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Role of imprint cytology in the intraoperative evaluation of sentinel lymph nodes for malignant melanoma

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Abstract

Controversy exists over the utility of different methods for intra-operative sentinel lymph node (SLN) evaluation in patients with malignant melanoma (MM). The aim of this study was to evaluate the role of intra-operative imprint cytology (IC) in patients with MM. 215 SLNs from 99 patients with MM were examined by IC and results compared with the results of permanent sections. 24 patients had MM deposits in their SLNs and this was confirmed by histological examination. Intraoperative IC was positive in 11 of these patients (46% sensitivity). In addition, there were three false-positive IC diagnoses (79% positive predictive value); one of these was due to contamination during the sectioning of the SLN. The specificity and the negative predictive values of the IC were 96 and 85%, respectively. IC is a valuable method of intra-operative SLN evaluation which can spare approximately half of the patients with clinically occult regional metastases from a second surgical procedure. However, special care must be taken to avoid false-positive results due to contamination.

Keywords: Malignant melanoma; Sentinel lymph node; Imprint cytology; Metastases

1. Introduction

The incidence of malignant melanoma (MM) is increasing at a faster rate than that of any other cancer [1]. Metastases to the regional lymph nodes are the most significant prognostic factor for patients with MM [2,3]. The treatment of patients with MM and clinically negative regional lymph nodes was a matter of some debate and controversy in the last century. Some have advocated an elective lymph node dissection, whilst others were more in favour of a 'watch-and-wait policy'. This dilemma was resolved approximately 10 years ago by the introduction by Morton of the sentinel lymph node (SLN) biopsy [4]. The SLN biopsy proved to assess reliably the status of regional lymph node basins and can identify the patients with clinically occult nodal metastases who may benefit from a complete regional lymphadenectomy. Metastases in SLN proved to be the most significant independent predictor of tumour-related death in the patients with early-stage MM, as well as in patients with thick primary MM [5–7]. Since the pathological result of SLN determines the need for additional surgery—complete regional lymphadenectomy—, the intra-operative evaluation of SLN would spare the patient a second surgical procedure. The most commonly used intra-operative pathological method is frozen section (FS) analysis. There are arguments against the intra-operative FS analysis of SLN because of the potential loss of SLN tissue containing small foci of micrometastases, the introduction of freezing artifacts and the low sensitivity of the method. In melanomas, intra-operative FS is generally not recommended [8]. An alternative method to FS is intra-operative imprint cytology (IC) which is faster, cheaper, and proved to be as reliable as the FS analysis in patients with breast cancer [9–11]. However, there is a paucity of data in the literature about the role of IC in the patients with MM, with only one major study being published recently by Creager and colleagues [12].

We describe our series of 99 patients with MM in whom IC was used intra-operatively for assessing their SLN status.

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2. Patients and methods

2.1. Patients

From November 2001 to November 2002, 102 consecutive patients with clinically localised cutaneous MM underwent lymphatic mapping at the Institute of Oncology, Ljubljana, Slovenia. In 1 patient, the mapping showed only an interval SLN in the scapular region which was not found on surgery and, in 1 patient, with a primary melanoma on the face, lymphoscintigraphy was negative. One patient had an *in situ* melanoma and was also excluded from the analysis. The remaining 99 patients form the basis of the present study. Their clinical and pathological characteristics are listed in Table 1.

Table 1
Clinical and pathological characteristics of the patients ($n = 99$)

Characteristic	*N*	% of total
Gender		
Male	46	46
Female	53	54
Age (years)		
60	66	67
>60	33	33
Median	51 (20–83)	
Primary site		
Head	11	11
Neck	2	2
Trunk	42	42
Extremity	44	44
Clark's level		
Unknown	9	9
II	1	1
III	39	39
IV	43	43
V	7	7
Breslow thickness (mm)		
Unknown	4	4
<1	8	8
1–2	33	33
2.01–4	38	38
>4	16	16
Ulceration		
Present	28	28
Absent	66	67
Unknown	5	5
Histological subtype		
Superficial spreading	33	33
Nodular	33	33
Acrolentiginous	1	1
Lentigo maligna	3	3
Spitzoid-nevoid	2	2
Subungual	1	1
Unclassified	26	26

N, number.

