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Sentinel node biopsy and ultrasound scanning in cutaneous melanoma: clinical and technical considerations

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Abstract

The aim of this study was to discuss the role of preoperative ultrasound (US) scanning and sentinel node biopsy (SNB) in melanoma patients. 100 patients underwent SNB following preoperative US scan and lymphoscintigraphy; patent blue dye (PBD) was injected before biopsy. Intra-operative lymphoscintigraphy (IL) was performed in 51 basins. All nodes were examined with histology and immunohistochemistry. Sensitivity and specificity of US scanning was 33% and 100%, respectively; 7% were true positives. The low sensitivity was mainly due to the resolution power of the US scanner (2 mm) which was unable to identify all the patients with microdeposits. PBD associated with IL identified SNs in all cases. In all patients with Breslow >1.5 mm and in all cases with two metastatic SNs, further positive additional nodes were found. The mean counts per 10 s (CP10S) ratio for SN and non-SN values was 5.62 (1.29–23.51) and 3.09 (1.03–10.99) in the intra-operative and extra-operative phases, respectively. US scanning and preoperative lymphoscintigraphy associated with PBD allows preoperative patient selection and accurate SN(s) identification. Breslow thickness and the number of metastatic SN(s), but not their type, are correlated with disease spread; CP10S contributed to the differentiation amongst the nodes and the determining of procedure's completion. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Cutaneous melanoma; Gamma probe; Patent blue dye; Radioguided surgery; Sentinel node; Ultrasound scanning

1. Introduction

No randomised study has demonstrated that patients with clinical stage I cutaneous melanoma (CM) benefit from elective lymph node dissection (ELND) [1–4]. Only in a USA Melanoma Intergroup Study, a patient subgroup (age <60 years, thickness 1.1–2 mm) seemed to benefit from ELND [5]. Since metastases are present in only 20% of ELND [6] and lymphadenectomy is a major surgical procedure, the sentinel node (SN) biopsy technique appears worthwhile in selecting patients to treat, as first reported by Morton and colleagues [7]. However, in the preoperative work-up for melanoma patients, ultrasound (US) scanning can help identify early lymph node metastases measuring more than 2 mm, as reported by us and others [8,9]. Nevertheless no data are available on the real impact of this procedure in selecting melanoma patients for SN biopsy.

Although some clinical studies [10–14] have confirmed Morton's experience with patent blue dye (PBD), and others [13,15–17] have reported even better results using associated intra-operative lymphoscintigraphy (IL), some aspects of this technique are still debated: (a) evaluation of feasibility and efficiency of SN biopsy in all major lymph nodal districts (laterocervical region, axilla, groin), also under local anaesthesia; (b) standardisation of technique and definition of SN by IL; (c) study of histoprognostic correlations between the primary and any SN(s) metastases and/or additional nodes (AN) with metastases derived from radical lymph node dissection. This might justify the use of this procedure

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alone in well defined subgroups of patients, without performing radical lymph node dissection.

We therefore report here on our experience with the associated US scanning and IL mapping in melanoma patients, and discuss the above aspects.

2. Patients and methods

100 patients with stage I CM (47 males, 53 females; mean age 50.5, range 14–77 years), were treated at Clinica Chirurgica II, Padova University, from May 1993 to February 1999. Sentinel node biopsy (SNB) was performed in 108 lymphatic basins, since 8 patients presented two drainage sites. The CM site was the upper limb in 23 (23%) patients, the lower limb in 48 (48%), the trunk in 25 (25%) and the head–neck region in 4 (4%) patients. The mean Breslow thickness was 3.09 (0.7–19) mm, 53 tumours (53%) were classified as Clark's IV. Regression and ulceration were present in 9 (9%) and 33 cases (33%), respectively.

Preoperatively 69 patients (69%) (74 lymphatic basins) underwent US scanning utilising an ESAOTE AU4 IDEA (Ansaldo-Genova, Italy) scanner, with a 10 MHz frequency probe. A modified version of Vassallo's parameters was used to differentiate between negative and positive lymph nodes [18]. All patients underwent SNB. On the basis of histological findings lymph node basins were classified as: true-positive (TP), false-positive (FP), true-negative (TN) and false-negative (FN). Preoperative lymphoscintigraphy (PL) was performed the day before surgery in all patients, using a LFOW gamma camera (Orbiter Siemens, Desplaines, IL, USA). A mean volume of 0.18 (range: 0.1–0.3) ml of 99mTc-human serum albumin (Nanocoll Amersham Sorin, Milano, Italy) was injected intradermally in two perilesional sites, a final dose of 104.7 (range: 99–150) MBq being administered. After injection and dynamic scanning of the lymphatic outflow, using an emission body profile technique with a 57 Co shield, early and late images were obtained at 20 min and 2 h, respectively. In one case, intravenous Hydroxy-Di-Phosphonate (HDP Mallinckrodt Medical B.V. Petten, The Netherlands) was used to delineate the deep bony structures. The skin corresponding to the highest emission point was marked.

