How morphometric analysis of metastatic load predicts the (un)usefulness of PET scanning: the case of lymph node staging in melanoma

G S Mijnhout, O S Hoekstra, A van Lingen, P J van Diest, H J Adèr, A A Lammertsma, R Pijpers, S Meijer, G J J Teule

**Background:** In primary cutaneous melanoma, the sentinel node (SN) biopsy is an accurate method for the staging of the lymph nodes. Positron emission tomography (PET) has been suggested as a useful alternative. However, the sensitivity of PET may be too low to detect SN metastases, which are often small.

**Aim:** To predict the value of PET for initial lymph node staging in melanoma based on morphometric analysis of SN metastatic load, without exposing patients to PET.

**Material and methods:** In 59 SN positive patients with melanoma, the sizes of tumour deposits in the SNs and subsequent dissection specimens were measured by morphometry and correlated with the detection limits of current and future PET scanners.

**Results:** The median tumour volume within the basin was 0.15 mm³ (range, 0.0001–118.86). Seventy per cent of these deposits were smaller than 1 mm³. State of the art PET scanners that have a resolution of about 5 mm would detect only 15–49% of positive basins. Logistic regression analysis revealed no pretest indicators identifying patients expected to have a positive PET. However, the SN tumour load was a significant and single predictor of the presence of PET detectable residual tumour.

**Conclusion:** Morphometric analysis of metastatic load predicts that PET scanning is unable to detect most metastatic deposits in sentinel lymph nodes of patients with melanoma because the metastases are often small. Therefore, the SN biopsy remains the preferred method for initial regional staging.

**Patients**

Data were analysed from a cohort of 308 consecutive patients undergoing SN mapping in biopsy confirmed primary cutaneous melanoma between September 1993 and January 1999.
The patient group consisted of 135 men and 173 women aged 18 to 85 years (mean, 48). SN mapping was performed by a triple technique, including lymphoscintigraphy followed by blue dye and γ probe guided surgery. In our hospital, a complete LND of the regional basin is performed in the case of a tumour positive SN. Patients were screened for distant metastases but none were found, so that LNDs were carried out in all but two SN positive patients who refused to agree to the procedure. Within one year after the initial investigations, recurrences were diagnosed by the finding of distant metastases in one patient within six months, and in another four patients in the remaining period.

Pathology
Lymph nodes were fixed in neutral buffered formaldehyde. SNs smaller than 0.5 cm were processed and paraffin wax embedded intact, those between 0.5 and 1 cm were halved, and SNs larger than 1 cm were lamellated into approximately 0.5 cm pieces. All SN blocks were step sectioned at five levels with 250 μm intervals. On each level, sections were stained with haematoxylin and eosin (H&E) and by immunohistochemistry (S100 and HMB-45 antibodies). Lymph nodes in LND specimens were analysed in a routine fashion, meaning that one H&E stained section was made for each block, so that depending on the size of the lymph node one to three sections were obtained from each non-SN.

The size of the metastases was determined using an interactive video overlay system (Q-PRODIT; Leica, Cambridge, UK). The surface areas of individual tumour deposits were measured and the total tumour area was calculated for each node. Because tumour deposits are usually spherical, surface areas were converted to diameter (mm) and volume (mm³), as follows: diameter (d) = 2/(tumour area/π) and volume = 4/3π(d/2)³. The total tumour volume for each nodal basin was calculated by summing up the volumes of tumour in all positive SN(s) and non-SN(s) in that basin.

It has been estimated that with a single central cross section and immunostaining, 69% of metastases are found, as opposed to 81% in the 10 step procedure used in SNs of 1 cm. In addition, with a limited number of slices, as used in non-SNs, the slice may not contain the maximum diameter: assuming that the section level is random, the average measured diameter in the case of a single section will be 8.7 mm (in the middle between the maximum and minimum diameter) in a spherical metastasis of 1 cm. In volume terms, this implies a potential average underestimation of 34%. Combined with the hit probability of 69%, this yields a mean potential underestimation of the real tumour volume by 50% (0.69* (1.0 – 0.34)) in non-SNs.

