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REVIEW ARTICLE

The Role of Sentinel Lymph Node Biopsy in the Diagnosis and Prognosis of Malignant Melanoma

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Abstract. Sentinel lymph node biopsy was introduced into the management of cancer patients 20 years ago. Most hospitals now currently use the technique as a routine diagnostic tool in patients with localized malignant melanoma. However, the technique is complex and numerous details need to be determined and assessed to provide reliable diagnostic and prognostic information. In addition, the introduction of immunohistochemical and molecular techniques in the last decade has extended the information provided by the study of sentinel lymph nodes and created valuable opportunities for investigating the pathogenesis of this type of cancer. The aim of this review is to offer the reader a detailed analysis of the most important studies in the literature and the factors that should currently be considered in determining the indication for sentinel lymph node biopsy, performing the procedure correctly, and interpreting the findings in patients with malignant melanoma.

Key words: melanoma, prognosis, sentinel lymph node.

LA BIOPSIA DEL GANGLIO CENTINELA: SU PAPEL DIAGNÓSTICO Y PRONÓSTICO EN EL MELANOMA MALIGNO

Resumen. La biopsia del ganglio centinela (BGC) en el manejo del paciente oncológico fue introducida hace casi 20 años y actualmente es ya considerada, en la mayoría de los centros hospitalarios, como una herramienta de diagnóstico de rutina para los pacientes con melanoma maligno (MM) localizado. Sin embargo la técnica es compleja, con numerosos detalles a conocer y a evaluar, ya que de ellos depende su fiabilidad diagnóstica y pronóstica. Además, en la última década, y gracias a la aplicación de técnicas inmunohistoquímicas y moleculares, la información que podemos obtener del estudio del ganglio centinela es cada vez más amplia, ofreciendo un interesante campo para la investigación de la patogenia del cáncer. Esta revisión tiene como objetivo ofrecer al lector un detallado análisis de aquellos factores y series publicadas más importantes a tener en cuenta hoy en día para la correcta indicación, realización e interpretación de los resultados de la BGC en los pacientes con MM.

Palabras clave: melanoma, pronóstico, ganglio centinela.

The Importance of Prognostic Factors in the Staging of Melanoma

The biologic behavior of malignant melanoma is determined by the interaction of a series of factors that influence patient prognosis and, as a result, therapeutic management. A large number of studies into this complex tumor have focussed and continue to focus on identifying and understanding the nature of these factors. Nevertheless, despite extensive efforts to identify independent prognostic factors for malignant melanoma, no molecular, chromosomal, immunohistochemical, or histopathologic marker for the primary tumor that accurately predicts its behavior has yet been identified.[1]

According to the sixth classification of the American Joint Committee on Cancer (AJCC) (Table 1), the prognosis of a patient diagnosed with malignant melanoma will largely depend on 2 factors: the thickness of the primary tumor, measured in micrometers according to the method described by Breslow,[2] and the presence or absence of metastasis in the regional lymph nodes. Without a doubt, the acceptance of sentinel lymph node biopsy as the most accurate diagnostic

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Table 1. American Joint Committee on Cancer 2002 Classification and Survival at 5 and 10 Yearsa

Clinical Stage				*Pathologic Stage*				*5-Year Survival, %*	*10-Year Survival, %*
	T	*N*	*M*		*T*	*N*	*M*		
0	Tis	N0	M0	0	Tis	N0	M0	99	95
IA	T1[a]	N0	M0	IA	T1[a]	N0	M0	95	90
IB	T1[b]	N0	M0	IB	T1[b]	N0	M0	91	85
	T2[a]	N0	M0		T2[a]	N0	M0	89	80
IIA	T2[b]	N0	M0	IIA	T2[b]	N0	M0	77	65
	T3[a]	N0	M0		T3[a]	N0	M0	79	65
IIB	T3[b]	N0	M0	IIB	T3[b]	N0	M0	63	50
	T4[a]	N0	M0		T4[a]	N0	M0	67	55
IIC	T4[b]	N0	M0	IIC	T4[b]	N0	M0	45	35
III	TX	NX	N0	IIIA	T1-4[a]	N1[a]	M0	67	60[b]
					T1-4[a]	N2[a]	M0	67	
				IIIB	T1-4[b]	N1[a]	M0	52	40[b]
					T14[b]	N2[a]	M0	52	
					T1-4[a]	N1[b]	M0	54	
					T1-4[a]	N2[b]	M0	54	
					T1-4[a]	N2[c]	M0	54	
					T1-4[b]	N2[c]	M0	52	
				IIIC	T1-4[b]	N1[b]	M0	24	20[b]
					T1-4[b]	N2[b]	M0	24	
					TX	N3	M0	28	
IV	TX	NX	MX		TX	NX	MX	10[b]	5[b]

Abbreviations: M, metastasis; N, node; T, tumor.
[a]Adapted from Balch et al.3
[b]Approximate survival data taken from the curves shown by Balch et al.[3]

procedure to determine the histologic stage of the regional lymph nodes in the current staging system marked a turning point in the management of patients with malignant melanoma. As a consequence, the pathologic stage of the sentinel node became the most important independent prognostic factor in terms of overall survival, and it has been widely used to harmonize criteria and results among different working groups.[3]

Nevertheless, in recent years, the current classification system proposed by the AJCC has been criticized for its complexity and the absence of a consistent correlation between stage and prognosis. For instance, the introduction of sentinel lymph node biopsy has led to some patients who were previously considered stage II (localized disease) to be included in stage IIIA. As a result, the prognosis for patients in stage III covers a large spectrum of disease-free survival at 5 years, ranging from 13% to 69%, making it difficult to accept the stage as a homogeneous group that should be considered equally.[4-7]

In addition, according to the current classification, a patient with stage IIC disease has a worse prognosis than one with stage IIIA or IIIB disease, and the prognosis for patients in stage IIB is equivalent to that for patients in stage IIIA. Logically, it would be expected that a patient with stage N0 disease (no regional lymph node metastasis, stages I and II in the classification) would have a more favorable prognosis that a patient with N1 or N2 disease (stage III). This suggests that there must be other prognostic factors that would help to better classify patients within both N0 and N1. Some such factors have been shown to have prognostic potential in various independent studies and should be taken into consideration for future

modifications of the classification. These include mitotic index,[8,9] expression of certain markers in the primary tumor[1,10] or in the blood,[1] or even such recognizable factors as the age and sex of the patients.[11] Others are currently being investigated, such as the use of microarray technology in the primary tumor or molecular studies of sentinal lymph nodes[12,13]; the latter will be reviewed here.

The Technique of Sentinel Lymph Node Biopsy in Melanoma

History and Development

The concept that lymph from a given area of the body drains directly into a lymph node before passing through other nodes was introduced by Virchow in the middle of the 19th century. However, in 1923, Braithwaite was the first to coin the term *sentinel* to refer to those nodes that received direct lymphatic drainage.[5] The application of this concept to surgical oncology began in 1960 at the hands of Gould and Cabanas, who published studies on the drainage of parotic and penile carcinoma, respectively, although without performing a lymphographic study to determine exactly which were the sentinel nodes in each patient.[14,15] But without doubt, the study published in 1992 by Morton et al[16] from the John Wayne Cancer Institute marked a turning point in the recognition of the potential use of sentinel node biopsy in surgical oncology.

