Value of sentinel lymph node study in malignant melanoma

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The sentinel lymph node (SLN) is the first station of the lymph drainage of a primitive lesion and is therefore the most likely to harbor metastasis. Isotopic tracers or dyes can be used to detect the SLN.
SLN biopsy reduces morbidity and the cost of unnecessary lymphadenectomies in patients with clinically non-metastasized melanoma and improves staging of SLN located outside the usual drainage area.
We studied 115 patients with the diagnoses of cutaneous malignant melanoma with a Breslow of 0.75 mm. To map the lymphatic drainage areas, all patients underwent lymphoscintigraphy prior to surgery with intradermal injection of Technetium 99m sulfur colloid around the biopsy scar. Twenty minutes before surgery, 1 cc of isosulfan blue (1% lymphazurin) was also injected. Dye was used in the intraoperative search for SLN in nine patients and both dye and a gamma probe (Navigator; USSC, Norwalk, CT) were used in 106.
The SLN was identified in 113 patients (98%). The SLN was infiltrated in 14 patients (12.4%) and the SLN was the only node involved in 14 of them (78.6%).
Selective lymphadenectomy in melanoma is associated with a low rate of morbidity and reduces the rate of unnecessary complete lymphadenectomies in patients with intermediate-risk cutaneous melanoma while allowing for appropriate staging according to current recommendations.

Key words: Sentinel lymph node, malignant melanoma, selective lymphadenectomy, lymphoscintigraphy, isosulfan blue.

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Valor del estudio del ganglio centinela en el melanoma maligno

El ganglio centinela (GC) es la primera estación de drenaje linfático de una lesión primitiva y, por tanto, la que presenta más probabilidad de metástasis. Para detectarlo se pueden utilizar trazadores isotópicos o colorantes.
La biopsia del GC permite ahorrar la morbilidad y coste de linfadenectomías innecesarias en pacientes con melanoma clínicamente no diseminado y mejorar el estadiaje por la localización del GC fuera del área de drenaje habitual.
Se han estudiado 115 pacientes con diagnóstico de melanoma maligno cutáneo con Breslow 0,75 mm. Para localizar las áreas de drenaje linfático a todos los pacientes se les realizó una linfogammagrafía mediante la inyección intradérmica pericicatricial de sulfuro coloidal-^{99m}Tc antes de la intervención. Veinte minutos antes de ésta se inyectó igualmente 1 cc de azul de isosulfán (lymphazurin 1%). La búsqueda intraoperatoria del GC se realizó en 9 pacientes con el colorante y en 106 con la técnica combinada del colorante y una sonda detectora de radiaciones gamma (Navigator; USSC, Norwalk, CT).
El GC se identificó en 113 pacientes (98%), estaba infiltrado en 14 de ellos (12,4%) y fue el único ganglio afecto en 11 (78,6%).
La linfadenectomía selectiva en el melanoma es una técnica de escasa morbilidad que evita linfadenectomías completas innecesarias en pacientes con melanoma cutáneo de riesgo intermedio y permite realizar un adecuado estadiaje, según las más recientes recomendaciones.

Palabras clave: ganglio centinela, melanoma maligno, linfadenectomía selectiva, linfogammagrafía, azul de isosulfán.

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INTRODUCTION

The sentinel lymph node (SLN) originally described by Cabañas in 1977 in penile carcinoma[1] is defined as the first station of lymph drainage of a primitive lesion and the one, therefore, that is most likely to har-

bor metastasis. The definition is based on the hypothesis that lymphatic drainage of a malignant lesion is an orderly process by which a tumor will metastasize first to the SLN[2-5], which can be identified by lymphoscintigraphic imaging techniques.

Morton demonstrated that lymphatic drainage of a primary cutaneous malignant melanoma (MM) could be predicted intraoperatively[6,7] by means of the perilesional injection of a vital dye with further visualization and removal of the stained lymph nodes, a technique that is less invasive than complete conventional lymphadenectomy (CLD). The SLN technique is considered to indicate the presence of regional lymph node involvement[8,9], such that CLD can then be reserved for cases with histologically confirmed involvement and avoided in other cases. Several procedures have been used to identify the SLN. In 1993, Krag[10,11] used a gamma probe after intratumoral injection of Technetium 99m (^{99m}Tc)sulfur colloid in breast cancer. In 1994, Giuliano[12] used vital blue dye intraoperatively and Albertini[9,13] combined both methods.

