Sentinel Node in Malignant Melanoma – The Pathologist's Point of View

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Summary

Overall, the benefit of accurate nodal staging obtained by SLN biopsy far outweighs the risks and has important implications for patient management. That is not to say that this test should be over-utilized. SLN biopsy is an extremely valuable tool to stratify a group of patients in which the standard prognostic factors have failed providing reliable information. Although SLN biopsy can still be considered a relatively "new" diagnostic tool, clear prognostic and therapeutic implications can be drawn when the samples are properly handle. The mRNA detection of melanoma-associated antigens (tyrosinase, MART-1, gp100, and others) remains experimental unless conclusive results on its clinical value are available. Finally, there are four major reasons to perform SLN biopsy. (1) SLN biopsy improves the accuracy of staging and provides valuable prognostic information. The "N classification" distinguishes between macroscopic and microscopic metastases. (2) SLN biopsy facilitates early therapeutic lymph node dissection for those patients with nodal metastases. (3) SLN biopsy identifies patients who are candidates for adjuvant therapy with interferon α-2b. (4) SLN biopsy identifies homogeneous patient populations for entry onto clinical trials of novel adjuvant therapy agents.

Introduction

Despite several efforts in the treatment of malignant melanoma (MM), surgery remains the standard of care. An excision of the primary tumor with prognosis-adapted side margins is recommended worldwide as a basic thera-

peutic approach, whereas the role of elective lymph node dissection (ELND) is still controversial. ELND has not resulted in longer patient's survival in prospective randomized trials and the side effects are relatively frequent. To minimize surgical damage, a selective lymph node evaluation ["sentinel node biopsy" (SLN biopsy), "lymphatic mapping", and "nodal staging"] based on the concept of step-wise metastatic cascade has been proposed.

The term "sentinel node" to describe the first draining lymph node was coined in 1977, the concept of SLN biopsy being widely tried in different tumors such as breast cancer, cutaneous B-cell lymphoma, cancer of the vulva, Merkel cell carcinoma of the skin, squamous cell cancer of the head and neck, pharyngeal and laryngeal carcinomas, and thyroid cancer.

How to Identify Sentinel Nodes. Is the Node Truly Sentinel?

The standard procedure labels the skin around the primary MM, because it is known that a particular site of the skin drains to specific initial lymph nodes (SLNs). The demonstration of SLNs is more efficient by preoperative lymphoscintigraphy and a combination of blue dye with radioactive tracers, such as technetium 99m, which increases the diagnostic accuracy of the SLN identification. Several authors reported successful SLN identification between 90% and 100%, depending on the team experience and the primary tumor location. SLN biopsy in the head and neck area is more difficult due to variations of the lymphatic drainage. Identification rates varied between 70% and 90% in this region of the body.

The SLN technique can also identify negative SLN in cases proven to be positive in non-SLN. Radical surgical excisions of the primary melanoma prior to SLN biopsy could destroy lymphatic channels, resulting in non-reproducible lymphoscintigraphy and false identification. An ideal scenario for reliable identification rates of the SLN are previously untreated melanoma patients. Tumor cell emboli obstructing true SLN will result in derivation of dye and radioactivity to surrounding lymph nodes through intercommunicating channels.

Pathologic Examination of SLNs Sampling

The lymph node is bisected through its longest dimension; both halves are placed cut-face down in cassettes and fixed for a minimum of 24 h, which also result in a significant radioactivity decay. The technician is instructed to minimize "facing up". As soon as full-faced sections can be obtained, serial sections are taken and stained (H&E, S-100, HMB-45, Melan-A).

Although during the development phase of the SLN biopsy technique, frozen sections have been used, fomalin-fixed paraffin embedded tissue sections provide more reliable results. This approach minimizes material loss during "facing up" and allows more accurate interpretations and optimization of immunostaining.

Role of Immunohistochemistry

Conventional histology underestimates by 14% the number of patients with clinically localized MM who have early metastases in regional lymph nodes and 30% tumor-positivity in ostensibly tumor free nodes of patients with node spread MM. The sensitivity of micrometastasis detection increases significantly with the addition of immunohistochemistry. The best results are obtained using a combination of mono- and polyclonal antibodies directed against melanoma-associated antigens (MAA). These antibodies can be used for routine evaluation of paraffin-embedded specimens and improve the diagnostic accuracy for MM.

