Melanoma

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MELANOMA

NEW INSIGHTS IN FOLLOW-UP AND STAGING

S. DAMUDE

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1 GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

INTRODUCTION

Melanoma Incidence

Exposure to ultraviolet light is known to be a prominent risk factor for developing cutaneous melanoma.[1] Tanning beds and the rising popularity of sun holidays contribute to this increased exposure. Sunburns in childhood account for the highest risk.[2] The incidence of cutaneous melanoma is rising in most European countries, probably as a result of increased public awareness, resulting in an increase in thinner melanomas at time of diagnosis since the last two decades.[3,4] Recently, a stabilization in incidence has been reported in Australia and North America.[5] This might be the result of long lasting educational awareness programs at schools and in the media.[6] Due to early detection and improved staging with sentinel lymph node biopsy, the 5-year survival rates reported are 92% for American Joint Committee on Cancer (AJCC) stage IB and 53% for stage IIC melanoma patients.[7] Increasing incidence and improved prognosis have resulted in an increased prevalence of melanoma. Consequently, the number of melanoma patients in clinical follow-up is rising.[8,9]

Risk Factors

Known risk factors independently associated with the development of a primary cutaneous melanoma are history of (severe) sun burns, number of naevi, family history, light or red hair color, male sex, and older age.[10] Very recently, smoking was found to be associated with sentinel lymph node metastasis, ulceration, and increased Breslow thickness.[11] Risk factors for the development of additional lymph node metastases, based on patient and tumor characteristics, have extensively been described in the literature, such as male sex, thicker Breslow, regression, ulceration, number of positive SNs, maximum size of SN-metastases, invasion depth (Starz-classification), non-subcapsular location (Dewar-classification), and extra-nodal growth.[12-17] Several prediction tools for survival and prognosis in melanoma have been described and some are used in clinical practice.[18] For SLNB patient selection, the Memorial Sloan Kettering Cancer Center (MSKCC) developed and validated a nomogram for SN-status prediction.[19] Although not yet included in clinical guidelines, prediction models based on independently associated parameters were developed and validated, to enable risk stratification for NSN-positivity.[12,13] However, to this date, the exact behavior of cutaneous melanoma remains unpredictable.

Staging

Primary cutaneous melanoma is staged according to the TNM classification, developed by the American Joint Committee on Cancer (AJCC) in 1977. This staging system is last updated in 2017, the 8th edition, and is implemented in 2018.[20] For this thesis the 7th edition, updated in 2009, was used (**Figure 1**).[21] The TNM classification defines tumor (T), nodal (N) and distant metastasis (M) staging. Based on this classification, melanoma can be classified from AJCC stage I to IV. Alexander Breslow introduced Breslow thickness as a measure for the total vertical depth of a melanoma in 1970, an important diagnostic and prognostic factor to this date.[22] The T-staging is mainly based on Breslow thickness, ulceration, and mitotic rate of the primary tumor. In the upcoming 8th AJCC staging edition, mitosis is excluded for T-staging.[20] Clinically localized disease is defined as stage I-II melanoma. The N-staging is determined by the involvement of melanoma in the regional lymph nodes. For this purpose, the sentinel lymph node biopsy (SLNB) was introduced by Morton in 1992 as an important staging procedure. During this procedure, a radioactive tracer and a blue dye are injected to identify the first lymph node to which afferent lymphatic vessels drain.[23] Regional lymph node involvement is classified as stage III, and distant metastases as stage IV melanoma. The use of serum Lactate Dehydrogenase (LDH) level to categorize stage IV patients is abolished in the 8th edition.[20]

Surgical Treatment

Narrow excisional biopsy with melanoma free margins is recommended by the AJCC for suspect lesions to achieve adequate pathological evaluation, thereby providing the best information for diagnosis and staging.[21] The margin of a therapeutic re-excision depends on the Breslow thickness as determined in the primary biopsy. To this date, the recommended margin of a therapeutic re-excision is 1 cm for Breslow thickness <2.0 mm and 2 cm for melanoma >2.0 mm.[24] However, with a lack of solid evidence for these margins, the MELMART trial was initiated in 2015, randomizing 1 cm and 2 cm margins to investigate the influence of smaller resection margins on quality of life, local recurrence and melanoma specific survival (NTC02385214; estimated completion date 2026).[25]

To this date, sentinel lymph node biopsy (SLNB) is considered as the standard prognostic procedure for accurate staging in melanoma patients with Breslow thickness >1.0 mm, with a minimal treatment related morbidity.[21,26,27] Although

FIGURE 1.

American Joint Committee on Cancer

Melanoma of the Skin Staging

7th EDITION

Definitions

Primary Tumor (T)

- **TX** Primary tumor cannot be assessed (for example, curettaged or severely regressed melanoma)
- **T0** No evidence of primary tumor
- **Tis** Melanoma in situ
- **T1** Melanomas 1.0 mm or less in thickness
- **T2** Melanomas 1.01–2.0 mm
- **T3** Melanomas 2.01–4.0 mm
- **T4** Melanomas more than 4.0 mm

NOTE: a and b subcategories of T are assigned based on ulceration and number of mitoses per mm^2, as shown below:

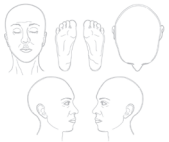
T CLASSIFICATION	THICKNESS (mm)	ULCERATION STATUS/MITOSES
T1	≤1.0	a: w/o ulceration and mitosis <1/mm^2 b: with ulceration or mitoses ≥1/mm^2
T2	1.01–2.0	a: w/o ulceration b: with ulceration
T3	2.01–4.0	a: w/o ulceration b: with ulceration
T4	>4.0	a: w/o ulceration b: with ulceration

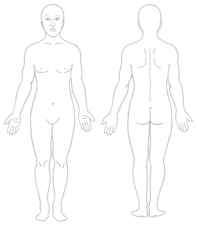
Regional Lymph Nodes (N)

- **NX** Patients in whom the regional nodes cannot be assessed (for example, previously removed for another reason)
- **N0** No regional metastases detected
- **N1-3** Regional metastases based upon the number of metastatic nodes and presence or absence of intralymphatic metastases (in transit or satellite metastases)

NOTE: N1–3 and a–c subcategories assigned as shown below:

N CLASSIFICATION	NO. OF METASTATIC NODES	NODAL METASTATIC MASS
N1	1 node	a: micrometastasis[1] b: macrometastasis[2]
N2	2–3 nodes	a: micrometastasis[1] b: macrometastasis[2] c: in transit met(s)/satellite(s) *without* metastatic nodes
N3	4 or more metastatic nodes, or matted nodes, or in transit met(s)/satellite(s) with metastatic node(s)	





Distant Metastatis (M)

- **M0** No detectable evidence of distant metastases
- **M1a** Metastases to skin, subcutaneous, or distant lymph nodes
- **M1b** Metastases to lung
- **M1c** Metastases to all other visceral sites or distant metastases to any site combined with an elevated serum LDH

NOTE: Serum LDH is incorporated into the M category as shown below:

M CLASSIFICATION	SITE	SERUM LDH
M1a	Distant skin, subcutaneous, or nodal mets	Normal
M1b	Lung metastases	Normal
M1c	All other visceral metastases	Normal
	Any distant metastasis	Elevated

ANATOMIC STAGE/PROGNOSTIC GROUPS

	Clinical Staging[3]				Pathologic Staging[4]			
Stage 0	Tis	N0	M0		0	Tis	N0	M0
Stage IA	T1a	N0	M0		IA	T1a	N0	M0
Stage IB	T1b	N0	M0		IB	T1b	N0	M0
	T2a	N0	M0			T2a	N0	M0
Stage IIA	T2b	N0	M0		IIA	T2b	N0	M0
	T3a	N0	M0			T3a	N0	M0
Stage IIB	T3b	N0	M0		IIB	T3b	N0	M0
	T4a	N0	M0			T4a	N0	M0
Stage IIC	T4b	N0	M0		IIC	T4b	N0	M0
Stage III	Any T	≥ N1	M0		IIIA	T1-4a	N1a	M0
						T1-4a	N2a	M0
					IIIB	T1-4b	N1a	M0
						T1-4b	N2a	M0
						T1-4a	N1b	M0
						T1-4a	N2b	M0
						T1-4a	N2c	M0
					IIIC	T1-4b	N1b	M0
						T1-4b	N2b	M0
						T1-4b	N2c	M0
						Any T	N3	M0
Stage IV	Any T	Any N	M1		IV	Any T	Any N	M1

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Financial support for AJCC 7th Edition Staging Posters provided by the American Cancer Society

Notes

[1] Micrometastases are diagnosed after sentinel lymph node biopsy and completion lymphadenectomy (if performed).

[2] Macrometastases are defined as clinically detectable nodal metastases confirmed by therapeutic lymphadenectomy or when nodal metastasis exhibits gross extracapsular extension.

[3] Clinical staging includes microstaging of the primary melanoma and clinical/radiologic evaluation for metastases. By convention, it should be used after complete excision of the primary melanoma with clinical assessment for regional and distant metastases.

[4] Pathologic staging includes microstaging of the primary melanoma and pathologic information about the regional lymph nodes after partial or complete lymphadenectomy. Pathologic Stage 0 or Stage IA patients are the exception; they do not require pathologic evaluation of their lymph nodes.

AJCC staging system for melanoma.

the first Multicenter Selective Lymphadenectomy Trial (MSLT-I), finished in 2014, found no difference in melanoma-specific survival or overall survival after ten years, disease-free survival was significantly better for patients in the SLNB-arm. SLNB identifies patients with nodal metastases, who may benefit from immediate completion lymph node dissection (CLND).[28]

In case of a positive sentinel lymph node, the current recommendation is to perform a subsequent CLND. However, in only about 20% of patients additional metastases in non-sentinel nodes (NSNs) are found, while the procedure is accompanied with significant morbidity and costs.[29,30] Despite this recommendation on performing CLND in all sentinel node (SN)-positive patients, its therapeutic value is highly debated.[14,15,31-34] The necessity of a routine CLND for SN-positive patients is still under investigation in the EORTC 1208: MiniTub (NCT01942603).[35] The (underpowered) DeCOG-SLT was not able to show survival benefit of CLND for unselected SN-positive patients.[33] The recently published MSLT-II results report slightly better disease free survival, but no benefit in overall or melanoma specific survival by performing CLND in SN-positive patients.[31] Therefore, it might become necessary to select only 'high-risk' SN-positive patients for CLND. A low risk could possibly justify CLND omission and ultrasonographic nodal observation.

Follow-up

For melanoma, there is currently no consensus on the adequate frequency of post-treatment follow-up visits, and surveillance intervals vary widely worldwide.[36-38] Most contemporary surveillance guidelines recommend intensive follow-up schedules.[39-41] Important reasons for surveillance frequency are patients' reassurance and anxiety reduction, early detection of recurrences or second primary melanoma, and evaluation of the quality of surgical treatment.[42-46] Patients' preferences regarding follow-up frequency, and follow-up methods are understudied. However, mixed feelings have been reported. It seems important to balance patients' reassurance without inducing additional anxiety.[47,48]

Self-inspection of the skin is probably the most important aspect of follow-up after being treated for melanoma. Skin self-examination (SSE) was already described in 1996 as a useful and inexpensive method for the early detection of a loco-regional recurrence or second primary.[49] The majority of melanoma

recurrences and 2nd primary melanomas occur within three years after initial treatment, with an increase in occurrence per AJCC stage.[43,50] Approximately 75% of the recurrences and almost 50% of the 2nd primaries are detected by patients themselves or their partners instead of by clinicians.[51,52] Patient education might even enlarge the number of patient-based detections of recurrent disease.[53] E-health videos could be of additional value for this purpose.[54] This implies that follow-up visits may currently be scheduled more frequently than necessary, possibly needlessly burdening patients and health care resources.[51,52]

Biomarkers

In the follow-up of melanoma patients, serum S-100B is increasingly used as tumor marker. It is mostly determined complementary to Lactate Dehydrogenase (LDH), to estimate tumor load, evaluate response to treatment, and as a prognostic tumor marker in advanced melanoma.[41,55-57] However, there is a wide variety in the use of biomarkers in melanoma worldwide.[38] To this date, the biomarkers S-100B and LDH are used mostly to evaluate response to systemic treatments in stage IV.