SLN removal is a minimally invasive technique that may provide an alternative to CLD. In this paper we report results from the treatment of patients with cutaneous MM at the Valencia Oncology Institute.

The objective was to demonstrate that SLN biopsy allows better staging and avoids unnecessary lymphadenectomies in patients with non-metastasized MM.

MATERIALS AND METHODS

From December 1997 to January 2001, 115 patients with MM were studied at Valencia Oncology Institute. Patients were classified according to the Breslow thickness scale: 97 with intermediate-risk MM (thickness 0.76-4 mm); 13 with high-risk MM (Breslow thickness > 4 mm); 5 with a Breslow thickness from 0.5 to 0.75 mm but with other histologic signs considered to indicate poor prognosis, such as ulceration, signs of regression or vascular embolization (table 1). All patients were diagnosed by excisional biopsy without margins and had no palpable lymph nodes. All patients underwent a preoperative study (hemogram, hepatic and renal profiles, S-100 beta-globulin, chest X-ray, abdominal ultrasound and/or abdominal and pelvic CAT), with negative results. The median age of patients was 50 years (range: 13-85 y). The locations of lesions are shown in (table 1).

To map lymphatic drainage channels, all patients first underwent lymphoscintigraphy by means of the intradermal injection at the four quadrants of the biopsy scar; 0.5 of Tc-99m sulfur colloid in a total volume of 0.5 mL was injected 1 to 3 hours before surgery. Dynamic study consisted of sequential images taken every 15-second during the first 10-15 minutes after administration of the tracer. Then, static images were obtained until SLN visualization (interval 1-3 hours) using a low-energy, general purpose resolution collimator and storing images in a 156 × 256 matrix. The focus of highest intensity uptake detected by the gamma camera, corresponding to the location of the SLN, was marked on the skin. Surgery began at the end of the study.

TABLE 1. **Tumor thickness by location**

Location	Breslow				Total
	0.5-0.75 mm	0.76-2 mm	2-4 mm	> 4 mm	
Lower limb	0	26	13	6	45
Trunk	6	29	6	2	43
Upper limb	0	12	4	2	18
Head and neck	0	5	0	3	8
Vulva	0	1	0		1
Total	6	73	23	13	115

Twenty minutes before surgery, 1 ml of isosulfan blue (1% lymphazurin) was intradermally injected at 3 or 4 points around the biopsy scar.

The intraoperative search for the SLN was guided by the dye in the first 9 patients by the dye and a handheld gamma probe (Navigator; USSC Norwalk, Connecticut, USA) in the next 106 patients. Lymph nodes were identified as SLNs and removed when stained blue and/or showing signs of isotopic activity.

Margins were widened according to Breslow thickness, as follows: 1 cm for MM with a Breslow thickness of 0.75-1 mm and 2 cm for MM with a Breslow thickness > 1 mm. In all cases the dyed area was included.

CLD was performed in the first seven patients, by way of control, and in all patients with non localized SLN or when there was histologically confirmed involvement.

SLNs were isolated and sent for histology fixed in 10% neutral formalin, sectioned in two halves and embedded in paraffin. Four serial sections 4 m thick were cut and stained with hematoxylin and eosin (H&E), without levels.

Fifteen samples were randomly selected from among SLN that were H&E negative patients of the SLN were randomly selected. Each paraffin block was emptied completely by cutting 4 µserial sections. A level of 14 sections was arbitrarily established. The first 10 sections were stained with H&E, the next sections were submitted for immunohistochemical study, and the last two blank sections were reserved for further potential studies.

The following immunohistochemical markers were used: S-100, HMB-45, NkC3, and MART1.

RESULTS

Lymphoscintigraphy located the SLN in 113 out of the 115 patients (98%), with uptake in only one lymph

node station in 95 patients (85%) and in two stations in 17 (15%). The primary lesion was located in the trunk in 13 of the 17 patients (10 cases with bilateral axillary uptake and three cases with bilateral inguinal uptake). Uptake was in the lower limbs in 3 patients (inguinal and popliteal uptake in 2 and inguinal and ilial in 1). In one patient with facial MM, there was simultaneous uptake in the supraclavicular fossa and parotidean cell.