- Polyclonal antibody to protein S-100 recognizes in the skin melanocytic lesions, neural cells, a subset of antigen presenting cells, and adipocytes. Thus, misinterpretations might occur in the examination of SLNs with S-100 alone.
- Antibodies to HMB-45 (gp 100) show higher specificity for melanoma and are used in parallel to S-100 staining. However, non-melanocytic lesions are HMB-45 positive such as breast carcinomas or angiomyolipoma.
- Melan-A, as a product of the MART-I gene and a melanocytic differentiation antigen and tyrosinase, an enzyme of the melanin production pathway, are alternative staining. Whereas Melan-A demonstrated clean and effective staining, tyrosinase gives weaker staining and higher background.
- MAGE-3 gene protein and reveals a high specificity (100%) but a low sensitivity (44%).

In conclusion, S-100 and HMB-45 remain as standard markers for immunohistology, but Melan-A is an attractive candidate for the detection of melanoma cells in paraffin-embedded tissues.

Molecular Examination of SLNs

A sensitive polymerase chain reaction (PCR)-based technique is able to detect isolated tumor cells. This technique can detect mRNA (after reverse transcription or RT-PCR) of melanocyte markers, but it does not assess criteria of malignancy. In addition, the lack of standardization has resulted

in considering RT-PCR detection of melanocyte markers as "experimental" technique.

The clinical significance of identifying isolated tumor cells is unknown. The relatively high number of RT-PCR positive SLNs (approximately 50%) in patients with MM of more than 0.75 mm is higher than the expected rate of progressive disease (disease relapse is about 30%). This finding highlights the need of validation and, eventually, quantification of molecular techniques that must be carried out under morphological control. This morphological control helps identifying nodal nevus cells, a potential cause of RT-PCR false positive results. The biological meaning of these solitary melanoma cells is also questionable. Since the definition of metastasis includes the capacity for angiogenesis and autonomous growth, the detection of tumor cells alone is unsatisfactory. The term "disseminated tumor cells", instead of "micrometastasis", should be preferred unless the biology of melanoma cells becomes clearer. Thus, interpretation of clinical studies on RT-PCR diagnosis should be considered with some caution. It appears too early to draw therapeutic decisions (i.e., radical lymphadenectomy of the entire basin) on RT-PCR findings alone. However, this experimental tool can significantly contribute to improve the knowledge and understanding of the metastatic cascade.

Different targets have been used. Tyrosinase, as the key enzyme of melanin biosynthesis, is exclusively expressed by melanocytes and their malignant variant, melanoma cells. Tyrosinase RT-PCR positivity (49%) is significantly higher than the number of immunohistochemically positive SLNs (18%). A clear correlation between RT-PCR status and Breslow's tumor thickness has been reported. In a multivariate analysis, histopathology and status of RT-PCR remain the only significant prognostic factors for predicting disease-free survival in observed melanoma patients. They concluded that tyrosinase RT-PCR in SLNs may serve as a powerful tool and furthermore might be of value in future classification systems of primary cutaneous melanoma.

More consistent results were reported from trials focusing on the detection of melanoma-associated antigens through RT-PCR in SLNs. Using standard histopathology, 10 patients (20%) showed positive SLN. All of these nodes were also positive for MART-1 and tyrosinase RT-PCR. Three negative patients based on HE staining examination demonstrated a positive RT-PCR status. The authors concluded that both markers are promising for the detection of occult metastatic disease. The mRNA expression of at least two of three melanoma-associated antigens (MART-1, MAGE-3, and tyrosinase) has been revealed in more than 90% HE/immunohistochemistry positive SLN and in 35% negative SLN by conventional techniques. HE- and immunohistochemical staining underestimate the pres-

ence of melanocytes in SLN and is a less powerful predictor of disease disease relapse thatn molecular diagnosis. However, the overall survival cannot be predicted using this technique.

Minimal Diagnostic Criteria of Positive SLN. Pitfalls

Reliable diagnosis of micrometastases requires strict diagnostic criteria and the recognition of potential pitfalls. The diagnostic criteria must include architectural and cytological features suggestive of malignancy along with evidence of melanocytic differentiation for those malignant cells. Currently, only the pathological diagnosis can fulfill all criteria in a single test. Micrometastases in SLNs show isolated and clustered atypical melanocytes in the subcapsular sinus, occasionally associated with stromal reaction. Melanocyte atypia is revealed by nuclear pleomorphism and hyperchromasia, frequently associated with nucleolus. Although the presence of melanin in malignant cells is variable, immunohistochemical evidence of melanocyte differentiation must be demonstrated in tumor cells (positivity for S-100 and at least one of HMB-45, Melan-A, NKI/C3).