For AJCC stage I and II, some studies did report that S-100B was not capable of predicting the SN status, due to low sensitivity.[58-60] Although S-100B has been described as a biomarker with prognostic capacities in cutaneous melanoma patients since the nineties, no consensus has been achieved on its value and implementation as detection marker for recurrences in clinical follow-up.[61] To date, only German and Swiss national guidelines recommend evaluation of serum S-100B in melanoma follow-up.[38] Biomarkers like LDH, S-100B, YKL-40, Melanoma Inhibitory Activity protein (MIA), and C-Reactive Protein (CRP) are reported as prognostic markers in different stages of melanoma.[62-66] However, these biomarkers are not yet implemented in prediction tools for NSN-involvement. Serum S-100B was found to be independently associated with NSN-involvement in SN-positive melanoma patients. Besides, elevated levels of S-100B appeared to be associated with recurrence risk and worse survival in patients presenting with palpable nodal metastases, suggesting a relation with melanoma tumor burden.[63]

Determination of S-100B

Melanoma studies that have tried to use S-100B for recurrence detection and prediction of sentinel-node positivity encountered problems due to the low sensitivity in these melanoma patients with minimal tumor load.[59,60] Another

frequently encountered problem with biomarkers is the undesirable presence of false-positive as well as false-negative results.[67] False-positive S-100B values may lead to unnecessary anxiety in melanoma patients, potential over-staging and mismanagement, and increased healthcare costs.
Determination of serum S-100B values in melanoma patients is performed by drawing a blood sample through a venipuncture and subsequent analysis of S-100B by immunoassay. Accurate analysis of this biomarker is important, as minor changes in serum S-100B levels might have clinical consequences.[62] Increased S-100B levels might be an expression of metastatic disease for which additional diagnostic tests and eventual further treatment, e.g. surgical and/or systemic therapy might be indicated. Multiple studies reported adipocytes to contain high levels of S-100B, suggesting S-100B values could be falsely elevated when mixed with subcutaneous cells, caused by adipocytes trapped in the needle during a venipuncture.[68-74]

OUTLINE

The unpredictable behavior of cutaneous melanoma results in the absence of consensus in national guidelines, regarding follow-up surveillance in AJCC Stage I-II melanoma patients. The studies in this thesis address differences in follow-up schedules and the possible implementation of a reduced follow-up surveillance schedule, practice variances regarding the sentinel lymph node biopsy, prediction tools for patient selection for completion lymph node dissection, and the use and accurate determination of the serum biomarker S-100B.

Part I - Aspects of follow-up in AJCC Stage I-II Melanoma focuses on different aspects of follow-up. The development and effects of an evidence-based reduced follow-up schedule, based on a currently still running multicenter randomized clinical trial, the MELFO-study (Melanoma Follow-up) is described in **Chapter 2**. Patients' preferred method for receiving information and education regarding melanoma and self-inspection of the skin and regional lymph nodes is investigated by distributing a web-based questionnaire among all AJCC stage I-II melanoma patients in follow-up (**Chapter 3**). The presence of practice variation

in performing a sentinel lymph node biopsy in the Netherlands is studied in a population based retrospective study (**Chapter 4**).

Part II - Prediction of nodal status in completion lymph node dissection using the biomarker S-100B addresses the necessity of performing a completion lymph node dissection in all sentinel node positive melanoma patients, as additional lymph node metastases are not found in about 80% of these patients. In **Chapter 5**, different clinico-pathological characteristics are tested for an association with finding additional positive lymph nodes in the completion lymph node dissection specimen. Based on the findings of this study, a potential prediction tool for additional positive lymph nodes is proposed in **Chapter 6**, with the aim to achieve adequate patient selection for additional completion lymph node dissection.

Part III - Accurate determination of the biomarker S-100B regards influences on falsely elevated serum S-100B values. With S-100B present in adipocytes, elevated levels of S-100B were found after performing a traumatic venipuncture in healthy volunteers (**Chapter 7**). **Chapter 8** describes a prospective study performed among AJCC stage II-III patients, implementing a dummy tube to flush away potential adipocytes in the first venipuncture, to verify this theory of falsely elevated S-100B values by adipocyte contamination.

A **Summary** of the studies performed is written in English and Dutch at the end of this thesis. Finally, new research developments regarding melanoma follow-up in Stage I-III cutaneous melanoma patients are discussed in the **Future Perspectives**.

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PART I

FOLLOW-UP IN AJCC STAGE I–II MELANOMA

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2

THE 2 MELFO-STUDY: PROSPECTIVE RANDOMIZED CLINICAL TRIAL FOR THE EVALUATION OF A STAGE-ADJUSTED REDUCED FOLLOW-UP SCHEDULE IN CUTANEOUS MELANOMA PATIENTS – RESULTS AFTER ONE YEAR

ABSTRACT

Background. Guidelines for evidence-based follow-up in melanoma patients are not available. This study examined whether a reduced follow-up schedule affects: Patient-Reported Outcome Measures (PROMs), detection of recurrences, and follow-up costs.

Methods. This multicenter trial included 180 patients treated for AJCC stage IB-II cutaneous melanoma, who were randomized in a Conventional follow-up Schedule Group (CSG, 4 visits first year, n=93) or Experimental follow-up Schedule Group (ESG, 1-3 visits first year, n=87). Patients completed the State-Trait Anxiety Inventory (STAI-S), Cancer Worry Scale (CWS), Impact of Events Scale (IES), and a Health-Related Quality of Life questionnaire (HRQoL, RAND-36). Physicians registered clinicopathologic features and the number of outpatient clinic visits.

Results. Socio-demographic and illness-related characteristics were equal in both groups. After one year follow-up, the ESG reported significantly less cancer-related stress response symptoms ($p=0.01$), and comparable anxiety, mental HRQoL and cancer related worry than the CSG. Mean cancer related worry and stress response symptoms decreased over time ($p<0.001$), while mental HRQoL increased over time ($p<0.001$) in all melanoma patients. Recurrence rate was 9% in both groups, mostly patient-detected and not physician-detected (CSG 63%, ESG 43%, $p=0.45$). Hospital costs of one year follow-up was reduced by 45% in the ESG compared to the CSG.

Conclusions. This study shows that the stage-adjusted, reduced follow-up schedule did not negatively affect melanoma patients' mental well-being and the detection of recurrences when compared to conventional follow-up as dictated by the Dutch guideline, at one year after diagnosis. Additionally, reduced follow-up was associated with significant hospital cost reduction.

INTRODUCTION

The incidence of cutaneous melanoma is rising in most European countries, probably as a result of increased public awareness, resulting in an increase in thinner melanomas at time of diagnosis since the last two decades.[1,2] Recently, a stabilization in incidence has been reported in Australia and North America.[3] Due to early detection and improved staging with sentinel lymph node biopsy, the 5-year survival rates reported are 92% for American Joint Committee on Cancer (AJCC) stage IB and 53% for stage IIC melanoma patients.[4] Increasing incidence and improved prognosis have resulted in an increased prevalence of melanoma. Consequently, there are more melanoma patients in clinical follow-up.[5,6]

For melanoma, there is currently no consensus on the adequate frequency of post-treatment follow-up visits, and surveillance intervals vary widely worldwide.[7-9] Most contemporary surveillance guidelines recommend intensive follow-up schedules.[10-12] Important reasons for surveillance frequency are patients' reassurance and anxiety reduction, early detection of recurrences or second primary melanoma, and evaluation of the quality of surgical treatment.[13-17] Patients' preferences regarding follow-up frequency are understudied. However, mixed feelings have been reported. It seems important to balance patients' reassurance without inducing additional anxiety.[18,19]

The majority of melanoma recurrences and 2nd primary melanomas occur within three years after initial treatment, with an increase in occurrence per AJCC stage.[14,20] Approximately 75% of the recurrences and almost 50% of the 2nd primaries are detected by patients themselves or their partners instead of by clinicians.[21,22] Patient education might even enlarge the number of patient-based detections of recurrent disease.[23] This implies that follow-up visits may currently be scheduled more frequently than necessary, possibly needlessly burdening patients and health care resources.[21,22]

There is a need for guidelines with an evidence-based follow-up frequency. The Melanoma Follow-up (MELFO)-study was designed to determine whether a stage-adjusted follow-up schedule adversely affects melanoma patients' mental well-being and the detection of 1st recurrences or second primary melanomas, and whether it decreases yearly costs per patient.

METHODS

2

Study Design

This randomized, controlled, multicenter trial was initiated by the University Medical Center Groningen (UMCG), conducted in six hospitals in the Netherlands in accordance with the Declaration of Helsinki, and approved by the central medical ethics committee (METc2004.127). Given the nature of the study, it was not possible to blind participants or physicians/nurse practitioners for group assignment. The conventional follow-up schedule was according to Dutch Melanoma guideline recommendations.[11] The experimental schedule was defined with an overall reduction of 27% of the number of conventional schedule visits during the first 5 years after diagnosis, based on the previously reported annual risk of recurrence development per AJCC stage: IB 18.4%, IIA 28.9%, IIB 41.0%, IIC 45.2% (Table 1).[21,24]

Primary endpoint was patients' mental well-being. Secondary endpoints were development of recurrence or 2nd primary melanoma, the person detecting it, and total hospital costs.

Patients and Procedure

All patients diagnosed with AJCC stage IB-II cutaneous melanoma, treated with curative intent between February 2006 and November 2013, were eligible for the study. Exclusion criteria were age <18 and >85 years, inadequate knowledge

TABLE 1.

Frequency of follow-up visits for conventional follow-up schedule, recommended by the Dutch Melanoma Working Party and reduced experimental follow-up schedule

"Conventional follow-up schedule"							"Experimental follow-up schedule"						
Years*	1	2	3	4	5	6-10	Years*	1	2	3	4	5	6-10
AJCC Stage							AJCC Stage						
IB	4	3	2	2	2		IB	1	1	1	1	1	1
IIA	4	3	2	2	2	1	IIA	2	2	1	1	1	1
IIB	4	3	2	2	2	1	IIB	3	3	2	1	1	1
IIC	4	3	2	2	2	1	IIC	3	3	2	1	1	1

of the Dutch language, and a history of previous malignancy. AJCC stage IA patients were also excluded, as the Dutch Melanoma guideline recommends only a single follow-up visit after treatment.[11] Physicians or nurse practitioners performing follow-up informed eligible patients about the trial immediately after diagnosis, and asked them to participate. After informed consent was given, randomization was performed into the conventional (CSG) or experimental (ESG) follow-up schedule group, stratified for AJCC stage, in random permuted blocks of four patients, generated by a validated system (Intrialgrator) with the use of a pseudo-random number generator and a supplied seed number. Randomization and data management were performed by the Netherlands Comprehensive Cancer Organization (IKNL). The first questionnaire (at inclusion; T1) and a pre-stamped return envelope were then sent to the patient's home address. All patients received oral and written information on melanoma and instructions on self-inspection of skin and lymph node bearing areas.[25] After 12 months (time point 2; T2), patients completed questionnaires again, excluding those with recurrent disease.