On the day of surgery, 20 min before skin incision, a mean volume of 1.1 (range: 0.6–3.3) ml of PBD (Patent V 2,5, Jacopo Monico, Laboratorio Chimico-biologico-Venezia/Mestre, Italy) was injected intradermally, with a mean of 8 (range: 3–16) injections surrounding the lesion or the scar. The mean interval between injection and surgery was 22.5 min (range: 6–41).

A hand-held CARE WISE gamma camera (Morgan Hill, CA, USA) was used to obtain intra-operative counts per 10 s (CP10S) of background and SN(s). Background and SN(s) emission values were recorded in preoperative, intra-operative and extra-operative phases. During the preoperative and intra-operative phase, the background and SN(s) values were acquired near the SN (at a distance of $\leqslant 3$ cm) and on the node, before and after the skin incision, respectively. The extra-operative evaluation was performed out of the operative field, close and in correspondence of the specimen. After SN(s) excision, the background activity at the wound site was reported as a postoperative value. After surgical incision (intra-operative time), the SN was identified as a hot spot and/or blue dyed node. The counts emission values, and the time required for SN(s) identification in the intra-operative phase were recorded.

The removed SN(s) were sent to the pathologist in buffered 10% formalin for paraffin-embedded sections for haematoxylin–eosin staining and for immunohistochemistry. The SN(s) were cut in half in correspondence of the hilum and then subdivided into 1–2 mm thick fragments including the whole node. All the fragments were embedded in paraffin; from each inclusion, 10 sections were prepared for histological and immunohistochemical (S-100 protein and HMB-45) examination.

All patients with positive SN(s) underwent radical lymph node dissection of the affected lymphatic basin, complete physical examination and US scanning of the draining lymphatic basins every 3 months for the first 2 years and every 6 months for the following 3 years. Standard chest X-ray and hepatic US scanning were repeated every 6 months.

3. Results

3.1. Lymph nodes US scanning

Seventy-four nodal basins were evaluated with US scanning. Thirty-eight (51%) were in the groin, 31 (42%) in the axilla and 5 (7%) in other sites. At histology, nine basins (12%) were found containing micrometastases not otherwise detectable (US resolution power $\leqslant 2$ mm of diameter). Five (56%) were found in the groin and 4 (44%) in the axilla. Of the residual 65 basins, 5 (7%) were found to be positive and confirmed at histology. Of the 60 (81%) lymphatic stations considered negative, 59 (80%) were confirmed as such at histology, but 1 (1%) contained metastases. Positive US scanning findings consisted of 5 TP (7%) and no FP, and negative US scanning cases consisted of 1 FN + 9 FN (10 FN = 14%) not detectable in any case and 59 TN (80%) (Table 1). The distribution of histological type for nodal metastases, excluding micrometastases, is reported in Table 2. US scanning for studying the lymph node basins showed a sensitivity of 33% (5/15) due to the inclusion of undetectable micropositive nodes, whilst the specificity was 100% (59/59), the

Table 1
Results of ultrasound (US) scanning and histological findings in 74 lymphatic basins from melanoma patients

Results of US scanning	Positive histology	Negative histology	Total
Positive	5 TP (7%)	0 FP (0%)	(7%)
Negative	9[a] + 1 FN (14%)	59 TN (80%)	69 (93%)
Total	15 (20%)	59 (80%)	74 (100%)

[a] Micrometastases.

positive predictive value 100% (5/5) and the negative predictive value 85.5% (59/69).

3.2. Vital dye method alone and in association with intra-operative lymphoscintigraphy

PBD was performed alone on 57 basins, and in association with IL on 51 basins. With the PBD alone 26 axillary, 30 inguinal, and 1 laterocervical basins were explored. The SN was identified in all the inguinal sites, in 23 of 26 axillary (88%) and none of the laterocervical basin sites, with a 93% overall success rate (Table 3). Of a total 92 SNs excised: 39 (42%) were in the axillary and 58 (58%) in the groin basins. When PBD and IL were associated, 19 axillary, 27 groin and 5 laterocervical (and other anomalous basins) were explored; in all cases the SN was found, with a success percentage of 100% (Table 3). Eighty-nine SNs were excised: 24 (27%) in axillary, 52 (58%) in groin and 13 (15%) in laterocervical basins. Overall, the SN was identified in 96% of cases.