Primary MM was diagnosed by excisional biopsy, with margins of 2–5 mm in all patients except 4 in whom the diagnosis of MM was clinical. SLN biopsy was performed together with radical re-excision (1–2 cm margins) of the site of the primary melanoma, except in the 4 patients mentioned before who underwent a SLN biopsy and radical excision synchronously.

2.2. Technique of lymphatic mapping and SLN biopsy

A standard protocol was used in all patients. Lymphoscintigraphy was performed on the morning of surgery, 2–6 h before the operation, to identify all basins at risk and the possible interval SLN. A total dose of 40–60 MBq ^{99m}Tc nanocolloid (Nanocoll; Nycomed Amersham Sorin, Italy) in a total volume of 0.4 ml normal saline was injected intradermally at four points around the biopsy site or primary MM. Dynamic scans of possible lymph drainage regions were obtained beginning immediately after a tracer injection and continued for 20 min. At that point, the first set of anteroposterior and lateral static images was taken. Another set of anteroposterior and lateral static images was taken after 2–5 h. The position of SLN was marked on the skin with indelible ink.

SLN biopsy was conducted under general anaesthesia 2–6 h after lymphoscintigraphy. 0.5–1 ml of Patent Blue (Blue Patente V; Laboratorie Guerbet, Aulnaysous-Bois, France) was injected intradermally at the same points as the ^{99m}Tc nanocolloid. All basins identified by lymphoscintigraphy were explored surgically. Incisions were fashioned in such a way that they could be incorporated into the incisions used for nodal dissection in the case of positive SLN. Surgical dissection was guided by a hand-held gamma probe (Navigator GPS System, USSC, USA) and by a blue-stained afferent lymphatic channel. The identified SLN was excised and measured for *ex-vivo* radioactivity. Additional hot nodes were removed until the ratio of the background radioactivity to the hottest *ex-vivo* SLN was less than 10%.

2.3. Pathological examination

Excised SLNs were bisected along the long axis. For each SLN half, two to four imprints were made by gently touching the cut surface of the SLN to a glass slide. Imprints were air-dried and stained with a quick stain (Hemacolor, Merck KgaA, Darmstadt, Germany). A cytopathologist examined the imprints and diagnosed them as negative or positive.

SLN were then fixed in 10% formalin and embedded in paraffin. A pair of sections (sections 1 and 2) was made from each block; the first was stained with H&E and the second for S-100 protein by immunohistochemistry (IHC). If initial review of these sections was negative, six additional consecutive sections were made (sections 3–8). Sections 3, 5 and 7 were stained with H&E,

sections 4 and 8 for S-100 protein, and section 6 for HMB45. IHC stainings were performed using the avidin–biotin–peroxidase complex method with commercially obtained antibodies—S100 (dilution 1:750) and HMB45 (dilution 1:200) (Dako, Glostrup, Denmark). For this study, all cases with positive findings by either IC or histology were reviewed.

3. Results

A total of 215 SLN were excised from 99 patients. All SLNs were radioactive, but only 114 SLN were also blue. The mean number of SLNs per patient was 2.2 (range 1–10). The majority of SLNs were from a single lymph node basin (in 80 patients, 81%), although in 15 patients, SLN were excised from 2, in 3 patients from 3 and in 1 patient from four lymph node basins. In permanent sections, melanoma deposits were observed in 24 patients (24%). Their clinicopathological characteristics are given in Table 2. According to the size of the largest deposit, they were classified as macrometastases (2 mm) in 9, micrometastases ($\leqslant$2 mm) in 8 and individual tumour cells or clusters (ITC) (0.2 mm) in 7 patients (Fig. 1). In 20 cases, metastases were already detected in the first pair of sections. In 3 cases, they became apparent in deeper H&E and S-100 sections, and in one case, the diagnosis of ITC was made from rare HMB45-positive cells that could not be identified on adjacent H&E sections.