Those authors demonstrated the feasibility of lymphatic mapping for the identification of sentinel lymph nodes in a feline model before going on to validate the results in a series of patients with malignant melanoma. In that study, the authors described a new technique that allowed identification of sentinel lymph nodes during surgery in patients with malignant melanoma using only blue stain as a marker and defined the sentinel node as the one closest to the site of the primary skin tumor that received direct lymphatic drainage. They also showed that the sentinel lymph node was the most likely to contain metastatic cells and that excision and intraoperative study of the sentinel node allowed accurate identification of the metastasis. In this way, they identified the patients (those with metastasis in the sentinel node) in whom complete lymphadenectomy should be performed. Using this technique, the authors successfully identified the sentinel lymph node in 194 out of 237 lymphatic basins (81.8%) and detected metastasis in 40 of them (20.6%).[16]

Following the study of Morton et al, the hypothesis that the histologic stage of the sentinel lymph node assessed by sentinel node biopsy reflected the condition of the other nodes in that lymph node station was confirmed by numerous studies, notably by Reintgen et al[17] in the United States and Thompson et al[18] in Australia. To validate the hypothesis, those studies included sentinel lymph node biopsy along with immediate complete lymphadenectomy in all cases, such that all the nodes in the station were examined. The different case series of patients analyzed in that way yielded results that coincided with the findings of Morton and colleagues, identifying around 20% of patients with occult metastasis (positive sentinel lymph node). The large majority of those were found to be limited to the nodes identified as sentinel nodes, indicating a failure rate for the technique (negative sentinel lymph node with another node positive for metastasis) of 1% to 2%.[17-19]

Based on those studies, the technique of sentinel lymph node biopsy has been perfected and standardized, and it is currently used routinely for the staging of malignant melanoma and breast cancer. It is also increasingly used in other solid tumors such as lung cancer, head and neck cancer, colon carcinoma, esophageal cancer, and other skin tumors such as squamous cell carcinoma or Merkel cell tumors.[20-23] In all these types of tumor, it is now widely accepted that the most important prognostic factor is the presence or absence of regional lymph node metastasis and that the technique of sentinel lymph node biopsy is the best tool for staging of those nodes, allowing lymphadenectomy to be performed selectively, in other words, only in those patients with sentinel nodes positive for metastasis.[3]

Current Debate Over Sentinel Lymph Node Biopsy: From Elective to Selective Lymphadenectomy

Since the publication of the study by Morton et al, and particularly in recent years, some authors have begun to dispute the use of sentinel lymph node biopsy as a standard technique for the management of patients with localized malignant melanoma.[24-26] The main argument against the routine use of the technique is that elective or prophylactic lymphadenectomy—offered to all patients with localized malignant melanoma—has not led to an increase in survival compared with therapeutic or delayed lymphadenectomy—reserved only for those patients with clinically palpable lymph node metastases—in the various studies designed to compare these approaches. It might therefore be expected that sentinel lymph node biopsy and subsequent selective lymphadenectomy also lack beneficial effects for the patient.

Table 2 summarizes the studies—2 from the World Health Organization, 1 from the Mayo Clinic, and 1 conducted by the Intergroup Melanoma Surgical Program, along with a metaanalysis of those 4 studies—showing the absence of benefit with elective lymphadenectomy.[27-30] However, a more detailed analysis of those studies suggests that certain subgroups of patients do in fact benefit to some extent from elective lymphadenectomy.

Table 2. Randomized Trials of Elective Lymphadenectomy and Therapeutic Lymphadenectomy Published to Date

Trial	*Authors*	*Starting Date*	*No. of Patients*	*Site*	*Breslow Depth*	*P*	*Follow-up, y*
WHO Melanoma Group							
No. 1	Veronesi et al[28]	1967	553	Limbs	Any	NS	10
No. 14	Cascinelli et al[27]	1982	227	Trunk	> 1.5 mm	.09a	11
Mayo Clinic Surgical Trial	Sim et al[29]	1972	171	Limbs	Any	.9a	4.5
Intergroup Melanoma Surgical Trial	Balch et al[30]	1983	737	All	1-4 mm	.11a	7.4

Abbreviation: WHO, World Health Organization.
aIn some subgroups there was a benefit of elective lymphadenectomy compared with therapeutic lymphadenectomy.

Table 3. Results and Objectives of the Multicenter Selective Lymphadenectomy Trial-I[a]

Primary Objective	*P*
Do SLNB and ICL increase overall survival compared with TL?	No
Secondary Objectives	
Do SLNB and SL increase disease-free survival compared with TL?	Yes P=.0065
Does the pathologic stage of the sentinel node have independent prognostic value?	Yes P=.0001
Do SLNM and SL identify those occult metastases that would develop into a palpable metastasisin the observation group?	Yes 19.8% vs 20.3%
For patients with positive sentinel nodes, does SL prolong overall survival compared with patients with clinically palpable metastasis who receive TL?	Yes P=.0034

Abbreviations: SLNB, sentinel lymph node biopsy; ICL, immediate complete lymphadenectomy; SL, selective lymphadenectomy; TL, therapeutic lymphadenectomy.
[a]Adapted from Morton et al.[32]

The defenders of the usefulness of sentinel lymph node biopsy argue that the conclusions of those studies regarding the benefit of elective lymphadenectomy cannot be applied to sentinel node biopsy, since elective and selective lymphadenectomy are not completely comparable. Elective lymphadenectomy without preoperative lymphoscintigraphy is a blind procedure that in some cases involves excision and analysis of lymph nodes that do not receive drainage from the primary tumor. It has been shown that around a third of malignant melanomas (particularly those located on the head and neck or trunk) drain into unexpected lymphatic basins or exhibit drainage into interval or aberrant nodes that are impossible to localize without lymphoscintigraphy.[31] Furthermore, sentinel node biopsy allows the pathologist to focus on a restricted number of lymph nodes, thus allowing a much more detailed analysis. In addition, elective lymphadenectomy is associated with high morbidity, indeed much higher than that of sentinel node biopsy.

The clearest argument in favor of the usefulness of sentinel lymph node biopsy in patients with malignant melanoma, however, comes from those who developed the technique. They designed the Multicenter Selective Lymphadenectomy Trial-I (MSLT-I) with the main objective to determine whether sentinel lymph node biopsy offers benefits in terms of survival in patients with localized malignant melanoma in which the Breslow depth is at least 1 mm or the Clark level ≥ IV. Table 3 shows the results according to objective for the first analysis of that study, which was published recently.[32] Survival was compared between 2 randomized groups of patients: sentinel lymph node biopsy plus immediate lymphadenectomy when the sentinel node was positive or observation plus therapeutic lymphadenectomy when clinically palpable lymph nodes appeared. A total of 18 centers in Europe, the United States, and Australia participated in the study, which involved 2001 patients, of whom 1973 were eligible for inclusion. The response to the question addressed in the primary objective (after a median follow-up of 59.5 months) was that sentinel lymph node biopsy clearly failed to improve survival compared with observation (overall survival of 87.1% and 86%, respectively; $P = .4$), although if survival was compared only between those patients with a positive sentinel node after biopsy followed immediately by lymphadenectomy and those patients who suffered recurrence and then underwent delayed lymphadenectomy, the differences were statistically significant (overall survival of 69.8% and 57.2%, respectively; $P=.01$). However, that analysis has been widely criticized, since from a statistical point of view it is not entirely clear that those 2 groups are comparable.