3.3. Sentinel lymph node(s) and metastatic lymph nodes derived from radical lymphadenectomy (additional nodes)

In the 100 patients (108 basins), 181 SNs were removed; 84 (78%) basins contained histologically negative SNs, whilst 24 (22%) basins (23 patients) had carried positive nodes. Radical lymphadenectomy was then performed and a total of 371 additional nodes (ANs) were examined. 23 (6%) of these, were histologically positive, so AN(s) were found to be positive in 43% of patients with positive SN(s). The type of SNs metastases in the groups of patients with and without positive AN(s) is reported in Table 4.

The percentage of patients with positive SN(s) and with positive ANs (Fig. 1) (Table 5) was found to be related to the Breslow thickness of the primary tumour.

Amongst 24 radically dissected basins, a single positive SN was found in 22 cases (92%) whilst two SNs were found in 2 cases (8%). These latter also had metastatic AN(s). In contrast, only 36% of basins had positive AN(s) when a single SN was metastatic.

3.4. Emission values (in CP10S) using a hand-held gamma camera

The emission values were recorded for 48 patients (48 basins). Twenty-two (46%) were in the axilla, 22 (46%) in the groin and 4 (8%) in the laterocervical area. Eighty-six lymph nodes were identified (80 intra-operatively and six after extra-operative specimen dissection), and only 48 were considered true SNs because they had higher emissions; 40 (83%) were hot and blue stained and 8 (17%) were hot, but without blue staining. Eight (17%) nodes were positive at histology. They were also the hottest nodes of the region. In 6 cases, single positive nodes were the only nodes found to be involved in the disease, whilst the other 2 cases had further positive nodes. As a whole, all of the highest emitting nodes were histologically positive. The mean intra-operative time required for the identification of the first SN was 33.5 (range: 29–38) min in the laterocervical basin, 11.3 (range: 2–55) min in the axilla and 5.2 (range: 1–17) min in the groin (Fig. 2). During the preoperative phase, the mean value (range in brackets) of the controlateral background was 1.6 (0–7) CP10S, the ipsilateral back-

Table 2
Results of ultrasound (US) scanning and type of metastases in the sentinel node specimen from six basins

US scanning	Partial/ pluriembolic (%)	Massive (%)	Total (%)
Positive	4 (67)	1 (17)	5 (83)
Negative	1 (17)	0	1 (17)
Total	5 (84)	1 (17)	6 (100)

Table 3
Comparison between patent blue dye (PBD) and PBD associated with pre-operative lymphoscintigraphy (PL) in 108 lymphatic basins from melanoma patients

Method	Axilla (%)	Groin (%)	Other (%)	Total (%)
PBD alone	23/26 (88)	30/30 (100)	0/1	53/57 (93)
PBD + PL	19/19 (100)	27/27 (100)	5/5 (100)	51/51 (100)
Total	42/45 (93)	57/57 (100)	5/6 (83)	104/108 (96)

Table 4
Distribution of type of nodal metastases in sentinel node/s (SN) in 23 melanoma patients with or without metastatic additional node/s (AN)

Type of metastasis	SN+ and AN– (%)	SN+ and AN+ (%)	Total
Micro	9 (69)	5 (50)	14 (61)
Partial	1 (8)	3 (30)	4 (17)
Massive	2 (15)	1 (10)	3 (13)
Beyond capsule	1 (8)	1 (10)	2 (9)
Total	13 (100)	10 (100)	23 (100)

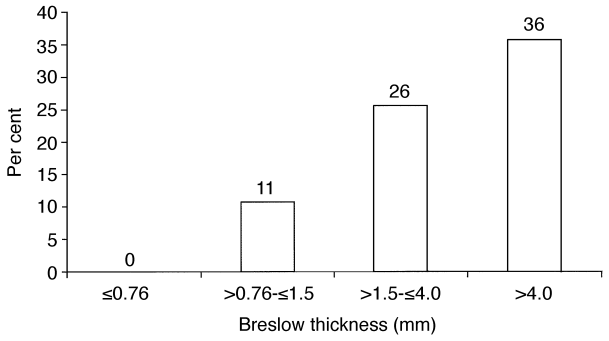

Fig. 1. Breslow thickness of primaries and percentage of positive sentinel nodes (SNs).