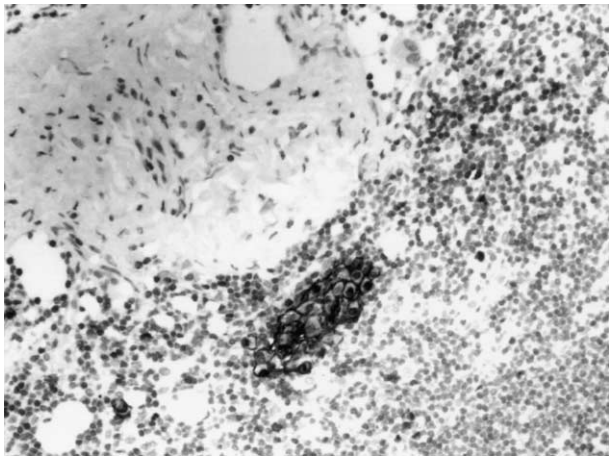

Fig. 1. Lymph node with a cluster of melanoma cells highlighted by immunostaining for S-100 protein.

Benign nevus inclusions were found in 12 patients (12%); none of them had metastases.

In all patients, except 1, a complete lymph node dissection was performed, which revealed additional posi-

Table 2
Clinicopathological characteristics of 24 patients with regional metastases

Patient	Lymph node basin	No. of positive SLN	Type of metastasis	Size of the largest metastasis in mm (sequence No. of slice with metastases)	No. of positive/no. of total non-SLN	IC
1	Axillary	1/1	mi	0.9 (1)	**1/14**	+
2	Axillary	1/1	mi	1.3 (1)	**1/16**	+
3	Inguinal	1/3	ITC (IHC)	0.0 (6)	NP	–
4	Inguinal	1/1	ma	4.4 (1)	0/25	–
5	Neck bilateral	3/3 +2/2	ma + ma	2.2 (1), 5.0 (1)	0/40 + 0/36	+
6	Inguinal	1/1	ITC	0.1 (1)	0/8	+
7	Inguinal	2/2	mi	1.8 (1)	0/13	+
8	Axillary bilateral	2/3 + 1/2	ma	4.4 (1), 2.5 (1)	**2/29** + 0/16	+
9	Axillary bilateral, inguinal bilateral	0/2 + 1/2 + 0/3 + 2/3	ma + ma	6.0 (1), 4.0 (1)	0/29 + **4/24**	+
10	Axillary	1/2	ma	4.5 (1)	**2/28**	+
11	Inguinal	1/3	mi	1.0 (3)	0/10	–
12	Inguinal	3/3	ma	2.5 (1)	0/33	+
13	Axillary	1/1	mi	0.4 (1)	0/12	–
14	Axillary	1/1	ma	5.2 (1)	0/23	–
15	Axillary, neck	1/3 +0/1	ma	6.0 (1)	0/10	+
16	Axillary	1/2	ITC	0.2 (1)	0/12	–
17	Axillary	1/2	ITC	0.2 (1)	0/37	–
18	Axillary bilateral	1/1 + 0/1	mi	1.0 (1)	0/20	–
19	Axillary bilateral	1/3 + 0/4	ma	11.0 (1)	0/18	+
20	Axillary	2/2	ITC	0.1 (1)	0/14	–
21	Neck	1/1	ITC	0.1 (3)	0/39	–
22	Neck	2/2	mi	0.8 (1)	1/25	–
23	Axillary	1/3	ITC	0.1 (7)	0/10	–
24	Inguinal	1/1	mi	0.6 (1)	0/12	–

ma, Macrometastasis (>2 mm); mi, micrometastasis ($\leqslant$2 mm); ITC, individual tumour cells or clusters $\leqslant$0.2 mm; IHC, immunohistochemistry; NP, not performed; IC, imprint cytology; SLN, sentinal lymph node.

tive non-sentinel lymph nodes in 6/23 patients (26%). The range of positive nodes was from one to four. There were 3 patients with macrometastases and 3 patients with micrometastases in SLN with additional non-sentinel lymph nodes metastases. There were not non-sentinel lymph nodes metastases in the group of patients with ITC in SLN (0/6).

Intra-operative IC was positive in 11 out of 24 patients with positive findings on permanent sections (Fig. 2), resulting in a 46% sensitivity. The sensitivity of IC was related to the size of metastasis in the permanent sections: IC was positive in 7/9 cases with macrometastasis, 3/8 cases with micrometastasis and 1/7 cases with ITC ($P=0.010$, chi-squared test for trend). A re-examination of the IC slides from the 13 false-negative cases did not reveal melanoma cells.