The main diagnostic problems in the interpretation of SLN are the distinction of MM micrometastases from hyperplastic interdigitating dendritic cells, melanin-laden macrophages, and nevus cells. (1) Interdigitating dendritic cells are located in the paracortical area and do not reveal melanocyte-specific markers. These cells are S-100 positive and become hyperplastic in immunosuppressed patients. The location and the absence of nuclear atypia help in the distinction. (2) Melanin-laden macrophages are located in subcapsular and medullary sinuses, show coarse melanin granules and mild nuclear atypia, and expressed CD68 instead of melanocytic markers. (3) Melanocytic nevi are a potential source for false-positive results in the examination of SLN for metastatic melanoma. The incidence varies from 0.3% (lymph node examination in patients with breast carcinomas) to 22% (nodes MM patients), probably reflecting methodological differences. Nodal nevi are located in the peripheral capsule (90%) and in the internal trabecula (10%), do not show cytological atypia, and do not induce stromal reaction. These lesions can also reveal features of blue nevus (pigmented spindle and dendritic cells). Thus, it is important that one is aware about the appearance and morphology of nodal nevi to avoid misinterpretation as melanoma metastasis in SLNs.

Patient Selection for SLN Biopsy

SLN biopsy is appropriate for patients with a significant risk of nodal metastasis, including those with melanomas 1.0 mm Breslow thickness or greater. SLN biopsy for melanomas less than 1.0 mm Breslow thickness

with poor prognostic features (eg, vertical growth phase, ulceration, and Clark's level IV) may be appropriate and requires further investigation. In addition, locoregional cutaneous recurrence appears to be highly predictable in the presence of histopathological signs of lymphatic invasion, including satellites and in-transit metastases. Lymphatic invasion is an important prognostic parameter and should be included as a stratification criterion when selecting patients for SLN biospsy and adjuvant (locoregional) therapy. In contrast, SLN status cannot predict the overall prognosis of patients with thick MM. The long-term survival of patients with T3-T4 MM is similar to the short-term survival of patients with metastatic disease, most likely correlating to the presence of latent metastases.

Prognostic Significance

SLN biopsy is not far away from being accepted as a standard care for melanoma patients. It has been clearly shown that the SLN biopsy status distinguishes melanoma patients with different prognosis (Table 1).

The prognostic significance of SLN biopsy status is complementary with known "conventional" prognostic factors for primary melanoma with regard to recurrences and survival times. Although tumor thickness and ulceration of the primary tumor influenced survival in SLN-negative patients, these markers provided no additional prognostic information in SLN-positive melanoma patients. The 3-year survival rates for SLN-positive patients accounted for 55.8%, in contrast to 88.5% for SLN-negative melanoma patients. Thus, an impressive distinction between patients with favorable and poor outcome is feasible using SLN biopsy. However, results from prospective studies are controversial and still in progress.

The new AJCC staging system differentiates micro- and macrometastases

Table 1 An estimation of prognosis in lymph node-"positive" (approximately) (%) melanoma patients according to the type of detection. ELND *elective lymph node dissection;* SLN biopsy *sentinel node biopsy;* LN *lymph node;* RT-PCR *reverse transcriptase polymerase chain reaction.*

Lymph node status		**Survival rates (%)**
Clinically visible/palpable lymph node metastasis		
Positive LN	1	40
	2-4	25
	5+	10
Lymph node metastasis detected in ELN		45-60
Lymph node metastasis detected in SLN biopsy		
With histology/immunohistology		50
With RT-PCR alone		60

and recognizes the prognostic significance of the tumor burden (microscopic vs. macroscopic metastases) for stage III melanoma patients. For that purpose, SLN biopsy has been proven an essential tool. Other prognostic factors for these patients are closely related and include the number of metastatic lymph nodes and the presence or absence of satellites and intransit metastases.