The SLN stained blue in 92 of the 115 patients in whom it was used (dye detection rate 80%). The SLN was located in 8 of the first 9 patients in whom only the dye was used (89%). The SLN was located in 103 of the 106 patients in whom the gamma probe was used (detection rate with the isotope technique 97%). In these 106 patients, the dye was also used along with the gamma probe and the combined technique located the SLN in 105 (detection rate with the combined technique 99%). In summary, the SLN was located in 113 out of the 115 patients (98%) (table 2) and a total number of 216 SLNs were removed (mean 1.9 per patient). Histology found 115 SLNs (7.5%) to be infiltrated in 14 patients. CLD was performed in 13 patients and was not performed in 1 patient in whom the SLN was located at the popliteal cavity. In 10 out of the 13 patients who underwent CLD (76.9%) the SLN was the only lymph node affected. Of the 3 remaining patients, 1 had infiltration in one non-sentinel lymph node (nSLN) and the other two patients had two nSLN infiltrated. Thus, metastasis was found in 5 of the 254 lymph nodes removed (2%). Involvement of the SLN was micrometastatic in 3 of the patients. All patients with nSLN metastasis had Breslow thicknesses exceeding 2 mm (table 3).

The SLN was histologically negative in 99 patients. CLD was performed in the first 7 of these patients, who served as controls. No infiltration was found in any of the nSLN cases. CLD was not performed in the 92 patients who underwent procedures later, if their SLNs were histologically negative.

The SLN was not located in 2 patients. A complete CLD was performed in 1 of them and all lymph nodes were histologically negative. The second patient did not undergo CLD because the lymph drainage channel was undefined (table 4).

In the immunohistochemical study performed in 15 randomly selected patients, micrometastatic involvement was found in 3 SLNs from 2 patients. Among the immunohistochemical markers used (S-100, HMB-45, NkC3, and MART1), only the melanosome marker (MART1) was expressed in the 3 SLNs. MART1 did not stain normal lymph node structures but was expressed strongly in all identified melanocytic lesions, whether benign or malignant.

CLD was not performed in these two patients as more than 12 months had elapsed between the initial procedure and the immunohistochemical study of the SLNs.

TABLE 2. **Detection rate of the sentinel lymph node by technique**

Technique	Patients (n.°)	Located	Detection rate (%)
Dye	115	92	80
Isotopic	106	103	97
Combined	106	105	99
Total	115	113	98

TABLE 3. **Patients with SLN+ (the two patients diagnosed by immunohistochemistry are not included)**

Patient	Breslow	SLN*	Non SLN**
1	1.2	1/3	0/13
2	4.2	1/1	2/11
3	2.13	1/1	1/17
4	1.5	1/2	0/5
5	2.6	1 mic/1	0/31
6	2	1/1	0/24
7	1.2	1/5	0/15
8	2.2	1/2	0/15
9	2.2	1/2	0/19
10	4.5	1 mic/2	0/12
11	3	1 mic/1	2 mic/11
12	1.7	2 mic/2	0/15
13	8	1/4	NP
14	4.2	1/3	0/18

SLN: sentinel lymph node (* No. involved SLNs/total number of removed SLNs); Non SLN: non sentinel lymph node (** No of non-SLNs involved/total number of removed SLNs); mic: micrometastases; NP: not performed. Patient with sentinel lymph node in the popliteal cavity.

DISCUSSION

Several randomized trials have demonstrated the inefficacy of routine regional prophylactic CLD to treat the early stages of MM. Lymph node involvement in these cases occurs in only about 20% of patients, meaning that 80% of patients would be subjected to an unnecessary surgery[14-17].

Moreover, several studies[5,18] support the hypothesis that lymphatic drainage from the site of a primary tumor progresses in an orderly way through lymphatic

TABLE 4. **Overall results**

	N.°	CLD	No CLD	Non SLN+
SLN?	2	1	1	0
SLN+	14	13	1	3
SLN–	99	7	92	0
Total	115	21	94	3

SLN: sentinel lymph node; Non SLN: non sentinel lymph node; SLN?: non identified sentinel lymph node; CLD: conventional lympha-denectomy.

vessels afferent to the SLN before disseminating to the other lymph nodes. This hypothesis has led to the development of less invasive techniques to identify patients that would benefit from regional CLD even though no palpable lymph nodes were present. One such technique is selective lymphadenectomy of the SLN.

