

Incomplete Sentinel Node Biopsy Is Not Clearly Related to Survival or Regional Recurrence in Cutaneous Melanoma Patients

Nicholas C. Lee, MBBS¹, Andrew J. Spillane, MD^{1,2,3,4,5}, Tony C. Y. Pang, MBBS, MBiostat¹, Lauren E. Haydu, BSCHE, MIPH^{2,3}, and Roger F. Uren, PhD^{2,6}

¹Royal North Shore Hospital, St. Leonards, Australia; ²Sydney Medical School, The University of Sydney, Sydney, Australia; ³Melanoma Institute Australia, Sydney, Australia; ⁴The Mater Hospital, North Sydney, Australia; ⁵Royal Prince Alfred Hospital, Camperdown, Australia; ⁶Nuclear Medicine and Diagnostic Ultrasound, RPAH Medical Centre, Camperdown, Australia

ABSTRACT

Background. In melanoma patients, we define incomplete sentinel node biopsy (I-SNB) as when fewer lymph nodes are removed during sentinel node biopsy (SNB) than identified on preoperative lymphoscintigraphy (LS). This study quantifies the frequency of I-SNB and evaluates any correlation with patient outcomes.

Methods. Evaluation of a prospective database of consecutive patients having LS and negative SNB from 1996 to 2006. Additional LS information was obtained from a nuclear medicine database. All statistical analyses were performed using the IBM SPSS Statistic 19.0 software package.

Results. I-SNB occurred in 20% of the cohort ($n = 2007$). For axillary ($n = 895$), groin ($n = 569$), and neck/axial patients ($n = 334$) I-SNB occurred in 12%, 26%, and 28% of cases, respectively ($P < .001$). On univariate analysis, there was a significant association between I-SNB and worse disease-free survival (DFS), $P = .007$ and trend toward worse melanoma-specific survival (MSS), $P = .056$. I-SNB was not associated with worse regional recurrence-free survival (RRFS), $P = .144$. There was no relationship between I-SNB and worse DFS, RRFS, or MSS on multivariate analysis. Sentinel node region (axilla better than groin and neck/axial) had a significant association with RRFS ($P = .039$) on univariate analysis and

DFS on univariate ($P = .009$) and multivariate analysis. Significantly worse outcomes for MSS, DFS, and RRFS were seen with male gender, increasing age, high mitotic count, ulceration, and increasing Breslow thickness.

Conclusion. This study demonstrates no statistically significant relationship between I-SNB and patient outcomes when adjusting for known prognostic factors. These data do not exclude the possibility that I-SNB may have a weak association with worse outcomes.

Most specialists consider sentinel node biopsy (SNB) to be a major step forward in the management of melanoma patients since Morton et al. described it nearly 20 years ago.¹ Multicenter Selective Lymphadenectomy Trial (MSLT) results suggest that early removal of involved regional lymph nodes (LNs) identified by SNB leads to improved survival when compared with similar percentage of patients that relapse in the regional LNs at a later date if they have not had a SNB.²

SNB aims to remove and histologically evaluate LNs that receive direct lymphatic drainage from the primary melanoma site. Preoperative lymphoscintigraphy (LS) is important in defining the site of sentinel nodes (SNs) for biopsy as there is lack of predictability of lymphatic drainage, especially primary melanomas on the trunk and head and neck areas.³ By combining LS with intraoperative gamma probe and blue dye, it should be possible to identify all SNs in the majority of patients. In melanoma patients, there is a well-documented false negative rate with SNB that can be attributed in various situations to errors or misinterpretations with regard to lymphatic mapping, surgical technique, or pathological assessment of LNs.⁴

Failure to remove all SNs identified on LS can be termed “incomplete SNB” (I-SNB). The frequency and impact of this has recently been the focus of a study by Richtig et al., who reported I-SNB in 42% of their cases.⁵ However their study was underpowered to show an impact on survival outcomes.⁶ Logically, if the number of SNs biopsied is less than the number identified on LS this may potentially mean missed opportunities to identify metastases. If there is an advantage to earlier removal of involved LNs, as many interpret the MSLT study to indicate, then failure to adequately remove all SN metastases may lead to increased regional recurrence and possibly worse survival outcome for these patients. To date there has been no other substantial literature on the frequency of I-SNB. We aim to test the hypothesis that I-SNB is associated with worse patient outcomes, in terms of regional recurrence and possibly worse survival.