A selective surgical approach will avoid ELND with the associated damage (i.e., lymph edema) in melanoma patients probably cured. Whether treatment decisions can be drawn from histologic and immunohistologic examination of the SLN is yet unclear. However, SLN biopsy status appears as the most attractive stratification factor for adjuvant treatment modalities. Interferon α-2b is registered for the adjuvant treatment of high-risk melanoma patients in most countries, but only roughly 10% of treated patients may profit from therapy. Interferon α-responding patients cannot be identified so far. Therefore, a careful patient selection for interferon treatment schedules is mandatory. Forthcoming clinical trials in the adjuvant setting will probably include SLN biopsy status as a stratification factor in the study design.

Conclusions

Overall, the benefit of accurate nodal staging obtained by SLN biopsy far outweighs the risks and has important implications for patient management. That is not to say that this test should be over-utilized. SLN biopsy is an extremely valuable tool to stratify a group of patients in which the standard prognostic factors have failed providing reliable information (see patient selection). Although SLN biopsy can still be considered a relatively "new" diagnostic tool, clear prognostic and therapeutic implications can be drawn when the samples are properly handle. The mRNA detection of melanoma-associated antigens (tyrosinase, MART-1, gp100, and others) remains experimental unless conclusive results on its clinical value are available. Finally, there are four major reasons to perform SLN biopsy.

(1) SLN biopsy improves the accuracy of staging and provides valuable prognostic information. The "N classification" distinguishes between macroscopic and microscopic metastases. With the increased use of SLN biopsy as a microscopic staging tool, a substantial number of patients are identified as having clinically occult lymph node disease. However, such sophisticated detection procedures may be incorporated into future staging criteria but are not sufficiently available or standardized to warrant their inclusion at this time. Immunohistochemical staining does help direct pathologists to suspicious areas and does help distinguish melanoma cells from other cell types in a lymph node.

Nevertheless, for the purposes of staging for nodal metastases, there must be histopathologic confirmation using standard hematoxylin and eosin staining.

(2) SLN biopsy facilitates early therapeutic lymph node dissection for those patients with nodal metastases.

(3) SLN biopsy identifies patients who are candidates for adjuvant therapy with interferon a-2b.

(4) SLN biopsy identifies homogeneous patient populations for entry onto clinical trials of novel adjuvant therapy agents.

Selected References

Balch, C. M., A. C. Buzaid, et al. (2001). "Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma." J Clin Oncol 19(16): 3635-48.

Balch, C. M., S. J. Soong, et al. (1996). "Efficacy of an elective regional lymph node dissection of 1 to 4 mm thick melanomas for patients 60 years of age and younger." Ann Surg 224(3): 255-63; discussion 263-6.

Balch, C. M., S. J. Soong, et al. (2001). "Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system." J Clin Oncol 19(16): 3622-34.

Bedrosian, I., M. B. Faries, et al. (2000). "Incidence of sentinel node metastasis in patients with thin primary melanoma (< or = 1 mm) with vertical growth phase." Ann Surg Oncol 7(4): 262-7.

Blaheta, H. J., U. Ellwanger, et al. (2000). "Examination of regional lymph nodes by sentinel node biopsy and molecular analysis provides new staging facilities in primary cutaneous melanoma." J Invest Dermatol 114(4): 637-42.

Blaheta, H. J., B. Schittek, et al. (1999). "Detection of melanoma micrometastasis in sentinel nodes by reverse transcription-polymerase chain reaction correlates with tumor thickness and is predictive of micrometastatic disease in the lymph node basin." Am J Surg Pathol 23(7): 822-8.

Bostick, P. J., D. L. Morton, et al. (1999). "Prognostic significance of occult metastases detected by sentinel lymphadenectomy and reverse transcriptase-polymerase chain reaction in early-stage melanoma patients." J Clin Oncol 17(10): 3238-44.

Brossart, P., U. Keilholz, et al. (1993). "Hematogenous spread of malignant melanoma cells in different stages of disease." J Invest Dermatol 101(6): 887-9.

Carson, K. F., D. R. Wen, et al. (1996). "Nodal nevi and cutaneous melanomas." Am J Surg Pathol 20(7): 834-40.

Cascinelli, N., A. Morabito, et al. (1998). "Immediate or delayed dissection of regional nodes in patients with melanoma of the trunk: a randomised trial. WHO Melanoma Programme." Lancet 351(9105): 793-6.

Cherpelis, B. S., F. Haddad, et al. (2001). "Sentinel lymph node micrometastasis and other histologic factors that predict outcome in patients with thicker melanomas." J Am Acad Dermatol 44(5): 762-6.