Instruments

Patients completed socio-demographic questions, two self-designed questions regarding follow-up schedule satisfaction, one on self-inspection and one on the number of melanoma related visits to the general practitioner (GP). Also, they filled in the following validated Patient Reported Outcome Measures (PROMs): (1) the 20-item State-Trait Anxiety Inventory-state version (STAI-S), measuring the transitory emotional condition of stress or tension perceived by respondents. Higher scores (range 20-80) indicate greater anxiety[26]; (2) the 3-item Cancer Worry Scale (CWS), assessing concerns about developing cancer (again) and their impact on daily functioning. Higher scores (range 3-12) indicate more concerns[27]; (3) the 15-item Impact of Event Scale (IES), assessing the extent to which people are bothered by memories of a major life-event in terms of intrusion and avoidance. Higher scores (range 15-75) indicate the presence of more intrusion/avoidance[28]; (4) the mental component summary (MCS) score of the RAND-36, a Health Related Quality of Life (HRQoL) questionnaire. The MCS score was standardized with a mean of 50 and a standard deviation of 10[29]. Surgical oncologists, dermatologists or nurse practitioners, performing follow-up, registered melanoma-related variables, and the actual frequency of melanoma related follow-up visits in the hospital. Follow-up consisted of a comprehensive patient history and physical examination. Laboratory testing

and diagnostic imaging was only performed in patients suspicious for recurrent disease, as appropriate.
Total follow-up costs of the first year were calculated for all participating UMCG-patients, data were received from the financial administration of the UMCG.

Statistical Analysis

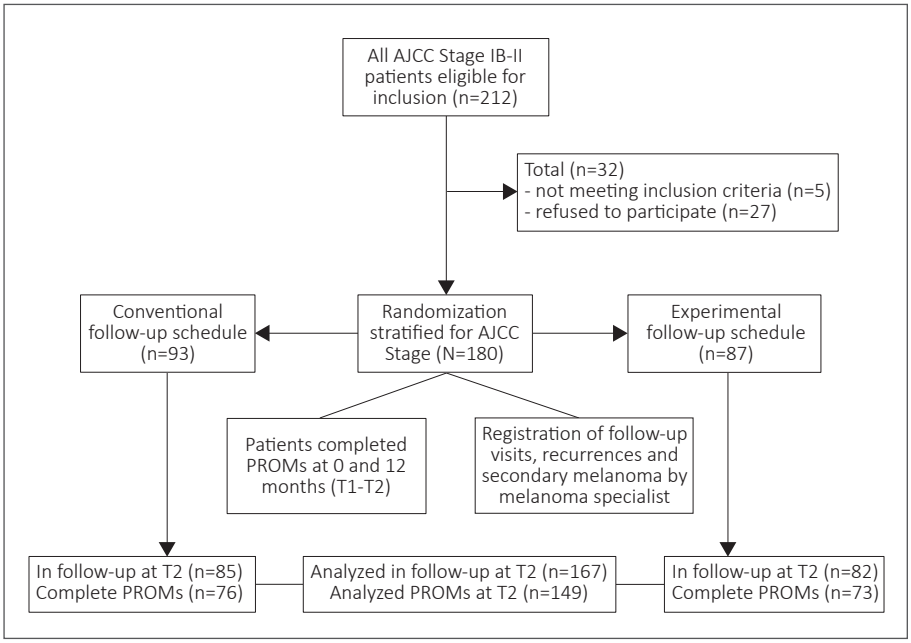
Power analysis for a two-sided test was performed on the STAI-state score with a power β=0.80 and α=0.05. The aim was to falsify the nil-hypothesis: no difference in STAI-state anxiety between patients in the ESG and the CSG. A sample size of 89 patients in each group was required to prove a difference between the groups of a minimum of 4 points (norm 36.5, standard deviation 9.4). The effect size of this outcome is 0.42.
Statistical analyses were performed on the questionnaires and physician/nurse-practitioner reports after one year of follow-up, using IBM SPSS statistics version 22 (SPSS Inc, Chicago, IL). Patient characteristics were compared between the groups using t-tests and chi-square tests as appropriate. Repeated measures ANOVA's were used to examine differences between study groups in PROM's, change over time, and interaction effects. Effect sizes (ES) were calculated to examine if significant differences found were clinically relevant. ES <0.2 were considered negligible, those between 0.2-0.49 small, those between 0.50-0.79 moderate, and those ≥0.80 large.[30] Statistical significance was achieved at $p<0.05$.

RESULTS

Patients

Of the 212 patients approached, 5 were not eligible and 27 refused participation (response 87%). A total of 180 patients were randomized, 93 patients were allocated to the CSG, and 87 patients to the ESG (Figure 1). Socio-demographic and clinicopathologic characteristics were comparable between groups. Median age was 57.4 years, 51.7% were females, 37.8% had completed high education (high vocational education or university), 84.4% had a partner, 47.2% had paid employment, and 38.9% reported other co-morbidity. Median Breslow thickness was 1.6 mm. The trunk was more commonly affected in males (54.0%) and the lower limbs in females (40.9%, $p<0.001$). At one year after enrollment

FIGURE 1.



Flow diagram of inclusion and randomization.

(T2), 84.5% of the CSG and 94.2% of the ESG reported being satisfied with the assigned schedule (p=0.60). Eight CSG patients preferred less frequent follow-up, whereas three CSG and four ESG patients desired more frequent follow-up (p=0.02). Fifteen patients had a recurrence, six before T2 and nine just after T2 questionnaire completion (Table 2).

A total of 19 patients (CSG: 11.8%, ESG: 9.2%, p=0.92) were lost to follow-up at T2. Before T2, 6 patients had recurrent disease (of whom 3 died), and 2 died of non-melanoma related causes. Eleven patients withdrew from the study before T2 because of dissatisfaction with the allocated schedule (CSG: n=5, ESG: n=3), or continuation of follow-up in another clinic (CSG: n=1, ESG: n=2). Excluding these 11 patients plus the 2 deceased of other cause, but including all 15 recurred patients, a total of 44 patients (26.3%) did not adhere completely to the assigned follow-up schedule. Thirteen patients (7.8%; CSG: n=10, ESG: n=3) attended less outpatient clinic visits than planned, while 31 patients (18.6%; CSG: n=12, ESG: n=19) paid extra visits, due to melanoma-related anxiety or physical complaints (no significant difference between groups, p=0.068). Besides outpatient clinic visits, some patients also reported melanoma-related

TABLE 2.

Baseline characteristics (CSG: n=93, ESG: n=87) and follow-up related questions; comparison between study groups

Characteristics	Conventional schedule No.	%	Experimental schedule No.	%	p-value
Gender					
Female	42	45.2	51	58.6	0.071*
Male	51	54.8	36	41.4	
Age (years)					
Median, range	55, 23-81		61, 20-85		0.285^
Level of education[a]					
High	37	39.8	31	35.6	0.524*
Intermediate	38	40.9	33	37.9	
Low	18	19.4	23	26.4	
Relationship status					
With partner	76	81.7	76	87.4	0.297*
Without partner	17	18.3	11	12.6	
Daily activities					
Employed for wages	49	52.7	36	41.4	0.129*
Not employed for wages	44	47.3	51	58.6	
Presence of co-morbidities					
No	62	66.7	48	55.2	0.114
Yes	31	33.3	39	44.8	
Primary melanoma site					
Lower extremity	32	34.4	23	26.4	0.517*
Upper extremity	17	18.3	15	17.2	
Trunk	34	36.6	41	47.1	
Head/neck	10	10.8	8	9.2	
Breslow thickness (mm)[b]					
Median, range	1.6, 0.3-8.0		1.7, 0.6-7.4		0.733^
<1.00	3	3.2	9	10.3	0.181*
1.00-2.00	56	60.2	42	48.3	
2.00-4.00	26	28.0	28	32.2	
>4.00	8	8.6	8	9.2	

TABLE 2.

Continued

Characteristics	Conventional schedule No.	%	Experimental schedule No.	%	p-value
Ulceration					
No	72	77.4	64	73.6	0.547*
Yes	21	22.6	23	26.4	
AJCC Stage					
Ib	56	60.2	47	54.0	0.820*
IIa	19	20.4	19	21.8	
IIb	12	12.9	15	17.2	
IIc	6	6.5	6	6.9	
Schedule satisfactionc (T2)					
Yes	60	84.5	65	94.2	0.064*
No	11	15.5	4	5.8	
Missing	14		13		
Reason for schedule dissatisfaction[c]					
Wish for less frequent visits	8	72.7	0	0.0	**0.016****
Wish for more frequent visits	3	27.3	4	100.0	
Frequency of self-inspectionc (T2)					
At least once a month	58	78.4	48	65.7	0.232*
Every 3 months	10	13.5	16	21.9	
Less than every 3 months	6	8.1	9	12.3	
Missing	11		9		
Number of outpatient clinic visits (T2)					
Median, range	4, 2-6		2, 1-4		**0.001**
Less than planned:	10	11.8	3	3.7	0.051*
• - 1 visit	8	9.4	1	1.2	
• - 2 visits	2	2.4	2	2.4	
According to assigned schedule	63	74.1	60	76.9	
More than planned:	12	14.1	19	23.2	0.133*
• + 1 extra visit	8	9.4	17	21.3	
• + 2 extra visits	4	4.7	2	2.5	

→ *Continued next page*

TABLE 2.

Continued

Characteristics	Conventional schedule No.	%	Experimental schedule No.	%	p-value
Reasons extra outpatient clinic visit					
Physical symptoms	9	56.3	11	52.4	0.956*
Anxiety	6	37.5	9	42.9	
Other	1	6.2	1	4.7	
Extra GP consultationsc (T2)					
No	68	80.0	71	86.6	0.255*
Yes	17	20.0	11	13.4	
1 melanoma related visit	16	18.8	10	12.2	0.498*
2 melanoma related visits	1	1.2	1	1.2	
Total extra visits T2 (hospital + GP)					
1 extra visit	20	23.5	19	23.3	0.930 *
2 extra visits	5	5.9	4	4.9	
3 extra visits	1	1.2	2	2.4	

Abbreviations: AJCC Stage; American Joint Committee on Cancer, GP; General practitioner, T2; after one year follow-up. T2: 167 patients included in analyses (CSG: n=85, ESG: n=82).
[a] *Highest level of education completed (high: high vocational education, university; intermediate: secondary vocational education, high school; low: elementary school, low vocational education).* [b] *Categories based on the publication of Hollestein et al.*[1]
[c] *Self designed questions. Level of significance p<0.05, printed in* **bold**. **Chi2-test,^Independent student T-test.*

visits to the GP. Summarizing outpatient clinic and GP visits, 26 patients (30.6%) in the CSG and 25 patients (30.5%) in the ESG paid extra visits during the first year after diagnosis, with a range of 1-3 extra visits per patient (**Table 2**). Adherence to schedule was not related to T2 schedule satisfaction. A comparable percentage of satisfied patients (20.5%, 25/122; CSG: 6 less, 7 extra, ESG: 12 extra) and dissatisfied patients (26.6%, 4/15; CSG: 1 less, 2 extra, ESG: 1 extra) did not adhere to the schedule as planned.

