The difficulty with measuring the largest melanoma tumour diameter in sentinel lymph nodes

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ABSTRACT Identification of sentinel node (SN) metastases can set the adjuvant systemic therapy indication for stage III melanoma patients. For stage IIIA patients, a 1.0 mm threshold for the largest SN tumour diameter is used. Therefore, uniform reproducible measurement of its size is crucial. At present, the number of deposits or their microanatomical sites are not part of the inclusion criteria for adjuvant treatment. The goal of the current study was to show examples of the difficulty of measuring SN melanoma tumour diameter and teach how it should be measured. Histopathological slides of SN-positive melanoma patients were retrieved using the Dutch Pathology Registry (PALGA). Fourteen samples with the largest SN metastasis around 1.0 mm were uploaded via tele-pathology and digitally measured by 12 pathologists to reflect current practice of measurements in challenging cases. Recommendations as educational examples were provided. Microanatomical location of melanoma metastases was 1 subcapsular, 2 parenchymal and 11 combined. The smallest and largest difference in measurements were 0.24 mm and 4.81 mm, respectively. 11/14 cases (78.6%) showed no agreement regarding the 1.0 mm cut-off. The median discrepancy for cases ≤5 deposits was 0.5 mm (range 0.24–0.60, n=3) and 2.51 mm (range 0.71–4.81, n=11) for cases with ≥6 deposits. Disconcordance in measuring SN tumour burden is correlated with the number of deposits. Awareness of this discordance in challenging cases, for example, cases with multiple small deposits, is important for clinical management. Illustrating cases to reduce differences in size measurement are provided.

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INTRODUCTION

Sentinel node (SN) biopsy is an important part of routine staging for clinically localised melanoma patients.[1] The identification of SN metastases significantly impacts clinical practice, as it implicates worse survival[2] and nowadays sets the indication for adjuvant systemic therapies for stage III patients.[3] In line with the inclusion criteria of the phase 3 adjuvant trials,[4–6] for a subset of these patients (stage IIIA according to the seventh edition of the American Joint Committee on Cancer melanoma staging system) a threshold of >1.0 mm of the largest tumour diameter in the SN biopsy (ie, 'SN tumour burden') sets the indication for adjuvant systemic therapy.[7] The European Organisation for Research and Treatment of Cancer (EORTC) Melanoma Group defines SN tumour burden as 'a single measurement of the maximum diameter of the largest lesion in any direction'.[8 9] However, in daily practice, it appears that this definition is not clear enough, because pathologists are frequently unsure how to measure the maximum diameter, since metastatic cells often present as discontinuous groups. For this reason, measuring the SN tumour burden is prone to observer variation, especially in challenging cases. Surprisingly, evidence-based studies in the melanoma literature assessing the interobserved agreement for measuring SN tumour burden are sparse,[10] and only one study has systematically re-evaluated measurements of positive SNs.[11] This is important, because part of these patients (stage IIIA) could nowadays be at risk of unjustified exposure to the severe and potentially fatal side effects of adjuvant systemic treatment in case of overestimation of SN tumour burden.[12] By identifying these patients, the high costs of these systemic therapies can be avoided.[13] On the other hand, patients are also at risk of losing their indication for systemic therapy in case their SN tumour burden is incorrectly measured below 1.0 mm. Therefore, uniform and reproducible assessment of SN tumour burden is crucial. The goal of the current study was to show examples of how the largest melanoma tumour diameter in sentinel lymph nodes should be measured. This was done by selecting a number of cases that were challenging, mainly because the largest tumour diameter in the SN biopsy was approximately 1.0 mm, and different measurement would have crucial therapeutic implications for the patient.

PATIENTS AND METHODS

Collection of data Data for this retrospective nationwide study were obtained from PALGA, the Dutch Nationwide Network and Registry of Histopathology and Cytopathology. Since 1991, PALGA has prospectively been collecting data from all pathology laboratories in the Netherlands.[14] All data were encoded and used anonymously.