Gershenwald, J. E., M. I. Colome, et al. (1998). "Patterns of recurrence following a negative sentinel lymph node biopsy in 243 patients with stage I or II melanoma." J Clin Oncol 16(6): 2253-60.

Gershenwald, J. E., W. Thompson, et al. (1999). "Multi-institutional melanoma lymphatic mapping experience: the prognostic value of sentinel lymph node status in 612 stage I or II melanoma patients." J Clin Oncol 17(3): 976-83.

Hofbauer, G. F., C. Schaefer, et al. (1997). "MAGE-3 immunoreactivity in formalin-fixed, paraffin-embedded primary and metastatic melanoma: frequency and distribution." Am J Pathol 151(6): 1549-53.

Jarrett, P., W. Thomson, et al. (1999). "Immunohistochemistry and sentinel node biopsy in melanoma: the use of tyrosinase and Melan-A." Br J Dermatol 141(2): 361-2.

Lukowsky, A., B. Bellmann, et al. (1999). "Detection of melanoma micrometastases in the sentinel lymph node and in nonsentinel nodes by tyrosinase polymerase chain reaction." J Invest Dermatol 113(4): 554-9.

McMasters, K. M., D. S. Reintgen, et al. (2001). "Sentinel lymph node biopsy for melanoma: controversy despite widespread agreement." J Clin Oncol 19(11): 2851-5.

Sim, F. H., W. F. Taylor, et al. (1986). "Lymphadenectomy in the management of stage I malignant melanoma: a prospective randomized study." Mayo Clin Proc 61(9): 697-705.

Veronesi, U., J. Adamus, et al. (1977). "Inefficacy of immediate node dissection in stage 1 melanoma of the limbs." N Engl J Med 297(12): 627-30.

Wick, M. R., P. E. Swanson, et al. (1988). "Recognition of malignant melanoma by monoclonal antibody HMB-45. An immunohistochemical study of 200 paraffin-embedded cutaneous tumors." J Cutan Pathol 15(4): 201-7.

Sentinel Node (SLN) in Malignant Melanoma. The Pathologist's View

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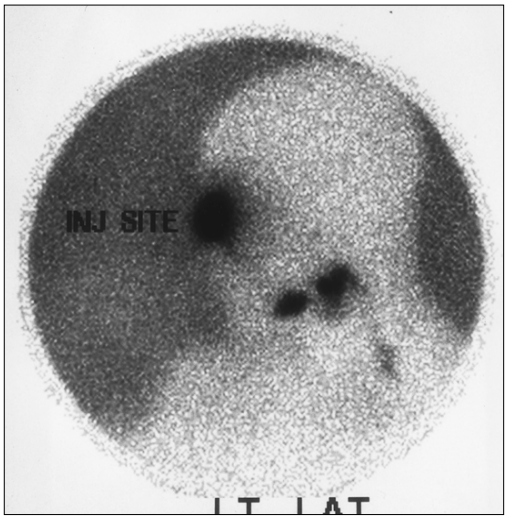
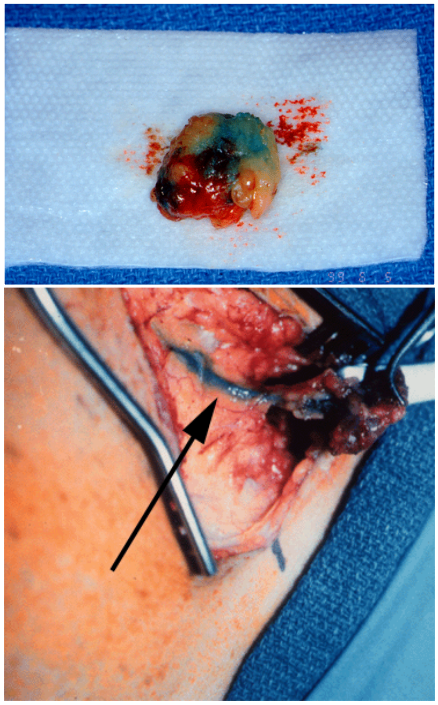
SLN Biopsy in MM

- **Identification of SLN**
- **Pathologic examination**
- **Indications**
- **Prognostic implications**

