In search of the sentinel node: validation and sophistication of lymphatic mapping and sentinel node biopsy in breast cancer and melanoma
van der Ploeg, I.M.C.

Citation for published version (APA):
van der Ploeg, I. M. C. (2009). In search of the sentinel node: validation and sophistication of lymphatic mapping and sentinel node biopsy in breast cancer and melanoma Amsterdam: Nederlands Kanker Instituut - Antoni Van Leeuwenhoekziekenhuis

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
CHAPTER 8

Comparison of three micromorphometric pathology classifications of melanoma metastases in the sentinel node

Van der Ploeg IMC, Kroon BBR, Antonini N, Valdés Olmos RA, Nieweg OE

Ann Surg, in press
Abstract

**Introduction:** Three micromorphometric parameters of melanoma sentinel node metastases were compared: invasion depth from the capsule (Starz-classification), maximum diameter (Rotterdam-criteria) and location within the node (Dewar-classification). The purposes of this study were to determine which classification best predicts additional lymph node disease and survival, and to suggest a threshold below which a completion dissection may be omitted.

**Methods:** The pathology slides of 116 patients with tumour-positive sentinel nodes were reviewed. The follow-up data were obtained from the prospectively kept database. The median follow-up duration was 53 months.

**Results:** Metastases with an invasion depth under 0.3 mm or diameter less than 0.1 mm were not associated with additional involved nodes. Four percent of the patients with metastases with an invasion depth of 0.3 -1.0 mm had other involved nodes and 3% of the patients with metastases with a diameter of 0.1-1.0 mm. Other nodes were involved in 3% of subcapsular metastases, 9% of both subcapsular and parenchymal metastases, and 33% in case of multifocal or extensive disease. The smallest tumour invasion depth and diameter associated with additional involved nodes was 0.4 mm. Only five-year overall survival differences in the three successive invasion depth categories were statistically significant: 92%, 83% and 68%. Five-year overall survival was 81% in patients with one involved sentinel node and 60% if there were more.

**Conclusions:** Invasion depth and diameter of the metastasis correlate best with the presence of additional nodal disease. Invasion depth best predicts overall survival. It seems justified to refrain from completion dissection in patients with a sentinel node tumour invasion depth up to 0.4 mm.
**Introduction**

Sentinel lymph node biopsy is nowadays widely used to identify melanoma patients who have lymph node metastases. Sentinel node-negative patients are spared further surgery and sentinel node-positive patients are generally subjected to a completion node dissection. No additional metastases are found in the dissection specimen in approximately 80% of the latter patients. They do not appear to benefit from the node dissection, yet they are exposed to the considerable risk of the associated morbidity.

A fair number of melanomologists have attempted to develop criteria to determine which patients with sentinel node metastasis have no other involved nodes and may be spared completion dissection. Some investigators based their criteria on primary tumour characteristics such as Breslow thickness and ulceration. Others used sentinel node features like the number of involved nodes, the size of the metastasis or its location within the node as predictive factors. A combination of different parameters has been described as well. Parameters based on the tumour extent in the sentinel node have been suggested to also predict recurrent disease and survival, implying both therapeutic and prognostic consequences.

In the present study, three micromorphometric parameters of sentinel node involvement in melanoma patients were compared: the invasion depth of a metastasis from the capsule (Starz-classification), the maximum diameter of a metastasis (Rotterdam criteria) and its location in the sentinel node (Dewar-classification). The purpose of this study was to assess which classification best predicts additional lymph node involvement and overall survival. In addition, it was attempted to determine a threshold below which it appears safe to refrain from completion dissection.

**Patients and methods**

At the Netherlands Cancer Institute, lymphatic mapping is performed in patients with a melanoma with a Breslow thickness of at least 1.0 mm or a Clark level of at least IV. Between October 1993 and July 2007, 152 of the 583 patients (26%) who underwent sentinel node biopsy at our institute had a tumour-positive sentinel node. Thirty-two patients were excluded because they did not undergo a node dissection and four patients were lost to follow-up. The remaining 116 patients are the study population and constitute 76% of the patients with involved sentinel nodes and 20% of the whole group. The median Breslow thickness was 3.0 mm (range 1.0 - 12.0 mm).

A two-day protocol using 99mTc-nanocolloid and blue dye for sentinel node identification was used. A sentinel node was defined as a lymph node to which the primary tumour drains directly. A median number of two sentinel nodes (range one to six) were excised. All sentinel nodes were formalin-fixated, bisected, paraffin-
embedded, and cut at a minimum of six levels at 50 to 150 µm intervals. Pathologic evaluation included haematoxylin and eosin staining as well as immunohistochemical staining using S-100 in combination with HMB-45 or MART-I. All slides were retrieved and reviewed for the present study.

