ORIGINAL ARTICLE

Intra-nodal nevi in sentinel node-negative patients with cutaneous melanoma does not influence survival

F.S.A. de Beer,[1,*] P.J. van Diest,[2] V. Sigurdsson,[1] M.-A. el Sharouni[1]

[1]Department of Allergology and Dermatology, University Medical Centre Utrecht, Utrecht, The Netherlands
[2]Department of Pathology, University Medical Centre Utrecht, University Utrecht, Utrecht, The Netherlands
*Correspondence: F.S.A. de Beer. E-mail: f.s.a.debeer-2@umcutrecht.nl

Abstract

Background Melanoma patients with intra-nodal nevi (INN) and without melanoma metastasis in the sentinel lymph node biopsy (SLNB) are generally treated as patients with negative SLNB. However, diagnosis of INN may be difficult and nodal melanoma metastases may falsely be regarded as INN.

Objectives Our aim was to evaluate the clinical significance of INN in the SLNB in patients with primary cutaneous melanoma on a nationwide level in The Netherlands by comparing survival between three groups: patients with INN and without nodal melanoma metastasis (INN group), patients without INN and without nodal melanoma metastasis (negative SLNB group) and patients with nodal melanoma metastasis irrespective of INN (positive SLNB group).

Methods Data were obtained from 'PALGA', the Dutch Nationwide Network and Registry of Histopathology and Cytopathology, yielding a cohort of adults with histologically proven, primary, invasive cutaneous melanoma patients in The Netherlands diagnosed between 2000 and 2014 who underwent SLNB. Clinical and pathological variables were extracted from the pathology text files. Differences between patients with INN, negative SLNB and positive SLNB were analysed using Kaplan–Meier analysis.

Results A total of 11 274 patients were eligible for inclusion. The prevalence of INN in the SLNB was 5.0%. Melanomas with INN had similar median Breslow thickness compared to melanomas with negative SLNB and were more frequently located on trunk and upper limbs and observed in younger patients compared to melanomas with negative and positive SLNB. Overall survival of patients with INN showed no significant difference compared with negative SLNB (median follow-up of 5.7 years of all patients).

Conclusions As there seems to be no difference in overall survival between patients with INN and negative SLNB, the diagnosis of INN seems to be reliable. Current practice to treat patients with INN as patients with negative SLNB appears to be appropriate.

Received: 1 April 2019; Accepted: 8 July 2019

Conflicts of interest

All authors declare they have no conflict of interest.

Funding source

None.

Introduction

Skin cancers (excluding basal cell carcinoma) are the second most common registered cancer in The Netherlands in both men and women, of which melanoma comprises 39.8%.[1] Additionally, melanoma accounts for the vast majority of skin cancer-related deaths with rapid and unpredictable metastasis where treatment opportunities are still limited and its incidence is expected to rise.[2,3]

According to current national and international guidelines, sentinel lymph node biopsy (SLNB) is advised for patients with primary cutaneous melanoma staged T1b or higher to optimally inform patients on their prognosis.[3] Some patients will have benign intra-nodal nevi (INN) in the SLNB for which two hypotheses have been described. One states INN are the result of benign dissemination of cutaneous melanocytes, and another hypothesis claims INN are settling neural cells during embryonal migration of cells from the neural crest.[4–6] INN are mostly located in the capsule or trabeculae of the lymph node and can be difficult to distinguish from nodal melanoma metastasis.[7,8] A misdiagnosis of melanoma metastasis as INN may lead to false

staging and inappropriate treatment. Previous studies have described an association between the presence of INN and primary cutaneous melanoma, and are also described in patients with other malignancies, including breast cancer, and in patients without malignancies.[7,9–11] The reported prevalence of INN in the SLNB in patients with melanoma described ranges from 3.9% to 25%.[7–9,11–,14] Patients diagnosed with INN (and without nodal melanoma metastasis) are treated as SLNB-negative patients in current practice. Only few studies have reported the clinical significance of INN and discuss the consequences of potential misdiagnoses.[12–14]

To the best of our knowledge, the prevalence of INN in the SLNB in patients with primary cutaneous melanoma in The Netherlands and the clinical significance of INN compared with negative and positive SLNB is unknown. The aim of this study was therefore to describe the frequency of INN in patients with primary cutaneous melanoma in The Netherlands and to compare survival of patients with INN to patients with negative and positive SLNB.

