report of De Cicco et al. [16] showed that in 90% of their patients the SLN was visualised within less than 15 min. In our series this was true in 83% of the cases. In our study the radioactive technique proved superior to the blue dye technique, with a higher rate of SLN identification (98% vs 80%). This higher identification rate has been confirmed by other authors such as De Hullu et al. [2, 17].

Metastatic involvement of SLN varies from 17% to 32% in different series. In the present study, metastases (either macro- or micrometastases) were found in 23 out of 68 (34%) patients with successful SLN identification (considering the overall group of patients). This high rate could be due to the inclusion of seven stage III patients in the validation group, as five of them presented at least one metastatic SLN. However, in the remaining patients no false negative SLNs were observed.

The literature available on squamous cell vulvar cancer is limited. The total number of cases reported until now is less than 500. Using lymphatic mapping with blue dye and/or the radiotracer technique seems to confirm that the SLN hypothesis is just as valid in vulvar cancer as in other types of tumour, with an equally high negative predictive value [18, 19]. The great majority of these studies were performed with consecutive lymphadenectomy; our study differs in that, after a suitable validation phase, the SLN biopsy allowed avoidance of lymphadenectomy providing the SLN was negative for metastasis. To our knowledge,

Table 2. Clinical, scintigraphic, surgical and pathological data of the patients included in the application group

Case	Age (yrs)	Lesion location/type	Stage	Lymphatic drainage	Blue dye staining	Radiotracer uptake	SLN pathological study	Lymphadenectomy
1	46	Left labium majus/SSM	1.5mm	Left groin	Yes	Yes	2 (−) L	Not performed
2	74	Periclitoris/SSM	5 mm	Bilateral	Yes	Yes	1 (−) R/1 (+) L	Bilateral/ 8 (−) R and 5 (−) L
3	49	Right labium minus/SSM	1.2mm	Right groin	Yes	Yes	1 (+) R	Right groin/29 (−)
4	63	Left labium majus/SSM	4.5mm	Left groin	Yes	Yes	1 (−)/1 (+) L	Left groin/12 (−)
5	54	Left labium minus/SSM	1.2mm	Left groin	Yes	Yes	3 (−) L	Not performed
6	49	Left labium minus/SSM	1.3mm	Bilateral	Yes	Yes	1 (−) R/ 2 (−) L	Not performed
7	79	Left labium minus/SSM	3.9mm	Not found	No	No	SLN not found	Bilateral/1 (+) and 3 (−) R and 2 (+) and 2 (−) L
8	46	Left labium majus/NMM	7 mm	Bilateral	Yes (right)	Yes	1 (+)/2 (−) R and 2 (−) L	Right groin/7 (−)
9	70	Right labium majus	Ib	Right groin	Yes	Yes	1 (−) R	Not performed
10	88	Left labium minus	II	Bilateral	Yes	Yes	1 (−) R and 1 (−) L	Not performed
11	77	Left labium minus	Ib	Bilateral	Yes	Yes	2 (−) L	Not performed
12	76	Right labium minus	Ib	Right groin	Yes	Yes	1 (−) R	Not performed
13	82	Right labium majus	Ib	Right groin	Yes	Yes	2 (−) R	Not performed
14	86	Right labium majus	II	Right groin	Yes	Yes	1 (−) R	Not performed
15	73	Left labium minus	Ib	Left groin	Yes	Yes	1 (+) and 1 (−) L	Not performed[a]
16	80	Right labium minus	II	Bilateral	No	Yes	2 (−) R and 1 (−) L	Not performed
17	78	Left labium majus	Ib	Left groin	No	Yes	1 (−) L	Not performed
18	61	Clitoris	II	Bilateral	Yes	Yes	1 (−) R and 1 (+) L	Bilateral/6 (−) R and 8 (−) L
19	75	Right labium majus	Ib	Right groin	Yes	Yes	1 (−) R	Not performed
20	58	Right labium majus/clitoris	II	Bilateral	Yes	Yes	1 (+) R and 1 (−) L	Right groin/1 (+) and 8 (−)

R right groin, *L* left groin, *(−)* negative for metastasis, *(+)* positive for metastasis, *SSM* superficial spreading melanoma, *NMM* nodular malignant melanoma

[a] Only radiation therapy was added

the present series of cases is also the largest number considered in a single study. In most protocols a false negative rate of less than 5% is considered acceptable. In order to get to 5% one has to evaluate 20 SLN-positive patients with a maximum of one false negative biopsy. So, the main limitation of this study is the low number of cases included in the validation group to reach these figures. Nevertheless, we consider that the scarcity of this malignancy and our knowledge of SLN biopsy based on its application in melanoma and breast cancer imply that smaller numbers should be acceptable. In the absence of SLN metastases, the possibility of finding involvement in the remaining nodes is low, given the negative predictive value achieved in our series.

Malignant melanoma of the vulva is less frequent than squamous carcinoma and, until recently, very few investigators have reported successful localisation of the SLN in such patients [20, 21]. We do not totally agree with De Hullu and co-workers that application of the SLN technique should be restricted to patients with intermediate thickness melanoma (1–4 mm) [22]. Although there is a high probability of more metastatic spread to the nodes in these patients, we think that early recognition of metastatic cells can avoid the growth of bulky nodal masses. However, further studies will be needed to assess this issue.

Finally, this study of the use of the SLN technique in patients with vulvar malignancies is the first to include a patient group without consecutive lymphadenectomy. This is probably still a controversial issue, but the previous validation by our team and the results of many others are encouraging. These patients can be spared the current high rate of morbidity by reducing the radicality of surgery, as suggested in some very recent publications. The contribution of the new results should be assessed in the light of the existing data [23, 24].

In summary, SLN identification permits the accurate pathological study of regional nodes, and its application on a routine clinical basis will reduce the high morbidity of current surgical treatment in patients with vulvar tumours. We believe that this technique is best applied in patients with squamous cancer of FIGO stages Ib and II with lesions of less than 3 cm in diameter and in patients with vulvar melanoma with a Breslow thickness exceeding 1 mm. The technique should not be offered, however, to patients with clinically palpable nodes.

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