The sentinel node slides were reviewed and metastases were classified based on penetration depth, maximum diameter and location within the node. The penetration depth was measured as maximum distance of tumour cells from the interior margin of the lymph node capsule, as described by Starz et al. Presence of tumour cells up to 0.3 mm was classified as Starz-I, invasion between 0.3 mm and 1.0 mm was Starz-II, and a deeper invasion meant Starz-III. The maximum tumour diameter was categorized according to the three Rotterdam groups described by Van Akkooi et al: tumour burden of at least eleven cells but less than 0.1 mm diameter, a diameter of 0.1 mm or more up to and including 1.0 mm, or a diameter of more than 1.0 mm. None of the 116 patients had less than eleven tumour cells in the sentinel node. The location of the metastatic deposit within the sentinel node was determined as described by Dewar: subcapsular (Dewar A), combined subcapsular and parenchymal (Dewar B), only parenchymal (Dewar C), multifocal (Dewar D), or extensive involvement of the sentinel node (Dewar E). Intracapsular disease was classified as Dewar A. Lymph nodes classified as Dewar C were not included in the 116 study patients because the exclusive parenchymal involvement prevents comparison with the Starz-classification. The highest of each classification prevailed when metastases were present in more than one sentinel node in a lymph node basin.

All 116 patients underwent a completion lymph node dissection. A median number of thirteen lymph nodes were recovered from the dissection specimens, ranging from one to 45 nodes. All lymph nodes harvested were examined in 4.0 mm sections stained by haematoxylin and eosin. The patients were followed at our institute. The median follow-up duration was 53 months ranging from two to 170 months.

The results of the three different classifications were analyzed and compared. The number of additional metastases was measured per category for each classification. Survival duration was calculated from the date of sentinel node biopsy to the date of death or last date of follow-up. Survival curves and rates were estimated by the Kaplan-Meier method and compared with the log-rank test. Univariable binary logistic regression was applied to correlate the number of involved sentinel nodes with the presence of additional metastases and survival. The threshold for identifying patients who may benefit from a completion dissection was calculated based on both the invasion depth and diameter for threshold values ranging from 0.1 mm to 4.0 mm by 0.1 mm increments and based on the five-year survival. All statistical analyses were performed using SPSS 15 (Version 15.01, for Windows, SPSS Inc, Chicago, IL, USA) and Statistical Analysis Software version 9.1 (SAS Inc, Bethesda Maryland, USA).
Table. Additional nodal involvement in the dissection specimens and survival rates related to the three different classifications.

**Results**

Histopathologic evaluation revealed additional metastases in fifteen of the 116 patients (13%) (table). None of the patients in the category with the smallest invasion depth or diameter had additional involved lymph nodes, whereas further involvement was found in one of the patients (3%) with the most favourable (subcapsular) location. In the intermediate categories, these percentages were 4, 3 and 9 respectively and in the least favourable categories 18, 18 and 33. Patients with multiple involved sentinel nodes were found to have a 1.8 times higher chance of having additional nodal disease than patients with a single metastasis (p= 0.293).

Five-year overall survival rates were 92% and 100% for the favourable invasion depth and diameter categories, and 83% and 84% for the intermediate categories respectively (figures 1 and 2). The five-year overall survival rates were 83% for the patients with a subcapsular metastasis and 71% for patients who also had a metastasis in the parenchyma of the node (figure 3). In the high-risk categories of the three classifications survival percentages were similar and varied from 67 to 69. Differences in the five-year overall survival were only statistically significant for the three categories of invasion depth (p=0.046). Pair-wise evaluation yielded a statistically significant difference between the low and high tumour extent groups of the invasion depth classification (p=0.024). Other than that, pair-wise comparison showed a statistically significant difference only between patients with subcapsular metastases and patients who had parenchymal metastases a well (p= 0.027). Five-year overall
survival was 81% in patients with one involved sentinel node and 60% if there were more (p=0.012).

The threshold at which patients were at risk of having additional metastases was an invasion depth or diameter of 0.4 mm. Survival at five-years was 94% in the patients with an invasion depth up to 0.4 mm and 70% in the patients with a 0.4 mm or deeper invasion (p=0.017). These rates were 90% and 73% respectively when the maximum diameter was less or more than 0.4 mm (p=0.118).

**Figure 1.** Overall survival in patients classified according to invasion depth of the metastasis (Starz-classification) (overall comparison: p-value= 0.046).

**Figure 2.** Overall survival in patients classified according to maximum diameter of the metastasis (Rotterdam criteria) (overall comparison: p-value= 0.094).
Discussion

This study of three micromorphometric classifications of sentinel node metastases shows that each predicts additional lymph node disease reasonably well. An important purpose of the study was to identify patients without additional involved lymph nodes. The classifications based on invasion depth and size of the metastases identified such a category, which the classification based on location failed to accomplish. The various categories of the classifications based on invasion depth and diameter also showed a better separation of survival rates compared to the classification based on metastasis location. Five-year survival in the most favourable diameter category was 100% compared to 83% in the most favourable location category. Invasion depth of the sentinel node tumour lesion predicted the five-year overall survival best. It should be noted that patients with only parenchymal metastases were not incorporated in the classification based on the location of the metastasis.