Study population

Cases were selected from a cohort (n=322) previously described.[15] Prior to selecting eligible cases for this study, the largest melanoma tumour diameter in the SN biopsy was measured in all cases as recommended by the EORTC as a measurement of the maximum diameter of the largest lesion in any

Table 1 Tumour burden measurements per individual case for all 14 cases that were sentinel node (SN)-positive

Case number	Median size of SN tumour burden measurement in mm (range)	Biggest difference of SN tumour burden measurement (mm)	Difference at 1.0 mm threshold
1	1.3 (0.8–5.3)	4.5	Yes
2	0.3 (0.1–3.4)	3.3	Yes
3	0.7 (0.5–1.2)	0.7	Yes
4	0.6 (0.5–2.2)	1.7	Yes
5	1.0 (1.0–1.5)	1.0	Yes
6	1.1 (0.2–1.2)	0.2	Yes
7	1.0 (0.8–1.4)	0.6	Yes
8	1.3 (1.0–1.5)	0.5	No
9	3.3 (2.1–6.9)	4.8	No
10	1.0 (0.2–2.8)	2.6	Yes
11	0.6 (0.8–1.2)	0.8	Yes
12	1.1 (0.7–3.2)	2.5	Yes
13	3.1 (2.5–3.8)	2.5	No
14	0.5 (0.4–3.0)	2.6	Yes

direction.[8 9] A total of 14 'challenging' cases where discrepancies were to be expected were selected for digital review. Cases with a largest tumour diameter in the SN biopsy of approximately 1.0 mm were preferentially selected. The number of deposits (1, 2–5, 6–10, 11–20 or >20) and their localisation (subcapsular, parenchymal, combined, extensive confluent or extensive multifocal) were categorised according to Dewar *et al*.[16] All whole-slide cases were digitally reviewed by 12 experienced pathologists on both H&E and immunohistochemically stained slides. All pathologists examined the same single H&E and single immunohistochemically stained slide that were selected per case. No further instructions were given, in order to simulate each pathologists' individual daily practice in evaluating SN tumour burden as much as possible. The tumour diameter was measured by a digital ruler. The absolute overall variation in measurement was defined as the difference between the smallest and the largest measurement of all 12 pathologists for a single case.

Statistical analysis

Categorical variables were summarised as numbers and percentages. Continuous variables were summarised as median with IQR for non-normally distributed data. Data were analysed using R V.3.6.1 and SPSS V.26. Because the aim of the paper was educational, and because of the relatively low number of cases, no further statistics were conducted.

RESULTS

Concordance among pathologists

Table 1 shows an overview of the 14 cases with melanoma metastasis. Of the 14 SN-positive cases, 14 had H&E staining available, 13 additional Melan-A staining and 11 S100 staining. The median difference between all cases was 2.09 mm (IQR 0.68–2.79). The smallest absolute overall variation in measurement was 0.24 mm (case 6), and the largest difference was 4.81 mm (case 9). In 11 of the 14 cases (78.6%) there were measurements of ≤1.0 mm and >1.0 mm (figure 1).Figure 2 shows an overview of each individual measurement per pathologist per case. Examples of three of the cases, along with the recommended method to measure the SN tumour burden, are shown in figure 3.

Number of deposits and microanatomical location

Microanatomical location of melanoma metastases was the following: 1 subcapsular (case 13), 2 parenchymal (cases 7 and 12) and 11 combined. The number of deposits (1, 2–5, 6–10, 11–20 or >20) represented 1, 2, 3, 5 and 3 of the cases, respectively, Cases 6, 7 and 8 harboured 5 or less deposits, with case 6 having only one solitary deposit and cases 7 and 8 harbouring 2–5 deposits. The median discrepancy for cases up to five deposits was 0.5 mm (range 0.24–0.60) and 2.51 mm (range 0.71–4.81) for cases with six or more deposits.