METHODS

A prospective database was used to identify a case series of 2525 consecutive patients who had LS and tumor negative SNB for cutaneous melanoma at the Melanoma Institute Australia (MIA), from January 1996 until December 2006. The follow-up period extended until January 2011. Patients who had multiple primary melanomas or SNB conducted for local recurrence were also excluded ($n = 518$), leaving a final cohort of 2007 patients.

Patient information was retrieved from the MIA Melanoma Research Database (MRD), which contains information systematically recorded and quality assured by MIA data managers regarding patient demographics, tumor characteristics, SN data, regional/distant disease, investigations, follow-up, survival as well as any surgeries performed. LS information was compiled from the database held at the nuclear medicine and ultrasound practice of one of the authors (RU). All SN dissections were performed by MIA surgeons.

The technique for LS used at MIA for this study has been previously described.⁷ In short, this involves the use of a small particle colloid (antimony sulfide) labeled with technetium 99 m. Injection of the isotope was performed on the day of surgery or the day before SNB. After tracer injection, dynamic imaging is used to identify the lymphatic collectors reaching possible SNs. Scanning for the SN in anteroposterior and lateral views is performed using a digital gamma camera with low-energy, super-high-resolution collimator. A delayed scan is performed to examine all regions that would drain the primary melanoma site. A tattoo is used to mark the skin overlying the SN(s). A high-resolution ultrasound examination of the SNs is performed

on some patients after their LS. SPECT/CT is used to assist in localization of SNs from May 2008 onward.

All SNB procedures used peri-tumoral Patent Blue V dye injected 15 minutes prior to surgery to assist with intraoperative SN localization. In addition, a handheld gamma probe (most often the Tyco Navigator) was used. Radioactive counts were performed on all excised nodes with the gamma probe. SNs excised at operation included all “hot” LNs consistent with the LS sites, any other “blue” nodes and any LNs with high radioactive counts comparable to the removed SNs even if they were not preoperatively identified on LS. To emphasize, early dynamic lymphoscintigraphy images are imperative to identifying SNs as getting direct drainage from the melanoma site. These are used to attempt to ensure second-tier nodes are not mistaken for SNs. If there is any ambiguity, a report is sent to the surgeon to check if there is a blue channel bypassing a SN during surgery.

Univariate survival analysis of categorical covariates was carried out using the Kaplan-Meier method together with the log-rank (Mantel-Cox) test to calculate statistical significance. Univariate survival analysis of continuous covariates and multivariate survival analysis was conducted with the Cox Proportional Hazards Model. For all analyses, 2-tailed P values less than .05 were considered statistically significant. All analyses were carried out with the IBM SPSS Statistic 19.0 software package.

For the purposes of this study, regional recurrence free survival (RRFS) was measured from date of SNB to date of recurrence in the regional LN basin or otherwise date of last follow-up. Disease-free survival (DFS) was measured from date of SNB until date of first recurrence or otherwise date of last follow-up. Melanoma-specific survival (MSS) was measured from date of SNB to death from melanoma or date of last follow-up. I-SNB was defined as when the number of LNs removed at surgery was less than the number of nodes noted on the preoperative LS.

RESULTS

The cohort was predominantly male (60%), and mean age was 55.8 years. Mean follow-up period was 54.9 months after SNB with 103 patients (5.1%) lost to follow-up. Most common LN basin biopsied was the axilla. A summary of patient and primary tumor characteristics is provided on Table 1. There were some missing data in the MIA database, which was less than 5% for each patient/tumor characteristic. Combining all the LS data, the median number of LNs identified in one person was 2, range 1–9. The higher number of nodes accounted for in the range is due to drainage to multiple LN fields in the same patient. For example, for melanoma around the nape of the

TABLE 1 Summary of patient clinicopathologic characteristics (*n* = 2007)