What is clear from this multicenter study is the predictive value of the histologic stage of the sentinel lymph node, since disease-free survival was better for the group treated with selective lymphadenectomy following sentinel node

biopsy (disease-free survival at 5 years of 78.5% compared with 73%; *P*=.006 by log-rank test) and the pathologic stage of the sentinel node was the most important independent prognostic factor (relative risk, 2.66; 95% confidence interval, 1.90-3.72). In addition, another interpretation of these results suggested by Morton was that sentinel node biopsy correctly identified those patients with occult metastasis, who would have developed clinically palpable lymph node metastases had they been in the observation group. This claim is based on the observation that, firstly, the percentage of patients with a positive sentinel lymph node following biopsy and the percentage of patients who developed lymph node metastasis in the observation group was very similar (19.8% vs 20.3%), and secondly, that the mean number of positive nodes per patient increased from 1.6 after sentinel node biopsy to 3.4 after therapeutic lymphadenectomy in the observation group.

Following the publication of these detailed results from the MSLT-I study, most authors and centers specialized in the management of patients with malignant melanoma feel that it is currently appropriate to perform sentinel lymph node biopsy in those patients with malignant melanoma who meet the criteria, so long as the patient is always informed of the risks and benefits of the technique and of how the information obtained will influence the management of their disease.

In addition, we should remember that, to date, sentinel lymph node biopsy has been performed for diagnostic rather than therapeutic purposes. Therefore, the apparent lack of benefit in terms of overall survival would be more related to the limited therapeutic options that are currently available, meaning that earlier diagnosis does not always translate into more effective treatment.

For the technique of sentinel lymph node biopsy to be performed correctly there must be cooperation among members of a multidisciplinary team that includes a dermatologist/oncologist who is able to determine whether the test is indicated or not, a surgeon, a radiologist specialized in nuclear medicine, and a pathologist. Each member of the team will be involved in 1 of the 5 steps in the technique that we will now describe: selection of patients, preoperative lymphatic mapping, intraoperative identification and excision of the sentinel node, microscopy and, occasionally, molecular analysis of the sentinel node, and early radical lymphadenectomy in those patients with a positive sentinel node.

Criteria for Selection of Patients Amenable to Sentinel Lymph Node Biopsy

As in any diagnostic or therapeutic intervention, it is important to carefully select patients in order to optimize the results. The recommended criteria for selection of patients with malignant melanoma who are amenable to sentinel lymph node biopsy are constantly being revised, largely because the staging of melanoma itself is also changing. In general, sentinel node biopsy should be recommended in all patients with primary malignant melanoma without evidence of local or distant metastasis and in whom the estimated risk of lymph node metastasis is at least 10% (clinical stages IB and IIA, IIB, and IIC of the sixth AJCC classification[33]). The risk of finding a positive sentinel node is correlated with a number of known factors associated with the primary tumor, such as thickness (measured by Breslow depth or Clark level) and the presence or absence of ulceration. Based on these factors and the current prognostic stratification for malignant melanoma published by the AJCC,[3] sentinel node biopsy would appear to be clearly justified and accepted in those patients with a localized primary malignant melanoma with a Breslow depth of at least 1 mm or those cases which, irrespective of thickness, have a Clark level of IV-V or ulceration.

For the moment, in tumors with a Breslow depth of less than 1 mm, the indication for sentinel node biopsy is less well accepted based on markers of the aggressiveness of the tumor such as a Breslow depth between 0.75 and 1 mm, the presence of regression, high mitotic index, vertical growth phase, or expression of certain genes.[1,5] These other possible criteria are not included in the AJCC classification or are difficult to standardize, particularly the presence of regression, for which the studies performed have yielded conflicting results.[5,33-35] Some authors have shown that thin tumors with complete regression (signs of regression in an area of the tumor in which melanoma cells are not identified) affecting more than 50% of the invasive malignant melanoma are correlated with a more aggressive course, and therefore, that sentinel node biopsy could be indicated in these cases.[35]

On the other hand, before performing the test, we should rule out a series of factors that could alter lymphatic drainage in the region, with the result that the sentinel node identified is not to the true sentinel node: primary tumor excised with wide margins (>1 cm), reconstruction with grafts or flaps, tumors with previous surgery or radiotherapy in the lymph node station to be examined, and those with acute infection of the surgical wound from simple resection of the tumor. Another factor to be taken into consideration is the site of the primary tumor, since certain areas such as the head and neck present particular difficulties for sentinel node biopsy, either due to difficulty locating the focal radioactivity when the malignant melanoma drains into the parotid gland or the difficulty of excising a sentinel node within the parotid due to the risk of damaging vital structures such as the facial nerve.[31] In addition, the general condition of the patient should also be assessed in all cases, along with age, quality of life, and associated surgical risk.[5,12]

Table 4. Most Commonly Used Sampling Protocols and Staining Methods

Authors	*No. of Patients*	*Primary Tumor, Breslow Depth*	*Processing Technique for the*	*Sectioning Level*
Spanknebel et al[46] (RPA)	49	3 mm, 27%	Bisection through the hilum	1 level
Spanknebel et al[46] (EPA)	49	3 mm, 27%	Bisection through the hilum	20 levels every 50 µm
Cook et al[42] (protocol 1)	416	2.03 mm, 25%	Bisection through the hilum	No
Cook et al[42] (protocol 2)	103	2.16 mm, 20%	Bisection through the hilum	2 levels every 50 µm
Cook et al[42] (protocol 3)	74	1.77 mm, 13%	Bisection through the hilum	5 levels every 50 µm
Abrahansen et al[39]	100	1.56 mm, 23%	Bisection through the hilum	Every 250 µm (entire lymph node)
Bostick et al,[41] Takeuchi et al[68]	72	1.8 mm, NA	Bisection through the hilum	80 µm frozen + 3 levels every 40 µm
Starz et al[44]	96	NA	Parallel to the long axis	1 mm with a scalpel
Li et al[43]	1152	2.1 mm, NA	Bisection through the hilum	1 level
Rimoldi et al[45]	57	1.9 mm, NA	Parallel to the short axis	2-3 mm with a scalpel

Abbreviations: EPA, exhaustive pathologic analysis; SLN, sentinel lymph node; H&E, hematoxylin-eosin; NA, not available; RPA, routine pathologic analysis.

