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Original Research

# High discordance rate in assessing sentinel node positivity in cutaneous melanoma: Expert review may reduce unjustified adjuvant treatment

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**KEYWORDS**
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**Abstract** ***Introduction:*** Identification of sentinel node (SN) metastases can set the adjuvant systemic therapy indication for patients with stage III melanoma. Studies re-evaluating the diagnosis of initially positive SN biopsies are scarce.
***Materials and methods:*** Dutch patients with melanoma who underwent SN biopsy between 2003 and 2014 were selected from PALGA, the Dutch Pathology Registry. Histopathological slides of SN-positive patients were retrieved for review. A random sample was reassessed by an expert melanoma pathologist. Recurrence-free survival (RFS) of patients who were misclassified (false-positive) was compared with those with a true positive SN status. For comparison, a group of SN-negative patients was included. Multivariable logistic analysis was performed to assess clinicopathological characteristics associated with misclassification of SN status.
***Results:*** Diagnosis was downgraded from melanoma metastasis to nodal nevus in 38 of the 322 reviewed patients (11.8%). Considering the inclusion criteria of phase III adjuvant trials, at least 4.3% of patients would have falsely qualified for adjuvant therapy. In multivariable analysis, patients with a low SN tumour burden and subcapsular SN tumour location had a significantly higher chance of being misclassified. The five-year RFS of the 38 downgraded

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patients was 86.7% (95% confidence interval [CI] = 72.6–96.6), similar to the 85.9% (95% CI = 84.9–86.8, p = 0.18) for 6413 SN-negative patients and better than the 53.2% (95% CI = 47.2–59.9, p = 0.009) of 284 patients who were truly SN positive upon review.

***Conclusion:*** More than 10% of originally positive SN biopsies of patients with melanoma concern misclassified nodal nevi. We advocate that when adjuvant treatment is considered in patients with stage III melanoma, SN biopsies should be reassessed by an expert melanoma pathologist.



## 1. Introduction

Sentinel node (SN) biopsy is an important part of routine staging for patients with clinically localised melanoma [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 patients with stage III disease [3]. In line with the inclusion criteria of the phase III adjuvant trials [4–6], for a subset of these patients (stage IIIA as per the 7th edition of the American Joint Committee on Cancer [AJCC] melanoma staging system), a threshold of >1.0 mm SN tumour burden is applicable for the indication for adjuvant systemic therapy [7]. Multiple studies have shown that pathological re-evaluation of initially negative SN biopsies by immunohistochemistry and serial sectioning may detect deposits of occult melanoma cells [8–15]. However, only one study seems to have systematically re-evaluated 'initially positive' SNs [16], while these are the patients who nowadays could be at risk of unjustified exposure to the severe and potentially fatal side-effects of adjuvant systemic treatment in case of a false-positive SN diagnosis [17]. Moreover, by identifying patients with a false-positive SN diagnosis, the high costs of these systemic therapies can be avoided [18]. Therefore, adequate assessment of SN positivity is crucial. The goal of the present study was to review SN biopsies of patients with melanoma that were initially diagnosed positive for melanoma metastases and determine concordance when reassessed by a dedicated melanoma pathologist. In addition, clinicopathological characteristics associated with misdiagnosis were assessed, and survival of patients who were initially misdiagnosed as having stage III disease upon review was compared with that of SN-negative patients and truly SN-positive patients.

## 2. Materials and methods

### 2.1. 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 [19]. All data were encoded and used anonymously. Ethical approval was granted by the board of PALGA.

### 2.2. Study population

Pathology reports of all newly diagnosed patients with invasive melanoma in the Netherlands between January 2003 and December 2014 for whom SN biopsy was performed were analysed. Patients presenting with stage III locoregional metastases (defined as in-transit, satellite or lymph node metastases other than in the SN biopsy) or stage IV disease (distant metastases) within 12 weeks of initial diagnosis were excluded. Patients with multiple primary melanoma, non-cutaneous melanoma, desmoplastic melanoma, microsatellites and melanomas occurring in children (age <18 years), were also excluded. For each patient, clinical and pathological variables were extracted from the pathology files, including the date of diagnosis, age, gender, Breslow thickness (in millimetre), presence of ulceration, melanoma subtype, anatomical localisation, recurrence (date, site and type, skin—local or in-transit, in regional nodes or at a distant site), SN status and the number of positive SN biopsies. Patients were classified as per the 7th AJCC melanoma staging system because this version was used in the randomised controlled trials studying the efficacy of adjuvant systemic therapy in patients with stage III melanoma [4–6].