SLN Identification





SLN Biopsy in MM Histopathology Processing

- **Proper fixation**
- **Bisection and complete embedding**
- **Serial sectioning (20) and review of the first 10**
  - **H&E (alternate sections), S-100, HMB45, Melan-A, CD68, CD31**
- **Chromogen Selection**

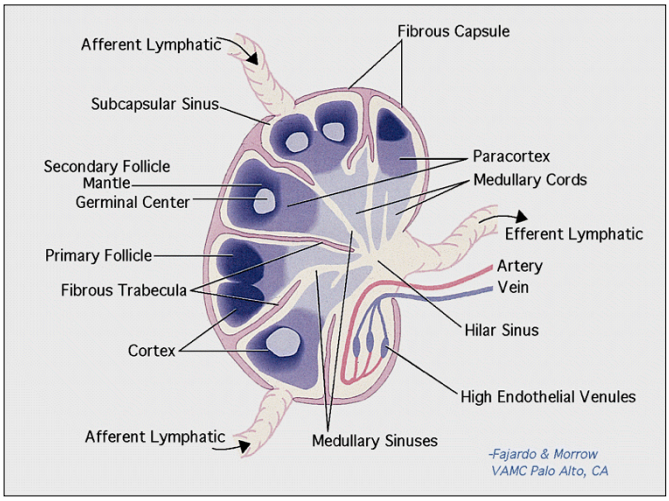
SLN Biopsy in MM Examination Techniques

- Histopathology and immunohistochemistry
- Molecular analysis: reverse transcription-polymerase chain reaction (RT-PCR)

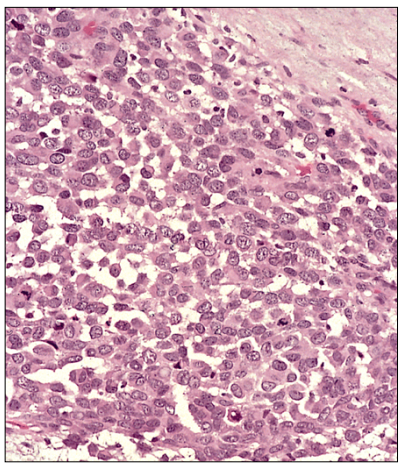
SLN Biopsy in MM Criteria of Positivity

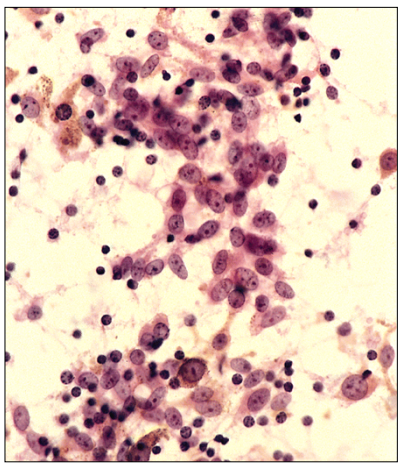
- Preferentially subcapsular sinus
- Cohesively noncohesive clusters
- Cytologic criteria of malignancy
  - Hyperchromatism
  - Nuclear pleomorphism ± pseudoinclusions
  - Prominent nucleoli
- Evidence of melanocyte differentiation