Finally, some authors also advocate the use of sentinel node biopsy in the management of some melanocytic lesions with unclear behavior, such as atypical Spitz tumors.[36]

Lymphatic Mapping and Intraoperative Detection of Sentinel Lymph Nodes

Although in initial studies only blue stain was used to identify the direct afferent trajectory to the regional lymph node station, it was soon found that preoperative mapping was more accurate when blue stain was combined with preoperative lymphoscintigraphy and the intraoperative use of a gamma ray detector.[37] In addition, the presence of multiple sentinel nodes in each node station, aberrant sentinel nodes (outside conventional lymph node stations), or in-transit sentinel nodes (in intermediate territories, such as in the poplitea or cubital region) were also not known with certainty before the introduction of lymphoscintigraphy.[5,12,19,31]

With the combination of preoperative injection of a colloid labeled with technetium-99m (^{99m}Tc) and intraoperative injection of blue dye, most authors report excellent rates of sentinel node identification (around 98% to 100%), indicating that at least 1 sentinel lymph node has been identified in almost all patients. This does not guarantee, however, that all the sentinel nodes for that patient have been excised. It is not very clear how many nodes should be considered as true sentinel nodes, but it seems that the number is greater than initially thought. Furthermore, there is still no clear agreement regarding the exact definition of a sentinel node on the basis of radioactivity. Accumulated experience shows that a sentinel node is not necessarily the one closest to the tumor (as initially suggested by Morton and colleagues[16]), nor is it the first to appear in the early lymphoscintigraphy image. Not all nodes that contain radioactivity are sentinels, nor are all sentinel nodes radioactive. Likewise, not all blue nodes (stained following injection of blue dye) are sentinel nodes, nor all sentinel nodes blue. The best-accepted definition of a sentinel node is one with in vivo radioactivity counts at least twice that of the background in the surgical field and with ex vivo counts at least 10 times higher than background.[12] The Sunbelt Melanoma Trial Group defined the "10% rule," which proposes that any blue node or any node that displays a radioactivity count at least 10% of that displayed by the node that captures the highest amount of radioactivity be considered a sentinel node.[38] This definition reduces the likelihood of missing a sentinel node and does not lead to an excessive increase in the number of nodes that are excised. In addition, it is the same as the criteria used for sentinel node biopsy in breast cancer.

Pathology Assessment of Sentinel Lymph Nodes

Sampling Techniques and the Introduction of Immunohistochemistry

The introduction of sentinel node biopsy provided the perfect opportunity for rapid and accurate pathologic staging of the lymph nodes. Nevertheless, current recommendations for histopathologic analysis of sentinel nodes are a long way from being rapid or easily standardized. Since a complete

No. of Sections	*Staining*	*Positive Patients, %*	*Patients With Nevus in the SLN*
1 frozen and 1 paraffin embedded	H&E	20%	0%
60	H&E, anti-S100, anti-HMB45	61%	3% (SLN)
8	H&E, anti-S100, anti-HMB45	18%	10.7%
12	H&E, anti-S100, anti-HMB45	25%	8.7%
20	H&E, anti-S100, anti-HMB45, Pan Melanoma Plus	34%	21.6%
Dependent upon size	H&E, ant-S100, anti-HMB45, MELAN A	28%	28%
4-16	H&E, anti-S100, anti-HMB45	24%	11%
Dependent upon size	H&E, anti-S100, anti-HMB45	38%	18%
4	H&E, anti-S100, anti-HMB45	15%	NA
12-20	H&E, ant-S100, anti-HMB45, anti-tyrosinase, MELAN A	24%	11%

histologic study of the entire lymph node is impossible, each study group has designed its own protocol. Table 4 shows some of the most commonly used protocols for sampling and subsequent histopathology. The protocols proposed share certain elements but differ significantly in others.[39-46]

For instance, most groups agree on the low accuracy of the intraoperative study of frozen tissue, since its sensitivity for detecting metastasis has been demonstrated on numerous occasions to be as low as 47%.[47] In contrast, the different protocols do not agree so strongly on how exhaustive the pathologic study of sentinel nodes should be. According to the hypothesis proposed by Cochran et al,[48] melanomas typically metastasize in the subcapsular space along the central plane of the node. Consequently, those authors proposed taking no more than 10 sections every 2 to 4 µm from each central face of the node cut longitudinally in half. It has been widely demonstrated, however, that the deeper the sectioning the greater the likelihood of finding metastasis. Consequently, other authors favor the use of alternate slices taken every 1 to 2 mm followed by analysis of a variable number of sections (between 3 and 20) from each slice.[40,42,44]

In terms of the staining technique used, it is generally accepted that conventional hematoxylin-eosin staining allows detection of a malignant melanoma cell among 10^4-10^5 cells, while immunohistochemistry allows detection of a melanoma cell among 10^5-10^6 cells. Consequently, most authors recommend using additional immunohistochemistry in sections adjacent to those analyzed with hematoxylin-eosin if metastatic cells are not observed with that stain.[47]

In addition, immunohistochemistry can help to differentiate between melanoma cells and other benign cells present in the lymph node. Most immunohistochemistry studies use an antibody against the cytoplasmic S100 protein (polyclonal anti-S100), which is highly sensitive for the detection of melanoma cells, although not very specific. Completion of the immunohistochemical study of the sentinel node is therefore recommended with more specific markers of melanocytes such as HMB45 (monoclonal HMB45), an antibody directed against the protein Pmel 17/gp100, expressed in immature melanosomes. Other authors prefer the use of antibodies against MELAN-A, another protein found in immature melanosomes, since it is more sensitive than and just as specific as the HMB marker.[45] Antibody cocktails are also available for the identification of melanocytes (the so-called pan-melanoma cocktails, which allow detection of HMB45, MART1, and tyrosinase, among others, in a single staining procedure).[1]

With a combination of studies involving multiple sections and the use of hematoxylin-eosin and immunohistochemistry in each slice, the accuracy of the pathologic study is increased by up to 15% compared with analysis of the central faces of each half of the node using hematoxylin-eosin alone, but with the drawback that the process is very painstaking and not very practical due to the costs involved.[42,46]

Differential Diagnosis of Suspected Melanoma Cells in a Sentinel Node

Lymph nodes often contain groups of cells that can be difficult to differentiate from metastatic melanoma cells. For instance, it is not uncommon to observe paracortical dendritic cells, macrophages, Schwann cells from the nerves

in and around the node, or ganglion cells, all of which are S100-positive and can be diagnosed erroneously as melanoma cells.[47] The presence of pigment from a tattoo or anthracite can be misleading, particularly in macroscopic analysis of the piece.[49]

There is also an added difficulty in the study of melanoma metastasis in the lymph node, namely the presence of benign (nonmelanoma) melanocytes in the lymph nodes. The true incidence of nodal nevus, as these groups of cells have been called, is not known, and the rates that appear in the literature are highly variable, with frequencies as different as 1% and 30% of patients with malignant melanoma having nevi in a lymph node.[44,50-52] Also, the presence of nevus cells has been described in nodes from the drainage areas of other malignant tumors, such as breast cancer, or benign lesions, such as blue nevi, though the incidence is notably lower.[50,51] Interestingly, nevus cells have been described exclusively in nodes from drainage areas of the skin and not in deeper lymph nodes such as the abdominal nodes.[50]

There are 2 theories that attempt to explain the phenomenon of nodal nevi: abnormal embryonic migration of melanocytes (embryologic theory) and transport of cells by embolization through the lymphatic vessels from a cutaneous nevus to the corresponding lymph node (benign metastasis theory). Two factors support the second theory: the observation that nevus cells are more common in sentinel lymph nodes than in non-sentinel nodes and that they are more common in nodes for melanomas associated with nevi than in de novo melanomas.[47]

Prognostic Significance of Melanoma Cells in Sentinel Nodes: The Importance of Metastatic Burden