For all positive SN biopsies, all anonymously coded histopathological slides (haematoxylin and eosin [H&E] and all available immunochemical staining) were requested at each individual hospital and/or pathology laboratory in the Netherlands for review (n = 26 pathology laboratories, n = 1279 cases). Not all patients were reassessed because of efficiency and time reasons. Only 8 pathology laboratories did not have their slides available for review, for reasons that they did not have time or personnel to retrieve their slides: they did not respond to our invite on multiple occasions, or there were additional costs that we could not account for. For the present study, of the 26 packages with slides that we received, we randomly selected all cases from one

pathology laboratory and all cases from the consecutive four pathology laboratories that we received thereafter (n = 322 from 5 pathology laboratories; two academic and three non-academic). These 322 cases were reviewed by a dedicated European Organisation for Research and Treatment of Cancer (EORTC) melanoma pathologist (W.A.M.B.), and the first two authors (M.-A.E.S. and A.E.L.), to review the diagnosis and to assess tumour burden (in mm) and localisation (subcapsular and non-subcapsular). Tumour burden was defined as the single measurement of the maximum diameter of the largest lesion in any direction, as per the EORTC protocol [20]. SN-negative patients were included to compare survival, and their pathology reports were analysed to see if nodal nevi were present. A nodal nevus was defined as a collection of non-atypical nevoid melanocytes located within the capsule, sometimes extending within the septa or trabecula of the capsule deeper within the lymph node. Melanoma metastasis was defined by the presence of morphological atypical melanocytes present within the subcapsular region or parenchyma of a lymph node. Patients with both subcapsular and non-subcapsular SN tumour deposits were classified as non-subcapsular. The primary melanoma was not available for comparison. We had access to the slides analysed in the initial evaluation of the SN biopsy (mostly H&E, S100 and Melan-A or MART staining). No PRAME, p16, HMB45 or Ki-67 staining was available.

To check if the downstaging to nodal nevus was justified, recurrence-free survival (RFS) was compared between patients with an initially positive SN status who were downgraded to a nodal nevus, those with a persistent positive SN status upon review, SN-negative patients and SN-negative patients with a nodal nevus. In patients with multisite first recurrences, the site associated with the worst prognosis was scored as the first site. RFS was calculated from the date of initial melanoma diagnosis to the date of diagnosis of recurrence. Patients without recurrence were censored at either their date of death or the last date known alive or 1st January 2018 (the data collection cut-off date), whichever occurred earlier.

### 2.3. Statistical analysis

Categorical variables were summarised as numbers and percentages. Continuous variables were summarised as median with interquartile range for non-normally distributed data or mean with standard deviation for normally distributed data. Differences in proportions and medians were analysed using chi-square tests or the Mann-Whitney U test, respectively. Differences in means were assessed using Student's t-test. Kaplan-Meier curves were generated to compare RFS using paired log-rank tests among SN-negative patients, SN-positive patients, patients with a nodal nevus upon review and all SN-negative patients with a nodal nevus reported in their histology report. A logistic regression analysis was performed for all reviewed patients to assess which variables predicted a downgraded diagnosis. The model included Breslow thickness, tumour burden, gender, age, Dewar localisation of tumour burden, ulceration status, anatomical location and melanoma subtype. A 'not known' category was created for missing status for ulceration and anatomical location.

Data were analysed using R, version 3.6.1, and SPSS, version 26. A two-sided p-value of $<0.05$ was considered statistically significant.

## 3. Results

### 3.1. Recruitment and review

A random sample of 322 slides of sufficient quality was reviewed (Supplementary Fig. 1). There were no statistically significant differences in clinicopathological variables between the reviewed and non-reviewed cases, except for localisation of the melanoma (Table 1). In 287 of the 322 (89.1%) reviewed cases, an additional S100 and/or Melan-A staining was available besides H&E staining.