DISCUSSION

The present study was performed in order to examine how pathologists measure the largest melanoma tumour diameter in sentinel lymph nodes in current clinical practice in selected cases around the critical threshold of 1.0 mm, and to provide examples of how it should be measured. Measurements of 11 cases (78.6%) showed no agreement with respect to the clinical cut-off of 1.0 mm, which would lead to different indications for adjuvant systemic therapy. The number of deposits was statistically significantly correlated with this interobserver variability, as the median discrepancy for cases up to five deposits was only 0.5 mm, and 2.5 mm for cases with six or more deposits.

Literature assessing the interobserver agreement for measuring SN tumour burden is sparse. In one study, 7 experienced pathologists reviewed 44 randomly selected cases containing metastatic melanoma. The interobserver agreement for SN tumour burden measured as the maximum size of the largest deposit, was good to excellent (intraclass correlation coefficient 0.88 (95% CI 0.82 to 0.93)).[10] However, a major difference is that we specifically selected cases with SN metastases around the clinical threshold of 1.0 mm, in which a possible disagreement in the measurement would result in a different indication for adjuvant systemic therapy. Thus, we evidently expect the percentage in 'real-life' practice to be lower than what we present in the current study (78.6%).

Only one previous study has systematically re-evaluated measurements of positive SN biopsies in melanoma patients.[11] Franke *et al* reviewed 159 patients who received adjuvant treatment based on the largest diameter of the SN tumour burden of larger than 1 mm. Fourteen cases proved not to be a melanoma metastasis at all, but concerned a capsular nevus, and two cases merely had melanophages. In the remaining 145 patients, the SN tumour diameter turned out to be less than 1 mm in 12 patients (8.3%), and in 4 patients the SN tumour burden proved larger than 1.0 mm after expert review. In line with our results,