Patient Reported Outcome Measures

Of the participants, 83% completed all questionnaires at T1 and T2 (CSG: n=76, ESG: n=73). PROMs were analyzed for these 149 participants. Repeated measures ANOVA's showed one significant between-group-effect: the ESG had significantly lower mean scores on the IES than the CSG (p=0.01). The effect size was small (ES=0.36). Significant time effects were found on the CWS, IES, and RAND-36 MCS scores (p=0.001). Patients' CWS and IES mean scores decreased over time, and the RAND-36 MCS score increased over time. Effect sizes were small (CWS and RAND-36: ES=0.41) and moderate (IES: ES=0.53). No significant interaction effects were found (Table 3).

TABLE 3.

Descriptives of Patient Reported Outcome Measures at baseline (T1) and one year (T2), comparison over time and between study groups

Questionnaire	Study group	T1 Mean (SD)	T2 Mean (SD)	ANOVA
STAI-S	Conventional	31.4 (8.8)	31.0 (9.9)	F=0.4; p=0.54 (group)
	Experimental	31.3 (8.0)	29.5 (8.8)	F=3.3; p=0.07 (time)
				F=1.5; p=0.23 (interaction)
CWS	Conventional	4.6 (1.5)	4.2 (1.4)	F=2.7; p=0.10 (group)
	Experimental	4.5 (1.6)	3.7 (1.1)	F=14.1; **p<0.001** (time), ES=0.41
				F=2.0; p=0.16 (interaction)
IES	Conventional	21.7 (13.9)	14.4 (13.1)	F=6.6; **p=0.01** (group), ES=0.36
	Experimental	14.8 (13.4)	9.9 (12.0)	F=34.7; **p<0.001** (time), ES=0.53
				F=1.4; p=0.25 (interaction)
RAND-36 MCS Score	Conventional	49.7 (11.4)	52.5 (8.8)	F=0.25; p=0.62 (group)
	Experimental	49.3 (10.9)	54.3 (7.6)	F=24.5; **p<0.001** (time), ES=0.41
				F=2.0; p=0.16 (interaction)

Abbreviations: T1; at inclusion, T2; after one year, STAI-S; State-Trait Anxiety Inventory-State (range 20-80), CWS; Cancer Worry Scale (range 3-12), IES; Impact of Event Scale (range 15-75), MCS; mental component summary (standardized mean 50), F; F-statistic, ES; effect size. Number (n) varies due to missing answers: STAI-S; n=144 (75/69), CWS; n=143 (74/69), IES; n=116 (58/58), RAND-36; n=149 (76/73). Level of significance p<0.05, printed in **bold**.

Detection of Recurrences

Total recurrence rate at one year after diagnosis was 8.6% in the CSG (n=8) and 8.0% in the ESG (n=7, p=0.89). Recurrences occurred as loco-regional or in-transit metastases, regional lymph nodes, 2nd primary melanomas or distant disease. More recurred (6/15=40%; CGS: n=3, ESG: n=3) than non-recurred patients (25/152=16.4%; CGS: n=9, ESG: n=16) paid extra outpatient clinic visits (p=0.025). Eight of the 15 recurrences (53.3%) were patient-detected and not physician-detected (CSG 62.5%, ESG 42.9%, p=0.45). Seven of the eight self-detecting patients (87.5%) performed self-inspection at least once a month, whereas in the physician-detected group this was 57.1% (p=0.35). Self-inspection was performed at least once a month by 78.4% of the CSG and 65.3% of the ESG at T2 (p=0.23) (Table 4).

Cost Analysis

Total costs of the hospital based melanoma follow-up in the first year after primary excision, including detection and treatment of recurrences and all registered visits, was only calculated for the 79 patients treated at the UMCG. The total expense for the ESG (n=38) was €15,871.11, with a mean of €417.66 per patient, and €31,240.67 for the CSG (n=41), with a mean of €761.97 per patient. This demonstrates a mean cost reduction of 45% (€344.31, 95%CI 85.9-602.7, p=0.01) per patient in the ESG. The differences in number of outpatient clinic visits, and the type of diagnostics and surgeries performed, are presented in Table 5. Expenses incurred for co-morbidities or GP consultations were not taken into account in this calculation.

DISCUSSION

The MELFO study is the first randomized clinical trial on the subject of follow-up frequency in AJCC stage IB-II melanoma patients. The results provide evidence that the frequency of follow-up visits in these melanoma patients can be reduced, as neither anxiety, cancer worry, stress response symptoms, and mental health, nor detection of recurrences and 2nd primaries, were negatively affected by a reduced follow-up surveillance schedule. Besides, this is accompanied with 45% cost reduction of overall melanoma care and outpatient clinic visits.

TABLE 4.

Development of recurrence or second primary (CSG: n=93, ESG: n=87); comparison between study groups

	Conventional schedule		Experimental schedule		
	No.	%	No.	%	p-value
Recurrence					
Total	8	8.6	7	8.0	0.893*
Locoregional	1	12.5	0	0.0	
In transit	1	12.5	1	14.3	
Regional lymph nodes	2	25.0	2	28.6	
Distant	3	37.5	1	14.3	
Second primary melanoma	1	12.5	3	42.9	
Detection of recurrence					
Patient	5	62.5	3	42.9	0.447*
Specialist/NP	3	37.5	4	57.1	
Cause of death					
Other cause	1	1.1	1	1.2	0.522**
Melanoma-related[a]	2	2.2	1	1.2	

Abbreviations: NP; nurse practitioner. [a] *Also included in the number of recurrences.*
**Chi2-test; **Cell count too low to perform valid Chi2-test.*

Patients' mental well-being was similar in both groups or even better in the group with a reduced follow-up schedule. Specifically, levels of anxiety, cancer worry and mental health-related quality of life were comparable in the study groups, and significantly reduced stress response symptoms were reported by the experimental group that received low intensity follow-up surveillance. A possible explanation for this last finding might be that high-intensity follow-up surveillance can provoke stress rather than provide assurance. Mixed feelings of melanoma patients regarding follow-up have previously been described, with the majority of patients thinking follow-up visits were worthwhile, but half found them anxiety provoking also.[18] Stress response symptoms and cancer worry decreased significantly over the first year of follow-up and patients' mental well-being improved in both groups, possibly because patients became

TABLE 5. **Baseline characteristics (CSG: n=93, ESG: n=87) and follow-up related questions; comparison between study groups**

Hospital costs 1 year, UMCG	Conventional schedule	n=41	Experimental schedule	n=38	p-value
Total (in euro's), based on:	€ 31,240.67		€ 15,871.11		
Follow-up visits	**€ 20,325.88**		**€ 11,127.17**		
By NP	€ 141.20	n=4	€ 176.50	n=5	
By specialist	€ 18,427.21	n=175	€ 8,873.65	n=83	
Telephone consultation	€ 1,757.47	n=22	€ 2,077.02	n=26	
Diagnostics	€ 6,651.91		€ 1,349.67		
Laboratory testing	€ 318.09	n=2	-	-	
Ultrasonography	€ 729.66	n=5	€ 228.40	n=1	
CT-scan	€ 836.89	n=4	-	-	
PET/CT-scan	€ 2,468.83	n=2	-	-	
Bone scan	-	-	€ 344.18	n=1	
Pathology: biopsy/cytology	€ 2,298.44	n=17	€ 777.09	n=7	
Surgery	€ 4,262.88		€ 3,394.27		
Melanoma related	€ 1,424.25	n=4	€ 2,167.44	n=2	
Benign skin lesion	€ 2,838.63	n=5	€ 1,226.83	n=4	
Total per patient, mean ±SD	€761.97	±683.37	€ 417.66	±452.74	**0.010**^

Abbreviations: UMCG; University Medical Center Groningen, NP; nurse practitioner. Level of significance p<0.05, printed in **bold**. *^Independent student T-test.*

accustomed to having melanoma, or due to the prolonged disease-free time after diagnosis and treatment. These results support our hypothesis that a reduced follow-up schedule does not negatively affect melanoma patients' mental well-being.

The clinicopathologic characteristics of the MELFO study group are representative for the Dutch melanoma population.[31] Recurrence rate after 12 months follow-up was approximately 9% in both study groups. In literature, recurrence rates for AJCC stage IB-II patients are described from 18% to 45%,

however, with a median time to detection of 28 months.[21] Patient-detected recurrences for stage I-III melanoma are reported to be 60-75%.[12,22,24,32] Of the small number of recurrences and 2nd melanomas in the first year after diagnosis in this study, slightly more than half was patient-detected (53%). The proportion of patients performing self-inspection at least once a month was higher in the patient-detected group, emphasizing the importance of patient education in relation to the detection of recurrences.
Schedule satisfaction was high in both groups, suggesting patients might not have a preference for a certain surveillance schedule, but rely on the recommendations of their clinician. Almost a third of the patients reported that they paid extra melanoma-related visits to the specialist or GP, demonstrating that some patients take action when they suspect a recurrence or experience anxiety, regardless of the assigned schedule.
As the prevalence of melanoma continues to rise, the intensity of surveillance strategies becomes important in the context of contemporary resource use. Melanoma follow-up is associated with a major financial burden.[32,33] With the increasing cost-consciousness in current healthcare, the mean cost reduction of 45% per patient per year found in the MELFO study is considerable.

This study was limited by the number of patients included. According to the power analysis 89 patients were needed in each study group, however, 87 were assigned to the ESG. Nevertheless, as no differences or trends were found between the groups, these two patients would not have made a significant difference. Also, the number of patients who completed all questions in the PROMs was less than required. However, refusal (13%) and dropout (7% for follow-up and 17% for PROMS) rates were rather low. Lastly, calculation of costs was only possible of patients treated at a University Medical Center, and may be slightly different from costs made in smaller hospitals.

Most current guidelines on follow-up frequency are based on low-level evidence, with unknown impact on patients' mental well-being.[8,9] Several potential benefits of reducing the existing frequency of follow-up visits for AJCC stage I-II melanoma patients have been proposed. According to these observational studies and in line with the present RCT, low-intensity surveillance strategies seem more efficient and do not appear to adversely affect patients' clinical outcomes.[17,24,32,34-36] A survey conducted among melanoma specialists in Australia concluded that extended intervals may even encourage patients

to return immediately in case of a suspicious lesion, rather than waiting for their next scheduled appointment.[16] All MELFO patients were educated about monthly self-inspection of the skin and regional lymph nodes, increasing patients' ability to detect a possible recurrence or 2nd primary.[12,23,37] More patients suspecting a recurrence paid a visit outside of the assigned schedule than those not suspecting a recurrence, underlining the relevance of providing patient-education materials.[23]

In conclusion, stage-adjusted reduced follow-up surveillance for AJCC stage IB-II melanoma patients does not appear to adversely affect patients' mental well-being and the detection of recurrences, and is economically favorable compared to currently conducted high-intensity surveillance. These results suggest that lower-intensity surveillance may be safely recommended in evidence-based melanoma follow-up guidelines. Prolonged follow-up regarding the effect of a reduced surveillance schedule is necessary to strengthen this recommendation. In addition, all surveillance programs should emphasize the importance of patient education at diagnosis, to increase the ability of patients to self-examine their skin and lymph node bearing areas for the timely detection of recurrences.

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Mr. Marc Moncrieff is the investigator of an identical follow-up study in melanoma patients in England, which is performed at the Norfolk and Norwich University Hospital. The goal of these studies is to gain insight into the optimal follow-up schedule of melanoma patients in the Netherlands and England.