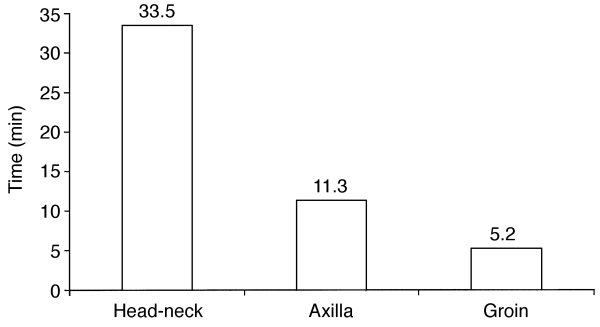

Fig. 2. Mean time required for sentinel node (SN) identification in melanoma patients, according to site.

ground 5.5 (0–26) CP10S, SNs 487 (0–2760) CP10S and non-SNs 98.25 (50–165) CP10S. During the intra-operative phase, the mean value (and the range) of background in the wound before the excision of lymph nodes was 30.2 (0–343.5) CP10S, SNs 774.3 (14–5386) CP10S and non-SNs 111.5 (3–229) CP10S. In the extra-operative phase, the mean value recorded for the background was 0.9 (0–10.5) CP10S, for the SNs 970.4 (8–6782) CP10S and for the non-SNs 164.3 (0–617) CP10S. In the postoperative phase, the mean background emission value in the wound after excision of all nodes was 16.7 (range: 0–69) CP10S. The mean ratio between SNs emission values and corresponding backgrounds was 88.5 in the preoperative, 25.6 in the intra-operative and 1078.2 in the extra-operative phases; the mean ratio between SNs and non-SNs emission values (only in patients with non-SNs), was 2.52 (range: 1.27–3.94), 5.62 (range: 1.29–23.51) and 3.09 (range: 1.03–10.99) in pre-, intra-, and extra-operative phase, respectively.

3.5. Patient status

So far, at a mean follow-up of 21.6 (range: 1.9–69.6) months, 11 (11%) patients have developed locoregional lymph node metastases. 7 (7%) of these patients had a negative SN biopsy at histology. 4 patients (4%) developed distant metastases and only one of these had previous positive SN(s) removal. At the moment, 77 (77%) patients are alive and tumour-free, 4 (4%) are alive with disease, 14 (14%) died of widespread disease from melanoma and 1 (1%) died of another disease. 4 (4%) patients have been lost to follow-up.

Table 5
Distribution of Breslow thickness of the primary tumour in sentinel node/s (SN)-positive melanoma patients with or without metastatic additional node/s (AN)

Thickness (mm)	*n* of patients	SN+ and AN– (%)	SN+ and AN+ (%)
⩽0.76	5	0	0
>0.76–⩽1.5	28	3 (11)	0
>1.5–⩽4.0	39	5 (13)	5 (13)
>4.0	28	5 (18)	5 (18)
Total	100	13	10

4. Discussion

The good results reported so far in the literature show that SN biopsy is a reliable diagnostic technique in the staging of melanoma, although it should still be considered an investigational procedure. Moreover, thanks to the high frequency probe, US scanning is increasingly reliable in detecting even infraclinic node metastases, though they must be greater than 2 mm. Thus, the association of the two methods may lead to a better work-up for the staging of these tumours, avoiding unnecessary SN biopsy in patients with US-positive nodes.

Our experience with US scanning for monitoring lymph node status in melanoma patients started in 1989, since false-negative rates up to 40% were reported for physical examination alone, at presentation and in follow-up of CM [3]; palpation, which mostly relies on the expertise of the physician, can reveal only advanced stage metastases [8]. In contrast, US scanning is a reliable method for the detection of nodal metastases, particularly in association with fine-needle aspiration biopsy, which allows false-positives to be ruled out [9]. Nevertheless, no data are yet available on the role of US scanning for lymph node evaluation in patients undergoing SNB.

This study demonstrated a sensitivity of 33% (correct identification of metastatic nodes), and a specificity of 100% (correct identification of non-metastatic nodes), but excluding those patients with 'micropositive nodes' (⩽2 mm), a limit imposed by the scanner resolution power that at the moment cannot be overcome, the sensitivity became 83% (5/6). Again, the negative predictive value was 85.5%, rising to 98.3% (59/60) in the same subset of patients, whilst the positive predictive value was 100%. US scanning correctly classified five clinical stage II cases (7% of the lymphatic basins

underwent US scanning), in which SNB was unnecessary, radical lymphadenectomy being performed immediately after US.