Lymph Node Architecture

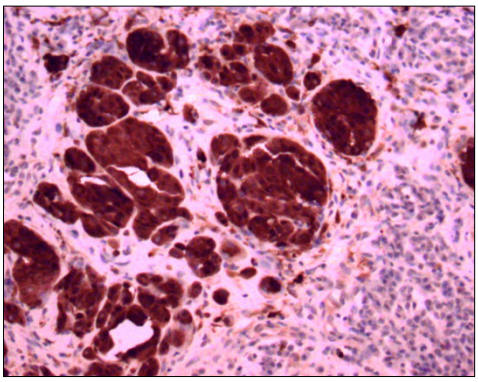


Lymph Node Metastasis of MM Criteria of Malignancy

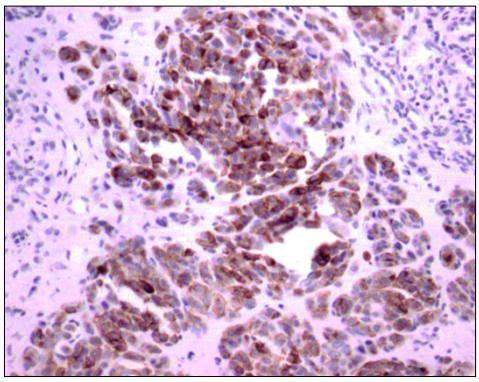




SLN Biopsy in MM
Melanocyte Differentiation

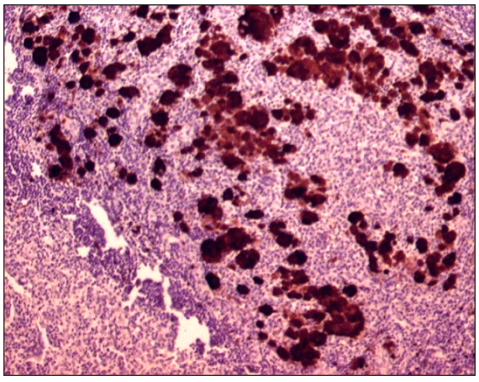


S-100

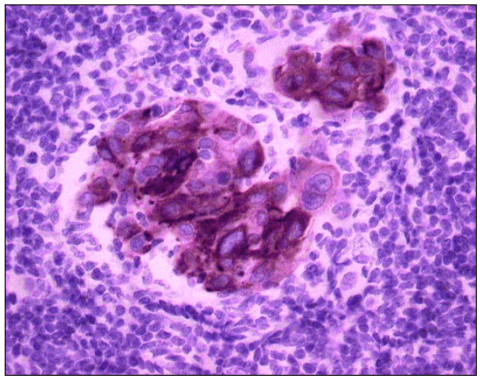


HMB45

SLN Biopsy in MM
Melanocyte Differentiation



S-100



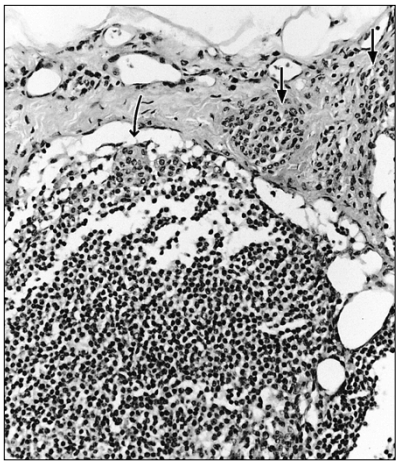
Melan-A

SLN Biopsy in MM Immunohistochemical Markers

Antigen/marker	Sensitivity	Specificity
S-100	+++	Moderate
HMB-45	++	Moderate
NKI/C3	+++	Moderate
MART 1/Melan-A	++	High
Tyrosinase	++	High
MAGE3	+	High

SLN Biopsy in MM Pitfalls

- **Capsular nevus cell aggregates**
- **Blue nevus**
- **Melanin-laden macrophages**



SLN Biopsy in MM Pitfalls

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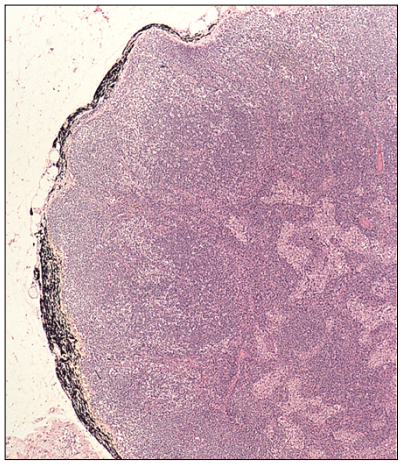

SLN Biopsy in MM Pitfalls

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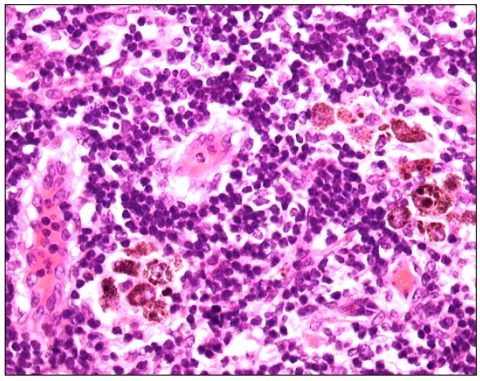
- **Melanin-laden macrophages**



SLN Biopsy in MM Molecular Processing

RT-PCR analysis

- RNA extraction. RNase-free conditions
- Reverse transcription to generate cDNA
- Amplification of the specific target