### 3.2. Downgraded diagnoses in relation to indication of adjuvant therapy

The diagnosis was downgraded from melanoma metastasis to nodal nevi in 38 patients (11.8%) (Table 2). S100 and/or Melan-A staining was available for all of these patients. The percentage of downgraded cases was comparable in each of the five pathology laboratories: 10.3%, 11.5%, 12.4% and 14.7%, except for one academic pathology laboratory that had 5.0% of their cases downgraded. Of the 322 reviewed patients, 175 patients were initially staged IIIA, 123 were staged IIIB/C and in 24, further determination was not possible because of missing ulceration status (Table 1) [7]. The size of the nodal nevus of the 38 downgraded patients ranged from 0.005 mm to 1.5 mm. Sixty-four of the 175 patients staged as IIIA (36.6%) had a SN tumour burden of $>1.0$ mm. Of the 38 misdiagnosed patients, 25 patients would have been incorrectly staged as IIIA if they would not have been reviewed. Of these 25 patients, 4 had an SN tumour burden $>1.0$ mm. Eight patients would have been incorrectly staged as stage IIIB/C, and in 5 patients, further determination was not possible because of missing ulceration status, but regardless, 2 had a SN tumour burden $>1.0$ mm. Thus, considering the inclusion criteria of the phase III adjuvant trials, at least 14 patients (4.3%) would have been falsely qualified for adjuvant therapy: 4 patients with stage IIIA disease with a nodal nevus of $>1.0$ mm, 8 patients with stage

Table 1
Baseline clinicopathological data of pathologically reviewed and non-reviewed patients with cutaneous melanoma with a positive sentinel node status.

| Clinicopathological characteristic | Reviewed cases (n = 322) | Non-reviewed cases (n = 957) | p-value |
|---|---|---|---|
| **Gender, n (%)** | | | 0.74 |
| Female | 147 (45.7) | 447 (46.7) | |
| Male | 175 (54.3) | 510 (53.3) | |
| **Mean age in years (SD)** | 54.1 (15.4) | 54.3 (14.7) | 0.88 |
| **Median Breslow thickness in millimetre (IQR)** | 2.5 (1.7–4.0) | 2.4 (1.6–3.7) | 0.27 |
| **Breslow thickness in millimetre, n (%)** | | | 0.17 |
| 0.1–0.7 | 1 (0.3) | 10 (0.1) | |
| 0.8–1.0 | 7 (2.2) | 42 (4.4) | |
| 1.1–2.0 | 106 (32.9) | 336 (35.1) | |
| 2.1–4.0 | 140 (43.5) | 396 (41.4) | |
| >4.1 | 68 (21.1) | 173 (18.1) | |
| **Ulceration, n (%)** | | | 0.38 |
| No | 179 (55.6) | 548 (57.3) | |
| Yes | 119 (37.0) | 320 (33.4) | |
| Unknown | 24 (7.5) | 89 (9.3) | |
| **Localisation, n (%)** | | | 0.01 |
| Head and neck | 8 (2.5) | 47 (4.9) | |
| Trunk | 145 (45.0) | 474 (49.5) | |
| Arms | 27 (8.4) | 88 (9.2) | |
| Legs | 124 (38.5) | 325 (34.0) | |
| Unknown | 18 (5.6) | 23 (2.4) | |
| **Subtype, n (%)** | | | 0.20 |
| Superficial spreading | 191 (59.5) | 545 (56.9) | |
| Nodular | 84 (26.2) | 270 (28.2) | |
| Lentigo maligna | 1 (0.3) | 0 (0) | |
| Acral lentiginous | 11 (3.4) | 21 (2.2) | |
| Unknown | 34 (10.6) | 122 (12.7) | |
| **Stage as per the 7th AJCC, n (%)** | | | 0.29 |
| IIIA | 175 (54.3) | 544 (56.8) | |
| IIIB/C | 123 (38.2) | 324 (33.9) | |
| Unknown | 24 (7.5) | 89 (9.3) | |
| **Stage as per the 8th AJCC, n (%)** | | | 0.40 |
| IIIA | 87 (27.0) | 287 (30.0) | |
| IIIB | 85 (26.4) | 270 (28.2) | |
| IIIC | 130 (40.4) | 330 (34.5) | |
| IIID | 0 (0.0) | 1 (0.1) | |
| Unknown | 20 (6.2) | 69 (7.2) | |

AJCC = American Joint Committee on Cancer, SD = standard deviation, IQR = interquartile range.