Characteristic	<i>N</i>	%
Patient sex		
Female	811	40
Male	1196	60
Lymph node field		
Axilla	895	45
Groin	569	28
Neck	334	17
Other	209	10
Ulceration		
Absent	1550	77
Present	432	22
Missing	25	1
Clark level of invasion		
I	4	0
II	42	2
III	549	27
IV	1219	61
V	138	7
Missing	55	3
Mitotic rate		
Absent	359	18
Present	1623	81
Missing	25	1
Breslow thickness		
0–1 mm	278	14
1.01–2 mm	907	45
2.01–4 mm	570	28
>4 mm	229	11
Missing	23	1
Histological subtype		
Superficial spreading (SSM)	674	34
Nodular (NM)	629	31
Acral lentiginous	24	1
Lentigo maligna	29	1
Desmoplastic	169	8
SSM/NM	104	5
In situ	2	0
Blue Naevus	3	0
Missing	373	19
Completeness of SNB		
Incomplete	403	20
Complete	1604	80
	Median	Mean (range)
Patient age (years)	57.0	55.8 (3.4–93.3)
Follow-up (months)	49.8	54.9 (0–169.7)

neck, lymph can flow to bilateral axillary fields and/or the neck LNs. Melanomas on the head and neck near the midline can drain up to 5 node fields. This pattern of single and multifield lymphatic drainage is shown in Table 2. For axillary SNB the median number of LNs identified was 2; for the neck SNBs the median number was 2; for groin SNBs median of 2; for other nodal areas the median number was 3. In the total assessed cohort 403 patients (20%) had an I-SNB. There was a significant association between I-SNB and the regional LN area the SNB was performed on, with a significantly lower rate of I-SNB occurring in the axilla (12%) than the neck (28%), groin (26%), and other areas (25%) ($\chi^2 = 65.8$, 3 degrees of freedom, $P < .001$).

Median survival was not reached at the conclusion of the study. Univariate analysis results for survival outcomes of interest are shown in Table 3. There was a significant relationship between I-SNB and worse DFS ($P = .007$) with a trend toward worse MSS ($P = .056$) (Fig. 1). I-SNB was not a significant predictor of RRFs ($P = .144$). Significant univariate analysis differences on DFS ($P = .0009$) and RRFs ($P = .039$) were seen with SN location (axilla better than groin and neck/axial) (Fig. 2). There was also a trend toward worse MSS for SN location ($P = .053$). Additional factors that were confirmed to be associated with worse DFS, RRFs, and MSS included male sex, increasing tumor thickness, presence of ulceration, and presence of mitoses. Further univariate results for survival for continuous variables confirmed other known factors associated with worse survival such as older age at time of SNB (DFS: HR = 1.03 [95% CI: 1.02–1.03] $P < .001$;

TABLE 2 Relationship between the number of LNs and LN fields identified on LSG, and the average number of LNs excised during the SNB

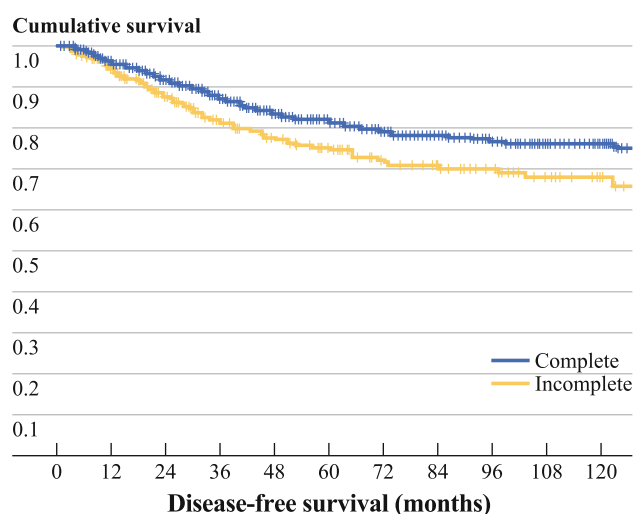
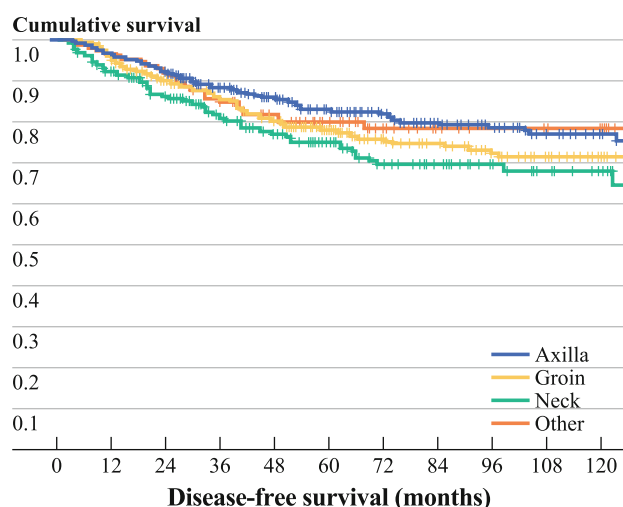
Nodes identified LSG	1-Field LSG		2-Field LSG		3-Field LSG		4-Field LSG	
	Mean nodes excised SNB	<i>N</i>	Mean nodes excised SNB	<i>N</i>	Mean nodes excised SNB	<i>N</i>	Mean nodes excised SNB	<i>N</i>
1	1.4	598	–	–	0	–	0	–
2	2.1	578	2.4	145	–	–	0	–
3	2.8	280	3.3	129	2.6	20	–	–
4	3.4	112	3.7	66	1.7	6	6	2
5	4.5	24	4.2	18	4.2	5	–	–
6	3.5	6	3.4	9	2	1	4	1
7	5	3	–	–	0	–	0	–
8	–	0	8	1	8	1	–	–
9	–	0	9	1	–	0	9	1