Materials and methods

Design and study population

Data for this retrospective nationwide study were derived from 'PALGA', the national Pathology registry that since 1987 prospectively collects all pathology data from all pathology laboratories in The Netherlands (http://palga.nl). For the present study, adults with histologically proven primary cutaneous melanoma of all stages with known Breslow thickness (BT) who underwent SLNB between 2000 and 2014 were included. We divided patients into three groups: patients with INN and without nodal melanoma metastasis in the SLNB (INN group), patients without INN and without nodal melanoma metastasis in the SLNB (SLNB-negative group) and patients with nodal melanoma metastasis in the SLNB (SLNB-positive group). In case of a positive SLNB, we did not consider any further the presence of INN, as it does not influence survival. Nodal nevi were recognized based on their localization mostly within the capsule of the lymph node, their often triangular shape with a broad base, lack of cellular atypia and mitosis, being S100/Melan A positive if stained by immunohistochemistry.

Data collection

For each patient, clinical and pathological variables were manually extracted from the pathology text files, including year of diagnosis, age, sex, BT (mm), T stage, ulceration (present or absent), type of cutaneous melanoma [superficial spreading melanoma (SSM), nodular melanoma, lentigo maligna melanoma (LMM) and acral lentiginous melanoma (ALM)] and body site [head and neck (H&N), trunk, arms or legs]. Patients with desmoplastic melanoma or patients without or unclear BT or unclear SLNB results were excluded. In addition, we excluded patients who had metastases within 14 days after diagnosis of melanoma, determined with a direct complete lymph node dissection (CLND), fine needle aspiration (FNA) or otherwise diagnosed positive lymph nodes, to ensure patients were free of metastases prior to their diagnosis of primary melanoma. Staging was based on guidelines at the time (2000–2002 5th AJCC, 2003–2009 6th AJCC, 2010–2014 7th AJCC).[15,16]

As guidelines do not comment on the desired time between primary excision and SLNB, which in practice is known to vary for different reasons, we arbitrarily decided to include all SLNB performed within 100 days after initial diagnosis.[17] Eventually, we excluded patients with multiple primary melanoma, as previous research showed these patients have worse overall survival (OS) as compared to single primary melanoma patients.[18] Follow-up, OS data and vital status (dead or alive) were obtained from The Netherlands Cancer Registry, which gathers information on every patient with cancer in The Netherlands, ending at date of death, date last known alive or 1 January 2018.

Statistical analysis

For continuous data, skewness and kurtosis tests were used to demonstrate normal or non-normal distribution (normal distribution for scores between −1 and 1). Normally distributed data were expressed in mean with standard deviation (SD), and non-normally distributed data were expressed as median with interquartile range (IQR). Depending on distribution, one-way ANOVA tests (normal distribution) or Kruskal–Wallis tests were used to compare differences for continuous variables. Chi-squared or Fisher's exact tests were used for comparison of categorical data. OS was illustrated using Kaplan–Meier curves and compared by log rank test. *P*-values < 0.05 were considered statistically significant. Statistical analyses were performed with Statistical Package for Social Sciences (SPSS) software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0., IBM Corp., Armonk, NY).

Ethical approval

All data were encoded and used anonymously. Ethical approval was granted by the board of PALGA.