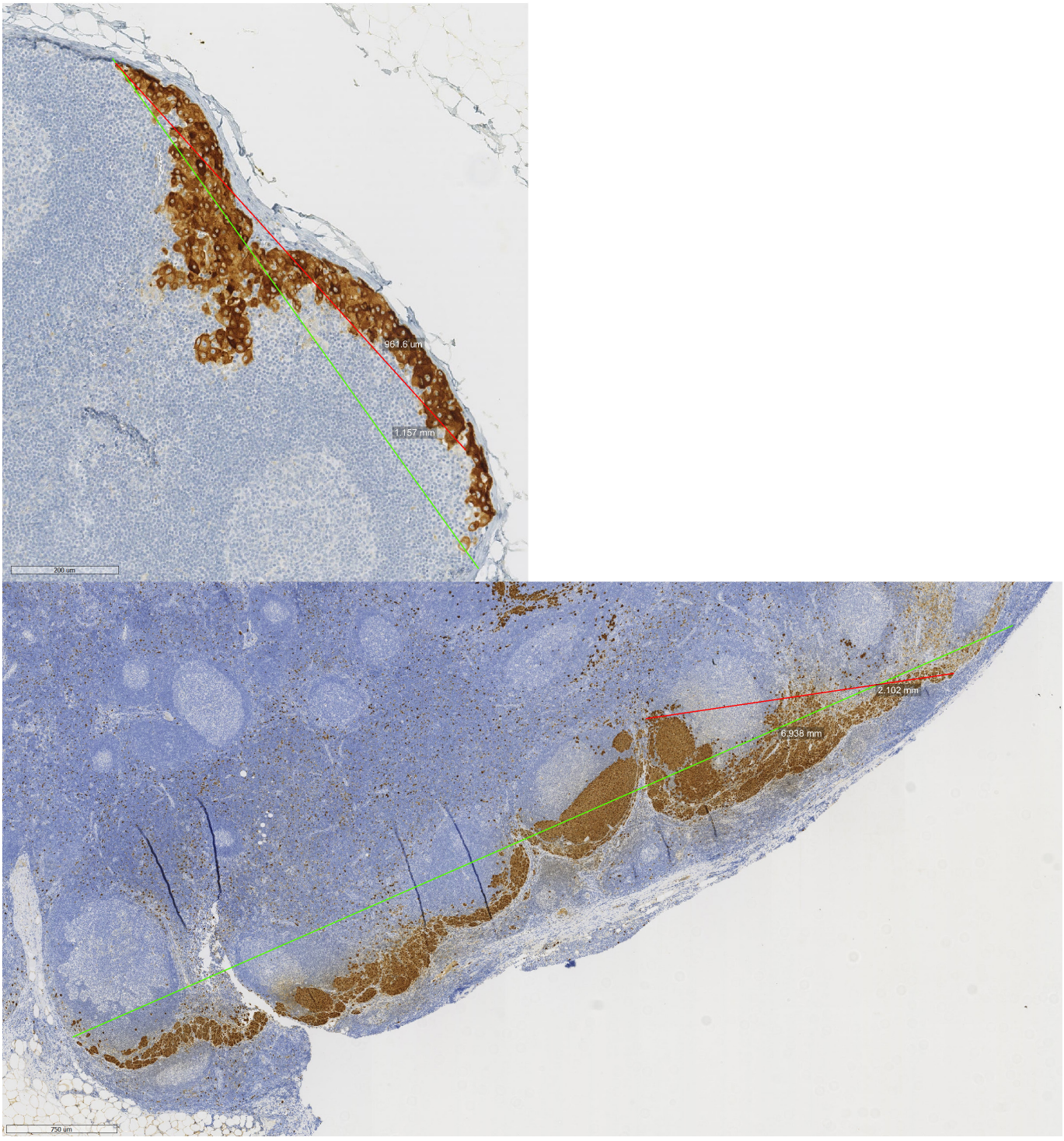

Figure 1 Illustrations of the case with the highest concordance (case 6, upper figure) and the case with the lowest concordance (case 9, lower figure). For case 6 the absolute difference was 0.24 mm and for case 9 the absolute difference was 4.81 mm.

this study also demonstrates the existence of relevant discrepancies in SN measurements among pathologists. Our study shows that cases with multifocal involvement, in a so-called 'shotgun' pattern, are more prone to discordant measurements.

A unique part of our study was that all measurements were digitised and saved. This enabled us to identify that the majority of especially large discrepancies were caused by adding up metastases for size measurement instead of measuring a single largest lesion.

In 2009, the EORTC Melanoma Group proposed recommendations how to measure SN tumour burden, stating that 'the most reproducible measure is a single measurement of the maximum diameter of the largest lesion in any direction'.[8] Among others, they recommended to only measure what you see; that is, 'if two lesions are interrupted by lymphocytes (or other cells or structures), these are to be considered as two separate lesions'. In case of smaller, curved lesions, it was recommended that the diameter should be measured by a straight