Once it has been confirmed that the sentinel lymph node contains melanoma cells, the patient undergoes complete lymphadenectomy. However, as mentioned, not all patients with metastasis in a sentinel node follow the same course. This suggests the possibility that not all metastases to the sentinel lymph nodes have prognostic implications for the patient. Once again, there is a lack of unanimous agreement regarding the minimum size or minimum metastatic burden in the sentinel lymph node that should be considered a true metastasis with prognostic significance for the patient with melanoma. In other solid tumors such as breast cancer there is sufficient scientific evidence to differentiate between the presence of isolated cells (deposits of less than 0.2 mm, often only seen with immunohistochemistry), the presence of micrometastasis (defined as deposits of cells smaller than 2 mm), and the presence of macrometastasis (deposits larger than 2 mm). Only this last type, the macrometastases, have demonstrated prognostic significance for the patient, and this is reflected in the TNM classification for breast cancer, according to which, complete lymphadenectomy is only indicated in cases of macrometastasis.[53]

In malignant melanoma, some studies have attempted to measure tumor size or metastatic burden in an effort to correlate it with clinical course, although this concept is not currently reflected in the AJCC classification, except in the distinction between macrometastasis (lymph node metastasis that is clinically palpable or involves extracapsular invasion) and micrometastasis (all those observed by histology) and the number of affected nodes (threshold of 3).[3]

A few years ago, Startz et al[44] proposed a new classification, the S stage, to stratify disease of sentinel lymph nodes based on the parameters n (number of 1-mm slices in which melanoma cells were observed) and d (maximum distance of tumor cells from the capsule towards the center). This staging method correlated well with other known prognostic factors such as the Breslow depth of the primary tumor or the number of lymph nodes positive for metastasis. In addition, in the multivariate analysis of prognostic factors, the S3 stage, defined as d > 1 mm, could be considered the most important negative prognostic factor. Following that study, Carlson et al[54] published another series of 104 positive sentinel lymph nodes and classified them using another method: isolated cell deposits in the subcapsular space or in the interfollicular zone, micrometastasis (considered as foci of cells ≤ 2 mm), and macrometastasis if >2 mm. Those authors found that only patients with macrometastasis had a significantly worse survival.

Computer-assisted analysis has recently been used to determine the relative area of metastasis within the sentinel lymph node and on that basis predict the existence of other positive nodes and survival.[55,56] However, these methods are currently not feasible in day-to-day diagnosis.[3,47]

Molecular Techniques for the Analysis of Sentinel Lymph Nodes. The Most Widely Used Markers: Which Ones and How Many Should We Use?

Molecular methods represent an alternative to pathologic studies since they have the capacity to detect very small quantities of tumor-related factors in different tissues, which can thus be analyzed in their entirety. Smith et al[57] were the first to report the use of reverse transcriptase polymerase chain reaction (RT-PCR) for the detection of metastatic cells in the blood of patients with malignant melanoma. Soon afterwards, Wang et al[58] adapted the technique for the detection of melanoma cells in lymph node tissue, and since then, various groups have reported their experience in the molecular detection of melanoma cells in sentinel lymph nodes.[41,43,59-69] It is worth noting, however, that the use of these molecular techniques is still under investigation

and is not yet recognized as a valid method for routine diagnosis.[70] One of the main problems to take into account when interpreting the results of these studies is the methodologic variability between different laboratories, in terms of the quantity and type of tissue used (paraffin-embedded or fresh tissue), the amplification technique (simple PCR, nested PCR, or real-time quantitative PCR), and the markers used. The most-valid and widely used molecular markers for the detection of malignant melanoma cells in sentinel lymph nodes can be classified in 2 groups: melanogenesis-related proteins (MRP) and cancer/testis antigens (CTA).

The MRP used by most groups is tyrosinase, the key enzyme in the synthesis of melanin. The gene *MART1* is also widely used as a marker of melanoma cells. It was originally described as the main melanoma antigen recognized by T lymphocytes[71] and it has also been implicated in the synthesis of melanosomes (regulation of Pmel 17), meaning that both melanoma cells and benign melanocytes can express it.

Among the CTA markers, so named since expression has been observed both in tumors and in testicular and placental tissue, the most used are the melanoma antigen genes (MAGE).

Recently, the group led by Hoon reported 2 new markers for the detection of melanoma cells in sentinel lymph nodes: GalNAc (beta1-4-N-acetylgalactosaminyl-transferase) and Pax3 (paired-box homeotic gene transcription factor 3).[68] GalNAc is an enzyme implicated in the synthesis of the ganglioside GM2/GD2 and is detected in melanomas and neuroblastomas. Pax3 has an important role in the regulation of melanin synthesis, but is also implicated in other processes such as cell migration and prevention of apoptosis.

Clinical Relevance of Molecular Studies of Sentinel Nodes: The Most Important Studies and Case Series

Various studies have been published on the usefulness of molecular methods for the detection of occult disease in sentinel lymph nodes from patients with malignant melanoma. Among them, we have identified the 13 most important studies, with independent patient series, that address the possible prognostic significance of detecting those markers (Table 5).[41,43,59-69]

Although most studies reported prognostic significance for the detection of tyrosinase by nested PCR, the length of follow-up was very short (less than 3 years).[41,43,59,60,67,69] Nevertheless, as highlighted by Kammula et al,[61] and also in a study published by our group,[63] it would be interesting to determine what would happen in the majority of these studies in terms of prognostic significance of molecular detection in sentinel lymph nodes if the period of follow-up were longer. Both in the study by Kammula and colleagues and in our own, an increase in the length of follow-up implied a loss of significance in terms of the risk of recurrence in both groups of patients (those who were positive and negative in the molecular study). Thus, if we compare the percentage of recurrence in patients with negative sentinel lymph nodes following pathology in the different studies, we find that longer follow-up is associated with larger numbers of recurrences: the risk of recurrence in this group of patients increases from around 10% after 3 years of follow-up to around 25% after 5 years. Consequently, the presence or absence of prognostic value for molecular detection in sentinel lymph nodes may also vary over time.

What is, without doubt, surprising in all these molecular studies (including our own experience) using a single molecular marker, tyrosinase, is the excessive sensitivity, with rates of positivity in patients in whom sentinel lymph nodes were negative in the pathologic study ranging from 25% to 31%, representing a high percentage of false positives.[70] Based on these results, various groups, including our own (unpublished data), have opted for a real-time quantitative PCR method with a combination of markers, allowing an increase in specificity compared with that obtained through nested PCR to detect tyrosinase, and in turn, offering a more objective and reproducible method.[66,68]

As a result of these various molecular studies, prospective multicenter trials have been initiated to determine the prognostic value of molecular detection. Figure 1 illustrates the design of those studies. The first of those was the Sunbelt Melanoma Trial, which sought to determine whether treatment with interferon (IFN) α 2B in combination with lymphadenectomy is more effective than lymphadenectomy alone to prolong disease-free survival and overall survival in patients with positive sentinel lymph nodes. Among the secondary objectives, the Sunbelt study was the first to include molecular results as a criterion for deciding on the treatment used, since patients with positive sentinel nodes following the molecular study were randomly assigned to 2 groups: observation or lymphadenectomy. The period for inclusion of patients ended in 2004 and the results of the molecular study have recently been published.[65] A total of 1446 patients with negative sentinel lymph nodes based on histology were included. Of those, 620 patients (42.8%) were positive for tyrosinase and 1 or more of 3 other markers (MART1, MAGEA3, and gp100). In addition, peripheral blood from 820 patients was analyzed using the same molecular methods. Following a median follow-up period of 30 months, the molecular analysis of sentinel lymph nodes did not succeed in identifying those patients at greater risk of relapse. The study of peripheral blood did show a statistically significant difference, but only in terms of disease-free survival, which was worse in patients who were positive for 2 or more markers (P=.006).