Table 2
Baseline data of 322 patients with melanoma who were originally diagnosed as sentinel node positive, stratified by pathology review status.

| Clinicopathological characteristic | SN negative upon review (n = 38) | SN positive upon review (n = 284) | p-value |
|---|---|---|---|
| **Gender, n (%)** | | | 0.42 |
| Female | 15 (10.2) | 132 (89.8) | |
| Male | 23 (13.1) | 152 (86.9) | |
| **Median tumour burden in millimetre (IQR)** | 0.3 (0.2–0.6) | 0.9 (0.3–2.3) | <0.001 |
| **Tumour burden in millimetre, n (%)** | | | 0.007 |
| <0.1 | 4 (18.2) | 18 (81.8) | |
| 0.1–1.0 | 27 (16.5) | 137 (83.5) | |
| >1.0 | 7 (5.1) | 129 (94.9) | |
| **Tumour burden location, n (%)** | | | <0.001 |
| Subcapsular | 12 (37.5) | 20 (62.5) | |
| Non-subcapsular[a] | 26 (9.0) | 264 (91.0) | |
| **Mean age at diagnosis in years (SD)** | 51.6 (13.9) | 54.5 (15.6) | 0.29 |
| **Median age at diagnosis in years (IQR)** | 52 (41–64) | 55 (43–67) | 0.23 |
| **Median Breslow thickness in millimetre (IQR)** | 1.8 (1.2–2.5) | 2.6 (1.8–4.0) | <0.001 |
| **Breslow thickness in millimetre, n (%)** | | | 0.01 |
| 0.1–0.7 | 0 (0.0) | 1 (100) | |
| 0.8–1.0 | 2 (28.6) | 5 (71.4) | |
| 1.1–2.0 | 21 (20.0) | 85 (80.2) | |
| 2.1–4.0 | 11 (7.9) | 129 (92.1) | |
| >4.1 | 4 (5.9) | 64 (94.1) | |
| **Localisation, n (%)** | | | 0.84 |
| Head and neck | 1 (12.5) | 7 (87.5) | |
| Trunk | 19 (13.1) | 126 (86.9) | |
| Arms | 2 (7.4) | 25 (92.6) | |
| Legs | 15 (12.1) | 109 (87.9) | |
| Unknown | 1 (5.6) | 17 (94.4) | |
| **Subtype, n (%)** | | | 0.64 |
| Superficial spreading | 26 (13.5) | 166 (86.5) | |
| Nodular | 9 (10.7) | 75 (89.3) | |
| Lentigo maligna | 0 (0.0 | 1 (100) | |
| Acral lentiginous | 0 (0.0) | 11 (100) | |
| Unknown | 3 (8.8) | 31 (91.2) | |
| **Ulceration, n (%)** | | | 0.06 |
| No | 25 (14.0) | 154 (86.0) | |
| Yes | 8 (6.7) | 111 (93.3) | |
| Unknown | 5 (20.8) | 19 (79.2) | |
| **Stage as per the 7th AJCC, n (%)** | | | 0.04 |
| IIIA | 25 (14.3) | 150 (85.7) | |
| IIIB/C | 8 (6.5) | 115 (93.5) | |
| Unknown | 5 (20.8) | 19 (79.2) | |

SN = sentinel node, AJCC = American Joint Committee on Cancer, SD = standard deviation, IQR = interquartile range.
[a] Patients with both subcapsular and non-subcapsular SN tumour deposits were classified as non-subcapsular.

IIIB/IIIC disease and 2 patients with unknown stage III, but with a SN tumour burden >1.0 mm.

### 3.3. Logistic regression

When assessing the association between clinicopathological characteristics and the chance of downgrading an initial SN-positive biopsy to a nodal nevus, on multivariable analysis, two predictors remained statistically significant: SN tumour burden (odds ratio [OR] = 0.39 [95% confidence interval {CI} = 0.19–0.78], p = 0.008) and non-subcapsular location of the nodal nevus (OR = 0.31 (95% CI = 0.13–0.72, p = 0.006) (Table 3). Examples of cases for which the diagnosis was downgraded from melanoma metastases to nodal nevi are displayed in Fig. 2. Some display unusual large nevi

Table 3
Multivariable logistic regression for misdiagnosis of nodal nevus as melanoma metastasis in 322 patients.