TABLE 3 Results of univariate survival analysis of categorical variables

Factor	Melanoma specific		Disease-free		Regional recurrence-free	
	N	5-year survival	N	5-year survival	N	5-year survival
Patient sex						
P value		<.001		.051		.009
Female	780	92.7%	781	–	781	94.5%
Male	1119	84.2%	1123	–	1123	90.3%
Thickness						
P value		<.001		<.001		<.001
0–1 mm	260	97.7%	260	95.4%	260	98.2%
1.01–2 mm	854	93.2%	858	85.5%	858	94.3%
2.01–4 mm	542	81.8%	543	72.0%	543	89.6%
>4 mm	223	71.8%	223	63.2%	223	83.2%
Ulceration						
P value		<.001		<.001		<.001
Absent	1462	91.0%	1466	83.7%	1466	93.7%
Present	415	76.2%	416	67.2%	416	86.4%
Mitoses						
P value		.038		<.001		.036
Absent	335	94.4%	335	90.2%	335	96.2%
Present	1542	86.4%	1547	78.1%	1547	91.3%
Sentinel node field						
P value		.053		.009		.039
Axilla (blue)	836	–	838	83.2%	838	93.7%
Groin (green)	538	–	539	78.1%	539	89.5%
Neck (tan)	324	–	325	75.1%	325	91.9%
Other (purple)	201	–	202	80.0%	202	93.0%
SNB completeness						
P value		.056		.007		.144
Complete	382	–	383	81.2%	383	–
Incomplete	1517	–	1521	75.1%	1521	–

RRFS: HR = 1.02 [95% CI: 1.01–1.03] $P = .002$ and MSS: HR = 1.03 [95% CI: 1.02–1.04] $P < .001$, increasing Breslow thickness (DFS: HR = 1.21 [95% CI: 1.17–1.26] $P < .001$, RRFS: HR = 1.21 [95% CI: 1.14–1.30] $P < .001$ and MSS: HR = 1.25 [95% CI: 1.19–1.32] $P < .001$), and increased mitotic rate (DFS: HR = 1.05 [95% CI: 1.04–1.06] $P < .001$, RRFS: HR = 1.04 [95% CI: 1.03–1.06] $P < .001$ and MSS: HR = 1.04 [95% CI: 1.03–1.06] $P < .001$).

Table 4 shows the results of the Cox regression multivariate analysis for survival. In summary, I-SNB did not remain significant for DFS. SN field (axilla better than others) remained a significant factor predicting DFS, however, not RRFS. All survival outcomes of interest (MSS, DFS, and RRFS) were significantly influenced by

**FIG. 1** Disease-free survival by sentinel node biopsy completeness**FIG. 2** Disease-free survival by sentinel node biopsy field

Breslow thickness, presence of ulceration, and mitotic rate. Age was a significant factor in MSS and DFS but not RRFS. Male sex was only significant for MSS.