Selective SLN biopsy is minimally invasive and allows the SLN to be located and removed for exhaustive study that yields the needed information without the drawbacks of CLD[13]. In the present study, lymphoscintigraphy had a 97% detection rate, similar to that found in other studies[19].

Preoperative lymphoscintigraphy allows lymphatic drainage channels to be identified up to the area of likely metastasization. Given that more than one drainage channel is present in a substantial percentage of cases (15% in our series)[20,21], preoperative lymphoscintigraphy is indispensable for characterizing lymph drainage patterns in patients after diagnosis of MM, particularly when the tumor is located in the trunk, head or neck[20,21].

Because a gamma radiation probe was initially unavailable in our study, we used the dye alone for the intraoperative detection, locating the SLN in 8 out of the 9 patients (89%). Later, when the technique became available and both techniques were used (isotopic tracer and dye), we were able to identify the SLN in 105 out of 106 patients (99%). The SLN was not located in one case because of radioactive interference from the tracer administered around the tumor near the axillary drainage area. If all patients are considered, the SLN was localized in 98% of cases, a percentage very similar to those reported by other authors[22-24].

Although we may have obtained the same results with the gamma camera alone, we preferred to use the combined technique, as most authors do so[25,26]. Using the combined technique adds almost no morbidity and often helps us to locate the blue-stained lymph node, particularly when the drainage area is very close to the primary lesion, when activity from the point of injection of the isotopic tracer interferes with the probe.

Regional lymph node recurrence of MM after removal of histologically negative SLNs varies from study to study, 0.8 to 4.1%[27-30] after a 2-year follow-up period. In our series, with a median follow-up of 12 months, recurrence of lymph node disease was observed in one patient, 7 months after SLN removal. The primary lesion was located in the lower limb and had a Breslow thickness of 4.22 mm. A retrospective immunohistochemical study was performed with negative result, either because it was a false negative or because new metastasis was involved. According to Gershenwald et al[30], SLNs might be incorrectly located during surgery, either due to technical error, or because of changes in the lymphatic drainage channels of the primary tumor due to infection and/or inflammation in the area of the previous biopsy. Alternatively, regional metastasis might have arisen from an occult, clinically undetectable focus of disease, although this possibility seems less likely. Patients with regional lymph node involvement have a poorer prognosis. In a study by Castinelli et al[14], the removal of micrometastasized lymph nodes improved survival in the group of patients with clinically negative lymph nodes. Survival at 5 years in the group of patients with micrometastases undergoing prophylactic CLD was significantly better than that of patients in whom CLD was performed on account of clinically detectable lymph nodes (48.2% versus 26.6%).

While SLN was the only lymph node affected in 78.6% of our cases, it is important to remember that other lymph nodes were not examined with the same technique, as serial sections were performed in SLNs whereas nSLNs were studied following the standard technique (bisection). Early in this study, immunohistochemical techniques were not available for use in all of our patients, although some authors[25,29,31] have demonstrated that the number of positive lymph nodes detected is higher when immunohistochemical analysis is used. Nevertheless, the proportion of SLNs that require immunohistochemistry for the identification of hidden metastasis decreases with the experience of the pathologist[32].

In our retrospective study of a control group of 15 randomly selected patients with H&E negative SLNs, immunohistochemistry detected micrometastasis in 2 (13%). Our view is that immunohistochemistry is essential for the accurate staging of patients with H&E negative SLNs. A noteworthy finding was that all patients with metastatic involvement of the SLN and nSLNs had a Breslow thickness higher than 2 mm, as found by other authors[19,33].

Thirteen of our patients had Breslow thicknesses >4 mm and negative preoperative studies. Although metastasis in these cases most likely occurs through the blood stream, it seems that the study of the SLN may be a relevant prognostic factor even in such cases[34].

In conclusion, selective lymphadenectomy of the SLN is associated with low morbidity and a high detection rate, particularly with a combined mapping technique is used. Patients when intermediate-risk melanomas can be appropriately staging according to the most recent recommendations of the American Joint Cancer Committee[35], while being spared unnecessary CLDs. Preoperative lymphoscintigraphy is essential to identify drainage channels so that the surgical approach can be planned. For a correct study of the SLN, serial sections at several levels must be made and immunohistochemistry analysis must take place.

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