Results

Patient characteristics

In total, 11 274 patients with melanoma were included: 568 with only INN (5.0%), 8165 with negative SLNB (72.4%) and 2541 with positive SLNB (22.5%; Table 1). Mean age of patients with only INN was significantly lower than in patients with negative or positive SLNB (resp. 51.8 vs. 54.2 vs. 53.8 years, $P < 0.001$). Patients with only INN had a median BT of 1.6 mm, which was comparable to patients with negative SLNB. Patients with positive SLNB were more often men (resp. 54.5% vs. 48.6% in INN vs. 46.2% in negative SLNB, $P < 0.001$). Patients with positive SLNB had significantly higher BT (2.4 mm vs. 1.6 mm in INN

and negative SLNB, $P < 0.001$) and were therefore more frequently T3 or T4 stage (43.1% and 20.2% compared to 28.6% and 7.7% in negative SLNB and 27.1% and 5.8% in INN, $P < 0.001$). Melanomas with positive SLNB were more often observed on the legs compared with melanomas with INN (33.4% vs. 25.9% in INN, $P < 0.001$) and were more frequently of nodular type (30.9% vs. 23.2% in INN vs. 22.0% in negative SLNB, $P < 0.001$), while INN and negative SLNB melanomas were more commonly SSM (65.5% vs. 63.9% vs. to 55.3% in positive SLNB, $P < 0.001$).

Melanomas with only INN and negative SLNB were similarly often ulcerated (19.2% vs. 20.3%), while positive SLNB were more frequently ulcerated (35.1%, $P < 0.001$). Additionally, only INN melanomas were more frequently observed on the trunk (52.5% vs. 41.1% in negative SLNB and 47.7% in positive SLNB, $P < 0.001$) and the upper limbs (16.5% vs. 16.1% in negative SLNB vs. 10.2% in positive SLNB, $P < 0.001$).

Overall survival

The median follow-up period was 5.7 years (Table 1). The OS curves showed significantly worse OS for patients with positive SLNB compared with patients with negative SLNB and only INN (Fig. 1, $P < 0.001$). No significant difference in OS was found for patients with only INN and negative SLNB ($P = 0.19$).

Discussion

In this study, we present the largest cohort on patients with primary cutaneous melanoma and INN in the SLNB in The Netherlands to date and demonstrate clinical differences between melanoma patients with only INN compared with patients with negative and positive SLNB.

We observed a prevalence of only INN in the SLNB of 5.0%, which is in line with other studies describing a prevalence of 3.9% up to 25%.[7–9,11–,13] Our relatively low prevalence could partly be explained by the retrospective nature of our study as we extracted all information on the SLNB from text files, assuming INN not to be present if not reported in the text files. Most previous studies provided single-centred data, where the same pathologists observed the histology of the SLNB, perhaps resulting in more awareness for INN.[9,10,12–14] INN mostly occur in the capsule or trabeculae of the lymph node,[13,19] but in rare cases INN occur in the parenchyma of the lymph node which is

Table 1 Baseline characteristics of all primary cutaneous melanoma patients undergoing SLNB in The Netherlands from 2000 to 2014