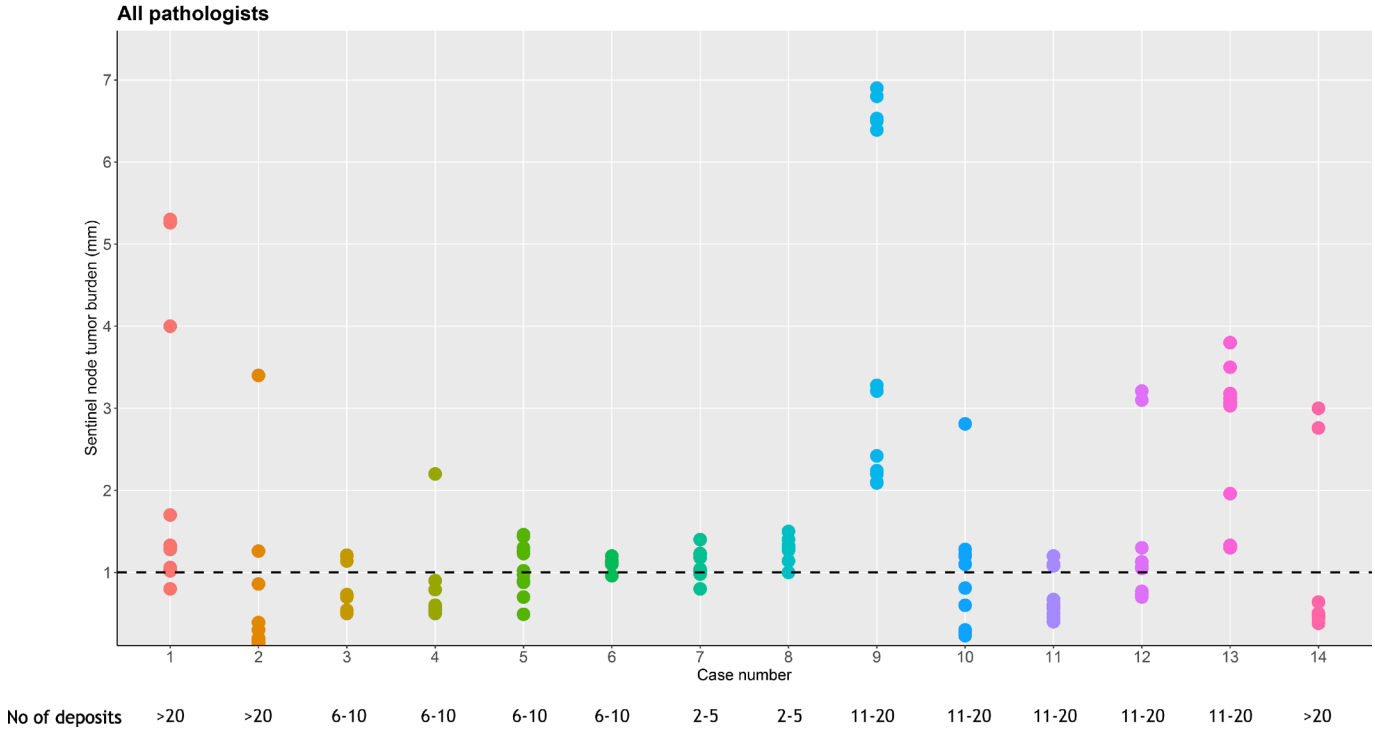

Figure 2 Overview of sentinel node tumour burden measurements for each case number per pathologist. The line on the Y-axis at 1.0 is shown to separate measurements ≤1.0 mm and >1.0 mm.

line between the furthest points. Ten years later, in 2019, the EORTC has re-emphasised the definition of SN tumour burden as the single measurement of the maximum diameter of the largest lesion in any direction.[9] Although a definition and recommendations on how to measure SN tumour burden have been proposed, the current results show that in clinical practice the current EORTC guideline apparently still leaves too much room for subjective interpretation, and may cause huge interobserver variability in measurement of the largest SN diameter between different pathologists. With the current study, we aim to renew attention on how to measure largest SN tumour diameter in patients with cutaneous melanoma. This is even more important now, because correct measurement of the largest SN tumour diameter has become of critical importance for the indication for adjuvant therapy.[3] A study by Madu *et al* showed excellent 5-year distant metastasis-free survival and melanoma-specific survival (both 91%) for stage IIIA melanoma patients with an SN tumour diameter less than 1.0 mm.[17] Therefore, discrepancies in its measurement can have great clinical consequences. As our data show, measurements tend to be overestimated, leading to potential unjustified adjuvant treatment, with high costs and potential side effects.[13] We have composed

1. Measure the maximum diameter of the largest, single lesion in any direction, regardless of its localization.
2. Measure only the lesions with confluent neoplastic cells. The lesions may not be interrupted by lymphocytes, other cells or structures.
3. Curved lesions are measured in a straight line between the furthest points of the lesion.