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MELANOMA PATIENTS' DISEASE-SPECIFIC KNOWLEDGE, INFORMATION PREFERENCE, AND APPRECIATION OF EDUCATIONAL YOUTUBE VIDEOS FOR SELF-INSPECTION

ABSTRACT

Background. Informing and educating melanoma patients is important for early detection of a recurrence or second primary. This study aimed to investigate Dutch melanoma patients' disease-specific knowledge, and their opinions on information provision and the value of e-Health videos.

Methods. All AJCC stage I-II melanoma patients in follow-up between March 2015 and March 2016 at a single melanoma center were invited to complete 19 online questions, addressing respondents' characteristics, knowledge on melanoma, and opinions on melanoma-specific information received and the educational YouTube videos.

Results. In total, 100 patients completed the survey (response=52%); median age was 60 years and 51% were female. Breslow tumor thickness was unknown by 34% and incorrectly indicated by 19%, for presence of ulceration this was 33% and 11%, for mitosis 65% and 14%, and for AJCC stage 52% and 23% respectively. Only 5% correctly reproduced all four tumor characteristics. Orally delivered information regarding warning signs, severity, treatment possibilities, and importance of self-inspection was clearest for patients, compared to information in the melanoma brochure. According to 77% of patients, YouTube videos regarding self-inspection of the skin and regional lymph nodes had additional value. Altogether, 63% preferred receiving information in multiple ways; 92% orally by their physician, 62% through videos, and 43% through brochures.

Conclusions. Patients' melanoma-specific knowledge appears to be limited. There is an urgent need for further improvement of providing information and patient education. In addition to oral and written information, e-Health videos seem to be a convenient supplemental and easy accessible method for patient education.

INTRODUCTION

Worldwide, the incidence of melanoma is still rising.[1] As a result of better staging, improved surgical techniques and the development of targeted drugs and immunotherapies, the ten-year relative survival is increasing.[2] Lower tumor stage at primary diagnosis and early detection of a recurrence are found to be prognostic factors for survival in melanoma patients.[3] Consequently, prevention of a primary melanoma and detection of primary melanomas, recurrences and second primaries have become an important issue in current healthcare systems.

Despite available prognostic systems, such as the American Joint Committee on Cancer (AJCC) staging system, the behavior of melanoma can be unpredictable, making it difficult for patients to get a grip on the disease. Therefore it is necessary for them to understand the basics of melanoma, the dissemination patterns, and how self-inspection should be carried out precisely.[4,5] Although melanoma patients are usually given oral and written disease-specific information, some patients indicate they have unmet information needs, and patient education for self-inspection is not always provided in follow-up.[5-7]

The reported rate of 70% patient-detected recurrences emphasizes the importance of patient education regarding self-inspection.[8] Skin self-examination (SSE) was already described in 1996 as a useful and inexpensive method for the early detection of a loco-regional recurrence or second primary.[9] Self-inspection is regarded as a crucial component of current follow-up. Detailed instructions about whole-body inspection as well as palpation of the scar area, in-transit route, and regional lymph nodes should be provided to patients and their relatives.[10]

In the present time in which the use of multimedia and e-Health technology is indispensable, the Internet and video-sharing sites like YouTube are commonly used sources for patients to obtain disease-specific information.[11] The use of videos for patient education has greatly increased since 1973, as this assures a standardized level of teaching and visual presentations may have a greater individual impact than oral or written information.[12] It appears that around 75% of patients acquire knowledge on their illness through web-based information searches, suggesting a platform like YouTube could be used for disseminating health-related information and as educational tool.[13-15]

The aims of this study were to examine: 1) Dutch melanoma patients' disease-specific knowledge, 2) opinions on oral and written information received and on the additional value of e-Health video-education for self-inspection, and 3) preferred information source.

METHODS

Procedure and Respondents

All AJCC stage I-II cutaneous melanoma patients in clinical follow-up at the UMCG between March 2015 and March 2016 were asked to participate. Patients were treated as recommended by the Dutch Melanoma Guideline.[16] According to this guideline, all patients received standardized oral and written information on melanoma and instructions on self-inspection during the first outpatient-clinic visit after diagnosis. Patients did not receive their pathological report. Additionally, they were informed about the Dutch Melanoma Patient Association.

An information letter was sent, explaining the goal of the study, with a hyperlink to the questionnaire, the web-links to two YouTube videos, and the melanoma brochure of the Dutch Cancer Society (DCS)[17] one week before the planned outpatient-clinic visit. Patients were asked to complete the online questionnaire after this outpatient-clinic visit, reading the brochure, and watching both YouTube videos. A reminder letter was sent after four weeks. The study was conducted in accordance with the Declaration of Helsinki, and approved by the central medical ethics committee (METc2015.031).

In collaboration with the DCS, a surgical oncologist, a psycho-oncological specialist, and a communication advisor of the University Medical Center Groningen (UMCG) developed two online instruction videos on self-inspection, in a format suitable for Dutch melanoma patients. The videos are available on YouTube: one explaining and visualizing self-inspection of the skin (5:06 minutes, https://www.youtube.com/watch?v=CYuBPSwuEU0) and another on self-inspection of the lymph node bearing areas (5:45 minutes, https://www.youtube.com/watch?v=vyE1o_tafiM). The purpose of these videos was to emphasize the necessity of self-inspection, to demonstrate how to perform self-inspection, and to increase patients' confidence in performing self-inspection.

Instrument

A self-developed 19-item, web-based questionnaire was created using SurveyMonkey®, addressing: respondent and tumor characteristics (10 questions), agreements and opinions on melanoma-specific information and education received (8 questions), and opinions on the value of video-education for self-inspection (1 question; 7 statements). To verify patients' responses, Breslow thickness, ulceration, mitosis, and AJCC stage were retrieved from pathological reports.

Statistical Analysis

Frequencies and percentages were calculated. Differences between responders (complete and incomplete) and non-participants were tested using chi-square tests or t-tests, as appropriate, with a significance level of 5%. Statistical analyses were performed using IBM SPSS statistics version 22. Figures were made using GraphPad Prism 5.04.

RESULTS

Of the 193 AJCC stage I-II melanoma patients approached, 124 started the survey, of which 14 did not complete the questionnaire and 10 did not watch the videos. Consequently, responses of 100 participants (response=52%) were analyzed. Of these, 51% were female and 42% had completed high vocational education or university. Median age was 60 (range 20-86) years and median time since diagnosis 32.5 (range 3-209) months. Of the primary melanoma, 76% had been detected by the patients (n=56; 22 male, 34 female) or relatives (n=20; 13 male, 7 female), and 24% during a medical check-up by general practitioner or specialist (n=24; 14 male, 10 female). Self-detection rate was significantly lower in male than in female patients (p=0.028). The manner of detection (self; relative; physician) was not related to level of education or age. The trunk was more commonly affected in males (55%) and the lower limbs in females (45%, p<0.001) (Table 1).

Those who did not complete the survey (n=24) were significantly lower educated (elementary school or low vocational education) than those who did (high vocational education or university; n=100; p=0.048), no differences were found in gender, age, or time since diagnosis. Of the non-participants

TABLE 1.

Characteristics of respondents and discrepancies between melanoma characteristics remembered by patients and melanoma characteristics according to the pathological report (n=100)

Characteristics	According to patient (n)	According to pathological report (n)	Unknown + incorrect (n, %)
Gender			
Female	51		
Male	49		
Age (years)			
Median, range	60, 20-86		
≤60	49		
>60	51		
Level of education[a]			
High	42		
Intermediate	32		
Low	26		
Time since diagnosis (months)			
Median, range	32.5, 3-209		
Person detecting primary melanoma			
Patient	56		
GP	14		
Specialist	10		
Other; friend, family	20		
Primary melanoma site			
Lower extremity	29		
Upper extremity	14		
Trunk	38		
Head/neck	19		
Breslow thickness (mm)			34 + 19 (53.5%)
Median, range	1.3, 0.1-12.0	1.2, 0.4-8.0	
T1: <1.00	16	20	
T2: 1.00-2.00	33	59	
T3: 2.00-4.00	11	15	
T4: >4.00	6	5	
Unknown	34	-	
Missing	-	1	

TABLE 1.

Continued

Characteristics	According to patient (n)	According to pathological report (n)	Unknown + incorrect (n, %)
Ulceration			33 + 11 (45.4%)
No	51	82	
Yes	16	15	
Unknown	33	-	
Missing	-	3	
Mitosis			65 + 14 (82.3%)
No	14	15	
Yes	21	81	
Unknown	65	-	
Missing	-	4	
AJCC Stage			52 + 23 (76.5%)
Ia	12	6	
Ib	12	67	
IIa	14	17	
IIb	10	4	
IIc	0	4	
Unknown	52	-	
Missing	-	2	
Unknown or incorrect			
1 tumor characteristic	14		
2 tumor characteristics	31		
3 tumor characteristics	26		
4 tumor characteristics	24		
All 4 known and correct	5		
Information on melanoma received before this study started? (yes)			
Oral (physician/NP)	85		
Written (melanoma brochure)	60		

Abbreviations: GP; General practitioner, AJCC Stage; American Joint Committee on Cancer, NP; Nurse Practitioner. Missing values were excluded for calculation of percentages.
[a] *Highest level of education completed (high: high vocational education, university; intermediate: secondary vocational education, high school; low: elementary school, low vocational education).*

(n=69), 55% were female and median age was 55 (range 18-89) years; level of education, and time since diagnosis were unknown. Respondents (n=124) and non-participants did not differ significantly in gender or age.

Patients' Melanoma Specific Knowledge

Of the 100 respondents, 34% replied not to know the Breslow thickness of their melanoma and 19% reported an incorrect Breslow thickness. Presence of ulceration was unknown for 33% and 11% answers were incorrect, presence of mitosis was unknown for 65% and 14% answered incorrectly, and AJCC stage was unknown for 52% and 23% answered incorrectly. Overall, only 5% correctly reproduced these four tumor characteristics (Table 1). No significant effect was found of gender, age, educational level, or time since diagnosis on correctly, not or incorrectly knowing these tumor characteristics.

Patients' Opinions on Information Provision

Fifteen percent of patients stated not having received oral information on melanoma from their physician/nurse practitioner (NP) and 40% replied they did not receive the melanoma brochure before they were approached for this survey.

Of the respondents, 89% (totally) agreed that the orally provided information about warning signs of melanoma was clear, stage and severity was clear for 66%, and treatment possibilities for 93%. Regarding (total) agreement with clarity of information gained from the brochure percentages were 82%, 65% and 74% respectively (Table 2). Regarding warning signs for a melanoma, 96% mentioned at least two aspects to be alert to: 77% mentioned a change in color, 81% changes in shape or size, and 66% physical changes (itching, bleeding, ulceration, raw surface). In total, 17% of patients indicated a wish for more information regarding melanoma. This was not associated with the number of unknown or incorrect tumor characteristics.

The physician/NP emphasized the importance of self-inspection for the detection of a recurrence, a second primary and nodular metastases according to 80%, 77% and 70% respectively, while respectively 45%, 46% and 38% remembered this information from the brochure. Instructions on how to perform SSE were provided by the physician/NP according to 87%, and through the brochure according to 78%. As for lymph-node self-examination (LNSE), 69% could recall receiving oral instructions, and 64% remembered this information from the brochure (Table 2).

TABLE 2.