	Overall	INN	Negative SLNB	Positive SLNB	*P*-value
Total no. of patients	11.274 (100)	568 (5.0)	8.165 (72.4)	2.541 (22.5)	
Mean age in years (SD)	54.0 (14.5)	51.8 (14.8)	54.2 (14.5)	53.8 (14.6)	<0.001
Male gender, *n* (%)	5.434 (48.2)	276 (48.6)	3.774 (46.2)	1.384 (54.5)	<0.001
Median Breslow in mm (IQR)	1.7 (1.2–2.8)	1.6 (1.2–2.2)	1.6 (1.2–2.4)	2.4 (1.6–3.8)	<0.001
T stage, *n* (%)					
T1	1.235 (11.0)	69 (12.1)	1.056 (12.9)	110 (4.3)	<0.001
T2	5.282 (46.9)	312 (54.9)	4.149 (50.8)	821 (32.3)	
T3	3.582 (31.8)	154 (27.1)	2.332 (28.6)	1.096 (43.1)	
T4	1.175 (10.4)	33 (5.8)	628 (7.7)	514 (20.2)	
Ulceration, *n* (%)					
Yes	2.655 (23.5)	109 (19.2)	1.655 (20.3)	891 (35.1)	<0.001
No	7.074 (62.7)	421 (74.1)	5.295 (64.8)	1.358 (53.4)	
Missing	1.545 (13.7)	38 (6.7)	1.215 (14.9)	292 (11.5)	
Localization, *n* (%)					
Head and neck	654 (5.8)	18 (3.2)	499 (6.1)	137 (5.4)	<0.001
Trunk	4.869 (43.2)	298 (52.5)	3.358 (41.1)	1.213 (47.7)	
Arm	1.664 (14.8)	94 (16.5)	1.311 (16.1)	259 (10.2)	
Legs	3.754 (33.3)	147 (25.9)	2.759 (33.8)	848 (33.4)	
Missing	333 (3.0)	11 (1.9)	238 (2.9)	84 (3.3)	
Type of melanoma, *n* (%)					
Superficial spreading	6.994 (62.0)	372 (65.5)	5.217 (63.9)	1.405 (55.3)	<0.001
Nodular	2.713 (24.1)	132 (23.2)	1.795 (22.0)	786 (30.9)	
Lentigo maligna melanoma	105 (0.9)	3 (0.5)	96 (1.2)	6 (0.2)	
Acro lentiginous	171 (1.5)	2 (0.4)	115 (1.4)	54 (2.1)	
Missing	1.291 (11.5)	59 (10.4)	942 (11.5)	290 (11.4)	
Median follow-up in years (range)	5.7 (3.5–9.8)	4.8 (3.0–7.6)	6.2 (3.7–10.4)	4.7 (2.7–8.0)	<0.001

Patients are divided into three groups: INN (patients with INN and without nodal melanoma metastasis), negative SLNB (patients without INN and without nodal melanoma metastasis) and positive SLNB (patients with nodal melanoma metastasis).
INN, intra-nodal nevi; IQR, interquartile range; SLNB, sentinel lymph node biopsy.

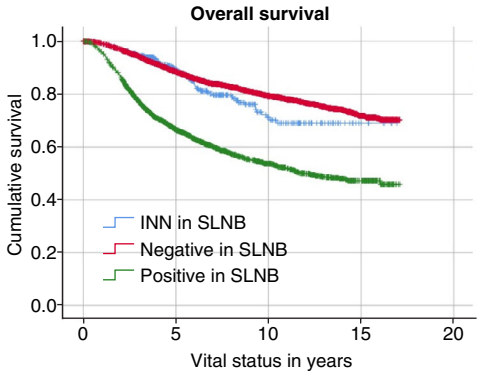

Figure 1 Overall survival in patients with primary cutaneous melanoma in The Netherlands from 2000 to 2014 who underwent sentinel lymph node biopsy (SLNB) stratified into three groups: INN (intra-nodal nevi), negative SLNB and positive SLNB.

similar to the location of metastatic melanoma.[8,20–22] Consequently, this could provide difficulties in distinguishing INN from metastatic melanoma, certainly for less experienced pathologists, and may lead to an underestimation of the true frequency of INN in our cohort.

Melanoma patients with INN are treated as negative SLNB patients due to the believed benign character of INN, underlined by immunohistological studies describing similarities between INN cells and benign cutaneous melanocytes.[9,21–23] Only three previous studies discuss the clinical significance of INN in the SLNB in patients with primary cutaneous melanoma.[12–14] Similar to our results, Gambichler *et al.*[13] described a significant association between the presence of INN and cutaneous melanoma localization on the trunk and upper limbs, the lower extremities being the strongest negative predictor of INN.[13] Smith *et al.*[12] showed a comparable association but the difference was not significant. Contrary to our results, Kim *et al.* described all INN were found in the lymph nodes of the lower extremities. Their study population, however, concerned Asian patients with acral lentiginous melanoma, thereby presenting a very different study cohort of moreover small sample size.[14] However, regarding both earlier mentioned hypotheses for the origin of INN we have no explanation for the association between the presence of INN and anatomical sites, as in both hypotheses we would expect no differences.