| Variable | Definition | Univariable | | Multivariable[a] | |
|---|---|---|---|---|---|
| | | OR (95% CI) | p-value | OR (95% CI) | p-value |
| Breslow thickness | Per millimetre | 0.64 (0.47–0.87) | 0.004 | – | – |
| Tumour burden[b] | Per millimetre | 0.32 (0.16–0.65) | 0.002 | 0.39 (0.19–0.78) | 0.008 |
| Dewar localisation | Subcapsular | 1 | | 1 | |
| | Non-subcapsular | 0.16 (0.07–0.37) | <0.001 | 0.31 (0.13–0.72) | 0.006 |
| Age | Per year | 0.99 (0.97–1.01) | 0.29 | – | – |
| Gender | Male | 1 | | – | – |
| | Female | 0.75 (0.38–1.50) | 0.42 | – | |
| Ulceration | No | 1 | | – | – |
| | Yes | 0.44 (0.19–1.02) | 0.06 | – | |
| | Missing | 1.62 (0.56–4.74) | 0.38 | – | |
| Anatomic location | Head and neck | 1 | | – | – |
| | Trunk | 1.06 (0.12–9.06) | 0.96 | – | |
| | Arm | 0.56 (0.04–7.12) | 0.66 | – | |
| | Legs | 0.96 (0.11–8.38) | 0.98 | – | |
| | Missing | 0.41 (0.02–7.55) | 0.55 | – | |
| Melanoma subtype | SSM | 1 | | – | – |
| | NM | 0.77 (0.34–1.72) | 0.52 | – | |
| | Other | 0.45 (0.13–1.54) | 0.20 | – | |

OR = odds ratio, CI = confidence interval, SSM = superficial spreading melanoma, NM = nodular melanoma.
[a] Only variables that were statically significant are shown. All variables that are shown in the univariable analysis were included in the multivariable analysis.
[b] Defined as the single measurement of the maximum diameter of the largest lesion in any direction.

some with paraseptal and/or focal parenchymal extension.

### 3.4. Survival comparison

A total of 6900 SN-negative patients were included for survival comparison, of which 487 had a nodal nevus. The five-year RFS was 85.1% (95% CI = 81.5–88.8) for the 487 SN-negative patients with a nodal nevus and 85.9 (95% CI = 84.9–86.8) for the remaining SN-negative patients (Fig. 1). The five-year RFS of the 38 patients with a downgraded diagnosis upon review was 86.7 (95% CI = 72.6–96.6), which was not statistically significantly different from that of the 487 SN-negative patients with a nodal nevus (p = 0.41) and from that of the remaining 6413 SN-negative patients (p = 0.18). There was a statistically significant difference in RFS between the 38 downgraded patients (86.7 [95% CI = 72.6–96.6]) and the 5-year RFS of the 284 patients that remained SN positive after review (53.2% [95% CI = 47.2–59.9], p = 0.009).