The current study shows that no other nodes were involved in patients with a tumour invasion depth or diameter up to 0.4 mm, which suggests that such patients can be spared completion node dissection. Only the invasion depth measurement was convincingly associated with a difference in overall five-year survival below (94%) and above (70%) the 0.4 mm cut-off value (p=0.012). This cut-off value is smaller than the 1.0 mm Starz and coworkers suggested in their publication on invasion depth.
but more liberal than the 0.1 mm value that Van Akkooi and coworkers proposed in their paper on maximum diameter as parameter.\textsuperscript{12,13} If the recommendation of Starz et al would have been used, metastatic disease would have been left behind in one of 40 patients (3\%) with an invasion depth up to 1.0 mm. If the recommendation of Van Akkooi et al. had been used to refrain from node dissection only in case of a diameter of less than 0.1 mm, six instead of eight patients would have been spared an unnecessary node dissection. If the recommendation of Dewar et al had been used, metastatic disease would have been left behind in one of 39 patients (3\%).\textsuperscript{6} The invasion depth cut-off point of 0.4 mm appears to spare most patients a useless node dissection while minimizing the risk of leaving disease behind.

The number of involved sentinel nodes has been suggested to be a significant predictive factor for further additional lymph node metastases in the dissection specimen.\textsuperscript{13,20} In the current study, there was indeed a trend for patients with more than one involved sentinel node to have a greater risk of having other nodes affected than patients with a single positive sentinel node, but the small numbers of patients may have prevented a statistically significant difference. Patients with one involved sentinel node were found to have a better survival than patients with multiple positive nodes (p=0.012).

Two comparative studies have shown that the invasion depth has a better predictive value for additional lymph node metastases than classifications based on the diameter of the metastasis.\textsuperscript{17,21} Comparing or combining results from different sources on the three micromorphometric classifications is difficult because most studies are retrospective and concern small numbers of patients.\textsuperscript{8,10,15,22,23} Different protocols of pathological examination of the sentinel nodes have been used. The number of sections and the levels at which the sections of the sentinel nodes were cut differed among investigators. Some investigators have used haematoxylin and eosin-staining and others have added immunohistochemical staining on both the sentinel nodes and the lymph nodes from the completion dissection specimen. The measurement of the invasion depth or diameter in case of multiple tumour lesions or lesions that are widely apart has been described to be complicated.\textsuperscript{22}

A drawback of the present study is that step-sectioning and immunohistochemistry staining were not done on the nodes retrieved from the dissection specimen. This may have caused underestimation of the number of additional involved nodes. Also, failing to find additional disease in other nodes does not necessarily mean that it is absent and finding additional disease does not necessarily mean that this is clinically relevant. The Dewar-classification was the least predictive for both additional nodal disease and overall survival. Also, localizing the tumour deposit has been associated with high inter-observer differences compared to measuring tumour size.\textsuperscript{22,24} The Dewar-classification did show that multifocal disease is associated with a high risk of additional metastases, irrespective of invasion depth and size of the lesions.

Choosing between the invasion depth and the diameter as best criterion is difficult. The observation that more patients can be spared a useless dissection and the prognostic
information on overall survival that the invasion depth provides give this parameter an edge. The diameter concerns only one aspect of the tumour lesion, i.e. size. The invasion depth takes into account both an aspect of size but also the location of the metastasis. The combination of both these features in one classification may explain why it appears to be the most attractive in this series, albeit by a slight margin. Larger observational studies may provide more information on criteria to guide the decision to carry out or omit node dissection. In the second Multicenter Selective Lymphadenectomy Trial, sentinel node-positive patients are randomized to dissection or nodal observation regardless of the size and location of the metastasis. The results of this trial are not expected for a considerable number of years but should provide important information in this respect.

The present study indicates that invasion depth and diameter of the sentinel lymph node metastasis best predict the risk of additional lymph node disease. It seems justified to refrain from completion dissection in patients with a tumour invasion depth or diameter up to 0.4 mm. The invasion depth best predicts overall survival. At The Netherlands Cancer Institute, patients with an involved sentinel node are offered participation in the second Multicenter Selective Lymphadenectomy Trial. Outside this trial, the above-mentioned guideline is followed.

Acknowledgement

The authors gratefully acknowledge the support of the collaborators of the Department of Pathology for their support in the revisions of all pathology slides of the sentinel nodes.

References


13. Van Akkooi AC, De Wilt JH, Verhoef C et al. Clinical relevance of melanoma micrometastases (<0.1 mm) in sentinel nodes: are these nodes to be considered negative? Ann Oncol 2006; 17:1578-1585.