SLN Biopsy in MM Molecular Analysis

- RT-PCR positive SLNs for tyrosinase (49%) outnumber immunohistochemically positive SLNs (18%)
- Results correlate with Breslow thickness.
- MAA RT-PCR positivity demonstrated in
  - 90% H&E-IHC positive SLN
  - 35% H&E-IHC negative SLN

SLN Biopsy in MM Examination Methods

	Histological	Molecular
Sensitivity	++	++++
Specificity	++++	+
False Positive	Very rare	Rare
False Negative	Rare	Very rare
Mel. Different.	Tested	Tested
Malign.Criteria	Tested	Non tested

Significance of Isolated Tumor Cells in SLN Biopsy

- **Clinically**
  - **Discrepancy between rate of positive SLN (50%) and rate of disease relapse (30%)**
- **Biologically**
  - **? First stage during dissemination or first defensive line**
  - **Importance of tumor burden and neoangiogenesis**

SLN Biopsy in MM Molecular Analysis

- **Highly sensitive method**
- **No specific genetic marker of malignant melanocytes is available**
- **Markers of melanocyte differentiation (gene expression)**
  - **Detection of Tyrosinase and MAA are good options**
- **Absolute need of morphologic validation!!**

SLN Status and Survival

MM Trial/group	Location/ Breslow	Improved Survival
WHO trial #1	Extremity/ Any Breslow	No
Mayo Clinic surgical trial	Mostly extremity/ Any Breslow	No
Intergroup surgical trial	All sites/ 1.5-4.0 mm	Patients < 60 years
WHO trial #14	Trunk/ > 1.5 mm	MM 1.5-4.0 mm

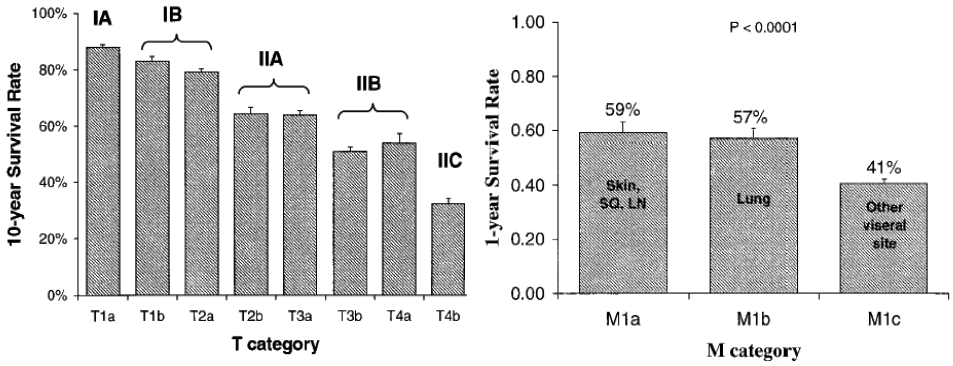
LN Status (%) Survival

Clinically visible/palpable lymph node metastasis		
Positive LN	1	40
	2-4	25
	5+	10
Lymph node metastasis detected in ELN		45-60
Lymph node metastasis detected in SLN biopsy		
With histology/immunohistology		50
With RT-PCR alone		60

Hematogeneous Spread of MM

- **Tyrosinase mRNA analysis of peripheral blood of MM patients demonstrate circulating tumor cells in:**
  - **1/10 patients with localized disease**
  - **6/17 patients with regional lymph nodes metastases**
  - **all 29 patients with distant metastases**

T Categories and M Categories



SLN Biopsy in MM Indications

- **Malignant melanomas Breslow < 1mm**
  - **Ulceration**
  - **Clark level IV**
  - **Vertical growth phase**
- **Malignant melanomas Breslow ≥ 1mm**
  - **Clark level III**

Sentinel Node in MM Unanswered Questions

- Importance of tumor burden in prognosis and patient stratification
- Role of vascularization of tumor deposits in MM progression and patient prognosis

SLN Biopsy in MM

- **Reasons to perform:**
  - Improves staging accuracy and provides prognostic information.
  - Facilitates early LN dissection in stage III MM
  - Identifies patients for adjuvant therapy
  - Identifies patients for entry onto clinical trials
- **Minimal requirements:**
  - Malignancy and melanocyte differentiation are the minimal criteria of positivity
  - At present, morphological validation is required to avoid false positive and increase the specificity