DISCUSSION

SNB has changed the way clinicians treat melanoma. It allows LN staging with a more conservative procedure compared with elective LN dissection and gives more prognostic and staging information than observation of regional LNs. The presence of a positive SN has been shown to be the most important prognostic factor for recurrence and survival.^{8,9} There is evidence that melanoma patients with LN metastases have improved survival if regional node dissection is done early on the basis of SN

TABLE 4 Multivariate survival analysis using Cox proportional hazards model

Melanoma-specific survival (<i>N</i> = 1787)	<i>P</i> value	Hazard ratio	95.0% confidence interval	
			Lower	Upper
Male sex	.008	1.556	1.123	2.156
Breslow thickness (mm)	.000	1.193	1.126	1.263
Ulceration present	.000	1.820	1.335	2.481
Age at sentinel node biopsy	.002	1.018	1.007	1.029
Mitotic rate (mitoses per mm ²)	.015	1.024	1.005	1.044
Disease-free survival (<i>N</i> = 1822)	<i>P</i> value	Hazard ratio	95.0% confidence Interval	
			Lower	Upper
Breslow thickness (mm)	.000	1.148	1.095	1.205
Ulceration present	.002	1.492	1.169	1.903
Mitotic rate (mitoses per mm ²)	.000	1.035	1.021	1.048
Age at sentinel node biopsy	.000	1.020	1.012	1.029
Sentinel node field				
Axilla	Reference			
Groin	.020	1.381	1.052	1.812
Neck	.031	1.393	1.031	1.881
Other	.850	1.039	.699	1.543
Regional recurrence-free survival (<i>N</i> = 1823)	<i>P</i> value	Hazard ratio	95.0% confidence interval	
			Lower	Upper
Breslow thickness (mm)	.000	1.160	1.079	1.246
Ulceration present	.001	1.838	1.264	2.672
Mitotic rate (mitoses per mm ²)	.003	1.031	1.011	1.051

status rather than not having a SNB and waiting for regional relapse at a later date.²

To maximize the utility of SNB, the false negative rate has to be kept to a minimum. It is therefore intuitively important to identify and remove all SNs. False negative SNB can stem from multifactorial causes related to nuclear medicine, surgery, and pathology components of the process.⁴ Strategies have been developed to minimize these causes. It is recommended that LS be performed prior to biopsy to reduce the likelihood of false negative SNB.³ However, the reproducibility of LS has been called into question for a variety of reasons. In patients who have undergone LS on 2 separate occasions, the same nodes are not always identified.^{10,11} In a group of 21 patients who had repeat LS prior to surgery it was found that the same SNs identified 94% of the time, and the authors estimate this leads to a SN-containing metastasis missed in >1% of patients subjected to LS and SNB.¹² It is reported that up to 10% of injected radiocolloid traverses SNs and lodges in adjacent non-SNs.¹³ Also, LS may not detect involved SNs because of lymphatic obstruction from metastases.¹⁴ The use of SPECT/CT can show more SNs than planar LS.¹⁵

About 15% of patients with negative SNB have disease recurrence due to lymphatic or hematogenous dissemination that bypasses the SN. Use of the gamma probe intraoperatively has been shown to increase the accuracy of SNB.¹⁶ Gamma probe count is subjective and may be altered with change of probe placement. Despite this, the SN with the highest intraoperative isotope count most often contains metastases.^{8,17} There is a learning curve for surgeons. According to the MSLT data, before SNs are identified with 95% accuracy, surgeons need to perform at least 55 cases.¹⁸ In light of the evidence presented in this study, these MSLT data are interesting, we suspect that this recommendation only refers to the surgeon's ability to remove at least 1 SN from each region identified on LS. The threshold number of cases before doing "complete SNB" in a suitable percentage of cases is yet to be determined.

This study has demonstrated no statistically significant relationship between I-SNB and survival on multivariate analysis. However, despite the size of this study population, it may still be underpowered to demonstrate such a relationship. Metastases are expected to develop in 20% of

intermediate-thickness melanoma.^{8,19} If we consider that there were only 403 cases of I-SNB, one would expect up to 20% ($n = 81$) of these to be positive. Thus a maximum of 81 patients (of 2007) would have their outcomes affected. However, the actual number of patients is likely to be significantly less as often there would be 1 of 2, 2 of 3, or another proportion of the sentinel nodes removed in the I-SNB group. There are also low event rates for the outcomes of interest (DFS, RRFS, and MSS) and relatively short follow-up. Thus, despite the large numbers of patients reported in the MIA database, the power of this study to determine significant outcome differences may be limited. This confirms the futility of the only other study published to date that has looked at this issue.^{5,6}