Table 5. Main Studies Published Evaluating Molecular Analysis of Different Markers in the Sentinel Lymph Node and Their

Authors	No. of Patients			Follow-up, mo	Recurrences, %		Type of Sample	Molecular Method
	Total (Inclusion)	Histo –	Histo +		Histo +	Histo –		
Takeuchi et al[68]	215 (1992-1996)	162	53 (24%)	60.4	60	24	Paraffin	qRT-PCR
Bostick et al[41]	72 (NA)	55	17 (24%)	12	29	5	Frozen	Single PCR
Kuo et al[62]	77 (NA)	40	37 (48%)	55	62	53	Paraffin	RT-PCR and ECL[b]
Kammula et al[61]	112 (1996-1997)	97	15 (13%)	67	53	14	Frozen Bisection	Nested PCR
Ulrich et al[69]	322 (1998-2002)	288	34 (10%)	37	44	10	Frozen Bisection	PCR
Ribuffo et al[64]	134 (NA)	119	15 (11%)	42	73	7.5	Frozen Bisection	Nested PCR
Goydos et al[60]	175 (NA)	141	34 (19%)	34	50	20.6	Frozen Bisection	Nested PCR
Blaheta et al[59]	116 (1996-1998)	101	15 (13%)	19	67	12.8	Frozen Bisection	Nested PCR
Schivers et al[67]	114 (NA)	91	17 (24%)	28	61	8	Frozen	Nested PCR
Li et al[43]	233 (1995-1997)	181	52 (22%)	20	34	6.6	Bisection	
Giese et al[66]	139 (1999-2002)	114	25 (18%)	29	36	3.5	Frozen Bisection	qRT-PCR
Mangas et al[63]	180 (1998-2003)	142	38 (21%)	45	31	7	Frozen Bisection Alternate sections	Nested PCR
Scoggins et al[65]	1446 (1997-2003)	1446	NA	30	NA	20 (approximately)	Frozen Bisection Alternate sections	PCR

Abbreviations: ECL, electrochemoluminescence (with labeled biotin); Histo, following pathologic study; NA, not available; PCR, polymerase chain reaction; qRT-PCR, real-time quantitative PCR.
[a]Independent patient series are shown in bold.
[b]Defined as positive for tyrosinase and any of the other 3 markers.

Following the Sunbelt study, another 2 multicenter studies with a similar design were initiated. The Florida Melanoma Trial (FMT) involved 10 institutions and included 3200 patients with malignant melanoma recruited between 1992 and 2002. Unlike the Sunbelt trial, in the FMT, patients with positive sentinel lymph nodes based on histology and/or molecular studies using nested PCR for tyrosinase were randomly assigned to 2 study arms: lymphadenectomy with IFN or IFN alone.[72]

Finally, the second part of the Multicenter Selective Lymphadenectomy Trial (MSLT-II), which is still recruiting, includes patients from the largest referral hospitals in the United States, Australia, and Europe to address, firstly, the possible therapeutic effect of sentinel lymph node biopsy per se and, secondly, the use of molecular techniques in patients with negative sentinel nodes to randomly assign patients to 2 study arms: observation and lymphadenectomy.[19]

Limitations of Sentinel Lymph Node Biopsy: False Negatives and False Positives in Histopathologic and Molecular Studies

As we have been discovering over the course of this review, the predictive potential of sentinel lymph node biopsy to identify those patients at greater risk of relapse is unquestionable, but it should also be recognized that the procedure has limitations. These limitations are not inappreciable, particularly if we define them in both directions: underestimation of the true incidence of metastasis (false negatives) and overestimation of that rate (false positives).

The existence of false positives due to the study technique refers in particular to molecular studies rather than pathologic studies, and they may be due to factors such as contamination of samples during the process of sentinel node biopsy (use

Prognostic Value[a]

Markers	*PCR Positive, %*		*P Histo –/PCR+ vs Histo –*	
	Histo +	*Histo*	*Disease-Free Survival*	*Overall Survival*
MART1/PAX/GAL/MAGEA3	79/74/70/47	6/17/17/5	<.0001 (≥2 vs ≤1 marker)	<.0001 (≥2 vs ≤1 marker)
Tyrosinase/MART1/MAGEA3	80/100/80	29/36/44	<.02 (≥2 vs ≤1 marker)	NA
Tyrosinase/MART1/TRP-1/TRP-2	89/92/35/43	62/43/10/10	<.09 (≥2 vs ≤1 marker)	<.08 (≥2 vs ≤1 marker)
Tyrosinase	100	52	> .05	> .05
Tyrosinase	100	14	< .0001	NA
Tyrosinase	100	58	.01	NA
Tyrosinase	100	48	.008	.02
Tyrosinase	100	35	.01	NA
Tyrosinase	100	52	.02	NA
	94	63	.06	
Tyrosinase/MART1	92	34	.01	NA
Tyrosinase	92	60	> .05	> .05
Tyrosinase/MART1/MAGEA3/gp100	NA	43b	> .05	> .05

of the same surgical tools for skin incisions and manipulation of the lymph node), laboratory contamination (less common), or the presence inside the lymph node of non-neoplastic cells that also express the markers used.[73,74] These situations can be avoided, however, through careful methodology.

The strict definition of a false-negative result in sentinel lymph node biopsy for malignant melanoma should only include those patients in whom there is metastasis in nonsentinel lymph nodes from a lymph node station in which a sentinel lymph node has been diagnosed as negative at the same point in time. False negatives defined in this way, also known as *skip metastasis*, were identified in the first studies to assess the effectiveness of sentinel node biopsy, and they occurred in around 2% of patients.[17-19] However, it is unlikely that such studies will continue to be performed given the morbidity associated with elective lymphadenectomy and the apparent lack of benefit for patients.[27-30]

Consequently, in order to facilitate comparison of the results from different studies, some authors have proposed other methods to calculate the rate of possible false negatives in sentinel lymph node biopsy.[5,34] In the broader sense of the term, we can define a false negative as any patient who suffers recurrence of the disease after negative sentinel lymph nodes are reported, whatever the site of the recurrence; this accounts for 24% of cases in studies with longer follow-up.[34,68]

However, it is also true that we cannot expect sentinel lymph node biopsy to be able to predict in any way how a tumor will behave, since it will always be a staging technique exclusively for the lymph nodes. For most groups that have published results on sentinel node biopsy, only those cases with recurrence in the same region studied by sentinel node biopsy, whether it be the only site of recurrence or it occurs simultaneously with other sites, should be considered as true false negatives for the technique.[34] Based on this