There were three false-positive IC diagnoses (79% positive predictive value). The presence of malignant cells was confirmed upon re-examination of the IC slides. In 1 of these cases, a cluster of malignant cells was found closely adjacent to the lymph node tissue in permanent histological sections. However, since these cells were negative for S-100 protein and HMB45 and were immunoreactive for cytokeratin, they were thought to result from contamination of the sample (Fig. 3). The specificity of the IC diagnosis was 96%, and the negative predictive value was 85%.

On a lymph node basis, intra-operative IC was positive in 18/32 positive lymph nodes (56% sensitivity). The specificity of the intra-operative IC was 97%, the positive predictive value was 90% and the negative predictive value was 97%.

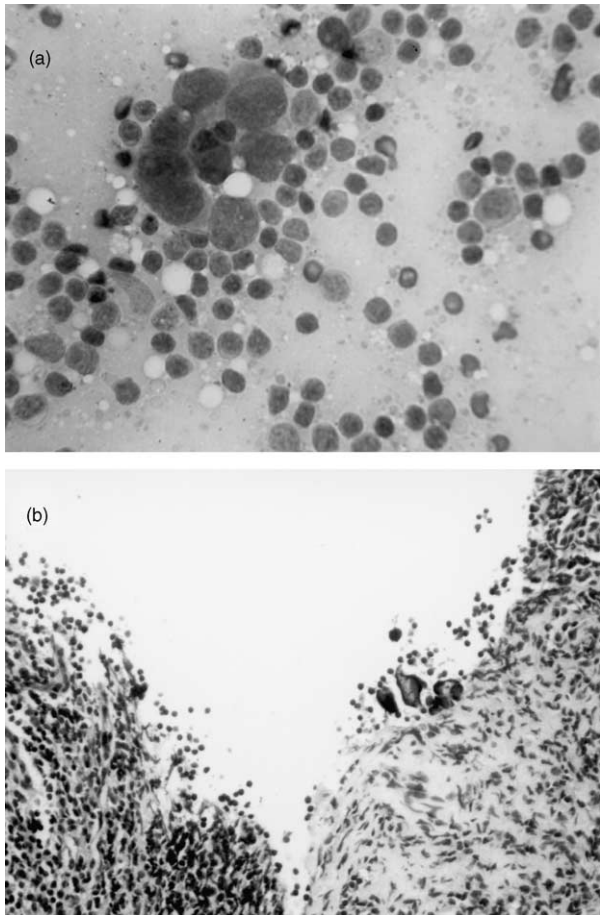

Fig. 3. A cluster of malignant cells is seen in imprint (a). The corresponding histological section shows a group of malignant cells that lie closely adjacent to (but not within) the lacerated lymph node tissue and are immunoreactive for cytokeratin (b).

4. Discussion

Lymphatic mapping with SLN biopsy has become the method of choice in the management of patients with MM. It enables an accurate regional staging of patients without subjecting them to the morbidity of elective lymph node dissections. The commonly accepted treatment of patients with micrometastases in SLN is removal of the remaining lymph nodes within the regional lymph node basin [13]. This raises the question of a possible intra-operative SLN evaluation that would spare the patient a second operation in cases of a positive diagnosis.

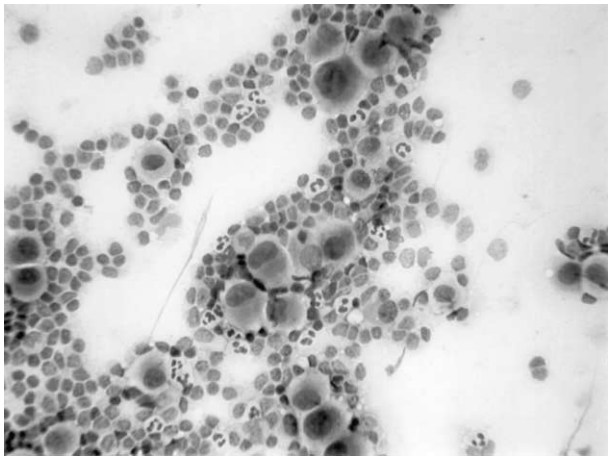

Fig. 2. Imprint of a sentinel lymph node wih metastatic melanoma.