Our study shows that PBD associated with IL leads to a correct identification of SN in 100% of cases, a higher percentage than that for the use of PBD alone (93%), with no differences observed amongst laterocervical, axillary or inguinal basins. In particular, the two methods combined play a more important role in the identification of SN(s) in difficult sites, such as laterocervical area. Our data are comparable with those reported in the literature, where a percentage from 96 to 99.5% and from 84 to 92% of SNs identification is reported using the two methods in association or the PBD alone, respectively [10,11]. It is now widely accepted that the two methods should be used in combination, except, perhaps, in patients with a melanoma of the face or neck where it is wiser to use IL alone, due to the possible persistence of blue spots on the skin after excision. In view of the small radioguided skin incision allowed by the combined approach, SNB appears to be feasible under local anaesthesia, at least in patients with inguinal and axillary SN(s).

To our knowledge, findings on the definition of SN by emission values from IL have not yet been published in the literature. Krag and associates defined SN as 15 CP10S [19], whereas Mudum and colleagues defined SN as 300–3000 CP10S [20]. Other authors have defined SN on the basis of the ratio between *in vivo* SN against background mean emission values, but the ratios have not yet been standardised. Glass and colleagues [21] initially identified an *in vivo* SN by a radioactive count ratio of 3:1, later changed to 2:1 because of high background counts. Bostick and coworkers [10] defined SN by an *in vivo* (intra-operative) or *ex vivo* (extra-operative) radioactive count ratio between SN and background $\geqslant$2:1. Albertini and colleagues [12] proposed different criteria for the identification of SN, considering an *in vivo* (intra-operative) SN to background ratio of 3:1, besides an *ex vivo* (extra-operative) SN to non-SN mean ratio of 10:1. In our experience the lowest ratio between the highest emitting node and non-SN was 1.29. It has been demonstrated that a large second-echelon node may accumulate more radiolabelled nanocolloid than a small first-echelon node [22]. Thus, at present only a blue node whose afferent lymphatic duct is clearly evident at dissection may be considered a 'true' sentinel node. This is usually the hottest node, and it is advisable to excise all blue stained and/or hot node/s and send to the pathologist.

Basically, SN biopsy procedure can be divided into three phases: the first (preoperative) in which the exact location of SN is determined by the hand-held probe progressively increasing the background level at which the detection produces its sounds and, consequently, decreasing the skin area overlying the node ('shrinking circle technique') [23] and leading to minimal dissection; the second phase (intra-operative) during which maximal attention should be paid to finding and not damaging the afferent lymphatic channel(s), to check radioactivity on SNs and, after its excision, in order to stop searching other nodes (generally when the residual background emission is lower than 50% in post-operative with respect to intra-operative phase); the third phase (extra-operative) when from the excised sample each lymph node can be accurately separated from the others.

The wide range in radioactive counts measured in the SNs by us and others depends on the doses and kinetics of the radiopharmaceuticals, of injection and recording count techniques and variation from patient to patient. Moreover, the site of the primary lesion, its distance from the lymph nodal basin and any modification in lymphatic channels due to primary excision could also explain the differences in radioactive counts [10,12,24].

The percentage of our patients with positive ANs was very high (43%) compared with those reported in studies by others (6.1–23%) [24–28]. This might be due to the observation that the percentage of positive ANs markedly increases with the primary tumour thickness. In our series it is more than 50% in sentinel node-positive patients when the Breslow was greater than 1.5 mm.

At present, few studies available in the literature report on micrometastases [14,15] in SN(s), whilst the type of metastases in nodes derived from radical dissection, or additional nodes (AN), are not described. We found no correspondence between histological type of metastases in positive SNs and the degree of positive ANs in the same lymphatic basin. However, we found an evident correlation between the number of metastatic SNs and the degree of involvement of ANs in the same lymphatic basin. The absence of a correlation between the type of metastases of SN and lymph node involvement in radical dissection may be due to the small number of patients in our series, so this aspect should be evaluated in a larger group.

In conclusion, US scanning is a reliable, not invasive and cheap method for the selection of melanoma patients before SN biopsy since it can obviate unnecessary procedures and some false-negative results (when a massive metastasis is present in SN). The SN biopsy technique should be based on the combination of lymphoscintigraphy and intra-operative PBD injection, but a more precise standardisation of this method is still required since it has been shown that many details, apparently of minor importance, may influence the final result. At present, local anaesthesia can be proposed for virtually all patients, taking into consideration the site and the number of the SN and the primary tumours characteristics.

Finally, with a view of selecting homogeneous subgroups of melanoma patients to submit to different

therapeutic options, further histoprognostic correlations amongst the primary and SN and/or AN(s) metastases should be investigated.

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