Patients' agreement on oral and written information received about melanoma and self-inspection (n=100)

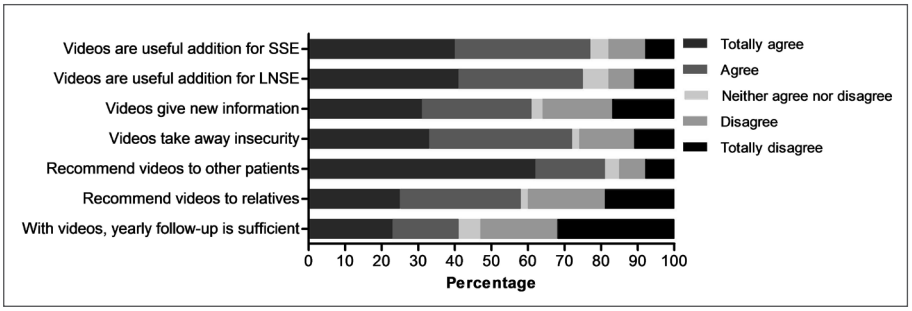
Question	Oral by physician (n)	Written in brochure (n)
Warning signs for melanoma clearly explained		
Totally agree	60	55
Agree	27	19
Disagree	6	-
Totally disagree	5	16
Missing	2	10
Stage and severity (Breslow, ulceration, mitosis, AJCC stage) clearly explained		
Totally agree	32	32
Agree	34	33
Disagree	15	11
Totally disagree	19	24
Treatment possibilities clearly explained		
Totally agree	74	47
Agree	19	27
Disagree	2	8
Totally disagree	5	18
Information received? (yes)		
A recurrence can be detected by SSE	80	45
A second primary can be detected by SSE	77	46
Nodular metastases can be detected by LNSE	70	38
Instructions received for SSE (yes)	87	78
Instructions received for LNSE (yes)	69	64

Abbreviations: SSE; skin self-examination, LNSE; lymph node self-examination.

Respondents' Appreciation of Educational YouTube Videos

The YouTube videos gave additional information to the oral and written instructions provided on SSE according to 77% of patients and 75% (totally) agreed the videos had additional value for LNSE. After watching both videos, 61% (totally) agreed to possess new information. The videos increased confidence in performing self-inspection according to 79%. Eighty-one percent would recommend the videos to other melanoma patients, and 58% would recommend their relatives to watch them. Overall, 53% (totally) disagreed that follow-up frequency could be decreased to once a year, with implementation of these videos (Figure 1). This disagreement was significantly related to shorter time since diagnosis (mean difference 16 months, p=0.005).

FIGURE 1.

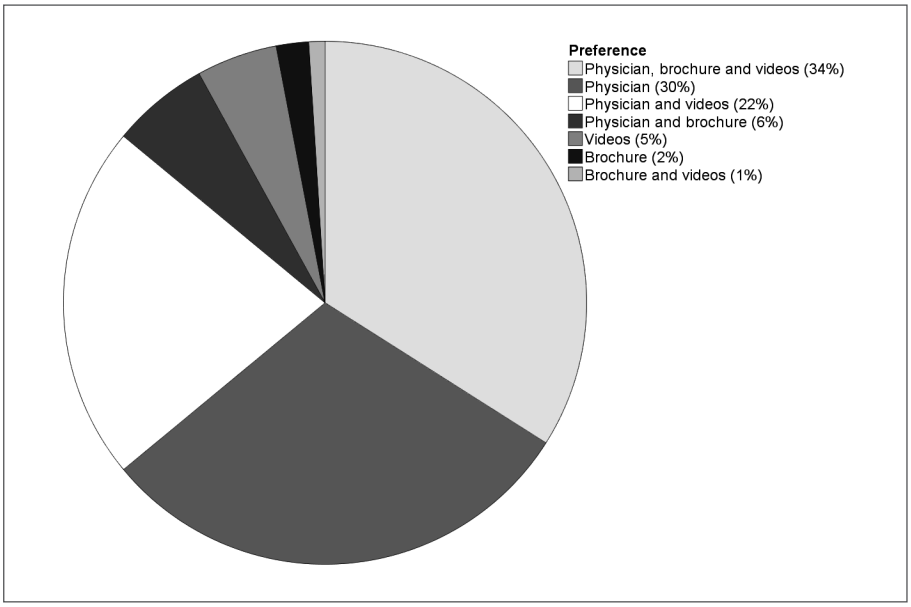


Additional value of e-Health instruction videos for skin self-examination (SSE) and lymph node self-examination (LNSE) on YouTube.

Information Source Preference

Of the patients, 63% preferred to receive patient education regarding self-inspection through more than one source. Summarized, 92% of all patients preferred their treating physician/NP to provide oral instructions, 43% preferred receiving instructions through a brochure, and 62% preferred the educational YouTube videos (Figure 2). No effect was found of gender, age, educational level, or time since diagnosis on preferred information source.

FIGURE 2.



Preference of information source regarding patient education for self-inspection. Preferred information source (total): 1) physician: 92%, 2) videos: 62%, and 3) brochure: 43%.

DISCUSSION

This study illustrates that the information currently provided to melanoma patients is insufficient. Two-thirds of patients prefer to receive information on melanoma and self-inspection in multiple ways, with the physician being the first source of preference, video transmission the second, and written information the third. Overall, e-Health education regarding self-inspection through YouTube seems to be considered a valuable supplement to instructions provided by the physician and the brochure, rather than as a substitute.

A small but significant percentage of patients (15%) indicated they did not receive oral information at all, and 40% stated not having received written information through a brochure, before study participation. This is worrying, as information should be provided to every patient according to the melanoma guideline as well as the Dutch law.[16,18] Possibly, some patients did not remember this information had been provided, being distracted by the message of having a malignancy. Nevertheless, providing adequate information and checking whether this is understood, must be the first issue to address.

Regarding specifics of their own melanoma, the vast majority of patients did not know one or more of their tumor characteristics (unknown: mitosis=65%, AJCC stage=52%, Breslow thickness=34%, ulceration=33%), or incorrectly remembered these characteristics (an additional 11-23%), suggesting that the oral information provided was lacking or unclear, or patients did not understand the relevance and forgot. These results emphasize the need for better quality, guidance, and greater consistency in providing information to patients. Remarkably, only 17% of patients expressed a wish for more information, and this was not associated with melanoma-specific knowledge. Dissatisfaction of melanoma patients regarding disease-specific information, and lack of patient education in follow-up have been reported before.[5,6]

The ignorance of patients concerning their own melanoma, might be a result of the discrepancy between the information needs of cancer patients in general, and the perception of physicians on how to inform patients.[19] In the United States of America it has already been suggested to offer every patient an individualized 'survivorship care plan' to increase patients' knowledge, including specifics of their disease, treatment, and possible side-effects, that can be updated regularly.[20] Almost all patients were able to mention warning signs indicative of a melanoma, suggesting the lack of awareness on their own prognosis is a result of inadequate information provision or understanding. Providing an individualized report to all patients, as proposed before, could improve patients' understanding. Nevertheless, the presence of a certain level of 'patient denial' might also be a factor to take into account. In literature, denial of diagnosis in cancer patients is reported to be between 4 and 47%.[21]

Information on warning signs, stage and severity, treatment options, detection of recurrent disease, and instructions for performing self-inspection was more clear to patients when received orally from the physician/NP, compared to the brochure. Apart from the necessity that medical specialists and nurse practitioners should further improve their skills to inform and educate patients, the present results underline the urgency that brochures should address these topics more explicitly as well, and that the currently used brochure on melanoma in the Netherlands might need a thorough revision. Besides, patients should be stimulated to read the brochure.

The finding that in more than three-quarter of patients the melanoma was self-detected by patient or partner/relative, emphasizes the importance of self-inspection. Male patients appear to detect a melanoma significantly

less frequent than female patients. Possibly, female patients perform more thorough self-inspection, or because melanoma in male patients is significantly more often located on the trunk. The trunk, particularly the back, may be harder to inspect than the leg. Although self-inspection for the detection of a recurrence or second primary is currently recommended for all melanoma patients, around 80% of patients recalled receiving instructions.[22] Nevertheless, this is much higher than the 13% reported in a previous survey, emphasizing the importance to improve educational strategies.[23] This difference may be explained by the instructions patients received orally and in writing shortly before study participation. Although of equal importance, more patients reported to have received instructions on SSE, suggesting less focus on LNSE. Possibly, LNSE is more difficult to explain to patients, as nodal recurrences don't usually present visually, but have to be detected by palpation.

The majority of patients appreciated the e-Health videos on YouTube as useful additional educational source and would recommend other melanoma patients to watch the videos. This appreciation could possibly increase by combining these two videos into one compact video. The use of instructional videos for effective patient education has been described before, as they can be delivered through different forms of multimedia, without requiring a high level of literacy.[24] Although more than two-third of patients in the present study felt more secure in performing self-inspection after watching the videos, a possible downside might be induction of anxiety, as reported for melanoma prevention television advertisements, graphically illustrating undetected spread of melanoma.[25] However, the use of videos to reduce patients' anxiety, while increasing knowledge and confidence in performing self-inspection, has been described since 1988.[12,26] Furthermore, video education has been reported to improve melanoma-specific knowledge among medical students, as well as protocol adherence for medical procedures.[27,28] This demonstrates the potential of videos for educational purposes. A video-based intervention designed to increase skin-awareness, SSE, and timely patient' presentation to a physician with suspicious skin lesions, was found to result in higher prevalence of self-inspection than written materials only.[29] Even though the current study shows that videos are of additional value for many patients, more than half disagreed that the frequency of outpatient-clinic visits could be lowered with use of these videos, suggesting a persisting level of insecurity and need for professional reassurance, especially in patients with shorter time since diagnosis.

The response rate (52%) in the present study is comparable with other questionnaire surveys among cancer patients. Patients who did not finish the survey were lower educated that those who did, possibly due to a certain complexity of an online survey. Educational level is another factor healthcare workers should be aware of, to achieve adequate information provision. However, education was not related to knowledge. Although the Internet might be a difficult accessible source for some older patients, the possible effect of age on Internet use is expected to diminish in the near future.

This study indicates that two-thirds of patients prefer to receive instructions for self-inspection through various sources combined. This is in line with previous literature, reporting patients prefer multiple information sources for knowledge acquisition, emotional coping, and health protection.[23] Nevertheless, the medical specialist/NP was found to be the preferred information-source, followed by e-Health videos, and lastly written information. Apparently, patients prefer the personal attention and expertise of their physician, rather than having to read a brochure. Healthcare providers have been reported to be the key source of health information for cancer survivors before.[11]
Many methods of providing information and education to patients are currently offered in clinical practice, however oral and written are still the most commonly used. Patients with chronic illnesses are found to increasingly rely on Internet-based resources to search for information and to manage their conditions.[11] In 2005, 39% of melanoma patients used the Internet to obtain information, this percentage is likely to only keep on rising.[30] Several advantages of web-based information have been reported, such as better-informed patients, improved communication between patient and physician, and time efficiency due to increased basic knowledge.[31] Of great importance is the contribution of healthcare professionals and organizations to the quality of the provided information, as web-based sources may also contain misleading or incomplete information.[13,32,33] Video-sharing sites are popular for retrieving health-related information by patients.[11] However, patients need to be assisted in finding comprehensive and accurate web-based information, and ideally, educational videos should focus on disease-specific information as well. Unfortunately, there is little attention for development of interventions for effective dissemination of e-Health videos for healthcare communication and education.[13]
With nearly two-thirds of Americans being smartphone owners, and an increasing number of patients using the internet to access health information, e-Health

tools (digital resources that facilitate self-management and information) may be effective for individual information needs and lead to improved melanoma-specific knowledge and quality of self-inspection, also longer after completion of treatment.[34-36] For example, YouTube videos can be watched as many times a patient needs and give consistent conceptualization of performing self-inspection, in contrast to healthcare providers. To increase patients' awareness and disease-specific knowledge, e-Health videos could be implemented as standard part of patient education. As videos are found to be a valuable addition to the oral and written information provided, more attention should be given to the development and publicity of online educational videos or smartphone apps, in the current era of Internet and social media.