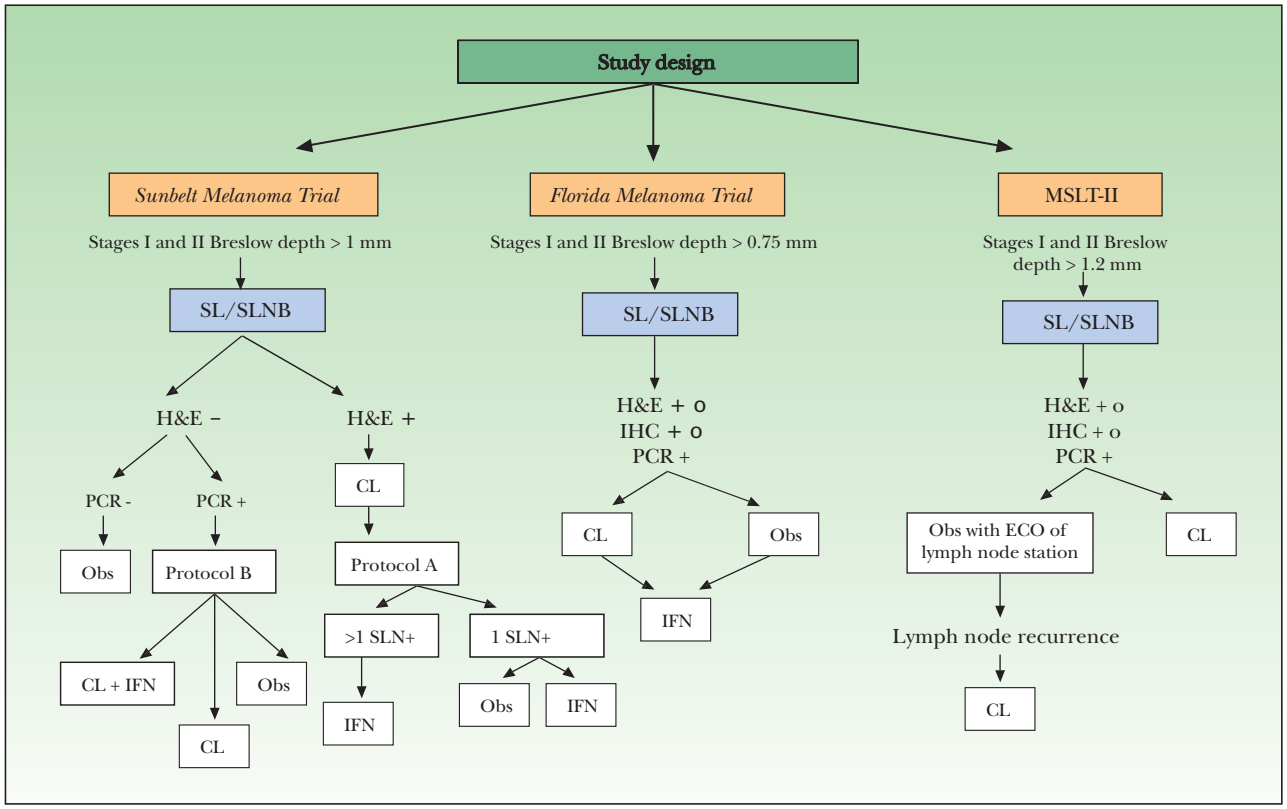

Figure 1. Design of ongoing multicenter studies of molecular detection in sentinel lymph nodes from patients with melanoma. CL indicates complete lymphadenectomy; H&E, hematoxylin-eosin; IFN, interferon; IHC, immunohistochemistry; MSLT-II, Multicenter Selective Lymphadenectomy Trial-II; Obs, observation; PCR, polymerase chain reaction; SL, selective lymphadenectomy; SLN, sentinel lymph node; SLNB, sentinel lymph node biopsy.

criterion, the percentage of false negatives is reduced by half to around 10% in the majority of case series (Table 6).

Whatever the definition in the different studies, these false negatives will be the consequence of 1 or more of the 3 mechanisms described below.

Technical Flaws

Technical flaws refer to those situations in which the biopsied lymph node does not correspond to the true sentinel node, either due to failures in the surgical technique itself or the prior lymphoscintigraphy. It can also occur due to changes in the drainage pattern of the primary malignant melanoma as a consequence of excision of the tumor with wide margins or inflammation or infection around the surgical wound. Two methods have recently been developed to confirm that the excised lymph node is a true sentinel node: the carbon dye method[75] and measurement of antimony concentration.[76] However, both methods have been criticized. The first because the use of carbon can hinder or mask the identification of melanoma cells in the lymph node, as these are sometimes very difficult to find, and the second because it allows retrospective checking but not at the time of sentinel node biopsy.[12] Furthermore, neither method allows the surgeon in the operating theater to be more confident that the excised lymph node is in fact a sentinel node and that no sentinel nodes have been left behind. Thus, despite a negative result following biopsy of a true sentinel node, other sentinel nodes may remain in the drainage basin and be a potential cause of recurrence. These cases are increasingly rare, since the definition of a sentinel lymph node is now more strict and the radiolabeled colloides used are smaller.[5,38]

Flaws in the Pathologic Study

Cases in which the pathologic study is flawed are understood to be those in which micrometastasis is missed by pathology or, less often, molecular studies of a lymph node correctly identified as a sentinel node. This occurs in particular as a consequence of inadequate analysis of the lymph node sample, since routine studies only analyze a small portion of the node. It was initially thought that this was the main cause of failure in studies of sentinel lymph nodes. However,

Table 6. Rates of Success and Lymph Node Recurrence in the Main Studies Published on Patients With Melanoma and Sentinel Node Biopsya

Authors	*Year*	*Total No. of Patients*	*No. of – Patients SLN*	*Median Follow-up, mo*	*No. of Lymph Node Recurrences*[b]	*Failure Rate, %*	*False Negatives, %*
Gershenwald et al[77]	1998	322	243	35	10	4.1	16.0
Essner et al[85]	1999	267	225	45	11	4.8	20.7
Gadd et al[86]	1999	NA	89	23	7	8	NA
Clary et al[78]	2001	308	252	24	11	4.4	16.4
Cascinelli et al[27]	2000	787	646	29	40	6	24.8
Statius Muller et al[87]	2001	263	204	42	3	0	7
Jansen et al[88]	2000	199	151	32	6	4	11.0
Harlow et al[89]	2001	329	297	36	10	3.3	20.4
Doting et al[90]	2002	200	150	47	6	4	10.0
Chao et al[91]	2002	1183	950	16	14	1.5	7.1
Vidal-Sicart et al[92]	2003	435	358	26	7	1.9	8.9
Morton et al[93]	2003	1599	1277	NA	33	2.6	9.2
Nowecki et al[94]	2003	726	579	34	274.7	13.6	
Yee et al[34]	2005	991	836	42	22	2.6	13.2
Berck et al[95]	2005	274	221	30	10	4.5	20.4
Rex et al[96]	2005	240	147[c]	318	5.4	13.8	
Wagner et al[97]	2003	408	323	31.4	11	3.4	11.5
Mangas et al[63]	2006	138	103c	45	4	3.8	10.2
Morton et al[32]	2006	769	603[c]	59.8	26	4.3	17.6
VanAkkooi et al[40]	2006	262	185	23	6	3.2	7.2