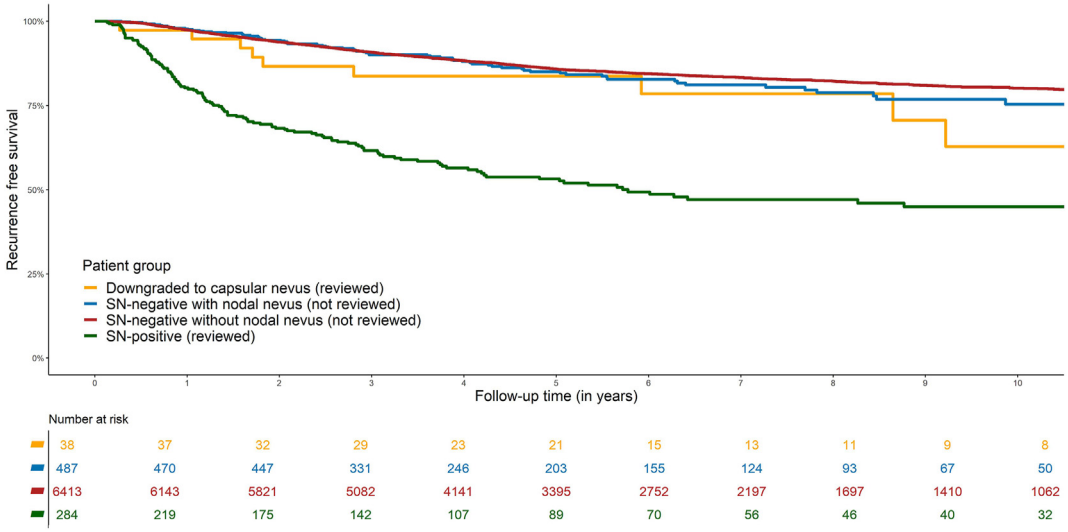


Fig. 1. Kaplan-Meier curves for recurrence-free survival of sentinel node false-positive, true-positive and sentinel node–negative patients with melanoma. SN = sentinel node.