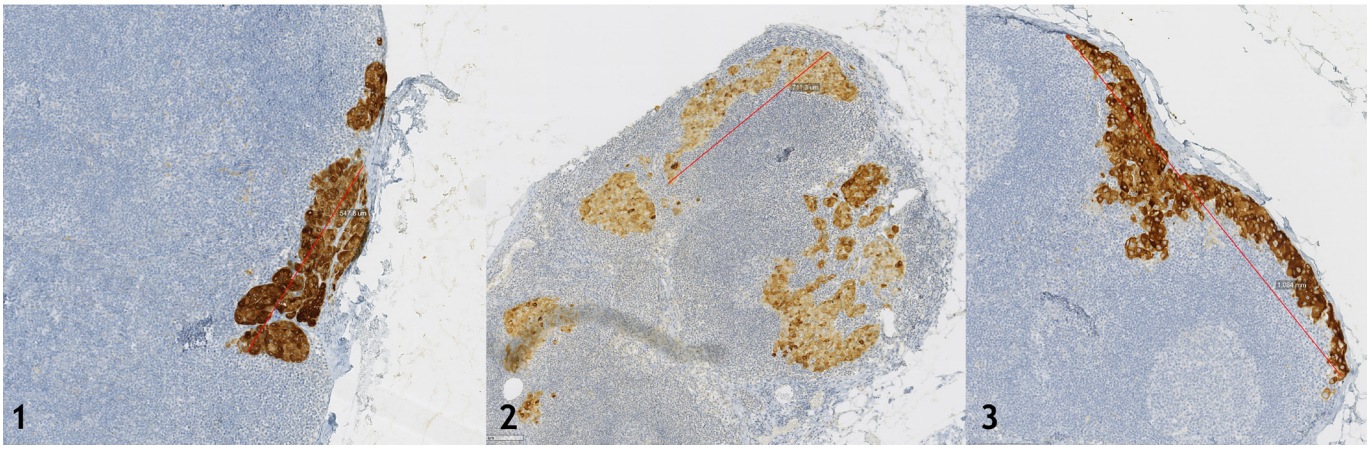

Figure 3 Recommendation box illustrated with histopathological slides of three different sentinel node-positive cases as educational examples, including measurements. All examples are Melan-A stainings.

several recommendations, illustrated by examples from clinical practice (figure 3).

Thus far there is no clear agreement on how to measure SN metastases that are comprised numerous scattered cells. In the updated EORTC-protocol for the evaluation of SN biopsy, it was suggested that for this reason 'in addition to measuring the maximum dimension of the largest metastatic deposit (and indicating its site) and the pattern of the distribution of metastases, all deposits should be counted in the most representative slides as 1, 2–5, 6–10, 11–20, or over 20'.[9] Murali *et al* also mention the 'occasional cases' of metastases with a scattered pattern that cause discrepancies in the measurements of SN tumour burden between different observers.[10] In the current study, no such case was included.

A strength of the current study is the experience of the pathologists that participated. It may therefore be expected that the interobserver agreement among general pathologists is even lower than was found in the current study. Another strength is the participation of pathologists from multiple European countries, reflecting the everyday clinical practice of measuring the SN tumour burden. Also, we did not give instructions in advance to the pathologists in order to simulate each pathologists' individual daily practice in evaluating SN tumour burden as much as possible. A suggestion for future research is to evaluate the implementation of the suggested recommendations through assessment of the learning curve of the pathologists. For the future, we would propose to do a similar study with cases of approximately 1.0 mm SN tumour burden, but this time with clear recommendations in advance to analyse whether this reduces the difference in interobserver agreement. A limitation is the relative small sample size of 14 SN-positive cases. This would have led to underpowered statistics if they were to be conducted. However, because the aim of the paper is educational, no further statistics were considered.

CONCLUSIONS

Our findings illustrate the need for refreshment of knowledge with regard to guidelines on how to measure the largest melanoma tumour diameter in the SN. The current results reveal potential large discrepancies that could have far-reaching clinical consequences. We propose to update the current EORTC guidelines, especially for challenging cases, with illustrated examples how to best measure SN melanoma tumour burden.

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