In-transit sentinel lymph nodes in malignant melanoma.  
What is their importance?

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Summary.—The sentinel lymph node (SLN) is the first node in a lymph node basin to receive direct drainage from the primary tumour. However, in some cases, lymphoscintigraphy images demonstrate the presence of lymph nodes located in the area between the primary tumour and the first regional lymph node basin. These nodes are called in-transit nodes and have to be considered SLNs as well.

Aim. It was to determine the incidence and location of in-transit SLNs in patients with malignant melanoma and to evaluate whether it is necessary to harvest them.

Method. Nine hundred patients with malignant melanoma were included. Lymphoscintigraphy was performed on the day before surgery following intradermal injection of 74-111 MBq of 99mTc-Nanocolloid in four doses around the primary lesion or the biopsy scar.

Results. The presence of in-transit SLNs was revealed in 80 patients. During surgery, in-transit SLNs were identified and excised in all but 3 patients (96.2 %). Metastatic cell deposits were identified in these in-transit SLNs in 15 patients (19.4 %), with 4 patients with no tumour involvement of the regional lymph node basin.

Conclusions. Lymphoscintigraphy is mandatory in the location of in-transit SLNs. Moreover, although the incidence of these nodes is relatively low in malignant melanoma, the presence of metastatic cells in these in-transit SLNs reaches a significant percentage. Therefore, excision of in-transit SLNs is necessary in all cases.

KEY WORDS: in-transit sentinel lymph node, lymphoscintigraphy, malignant melanoma.

INTRODUCTION

The technique for locating sentinel lymph nodes (SLN), using the combination of preoperative lymphoscintigraphy and intraoperative detection with a detection probe, continues to be widely used in a considerable number of patients1. Since the description of the procedure by Cabanas2 and its introduction in malignant melanoma patients by Morton3, this technique has been rapidly implemented in daily clinical use.

In the case of malignant melanoma in the initial stages, the use of SLN biopsy has become an essential requisite in clinical practice and routine surgery in order to obtain accurate stratification and to select
patients for an elective lymphadenectomy, avoiding the side effects of more invasive surgery, as well as unnecessary lymphadenectomies in a high percentage of cases\textsuperscript{4,5}.

In most patients, metastatic spread of malignant melanoma follows an orderly progression through the lymph channels\textsuperscript{6,7}. Nevertheless, although a large proportion of malignant melanomas have drainage in the lymph regions anatomically described in function of the location of the primary tumour, in some cases the presence of lymph nodes outside the expected lymph node stations can be observed. These lymph nodes situated on the pathway between the primary tumour and the lymph node regions are called in-transit or aberrant SLNs. In-transit SLNs meet the definition of SLNs as they are the first lymph nodes to receive lymph drainage directly from the primary tumour, although their location differs from classic drainage patterns\textsuperscript{8-11}.

In-transit SLNs theoretically have the same probabilities of presenting metastatic disease as SLNs located in a regional lymph node station. Herein lies the importance of taking lymphoscintigraphic images which allow drainage to in-transit SLNs to be identified, since their non-identification/resection could overlook the presence of potentially avoidable lymph node metastases.

The aim of our study was to determine the percentage and location of in-transit SLNs identified in our malignant melanoma patients, and to assess the necessity for resecting this type of SLN.

**MATERIALS AND METHODS**

**Patients**

Nine hundred patients were included prospectively in the study between January 1997 and October 2006 (428 men and 472 women; mean age 55 years, range 18-89 years). All were diagnosed with malignant melanoma and referred to the nuclear medicine department to have the SLN identification technique performed. The inclusion criteria were patients with melanoma in stages IB and II following AJCC guidelines (American Joint Committee on Cancer) (clinically negative; non-palpable lymph nodes; Breslow equal to or greater than 0.76 mm). A total of 793 patients previously had a diagnostic biopsy while 107 patients had the primary tumour in situ. The locations of the primary tumour are shown in table 1. The histological classes of the primary tumour were 500 patients with superficial spread malignant melanoma (56.1%), 235 with nodular malignant melanoma (26.2%), 66 with acral lentiginous melanoma (7%), 29 with lentigo maligna melanoma (3.3%) and 70 (7.4%) with other tumour variants.