Conclusion

This study shows the importance of providing adequate information and education to melanoma patients, as patients' knowledge on melanoma, their own tumor characteristics in specific, appears to be insufficient. Healthcare providers in oncology should be stimulated to not only provide patients oral information, but also in writing, addressing all individual aspects of their disease. The majority of patients wish to receive information in multiple ways, with the treating physician being the preferred source, followed by educational videos. Provided that the quality is guaranteed and recognizable for patients, e-Health videos may additionally contribute to patients' melanoma-specific knowledge, provide information on melanoma prevention, and encourage self-inspection of the skin and regional lymph nodes, as part of a multimedia patient education library. If regulated nationally, every country could develop e-Health videos on melanoma and other topics. Better informed and educated patients can make sincere decisions, which could have positive effects on adherence to treatment, follow-up, and the performance of self-inspection.

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PRACTICE VARIATION IN SENTINEL LYMPH NODE BIOPSY FOR MELANOMA PATIENTS IN DIFFERENT GEOGRAPHICAL REGIONS IN THE NETHERLANDS

ABSTRACT

Background. Due to the lack of solid evidence for treatment benefit of Sentinel Lymph Node Biopsy (SLNB) as part of loco-regional surgical treatment of non-distant metastatic melanoma, there might be variation in surgical treatment strategies in the Netherlands. The objective of the current study was to assess differences in the performance of SLNB, in geographical regions in the Netherlands, of non-distant metastatic melanoma patients (American Joint Committee on Cancer (AJCC) stage I-III).

Materials and Methods. A total of 28,550 melanoma patients, diagnosed between 2005 and 2013, were included in this population based retrospective study. Data were retrieved from the Netherlands Cancer Registry (NCR). Treatment strategies in 8 regions of the Netherlands were compared according to stage, excluding patients with distant metastasis (AJCC stage IV).

Results. Throughout the Netherlands, there was substantial practice variation across the regions. The performance of SLNB in patients with clinically unsuspected lymph nodes and Breslow thickness >1.0 mm was significantly different between the regions. In a post hoc analysis, we observed that patients aged over 60 years, female patients and patients with a melanoma located in head and neck have lower odds to receive a SLNB.

Conclusion. There is considerable loco-regional practice variation which cannot completely be explained by the patient and tumor characteristics, in the surgical treatment of non-distant metastatic melanoma patients in the Netherlands. Although national guidelines recommend considering SLNB in all patients with a melanoma thicker than 1 mm, only half of the patients received a SLNB. Future research should assess whether this practice variation leads to unwanted variations in clinical outcome.

INTRODUCTION

The incidence of melanoma in the Netherlands has increased at a high rate over the last decades. In 2001, 2,852 patients were newly diagnosed with invasive melanoma; this has increased to 6787 in 2016.[1] Although the rising trend in incidence is stabilizing or declining in Australia, New Zealand, North America, Israel and Norway, the incidence rates of melanoma in western European countries are expected to increase.[2-4]

Guidelines in the Netherlands recommend a narrow local excision followed by a wide local excision with proper resection margins of 1 or 2 cm, depending on the thickness of the melanoma.[5-7] In melanoma thicker than 1 mm or with unfavorable characteristics such as ulceration or mitoses, Sentinel Lymph Node Biopsy (SLNB) is advised based upon level II evidence.7 SLNB is a minimal invasive method to detect the presence of occult nodal metastasis. It has been shown to be the most powerful prognostic factor for survival in clinically node negative patients.[8,9] While following these guidelines is important to achieve the optimal staging for the majority of the patients, the surgical treatment of non-distant metastatic melanoma is still surrounded with clinical uncertainty.

The final results of the Multicentre Selective Lymphadenectomy Trial I (MSLT-I) showed no 10-year melanoma specific survival benefit of wide excision and SLNB with immediate Complete Lymph Node Dissection (CLND), compared to wide excision and nodal observation with delayed CLND. However, biopsy-based management did prolong disease-free survival rates for patients with intermediate-thickness melanomas.[10] These results are also criticized by others.[11]

The Multicentre Selective Lymphadenectomy Trial II (MSLT-II) showed that immediate completion lymph-node dissection did not increase melanoma specific survival in melanoma patients with sentinel-node metastases, but did increase the rate of regional disease control.[12] Also, there is evidence that treatment preferences of the medical specialist influence the decision to perform a SLNB.[13]

Given the recent developments, new adjuvant treatment options for non-metastatic melanoma patients might improve the recurrence-free survival, staging these patients properly will become more and more important.[14-15] This proper staging can lead to a more specific patient and tumor treatment in well informed melanoma patients.[16]

The aim of the present study is to investigate and describe regional differences in loco-regional surgical treatment strategies of non-distant metastatic melanoma patients, American Joint Committee on Cancer (AJCC) stage I-III in the Netherlands.

MATERIALS AND METHODS

In this population-based retrospective study, data from the Netherlands Cancer Registry (NCR) were used. The NCR registers data of all patients diagnosed with cancer and covers all hospitals in the Netherlands, which is geographically divided in 9 regions (Figure 1). The following data were extracted from the database: sex, year of birth, age at diagnosis, incidence year, localization, morphology, Breslow thickness, number of lymph nodes assessed, number of positive lymph nodes, local resection, SLNB, CLND, radiotherapy, follow-up time, survival status (death or alive) and regions. As registration rules for SLNB were different in one region, treatment strategies in this region could not be compared, this region was excluded from the analyses.

All new diagnosed patients with primary invasive non-distant metastatic melanoma, excluding patients with distant metastasis (AJCC stage IV), patients with morphology of the melanoma: nodular melanoma, superficial spreading melanoma and malignant melanoma Not Otherwise Specified (NOS) were selected. Data were collected at primary presentation only. A total of 28,550 non-distant metastatic melanoma patients diagnosed between 2005 and 2013 were included. Missing data on Breslow thickness (6.9%) were considered missing completely at random; these patients were excluded and the analyses were further stratified on Breslow thickness and lymph node status. Tumor Node Metastasis stage (TNM) classification at time of diagnosis was used. Patients were categorized in three groups: 1: Breslow thickness ≤1.0 mm without nodal metastasis (N0), 2: Breslow thickness >1.0 mm with non-palpable lymph nodes or unknown lymph node status (cN0 or cNx) and 3: patients with clinically suspicious lymphadenopathy (cN+) with any melanoma Breslow thickness.

Statistics

Statistical analyses were performed using STATA/SE version 12.0. For comparison of the patient characteristics and differences in treatment strategies in the regions, Chi squared tests were used. All analyses were stratified for stage.

Loco-regional surgical treatment for non-metastatic melanoma (local resection, SLNB, CLND and TLND) in the 8 regions in the Netherlands, according to stage, was compared using Chi squared tests. A sensitivity analysis was performed excluding patients with cNx. A difference was considered statistically significant if the p-value was ≤0.05.

FIGURE 1.

Geographical regions in the Netherlands and the number of patients included per region. Differences in loco-regional surgical treatment strategies across the regions

In post-hoc analysis, a multivariable logistic regression analysis was performed to explore which variables were associated with the performance of a SLNB. The explanatory variables were sex, age, location, morphology, Breslow thickness, incidence year and region. In this post hoc analysis the variable age was divided in smaller categories of five year to more accurately assess a possible cut-off value for the association of age with SLNB performance. To explore possible underlying mechanisms for variation in SLNB performance among the regions, differences in patient and melanoma characteristics within the intermediate thickness melanomas were assessed for linear trend.

TABLE 1.

Characteristics of all patients diagnosed with melanoma in the population-based Netherlands Cancer Registry 2005-2013

Characteristic	Number	Percentage
Sex		
Male	12,787	44.8
Female	15,763	55.2
Age (years)		
<45	7,023	24.6
45-54	5,881	20.6
55-64	6,407	22.4
>65	9,239	32.4
Incidence years		
2005	2,962	10.4
2006	2,485	8.7
2007	2,660	9.3
2008	2,876	10.1
2009	3,035	10.6
2010	3,270	11.5
2011	3,554	12.5
2012	3,714	13.0
2013	3,994	14.0
Localization melanoma		
Head & Neck	3,025	10.6
Trunk	11,429	40.0
Upper Extremities	6,223	21.8
Lower Extremities	7,799	27.3
Other	74	0.3
Morphology		
Nodular	3,769	13.2
Superficial Spreading	21,210	74.3
Malignant NOS	3,571	12.5

TABLE 1.

Continued

Characteristic	Number	Percentage
Stage		
Breslow thickness ≤1.0, N0[a]	16,152	56.6
Breslow thickness >1.0, cN0 or cNx[b]	12,070	42.3
Breslow thickness >1.0, cN+[c]	328	1.2
Regions		
1	1,734	3.1
2	3,224	11.3
3	4,722	16.5
4	2,655	9.3
5	5,413	19.0
6	2,506	8.8
7	3,788	13.3
8	4,508	15.8

Abbreviations: NOS, Not Otherwise Specified. [a] *no lymph node metastases;* [b] *clinical N-stage (no lymph nodes (cN0) or unknown (cNx));* [c] *clinical suspicious lymphadenopathy.*

RESULTS

Sociodemographic and Clinical Characteristics

Of the 28,550 melanoma patients included in this study, 15,763 (55.2%) were female (Table 1). The largest age-category was >65 years (n = 9,239, 32.4%). Median age was 57 years (Interquartile range (IQR) 45-68 years). The number of newly diagnosed patients increased during the studied time period, from 2,962 patients in 2005 to 3,994 patients in 2013. The trunk was the most commonly affected body site (n = 11,429), (40.0%). The histological type was superficial spreading melanoma in 74.3% of the patients (n = 21,210). Most of the patients (n = 16,152, 56.6%) were diagnosed with thin melanomas, Breslow thickness ≤1.0 mm. Median Breslow thickness was 0.9 mm (IQR 0.54mm-1.75 mm). Over the regions, the number of included patients varied from 1,734 (6.1%) in region 1 to 5,413 (19.0%) in region 5 (Table 1, Figure 1).

Thin Melanomas

In patients with Breslow thickness ≤1.0 mm, N0, all patients (n = 16,152) in all regions underwent local excision. The proportion of patients receiving SLNB in this patient group differed statistically significant ($p < 0.001$) between the regions, varying from 0.8% in region 5-8.6% in region 1 (Table 2). The percentage of patients with a positive SLNB differed from 2.3% in region 1 versus 16.3% in region 6 but this difference was not statistically significant ($p = 0.22$) (Table 2). SLNB proportions were in the same range after excluding patients with cNx.

Intermediate and Thick Melanoma

All patients with a Breslow thickness >1.0 mm, cN0 or cNx (n = 12,070) underwent local resection. The performance of SLNB was significantly different across the regions ($p < 0.001$), ranging from 22.5% in region 5-56.5% in region 6. Of these patients, 21%-25.8% had a positive SLNB; this proportion was not significantly different across the regions ($p = 0.21$). The proportion of patients receiving CLND after a positive SLNB was significantly different across the regions ($p < 0.001$), varying from 51.2% in region 1-75.6% in region 6 (Table 2).