Another issue is that there is no way to determine with certainty that the LNs identified on LS were definitely removed. The number of LNs on LS compared with surgical yield was used in this study as a surrogate marker of completeness of SNB. This is clearly imperfect for a number of reasons including limitations in the ability of LS to identify all the SNs. LS reports sometimes state that a certain number of definite SNs were identified as well as possible further SNs or second-tier nodes that can be assessed and confirmed at surgery. This leaves doubt about how much dissection should be done to try to confirm whether probable second-tier nodes are really SNs with direct blue dye drainage. A good example is when this situation is described in the groin area where the definite SNs are in the inguinal area but the possible sentinel or second-tier nodes are in the pelvic LNs. To retrieve these pelvic LNs requires a substantial additional surgery for what may be a second-tier node, and hence this may not be done unless there is compelling intraoperative evidence that it is a SN. Another possibility is in difficult procedures, such as in the neck area where there is often a high density of small LNs, that on occasion LNs that were not the true SNs may have been removed, falsely elevating the SN retrieval. Despite these and other subtleties it is important to note that I-SNB was significantly associated with worse DFS on univariate analysis. This relationship, was not statistically significant on multivariate analysis. There was a nonsignificant trend for I-SNB to be associated with worse MSS. These findings may suggest a possible weak association between I-SNB and outcome that has not reached statistical significance because of the factors discussed previously.

The issue of a lower rate of I-SNB in the axilla compared with other areas that SNB is commonly performed is a predictable finding but warrants further discussion. Noteworthy is an improved DFS for axillary SNB patients compared with other regions on the multivariate analysis. Both these findings are also likely to be complex in causation. Issues such as the anatomical complexity of the

neck and deep pelvic anatomy compared with axillary anatomy, the more frequent axillary procedures compared with other areas, internal referral bias of aesthetically sensitive head and neck cases to surgeons who do not perform neck dissections, and internal referral of low leg cases requiring flap reconstruction to surgeons who do not perform pelvic LN dissections. These issues are likely to be a reflection of a differential ability of surgeons to retrieve all LNs and again confound the ability to answer the study question.

Other risk factors that were shown to affect DFS, RRFS, and MSS in this study were male gender, higher mitotic count, increasing Breslow thickness, presence of ulceration, and increasing age. These associations are consistently shown in the literature.^{20,21}

There were some limitations on our study design and data. These included:

- This study question was addressed by combining several data sets; however, the sample size was large.
- The relatively short mean follow-up of 54.9 months, which is reflected by the fact that median survival was not reached during the study period.
- The patient sample was derived from a dedicated melanoma unit that may have a different population than nonspecialist centers. This may affect the external applicability of this study.

In conclusion, this study demonstrates no statistically significant relationship between I-SNB and patient outcomes when adjusting for known prognostic factors. The significant relationship with DFS and trend with MSS seen on the univariate analysis does not exclude the possibility that I-SNB may have a weak association with worse outcome. Factors such as variable rates of I-SNB in different LN fields, which relate to different anatomical accessibility and differences in individual surgeons' surgical skill set, may confound the analysis.

ACKNOWLEDGMENT A.S.'s research is funded in part by a grant from the Friends of the Mater Hospital.

REFERENCES

1. Morton DL, Wen DR, Wong JH, Economou JS, Cagle LA, Storm FK, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. *Arch Surg*. 1992;127:392–9.
2. Morton DL, Thompson JF, Cochran AJ, Mozzillo N, Elashoff R, Essner R, et al. Sentinel-node biopsy or nodal observation in melanoma. *N Engl J Med*. 2006;355:1307–17.
3. Thompson JF, Uren RF. Teaching points on lymphatic mapping for melanoma from the Sydney Melanoma Unit. *Semin Oncol*. 2004;31:349–56.
4. Karim RZ, Scolyer RA, Li W, Yee VS, McKinnon JG, Li LX, et al. False negative sentinel lymph node biopsies in melanoma may result from deficiencies in nuclear medicine, surgery, or pathology. *Ann Surg*. 2008;247:1003–10.