**Lymphoscintigraphy**

The day prior to surgery, a lymphoscintigraphic study was performed by administering a \textsuperscript{99m}Tc-labelled albumin nanocolloid (Lymphoscint or Nanocoll, Amersham Health-Sorin, Saluggia, Italy). A total of 74-111 MBq were injected distributed in 4 different sites (0.1 ml per site) around the primary lesion or biopsy scar. Dynamic images were obtained (128 × 128 matrix, with images every 30 seconds for 10 to 20 minutes) immediately after the injection, followed by static images (256 × 256 matrix with an acquisition time of 300 seconds per image) at 30 minutes and 2 hours and later when required. Anterior and lateral projections were obtained, and complemented with oblique or posterior projections when necessary. From a scintigraphic point of view, SLNs were considered as the first to appear on the dynamic study or in the sequential images in a specific lymph node region, those connected directly with an injection area by a lymph channel or when a combination of these criteria was present. An in-transit SLN was defined as all that uptake which was persistent in time located between the injection area (primary tumour) and the usual lymph drainage regions. The location of the SLN was marked on the skin using an indelible ink pen.

**Table 1**

<table>
<thead>
<tr>
<th>Location of the primary melanoma</th>
<th>Cases (%)</th>
<th>Patients with in-transit SLN (%)</th>
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</thead>
<tbody>
<tr>
<td>Upper limbs</td>
<td>142 (15.4%)</td>
<td>18 (12.7%)</td>
</tr>
<tr>
<td>Thorax and abdomen</td>
<td>389 (39.3%)</td>
<td>44 (11.3%)</td>
</tr>
<tr>
<td>Upper limbs</td>
<td>241 (30.5%)</td>
<td>15 (6.2%)</td>
</tr>
<tr>
<td>Head and neck</td>
<td>120 (13.6%)</td>
<td>3 (2.5%)</td>
</tr>
<tr>
<td>Genitals</td>
<td>8 (1.2%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

SLN: sentinel lymph node.
Intraoperative localisation

Surgery was performed the day after the lymphoscintigraphy. Prior to the intervention, an intradermal injection of patent blue or methylene blue (Laboratoire Guerbet, Aulnay-Sous Bois, France; Laboratorio Dr. Carreras, Barcelona, Spain) was applied in most patients (572) in a volume of 1 ml coinciding with the radiotracer injection sites. Next, the SLNs were identified and excised by use of a detection probe (Neoprobe 1000 or Navigator, USSC, Norwalk, USA) to guide the resection. From a surgical point of view, SLNs were considered as those with blue staining, the lymph node with most activity detected by the probe in a lymph region and lymph nodes with activity greater than 10% of the lymph node with greatest uptake.

Histological analysis

All the SLNs were sectioned and sent for processing and study to the Pathology Department. The procedure was performed using serial sections and staining with haematoxylin-eosin at multiple levels, and with immunohistochemical staining with S-100, HMB-45 and MART-1 antibodies.

Regional lymphadenectomy

A complete regional lymphadenectomy was carried out when the histological analysis revealed metastatic infiltration in any SLN. The lymph nodes resected with the SLNs or the lymph nodes from the complete regional lymphadenectomy (non-SLN) were evaluated routinely using haematoxylin-eosin staining.

RESULTS

Lymphoscintigraphy

The presurgical study using lymphoscintigraphy detected the presence of in-transit SLNs in 80 of the 900 patients studied (8.9%). All were considered SLNs, as they corresponded to the first lymph node receiving lymph drainage from the primary tumour (figs. 1 and 2). The number and percentage of patients with in-transit SLNs in function of the location of the primary tumour are shown in table 1. In all cases, drainage towards regional lymph nodes was identified, in the territory of which the SLN was also considered.