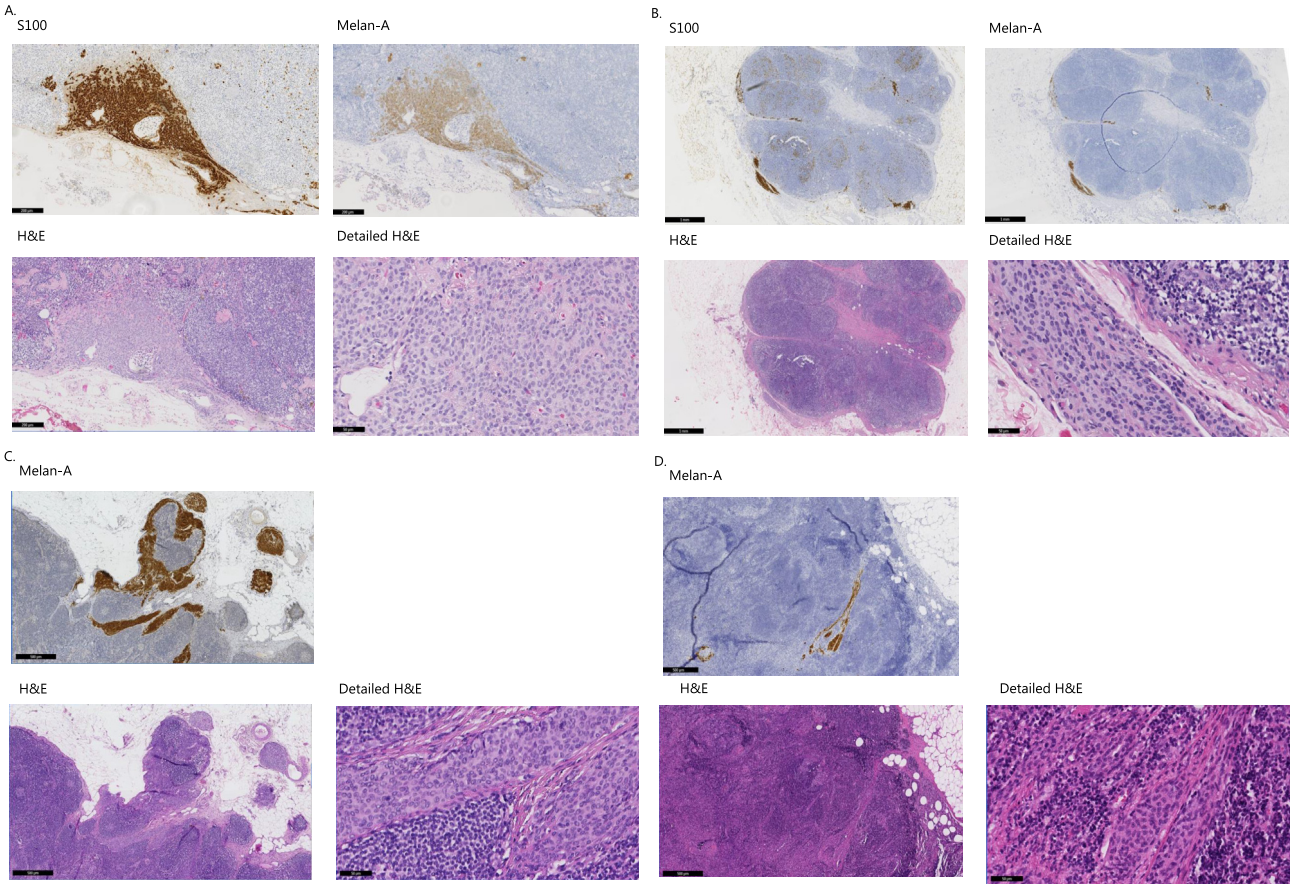


Fig. 2. (A–D) Illustrating examples of four cases in which diagnosis was downgraded from melanoma metastases to nodal nevus. (A–C) Nevus typically located in the capsule. The melanocytic cells lack atypia or mitoses precluding a diagnosis of melanoma metastasis. (C) Extensive and large nodal nevus with however typical capsular location. (B–D) Paratrabecular or septal deeper extension of nevus cells along fibrous bands originating from the capsule. These nevus cells can easily be misdiagnosed as melanoma metastases if pathologists misinterpret septal extension as parenchymal location of melanocytes. Immunostaining (S100 and/or Melan-A) confirms the melanocytic nature of the nevus cells and highlights the nodal/septal location of melanocytes. H&E = haematoxylin and eosin.

## 4. Discussion

This study was undertaken to reassess SN biopsies of patients with melanoma who were initially diagnosed positive for melanoma metastases and determine concordance when reassessed by an expert melanoma pathologist. Our results show that more than 10% of originally positive SN biopsies in patients with melanoma concerned capsular nevi that were misclassified as melanoma metastasis and that potentially at least 4.3% of patients with stage III disease nowadays would receive unjustified adjuvant treatment based on an overdiagnosed SN biopsy [7].

Recently, adjuvant therapy for patients with stage III melanoma has been proven to increase relapse-free survival for patients with melanoma [3] and is currently being implemented worldwide. Because a positive SN status is generally considered to be an indication for adjuvant therapy, the number of SN biopsies performed in patients with melanoma, which currently ranges from 40% to only 60% in large nationwide data, is likely to increase [21,22]. Therefore, adequate assessment of SN positivity (and its tumour burden) is more important than ever.

For a subset of these patients (stage IIIA), most adjuvant therapy guidelines apply a threshold of >1.0 mm SN tumour burden, e.g. as approved by the Food and Drug Administration [7]. This is because in the adjuvant setting, all studies with regard to patients with stage IIIA disease have been performed on patients with an SN tumour burden >1.0 mm [4–6]. In line with this, the European Society for Medical Oncology also advocates that treatment decisions for patients with stage IIIA disease and SN ≤1.0 mm should be made on an individual basis, and the European Association of Dermato Oncology and EORTC state it should be carefully discussed with these patients [23,24]. If we would not account for a >1.0 mm SN tumour burden threshold for patients with stage IIIA disease, all 38 misdiagnosed cases (11.8%) would have falsely qualified for adjuvant treatment.

Most studies that have shown that pathological review of 'initially negative' SN biopsies could lead to the detection of melanoma metastases reviewed only SN

biopsies of patients who developed metastatic disease during follow-up [8,10–13]. This led to percentages of upgraded diagnoses from 20% [11] to 43% [12]. We could identify only one study that reviewed an unbiased population of negative SN biopsies, which found a 5% upgrade rate [9]. However, to the best of our knowledge, only one study has previously re-evaluated 'initially positive' SNs and found a downgrade rate of 10.1% (16 out of 159 patients) [16]. Identifying falsely positive SNs is of importance because they put patients at risk of unjustified exposure to the potentially fatal side-effects of adjuvant systemic treatment [17]. Moreover, by identifying patients with a false-positive SN, the high costs of these systemic therapies can be avoided [18]. As the present results show a high downgrade rate (11.8%) of initially positive SN biopsies and that 44 of 1000 patients might receive unjustified adjuvant therapy, we advocate that in case adjuvant treatment is considered in patients with stage III melanoma, SN biopsies should be reviewed by an expert melanoma pathologist.