5. Richtig E, Komericki P, Trapp M. Ratio of marked and excised sentinel lymph nodes and scintigraphic appearance time in melanoma patients with negative sentinel lymph node. *Eur J Surg Oncol.* 2010;36:783–8.
6. Lee NC, Spillane AJ. Underpowered conclusions can be potentially misleading with regards to the ratio of number of lymphoscintigraphy identified and surgically excised sentinel nodes in melanoma patients, *Eur J Surg Oncol.* 2011;37:454–5.
7. de Wilt JH, Thompson JF, Uren RF, Ka VS, Scolyer RA, McCarthy WH, et al. Correlation between preoperative lymphoscintigraphy and metastatic nodal disease sites in 362 patients with cutaneous melanomas of the head and neck. *Ann Surg.* 2004;239:544–52.
8. Jacobs IA, Chang CK, DasGupta TK, Salti G. High isotope counts and sentinel node positivity in patients with melanoma. *Arch Surg.* 2003; 138:63–6; discussion 67.
9. Testori A, De Salvo GL, Montesco MC, Trifiro G, Mocellin S, Landi G, et al. Clinical considerations on sentinel node biopsy in melanoma from an Italian multicentric study on 1,313 patients (SOLISM-IMI). *Ann Surg Oncol.* 2009;16:2018–27.
10. Kapteijn BA, Nieweg OE, Valdes Olmos RA, Liem IH, Panday RK, Hoefnagel CA, et al. Reproducibility of lymphoscintigraphy for lymphatic mapping in cutaneous melanoma. *J Nucl Med.* 1996;37:972–5.
11. Valdes Olmos RA, Nieweg OE. Reproducibility of cutaneous lymphoscintigraphy: same or different lymphatic routes and sentinel nodes after reinjection? *J Nucl Med.* 2001;42:430–1.
12. Uren RF, Howman-Giles R, Chung DK, Morton RL, Thompson JF. The reproducibility in routine clinical practice of sentinel lymph node identification by pre-operative lymphoscintigraphy in patients with cutaneous melanoma. *Ann Surg Oncol.* 2007;14:899–905.
13. Nathanson SD, Anaya P, Karvelis KC, Eck L, Havstad S. Sentinel lymph node uptake of two different technetium-labeled radio-colloids. *Ann Surg Oncol.* 1997;4:104–10.
14. Lam TK, Uren RF, Scolyer RA, Quinn MJ, Shannon KF, Thompson JF. False-negative sentinel node biopsy because of obstruction of lymphatics by metastatic melanoma: the value of ultrasound in conjunction with preoperative lymphoscintigraphy. *Melanoma Res.* 2009;19:94–9.
15. van der Ploeg IM, Valdes Olmos RA, Nieweg OE, Rutgers EJ, Kroon BB, Hoefnagel CA. The additional value of SPECT/CT in lymphatic mapping in breast cancer and melanoma. *J Nucl Med.* 2007;48:1756–60.
16. Albertini JJ, Cruse CW, Rapaport D, Wells K, Ross M, DeConti R, et al. Intraoperative radio-lympho-scintigraphy improves sentinel lymph node identification for patients with melanoma. *Ann Surg.* 1996;223:217–24.
17. McMasters KM, Reintgen DS, Ross MI, Wong SL, Gershenwald JE, Krag DN, et al. Sentinel lymph node biopsy for melanoma: how many radioactive nodes should be removed? *Ann Surg Oncol.* 2001;8:192–7.
18. Morton DL, Cochran AJ, Thompson JF, Elashoff R, Essner R, Glass EC, et al. Multicenter Selective Lymphadenectomy Trial Group. Sentinel node biopsy for early-stage melanoma: accuracy and morbidity in MSLT-I, an international multicenter trial. *Ann Surg.* 2005;242:302–11.
19. Amersi F, Morton DL. The role of sentinel lymph node biopsy in the management of melanoma. *Adv Surg.* 2007;41:241–56.
20. Scoggins CR, Ross MI, Reintgen DS, Noyes RD, Goydos JS, Beitsch PD, et al. Gender-related differences in outcome for melanoma patients. *Ann Surg.* 2006;243:693–8; discussion 698–700.
21. Roka F, Kittler H, Cautzig P, Hoeller C, Hinterhuber G, Wolff K, Pehamberger H, Diem E. Sentinel node status in melanoma patients is not predictive for overall survival upon multivariate analysis. *Br J Cancer.* 2005;92:662–7.