The location of the in-transit SLNs was as follows: scapular region in 23 cases, popliteal location in 15 patients, epitrochlear in 11, thoracic in 10, abdominal in 9, brachial in 7, temporal in 2, lumbar in 2 and occipital in one single case.

Surgery

During surgery, of the 80 patients with in-transit SLNs visualised on lymphoscintigraphy, these nodes were removed in 77 of them (96.5%). In these 77 patients, a total of 91 in-transit SLNs were removed (1.2 per patient). The dye showed staining in at least one in-transit SLN in 65 of the 77 patients (84.4%), while all showed significant activity and were located with the detection probe (100%).

In three cases, despite correct visualisation and significant activity with the detection probe, the in-transit SLN could not be removed (one case in the epitrochlear region and 2 in the popliteal fossa). Nonetheless, in these three cases, it was possible to

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**FIG. 1.—Classical groin drainage in a patient with malignant melanoma in the right gastrocnemius area. The image on the left shows various uptakes in the groin area. In the image on the right, uptake of a radiotracer in a popliteal lymph node can be observed (in-transit sentinel lymph node, see arrow).**
excise a SLN in the regional lymph areas (axilla and groin, respectively).

The greatest difficulty during surgery was the abscission of SLNs located in the epitrochlear and popliteal regions (fig. 3), where the localisation and subsequent resection of the in-transit SLN exceeded 15 minutes.

**Histological study**

Histological analysis of the in-transit SLNs showed metastatic infiltration in 15 patients (19.5%), 4 of them presenting micrometastasis. The regional lymph node stations also showed lymph nodes with tumour infiltration in 11 of the 15 patients with positive in-transit SLNs (73.3%), while in the remaining 4 patients (26.6%), metastatic disease was not observed.

The location of the metastatic SLNs was in 5 cases in the scapular region, 4 in the thoracic region, 3 in the epitrochlear region, 2 in the popliteal regions and 1 in the temporal region. In these cases, a complete regional two-stage lymphadenectomy was carried out.

The mean Breslow index in the 15 patients with in-transit lymph node involvement was 2.7 mm (table 2) compared to 1.8 mm in the remaining 65 patients.

In the 4 patients with metastatic in-transit SLN, but without involvement in the lymph node region, the Breslow index of the primary tumour was 2.1 mm.

In the 3 patients in whom it was not possible to resect the in-transit SLN, the regional SLNs (2 axillary and 4 groin) showed metastasis in 1 case (groin). During follow-up of the cases with negative regional SLN, no recurrences or in-transit metastasis have been observed until the present time.

**DISCUSSION**

In most cases, lymphoscintigraphy detects the presence of SLNs in regional lymph node stations following marked (classical) lymph drainage patterns. However, in a not inconsiderable percentage of cases, lymphoscintigraphy can show previously unpredictable drainage areas, as has been described by other investigators\(^\text{12,13}\). In-transit SLNs are an example of this.

Some studies have published results in relation to the incidence of in-transit SLNs during lymphoscintigraphy, with figures varying between 3.1% and 7.8%.\(^\text{14,15}\) In our study, the incidence of in-transit SLNs was 8.9%, closer to the results of Uren et al\(^\text{15}\). Both the methodology of application of the technique and the type of size of the particles used may be the cause of this variation in the percentages, as has been suggested in the literature\(^\text{16}\). On the other hand, lymphoscintigraphy can present falsely positive images of in-transit SLNs, due mainly to lymphangiomas or lymphatic lakes (focal dilations of the lymph vessels), skin folds or other tissues with activity and especially due to skin contamination. In our group, one lymphangioma was identified in the epitrochlear area (excluded from the results analysis).

With respect to the location of the primary tumour, melanomas of the upper limbs and trunk are those...
which presented in-transit SLNs with greatest frequency (12.7 and 11.3%, respectively). These figures are similar to those published by Uren et al, who showed in-transit SLNs in 12.7% of patients with malignant melanoma located in the trunk area\(^{15}\). On the other hand, those which presented a lower number of in-transit SLNs were tumours located in the head and neck and in the genital area.