Abbreviation: SLN, sentinel lymph node.
[a]Adapted from Yee et al.[34][b]Lymph node recurrence is considered as any patient in whom a first recurrence occurs in the lymphatic basin of the biopsied sentinel lymph node, associated or not with other sites. [c]Difference between initial patients and subsequent ones: failure rate, number of lymph node recurrences/number of patients with SLN–; false negatives, number of lymph node recurrences/number of patients with SLN+ plus number of lymph node recurrences.

with the introduction of immunohistochemistry and techniques involving multiple sections, the sensitivity of the pathologic study has improved notably, but the problem is that the protocols are laborious (Table 4).[42] Thus, it has been observed that reanalysis of patients with presumed negative sentinel nodes following an initial pathologic study with hematoxylin-eosin but who suffer lymph node recurrences in the same lymphatic basin allows detection of a larger number of metastatic lymph nodes, thereby supporting the presence of false negatives in the initial study. Gershenwald et al[77] and Clary et al[78] found that 80% to 90% of patients with lymph node recurrences after an initial negative result for sentinel lymph node biopsy had metastatic cells in the sentinel nodes following more detailed analysis. Other authors such as Li et al[43] and Yee et al,[34] however, found metastasis in the sentinel lymph nodes following reanalysis in only 30% of cases. In this context, molecular diagnostic techniques, which are of great help in complementing the pathologic analysis of the node, will depend to a large extent on the sensitivity of the prior histologic analysis.[42,46,47]

In terms of false negatives in the results of the molecular study, we should include sampling errors or samples in which the RNA may have degraded, along with those in which the RT-PCR reaction has been ineffective.[74] Nevertheless, there are controls that can be routinely performed in the laboratory, such as the inclusion of an internal control gene that will be proportional to the quantity of RNA added to the reaction and the use of reference RNA as a control for correct reverse transcription. Another possible explanation of false-negative molecular results is tumor heterogeneity. Various studies have shown that no marker

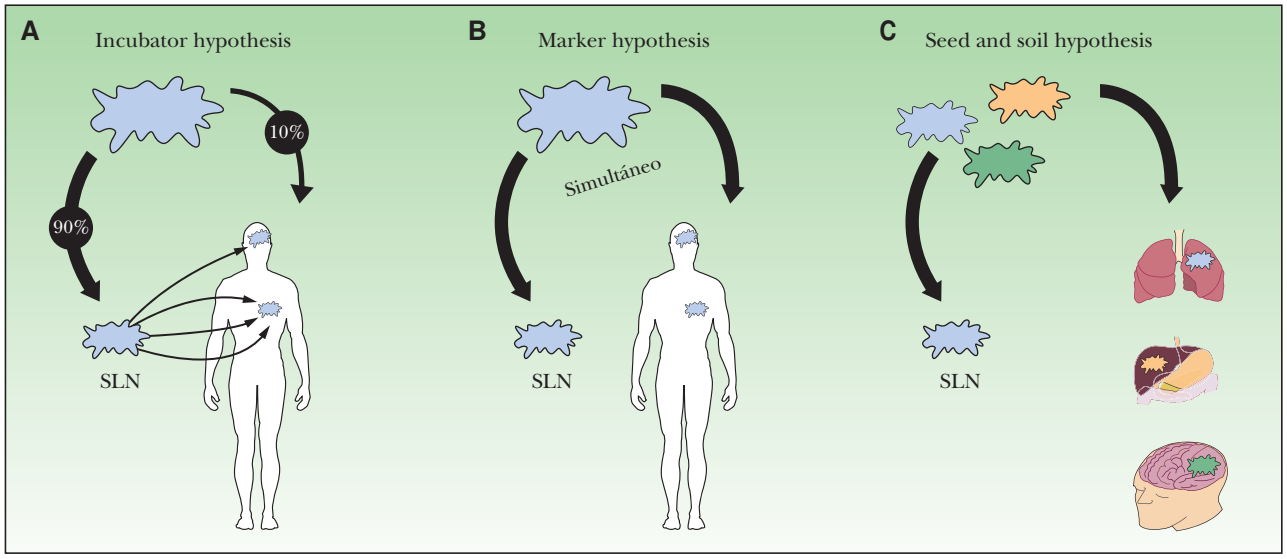

Figure 2. Theories to explain metastatic spread. SLN indicates sentinel lymph node.

will detect 100% of primary tumors; however, the heterogeneity of lymph node micrometastases has not been studied in depth. The current focus on the use of a combination of molecular markers rather than a single marker, as was previously employed, goes a long way toward resolving this problem.[73,74]

Biologic Flaws

Biologic flaws explain those cases that may be due to a process of biologic spread of the malignant melanoma that differs from that covered by the hypothesis used to explain the spread of metastasis on which sentinel lymph node biopsy is based (Figure 2A). This hypothesis, known as the *incubator hypothesis*, is supported by Morton and Cochran[79] and is based on the idea of ordered or stepwise spread of metastasis. According to this theory, in the large majority of tumors the first metastasis would develop in the sentinel lymph node, and only then would it spread to other regional lymph nodes during an initial phase before later developing the potential to spread to more distant sites.[79] The observations that size of metastasis in the sentinel lymph node and number of affected nodes are both prognostic factors for lymph node involvement are consistent with this hypothesis. In contrast, the apparent lack of therapeutic benefit associated with early detection of lymph node metastasis, either by sentinel node biopsy or elective lymphadenectomy, would not be explained by this hypothesis. An alternative model suggests that lymphatic and blood-borne spread occur simultaneously; this is the so-called *marker hypothesis* and is illustrated in Figure 2B.[24] In this case, the presence of lymph node metastasis would act as an irrefutable marker for distant disease, and as mentioned, this is not always the case. In addition to these 2 theories (partly considered to be opposing in nature), other authors have proposed a different model of spread in cancer that is characterized by the presence of different routes of spread according to the characteristics of each tumor.[10,80,81] According to this model, partly based on the "seed and soil" hypothesis (Figure 2C) proposed in 1889 by Paget,[82] some tumors, independently of traditional prognostic factors, would never have the biologic potential to form a metastasis, while others, despite an absence of known negative prognostic factors at the time of diagnosis, will retain a capacity to seed and grow in certain tissues (lymph nodes, lungs, liver, brain, etc).[80]

Finally, another biologic factor to take into consideration when interpreting the results of sentinel lymph node biopsy would be those metastatic cells that may remain in the lymphatic system outside the lymph node at the time of sentinel node biopsy. This concept would include those regional metastases occurring as a consequence of spread of the disease from local or in-transit disease that is hidden, from a clinical point of view, at the time of sentinel node biopsy. In such situations, a negative result for the excised sentinel lymph node only reflects the pathologic status of the sentinel node at that point in time, in other words, when the biopsy is performed. It was suggested that the technique of sentinel node biopsy would itself accentuate this phenomenon based on the observation in some case series that the percentage of patients with in-transit metastasis was increased in patients subjected to this test.[83] However, the literature is not clear on this point and the most recently published studies do not confirm the earlier finding.[84]

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Conflicts of Interest
The authors declare no conflicts of interest.

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