Gamblicher *et al.*[13] reported that females had more often INN, while Smith *et al.*[12] reported the opposite. In the present study, no significant association was found between gender and the occurrence of only INN in the SLNB. Additionally, our patients with only INN were significantly younger compared with patients with negative and positive SLNB, a finding also described by Gamblicher *et al.*[13] We hypothesized this might be the resulting from ageing and disappearance of INN during ageing, which is a well-known phenomenon in cutaneous nevi as well.[24]

Similar to most other studies, no significant association was found between BT and the occurrence of INN.[12,13] Only one study described a significant positive correlation between BT and presence of INN.[11] However, this study only included eight INN patients.

Regarding ulceration, we found a nearly similar ulceration rate between INN- and SLNB-negative patients (respectively 19.2% and 20.3%), compared with 35.1% in SLNB-positive patients ($P < 0.001$). This is no surprising finding, as it is known that ulceration is a negative prognostic factor and associated with higher BT as well, both of which is known to increase the likelihood of a positive SLNB.[25,26]

No significant difference in OS was found between patients with only INN and patients with negative SLNB, corroborating previous studies,[12,13] while patients with positive SLNB had significantly worse OS as expected. However, patients with INN in our study were significantly younger compared with patients with negative and positive SLNB, which may have affected OS. However, as there was no significant difference in OS between patients with INN and patients with negative SLNB, correction for confounders was not deemed necessary. The fact that OS for patients with INN and patients with negative SLNB is similar indicates that INN and melanoma metastases can be accurately discriminated by pathologists in The Netherlands.

Strengths of our study are the large sample size of included patients with single primary cutaneous melanoma and the relatively long median follow-up time of 5.7 years. A limitation is the retrospective study design, consequently leading to a risk of information bias with possible underreporting of INN, as we explained earlier. The similar OS of patients with INN and negative SLNB patients indicate that our findings may yet be representative.

In conclusion, our findings suggest that there is no significant difference in OS between cutaneous melanoma patients with INN or negative SLNB, indicating that diagnosis of INN is reliable and it is appropriate to regard and treat patients with INN and without nodal melanoma metastasis as patients with negative SLNB.[12,13]

References

1 IKNL. Nederlandse Kankerregistratie, beheerd door IKNL '© September 2018.
2 Arnold M, Holterhues C, Hollestein LM *et al.* Trends in incidence and predictions of cutaneous melanoma across Europe up to 2015. *J Eur Acad Dermatol Venereol* 2014; **28**: 1170–1178.
3 Nederland NMWIk. Melanoom Landelijke richtlijn, versie 2.1 (last accessed: 1 March 2016).
4 McCarthy SW, Palmer AA, Bale PM, Hirst E. Naevus cells in lymph nodes. *Pathology* 1974; **6**: 351–358.
5 Shenoy BV, Fort 3rd L, Benjamin SP. Malignant melanoma primary in lymph node. The case of the missing link. *Am J Surg Pathol* 1987; **11**: 140–146.