Just as important as the need for preoperative identification of the SLNs is their identification and resection in surgery. An in-transit SLN can reflect regional lymph bed infiltration, but it can also be the only site with metastatic cells present. Hence, the incidence of metastasis in in-transit SLNs is quite similar in most studies, varying between 14 and 22%\(^{14,15,17,18}\). In our study, it reached a percentage of 19.5%, consistent with other data from the literature. Of the 15 patients in whom metastatic involvement of the in-transit SLNs was identified in the histological analysis, 11 had added involvement of the lymph node bed. In the remaining 4, there was only involvement of the in-transit SLN. These figures reinforce the need to identify and excise in-transit SLNs, as occasionally they represent the only metastatic lymph node focus\(^{19}\).

The surgical difficulty was greater in the in-transit SLNs located in the epitrochlear area and popliteal region, with a mean time necessary for localisation and excision of in-transit SLNs of 15 minutes (compared to 5 minutes in other areas). This is because they are areas of reduced space, relative depth and significant vascular-nerve fibres. The rate of identification and resection of the SLNs was 96.5%; the areas previously described were those in which the in-transit SLNs could not be resected in 3 of the 80 patients in whom the presurgical lymphoscintigraphy showed significant activity in these specific locations.

Another aspect worth noting is the higher mean Breslow index in patients with metastatic involvement of in-transit SLNs. In comparison to the mean 2.7 mm in those with tumour infiltration, the 65 patients with negative in-transit SLNs showed a mean Breslow index of 1.8 mm.

In our study, patent blue or methylene blue was injected minutes before the surgery in all the cases with visualisation of in-transit SLNs on the lymphoscintigraphy. Staining with blue dye offers the surgeon a complementary aid, allowing the progression of lymph drainage from the primary tumour to the regional lymph node bed to be identified and therefore helps visualise the lymph node stations. However, staining in the lymph nodes is not achieved in all cases. The percentage of patients who showed staining of the in-transit SLNs was 84%. The rest did not show blue staining and therefore the isolated use of this technique is not recommended. On the contrary, all the in-transit SLNs visualised preoperatively on the scintigraphic images were identified by using a detection probe during surgery, although in 3 cases it could not be excised.

The limitations of this study are that in routine practice, a regional lymph node is always removed, as well as the in-transit SLN, and if we “strictly” apply the theory of the sentinel lymph node, in a large majority of cases, only the in-transit SLN should be resected. However, this fact is not completely accepted in the surgical community and in practice the in-transit SLN and the lymph node found in the usual lymph drainage area are removed. This procedure could slightly alter the results obtained as regards the number of regional lymph node recurrences, because if only the in-transit SLN is removed without altering the regional lymph area, recurrences or metastases could appear in them, which would modify the rate of false positives of the technique.

**CONCLUSION**

In view of these data, we can conclude that lymphoscintigraphy is essential for correct localisation of in-transit sentinel lymph nodes, and cannot be substituted by blue dye injection. Furthermore, although the percentage of in-transit SLNs in patients with malignant melanoma is low, they have tumour involvement, in a significant percentage of cases, similar to that of the lymph nodes in the regional lymph bed. Therefore, we believe that resection of in-transit SLNs is necessary in all cases.

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**Table 2**

**DESCRIPTION OF THE CASES WITH METASTATIC IN-TRANSIT SENTINEL LYMPH NODES**

<table>
<thead>
<tr>
<th>Location of the SLNs</th>
<th>Number of patients</th>
<th>Breslow (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scapular</td>
<td>5</td>
<td>1.8/2.1/2.2/3/3</td>
</tr>
<tr>
<td>Thoracic</td>
<td>4</td>
<td>1.6/2.6/3.2/4</td>
</tr>
<tr>
<td>Epitrochlear</td>
<td>3</td>
<td>1/4/3.3</td>
</tr>
<tr>
<td>Popliteal</td>
<td>2</td>
<td>3/2/4/1</td>
</tr>
<tr>
<td>Temporal</td>
<td>1</td>
<td>3.1</td>
</tr>
</tbody>
</table>

SLN: sentinel lymph node.