Statistical analysis
Logistic regression analysis was used to predict the usefulness of PET: (1) at initial regional staging and (2) after a tumour positive SN biopsy to identify patients with additionally involved regional lymph nodes. In the first situation, we studied whether the established prognostic variables—age, sex, Breslow thickness, and melanoma localisation—could predict the presence of a PET detectable amount of tumour. In the second situation, the feature “tumour volume in the SN” was added to this set of variables. In both situations, a range of PET detection limits was taken into account (2–10 mm). The significance of the correlations was evaluated by Pearson’s test. Tumour volumes in SNs and non-SNs were compared with the Wilcoxon signed ranks test. Significance was set at 0.05.

RESULTS
Of 308 patients, 59 (19%) had a tumour positive SN (table 1). SN positivity increased with Breslow thickness (table 1). In addition, the amount of tumour in the SN was significantly related to Breslow thickness (Pearson’s correlation coefficient = 0.397; p = 0.002). Thirteen SN positive patients had positive non-SNs, as shown by histological examination of 57 LND specimens and by (uneventful) follow up (at least two years) in two patients who refused LND. A weak positive correlation was found between the total amount of tumour in the regional basin and the Breslow thickness of the primary tumour (Pearson’s correlation coefficient = 0.286; p = 0.028). Table 1 shows that the average size of tumour deposits in SNs is extremely small, even in patients with Breslow thickness > 4.0 mm.

For a state of the art PET scanner the detection rate for metastases positive lymph nodes would be between 15% and 49% only. Figure 1 shows the predicted yield of scanners with alternative resolution parameters (see also appendix 1).

Table 1 Tumour volumetry of regional lymph nodes in 59 patients with cutaneous melanoma in relation to Breslow thickness and sentinel node status

<table>
<thead>
<tr>
<th>Breslow thickness (mm)</th>
<th>Patients with positive SN(s)</th>
<th>Patients with positive non-SN(s)*</th>
<th>Median total tumour volume in regional node+ basin (mm³) (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.76–1.5</td>
<td>19/122 [16%]</td>
<td>4/19 [21%]</td>
<td>0.01 [0.0001–1.18.86]</td>
</tr>
<tr>
<td>1.5–4.0</td>
<td>27/118 [23%]</td>
<td>5/27 [19%]</td>
<td>0.29 [0.0006–70.44]</td>
</tr>
<tr>
<td>&gt;4.0</td>
<td>13/22 [59%]</td>
<td>4/13 [31%]</td>
<td>1.57 [0.004–55.06]</td>
</tr>
<tr>
<td>Total</td>
<td>59/308 [19%]</td>
<td>13/59 [22%]</td>
<td>0.15 [0.0001–118.86]</td>
</tr>
</tbody>
</table>

*Assuming that the 2 patients refusing lymph node dissection (with uneventful follow up) had no involved nodes, 195% confidence interval, 15% to 24%, 195% confidence interval, 12% to 34%.

![Figure 1](http://jcp.bmj.com/ on June 9, 2022 by guest. Protected by copyright.)

The predicted yield of 18F-Fluorodeoxyglucose positron emission tomography (PET) scanners with different resolutions (1–10 mm), using the histopathological data from 59 patients with cutaneous melanoma and lymph node involvement. For each resolution, the percentage of PET positive tumour basins is expressed as a detection range corresponding to published target to background ratios of 1.5–10 (appendix 1).
With logistic regression analysis, we found that no established preoperative diagnostic indicator (age, sex, Breslow thickness, site of the primary melanoma) could predict PET positivity at any presumed detection limit. However, if the SN biopsy information (the tumour volume in the SN) was included, the amount of tumour in the SN was the only significant predictor for the presence of PET detectable tumour in non-SNs, but only at detection limits of < 65.4 mm³. It is predicted that at the 14.1 mm³ (3 mm) detection limit (p = 0.006), PET will miss more than 29% of the positive lymph nodes in patients with SN tumour volumes exceeding 19 mm³.