Intrao-perative evaluation of SLN from the patients with breast cancer with both frozen sectioning and IC has resulted in sensitivities as high as 91% and specificities of around 100% [14,15]. However, there were only a few studies examining frozen sections for SLN in patients with MM and an even smaller number of studies using IC techniques [12,16–19]. The sensitivity of the reported results range from 33 to 59% [12,16–19]. Additionally, the incidence of SLN metastases in the patients with MM is low in comparison with the overall incidence of SLN metastases in the patients with breast cancer [20–23]. Low sensitivity is an important factor to consider when we try to justify the extra cost and time of intra-operative evaluation.

We report here, a study of 99 melanoma patients with intra-operative IC evaluation of the SLN. The results of the SLN biopsy procedure in our series are compatible with results of other reported series [6,13,19,24,25]. The SLN identification rate was 98% (100/102), with all SLNs being radioactive, but with only 53% of SLNs also being blue. The overall incidence of nodal metastases was 24%, which is in keeping with the results of other series [6,13,19,24,25]. The observed incidence of 26% of the non-sentinel lymph node metastases justifies the approach of the complete lymph node basin dissection after a positive SLN biopsy.

The sensitivity of the intra-operative IC was 46%, which is among the highest reported for intra-operative evaluation of SLN in patients with MM. Creager was the only author who recently reported a study with a comparable number of patients [12]. In his report, the sensitivity was 39%. The high sensitivity in our series can be in part attributed to the dedicated cytopathologists used in our study who had substantial prior experience in cytopathology. The specificity and negative predictive value of IC in our study were 96 and 85%, respectively, which is still consistent with other reported data [12]. However, we observed 3 cases of false-positive intra-operative IC, which resulted in a positive predictive value of only 79%, whereas this was 100% in all other reported series [12,16,18,19].

One of the 3 false-positive IC cases in our series could be attributed to a contamination of the cut surface of SLN with carcinoma cells from another specimen, which was confirmed by IHC stainings. The reasons for the other two "false-positive" IC cases are not clear. Since the presence of malignant cells was confirmed on re-examination, they were not due to overdiagnosis. It may well be that these were actually false-negative cases by histology. The fact that on several occasions metastatic deposits were found in the first histological section of one half of the bisected lymph node, but not in the first section of the other, clearly shows that some tissue is lost during sectioning and this tissue may contain malignant cells that can be detected by IC.

The presence of benign nodal nevus inclusions could be a potential source of a false-positive intra-operative diagnosis, especially on FS. However, although present in 12 patients in our series, they were never detected in IC.

The analysis of the 13 false-negative imprint diagnoses in our series showed that in 4 cases metastatic cells were not found in the initial histological sections; therefore, malignant cells were not present in the imprint cut surface. In the remaining false-negative cases, metastatic deposits in the histological sections were most often in the form of micrometastases or ITC, whose detection required a careful comparison of H&E and IHC slides. Their recognition in cytology is problematic, since isolated metastatic melanoma cells may mimic lymph node histiocytes, especially when they contain haemosiderin or melanin pigment. The differentiation between these two cell types is difficult, and it is much safer not to recognise micrometastases than to make too many false-positive diagnoses. Micrometastases and ITC were usually located in the subcapsular sinus, and comparison of imprints and respective histological sections indicated that in several cases this part of the lymph node may not have been imprinted.

Another important consideration in the patients with MM and positive intra-operative SLN diagnosis is the required flexibility in surgical scheduling. In the case of the axillary metastases, this is not a major problem since a one-step, axillary lymph node dissection is not a time-consuming procedure. However, in the case of parotid/neck or groin dissections, scheduling can become a real problem. In contrast, especially in the parotid/neck dissections, a one-step procedure is often safer since it reduces the likelihood of facial or accessory nerve injury.

In conclusion, our results show that, despite its limitations, IC is a valuable method of intra-operative SLN evaluation in patients with MM, especially when in the hands of an experienced and dedicated cytopathologist. It can spare approximately half of the patients with clinically occult regional metastases from a second surgical procedure. However, special care must be taken when handling the SLN to avoid false-positive results due to contamination.

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