In most cases, differentiation between a nodal nevus and melanoma metastasis is straightforward, based on location and cytomorphological features of the melanocytic cells in the lymph node. However, in a subset of cases, nodal nevi may be difficult to discriminate from melanoma metastasis. Small melanoma metastasis or metastasis from a primary nevoid melanoma can be difficult to discriminate from a nodal nevus [25]. In typical cases, nodal nevi are located within the capsule; they are small, are often triangular shaped and lack the cytonuclear atypia and mitotic activity of melanoma cells. However, capsular nevi may be quite extensive and may show some parenchymal and paratrabecular extension mimicking localisation within the lymph node parenchyma and therefore melanoma metastasis. Indeed, subcapsular location of the nodal nevus was, besides low SN tumour burden, one of the predictors of misdiagnosis as metastases. In cases of subcapsular location, differential diagnosis may therefore be difficult and mostly relies on cytomorphology. Melanomas can have nevoid cytomorphology and bland appearance. In such cases, the discrimination of a metastasis from nevus is difficult especially when metastasis is small, e.g. as isolated cells instead of nests [26]. S100, Sox-10 and Melan-A/MART1 immunohistochemistry help to identify nevoid cells, but do not differentiate between nodal nevi and metastatic melanoma cells [26–29]. Weak or absent immunohistochemical staining for HMB-45, low Ki-67 proliferation, expression of p16 or absence of PRAME staining all favour a diagnosis of nevus [27–29].

One of the strengths of the present study is the generalisability of the results because we randomly selected cases from five different pathology laboratories all over the Netherlands. The rate of downgraded cases was comparable for four of the laboratories, except for one that had only 5.0% of their cases downgraded. One could argue that pathologists working in laboratories with less experience or lower volume of cases are more likely to have a high downgraded rate in the present study. However, we reviewed cases of two academic laboratories and three non-academic laboratories. Although the laboratory with the 5.0% downgraded rate was academic, the other academic laboratory was in between the 10.3–14.7% downgraded rate of the non-academic laboratories. Therefore, in addition, in academic laboratories, there is still a significant number of misdiagnosis of SN biopsies, which might be related to the fact that also in these pathology departments, SN biopsies are not always seen by a dedicated melanoma pathologist.

Another strength is the review by an expert EORTC melanoma pathologist and the comparison of RFS of the downgraded cases with that of other patients with melanoma. No statistically significant difference in RFS was found between the 38 patients who were downgraded upon review and SN-negative patients, implying that the downgrading of these patients is justified. Moreover, there was a statistically significant difference in RFS between the 38 downgraded patients and the remaining 284 SN-positive patients ($p = 0.009$). A limitation is that all cases were reviewed by a single expert pathologist, which may be related to differences in interpretation, even at the expert level. Another limitation is that not all 1279 cases were reviewed, but a random sample. To minimise bias and to optimise efficiency, all cases from one randomly selected pathology laboratory and all cases from the consecutive four pathology laboratories that were received thereafter were reviewed. However, we cannot completely exclude any bias in this approach, although comparison of clinicopathological characteristics between the 322 reviewed cases and the 957 non-reviewed cases showed no statistically significant differences, except for localisation of the melanoma (Table 1). A final limitation is that we were not able to explore the mitotic rate as it was not systematically recorded in the database.

## 5. Conclusion

A large number of originally positive SN biopsies in patients with melanoma are misclassified, indicating that some patients with melanoma might receive unjustified adjuvant treatment. We therefore advocate that when adjuvant treatment is considered in patients with stage III melanoma, SN biopsies should be reassessed by an expert melanoma pathologist.

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## Author contribution statement

**Mary-Ann El Sharouni**: Conceptualisation; Data curation; Formal analysis; Investigation; Methodology; Visualisation; Roles/Writing - original draft; Writing - review & editing. **Annelien E Laeijendecker**: Conceptualisation; Data curation; Formal analysis; Investigation; Roles/Writing - original draft; Writing - review & editing. **Karijn PM Suijkerbuijk**: Conceptualisation; Methodology; Formal analysis; Writing - review & editing. **Arjen J Witkamp**: Conceptualisation; Writing - review & editing. **Vigfús Sigurdsson**: Conceptualisation; Resources; Writing - review & editing. **Paul J van Diest**: Conceptualisation; Methodology; Resources; Writing - review & editing. **Carla H van Gils**: Conceptualisation; Methodology; Formal analysis; Writing - review & editing. **Willeke AM Blokx**: Conceptualisation; Methodology; Formal analysis; Investigation; Roles/Writing - original draft; Writing - review & editing.

## Conflict of interest statement

K.P.M.S. reports consulting fees/an advisory role/honoraria received (paid to the institution) from Novartis, Roche, MSD, BMS, Pierre Fabre and AbbVie. All other authors declare no conflict of interest.

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## Appendix A. Supplementary data

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