**DISCUSSION**

Our present morphometric study shows that the average total size of metastatic deposits in regional lymph node basins of patients with cutaneous melanoma is quite small. Within a broad range of detection limits for FDG-PET, most of these deposits remain undetectable. Earlier claims that PET would be of value²⁴–²⁵ have therefore been too optimistic.²⁶ Large patient studies to validate this conclusion in the clinical setting would result in unnecessary costs. Such studies may only be useful in a specific subset of patients—for example, those with a large (> 19 mm³) tumour load in the SN.

This is the first study using histomorphometric data to predict the usefulness of a new imaging technique for a certain indication instead of performing a traditional accuracy study. We first assessed what could be expected from FDG-PET. Our method provides a clear answer to this question and makes further patient studies superfluous, which could save much unnecessary cost. In addition, this method is probably useful in other cancers with predictable lymphatic spread, such as breast cancer, where similar optimistic expectations of PET sensitivity have been postulated,²⁷ but which have also been challenged.²⁸

The use of a “cumulative tumour volume” in separate lymph nodes to represent the total tumour burden in the basin obviously leads to some overestimation of PET sensitivity. On the other hand, PET sensitivity may be underestimated because of tissue shrinkage during fixation leading to reduced volume measurements, and the fact that histopathology does not identify 100% of tumour deposits,²⁴ as explained in the Methods section. However, we have tried to correct for this as much as possible. A further refinement could have been to use a sphere model approach instead of assuming that the metastases are round, but it is unlikely that this would have influenced the final results. Overall, we feel that our approach has resulted in realistic tumour volume assessments and resulting PET sensitivity estimations allowing a clear conclusion.

“The average total size of metastatic deposits in regional lymph node basins of patients with cutaneous melanoma is quite small”

Our prediction of the value of PET for initial regional staging (fig 1) is in agreement with in vivo data of four studies.²⁴–²⁷ The results of the study by Crippa et al.,²⁶ in which 23% of the nodal metastases < 5 mm were detected by PET, are comparable because in our database 95% of the tumour diameters were < 5 mm. The SN status appears to be the most important prognostic factor for recurrence;²⁶,²⁷ and the best method to select patients with melanoma for adjuvant trials.²⁸–²⁹ Because FDG-PET is an established method to monitor the early response to systemic treatment, PET could then provide a tool to identify subclinical tumours and to monitor their response to treatment in this specific subset of SN positive patients. In our opinion, the next step should be to study this specific subset of SN positive patients and to perform LND regardless of the PET results.

PET scanning is unable to detect small metastatic deposits in SNs of patients with primary melanoma. FDG-PET cannot replace the SN biopsy, which is still the preferred method for initial regional staging.

We conclude that morphometric analysis of metastatic load predicts that PET scanning is unable to detect most metastatic deposits in the sentinel and regional lymph nodes of patients with cutaneous melanoma because these metastases are often small. Therefore, the SN biopsy remains the preferred method for initial regional staging. Thus, PET studies where patients are scanned for this indication are superfluous.

**APPENDIX 1 LESION DETECTABILITY IN EMISSION TOMOGRAPHY**

Detectability is a function of resolution and contrast. According to standard principles, at a 5 mm system resolution (R), maximally 50% of the activity (emitted counts) from a lesion of 5 mm diameter (D) will be registered because recovery (the ratio between registered and emitted counts) is as follows: 1 – exp[-ln2*(D/R)²].²⁴ For various target to background (T/B) ratios, the contrast resulting from this ratio and the resolution of the imaging system can be determined because contrast = 1 + (T/B – 1) exp [-ln2*(D/R)²].

**REFERENCES**


**Take home messages**

- The morphometric analysis of metastatic load predicts that positron emission tomography scanning cannot detect most metastatic deposits in the sentinel lymph nodes of patients with melanoma because the metastases are usually too small.
- The sentinel node biopsy is therefore still the preferred method for initial regional staging in melanoma.
286 Mijnhout, Hoekstra, van Lingen, et al