6 Hart WR. Primary nevus of a lymph node. *Am J Clin Pathol* 1971; **55**: 88–92.
7 Patterson JW. Nevus cell aggregates in lymph nodes. *Am J Clin Pathol* 2004; **121**: 13–15.
8 Chakera AH, Hesse B, Burak Z *et al*. EANM-EORTC general recommendations for sentinel node diagnostics in melanoma. *Eur J Nucl Med Mol Imaging* 2009; **36**: 1713–1742.
9 Carson KF, Wen DR, Li PX *et al*. Nodal nevi and cutaneous melanomas. *Am J Surg Pathol* 1996; **20**: 834–840.
10 Fontaine D, Parkhill W, Greer W, Walsh N. Nevus cells in lymph nodes: an association with congenital cutaneous nevi. *Am J Dermatopathol* 2002; **24**: 1–5.
11 Holt JB, Sangueza OP, Levine EA *et al*. Nodal melanocytic nevi in sentinel lymph nodes. Correlation with melanoma-associated cutaneous nevi. *Am J Clin Pathol* 2004; **121**: 58–63.
12 Smith OJ, Coelho JA, Trevatt AE, Ross GL. Clinical significance of intranodal naevi in sentinel node biopsies for malignant melanoma. *Eur J Surg Oncol* 2016; **42**: 1427–1431.
13 Gambichler T, Scholl L, Stucker M *et al*. Clinical characteristics and survival data of melanoma patients with nevus cell aggregates within sentinel lymph nodes. *Am J Clin Pathol* 2013; **139**: 566–573.
14 Kim HJ, Seo JW, Roh MS, Lee JH, Song KH. Clinical features and prognosis of Asian patients with acral lentiginous melanoma who have nodal nevi in their sentinel lymph node biopsy specimen. *J Am Acad Dermatol* 2018; **79**: 706–713.
15 Balch CM, Buzaid AC, Soong SJ *et al*. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. *J Clin Oncol* 2001; **19**: 3635–3648.
16 Balch CM, Gershenwald JE, Soong SJ *et al*. Final version of 2009 AJCC melanoma staging and classification. *J Clin Oncol* 2009; **27**: 6199–6206.
17 Oude Ophuis CM, van Akkooi AC, Rutkowski P *et al*. Effects of time interval between primary melanoma excision and sentinel node biopsy on positivity rate and survival. *Eur J Cancer* 2016; **67**: 164–173.
18 Pardo LM, van der Leest RJ, de Vries E, Soerjomataram I, Nijsten T, Hollestein LM. Comparing survival of patients with single or multiple primary melanoma in the Netherlands: 1994-2009. *Br J Dermatol* 2017; **176**: 531–533.
19 LeBoit PE. What sentinel node biopsy in patients with melanoma (or patients whose doctors worry that they could have melanoma) might and might not do. *Clin Dermatol* 2009; **27**: 588–593.
20 Davis J, Patil J, Aydin N, Mishra A, Misra S. Capsular nevus versus metastatic malignant melanoma – a diagnostic dilemma. *Int J Surg Case Rep* 2016; **29**: 20–24.
21 Biddle DA, Evans HL, Kemp BL *et al*. Intraparenchymal nevus cell aggregates in lymph nodes: a possible diagnostic pitfall with malignant melanoma and carcinoma. *Am J Surg Pathol* 2003; **27**: 673–681.
22 Lohmann CM, Iversen K, Jungbluth AA, Berwick M, Busam KJ. Expression of melanocyte differentiation antigens and ki-67 in nodal nevi and comparison of ki-67 expression with metastatic melanoma. *Am J Surg Pathol* 2002; **26**: 1351–1357.
23 Fisher CJ, Hill S, Millis RR. Benign lymph node inclusions mimicking metastatic carcinoma. *J Clin Pathol* 1994; **47**: 245–247.
24 Stegmaier OC. Natural regression of the melanocytic nevus. *J Invest Dermatol* 1959; **32**: 413–421.
25 Santos FMD, Silva FCD, Pedron J, Furian RD, Fortes C, Bonamigo RR. Association between tumor-infiltrating lymphocytes and sentinel lymph node positivity in thin melanoma. *An Bras Dermatol* 2019; **94**: 47–51.
26 Conic RRZ, Ko J, Damiani G *et al*. Predictors of sentinel lymph node positivity in thin melanoma using the National Cancer Database. *J Am Acad Dermatol* 2019; **80**: 441–447.