The post-hoc analysis (Table 3) in patients with Breslow thickness >1.0 mm, cN0 or cNx showed that patients aged >60 years received significantly fewer SLNB's than younger patients. Also female patients had a significantly lower odds of receiving a SLNB (OR 0.85, 95%CI 0.78-0.94; $p = 0.001$). Patients with a melanoma located in the head and neck area had about a 3 fold lower likelihood of receiving SLNB compared to patients with a melanoma on the trunk or extremities.

Patients with a melanoma with Breslow thickness between 2 and 4 mm had a higher odds of receiving a SLNB (OR 1.54, 95%CI1.37-1.72; $p < 0.001$ for 2.1-3.0 mm and OR 1.55, 95%CI 1.32-1.82; $p < 0.001$ for 3.1-4.0 mm). During the studied time period the proportion of patients who received SLNB increased, with an odds ratio in 2006 of 1.34 (CI 1.09-1.65; $p = 0.006$ proportion: 16%) to an odds ratio of 3.75 (CI 3.10-4.55; $p < 0.001$, proportion: 23%) for patients diagnosed in 2013 when compared with patients whose incidence year was 2005(proportion: 12%). Patients living in the regions 5, 7 and 8 have a significantly lower odds for performance of SLNB in comparison with patients living in region 1 (respectively OR 0.38, 95%CI0.31-0.46; $p < 0.001$, OR 0.69, 95%CI0.56-0.84; $p < 0.001$, OR 0.73, 95%CI0.59-0.89); $p = 0.002$).

There were significant differences in patient and tumor characteristics, in line with the proportion SLNB; however the differences do not fully explain the geographical variation in SLNB (Table 4).

TABLE 2.

Treatment of the melanoma patients and proportion of patients with a positive SLNB, according to regions in the Netherlands and stage

	Regions n (%)								
Treatment	1	2	3	4	5	6	7	8	p-value[a]
Breslow thickness ≤ 1.0, N0									
Local resection	995 (100)	1920 (100)	2642 (100)	1598 (100)	2973 (100)	1397 (100)	2029 (100)	2598 (100)	N.A.
SLNB	86 (8.6)	42 (2.2)	128 (4.8)	50 (3.1)	23 (0.8)	43 (3.1)	54 (2.7)	90 (3.5)	<0.001*
SLNB positive	2 (2.3)	3 (7.1)	13 (10.2)	4 (8.0)	2 (8.7)	7 (16.3)	3 (5.6)	6 (6.7)	0.22
Breslow thickness > 1.0, cN0 or cNx[b]									
Local resection	716 (100)	1270 (100)	2029 (100)	1022 (100)	2384 (100)	1070 (100)	1714 (100)	1865 (100)	N.A.
SLNB	326 (45.5)	601 (47.3)	936 (46.1)	482 (47.2)	536 (22.5)	604 (56.5)	634 (37.0)	674 (36.1)	<0.001*
SLNB (selection cN0)[c]	288 (49.8)	501 (48.8)	731 (45.5)	368 (46.5)	308 (25.5)	380 (56.2)	498 (42.4)	466 (36.0)	<0.001*
SLNB positive	84 (25.8)	134 (22.3)	234 (25.0)	101 (21.0)	136 (25.4)	127 (21.0)	154 (24.3)	142 (21.1)	0.21
CLND after SLNB+	43 (51.2)	93 (69.4)	138 (59.0)	70 (69.3)	100 (73.5)	96 (75.6)	98 (63.6)	106 (74.7)	<0.001*
Breslow thickness > 1.0, cN+[d]									
Local resection	22 (95.7)	34 (100)	51 (100)	35 (100)	56 (100)	38 (97.4)	44 (97.8)	43 (95.6)	0.44
TLND	21 (91.3)	20 (58.8)	41 (80.4)	25 (71.4)	41 (73.2)	29 (74.4)	27 (60.0)	35 (77.8)	0.067

Abbreviations: N.A., Not Applicable; SLNB, Sentinel Lymph Node Biopsy; CLND, Completion Lymph Node Dissection; TLND, Therapeutic Lymph Node Dissection. [a] *p-value for differences between all the regions,* [b] *clinical nodal stage,* [c] *sensitivity analysis excluding cNx,* [d] *clinical suspicious lymphadenopathy. *significant p-value.*

TABLE 3.

Association of patient and melanoma characteristics on the SLNB-rate (multivariable analysis)

Patients with a melanoma Breslow thickness >1.0, cN0 or cNx[b]		
Characteristic	**Adjusted OR (95%CI)**	**p-value**
Age (years)		
≤20	Reference	
21-25	0.96 (0.52-1.78)	0.89
26-30	0.95 (0.54-1.70)	0.87
31-35	0.89 (0.51-1.54)	0.67
36-40	0.84 (0.49-1.43)	0.52
41-45	0.84 (0.50-1.42)	0.52
46-50	0.91 (0.54-1.53)	0.72
51-55	0.64 (0.38-1.08)	0.10
56-60	0.67 (0.40-1.12)	0.26
61-65	0.55 (0.33-0.93)	0.03*
66-70	0.48 (0.28-0.80)	0.005*
71-75	0.39 (0.23-0.66)	<0.001*
76-80	0.24 (0.14-0.41)	<0.001*
81-85	0.08 (0.04-0.14)	<0.001*
86-90	0.03 (0.01-0.06)	<0.001*
>91	0.006 (0.0008-0.05)	<0.001*
Sex		
Male	Reference	
Female	0.85 (0.78-0.94)	<0.001*
Localization[a]		
Head and Neck	Reference	
Trunk	3.48 (2.92-4.14)	<0.001*
Upper Extremities	3.95 (3.28-4.77)	<0.001*
Lower Extremities	4.19 (3.49-5.04)	<0.001*
Morphology		
Nodular	Reference	
Superficial Spreading	0.92 (0.83-1.03)	0.14

TABLE 3.

Continued

Patients with a melanoma Breslow thickness >1.0, cN0 or cNx[b]		
Characteristic	**Adjusted OR (95%CI)**	**p-value**
Breslow thickness		
1.0-2.0	Reference	
2.1-3.0	1.54 (1.37-1.72)	<0.001*
3.1-4.0	1.55 (1.32-1.82)	<0.001*
4.1-5.0	1.04 (0.84-1.28)	0.74
5.1-6.0	1.00 (0.76-1.32)	0.99
6.1-7.0	1.18 (0.83-1.70)	0.35
7.1-8.0	0.89 (0.57-1.40)	0.62
8.1-9.0	0.68 (0.39-1.18)	0.17
9.1-10.0	0.54 (0.25-1.13)	0.10
>10.1	0.44 (0.30-0.65)	<0.001*
Incidence Year		
2005	Reference	
2006	1.34 (1.09-1.65)	0.006*
2007	1.54 (1.26-1.90)	<0.001*
2008	1.83 (1.49-2.24)	<0.001*
2009	2.15 (1.76-2.63)	<0.001*
2010	2.16 (1.78-2.63)	<0.001*
2011	3.01 (2.48-3.66)	<0.001*
2012	3.19 (2.63-3.88)	<0.001*
2013	3.75 (3.10-4.55)	<0.001*
Region		
1	Reference	
2	1.14 (0.92-1.41)	0.24
3	1.11 (0.92-1.36)	0.28
4	1.24 (0.99-1.54)	0.06
5	0.38 (0.31-0.46)	<0.001*
6	1.70(1.36-2.14)	<0.001*
7	0.69(0.56-0.84)	<0.001*
8	0.73(0.59-0.89)	0.002*

[a] *Localization 'other' and morphology 'NOS' excluded for this analyses;* [b] *Clinical nodal stage; *=significant p-value*

Clinically Suspicious Lymphadenopathy

Only a few patients (n = 5) with macro metastasis in the lymph node (cN+, M0) did not receive local resection. There was a non-significant variation across the regions in performance of TLND in these patients, with 60.0% in region 7-91.3% in region 1 (p = 0.067) (Table 2).

TABLE 4.

Differences in patient and melanoma characteristics (n and %)

Region		5	8	7	1	3	4	2	6	p-value
SLNB	Yes	536 (22.5)	674 (36.1)	634 (37.0)	326 (45.5)	936 (46.1)	482 (47.2)	601 (47.3)	604 (56.5)	<0.001
Sex	Male	1.015 (50.9)	744 (52.0)	760 (48.9)	340 (53.3)	865 (47.5)	456 (51.4)	451 (46.1)	394 (48.2)	0.008
	Female	981 (49.2)	688 (48.0)	794 (51.1)	298 (46.7)	957 (52.5)	431 (48.6)	527 (53.9)	423 (51.8)	
Age	≤60	923 (46.2)	736 (51.4)	783 (50.4)	312 (48.9)	888 (48.7)	445 (50.2)	554 (56.7)	411 (50.3)	0.002
	>60	1.073 (53.8)	696 (48.6)	771 (49.6)	326 (51.1)	934 (51.3)	442 (49.8)	424 (43.4)	406 (49.7)	
Localization	Head & Neck	289 (14.5)	186 (13.0)	181 (11.7)	73 (11.4)	237 (13.0)	114 (12.9)	103 (10.5)	103 (12.6)	0.07
	Other	1.707 (85.5)	1.373 (88.4)	1.373 (88.4)	565 (88.6)	1.585 (87.0)	773 (87.2)	875 (89.5)	714 (87.4)	
Breslow	Median	2.0	1.855	1.95	2.0	2.0	1.7	1.8	1.9	0.03

Abbreviations: SLNB, Sentinel Lymph Node Biopsy.

DISCUSSION

This large observational study shows large differences in sentinel lymph node biopsy in stage I and stage II melanoma patients among regions in the Netherlands. In only half of the patients with a melanoma >1.0 mm (and clinically unsuspected lymph nodes) SLNB was performed. In case of a positive SLNB, a consecutive CLND was performed in half to three quarters of the patients. During the studied time period, the Dutch melanoma guideline did not recommend to perform a SLNB for melanoma patients with a thin melanoma of less than 1 mm.[7] However in the revised guidelines (revised on 01-03-2016 version 2.1)[7] SLNB is recommended in patients with ulcerations or mitosis ≥1/mm^2, this would explain the small chance to receive SLNB. Current study confirms that, in general, this guideline is followed for these patients; however they still have a small chance (between 0.8% and 8.6%, dependent on the region) to receive SLNB. The SLNB positivity rate in these patients was between 2.3% and 16.3%. Possibly these are the patients for whom a SLNB is recommended in the revised guideline. In a large retrospective study where 32,527 cases of T1 melanoma were included, the overall SLN positivity rate was 7.8%. Performing a SLNB was correlated with T-stage, thickness, level, ulceration, age, and geographic region. Patients with SLNB + had a significant diminished cancer-specific survival.[17]
For patients with thicker melanomas of 1.0 mm or more, the Dutch guideline recommends to consider performance of SLNB. However, we observed that only a quarter to half of the patients with a Breslow thickness >1.0 mm and clinically unsuspected lymph nodes indeed received SLNB during the observed period (Table 2). This finding is in line with the results of a previous observational study that reported a low performance of SLNB (45.2%) for patients with a melanoma of 1 mm or thicker between 2004 and 2011 in the north eastern part of the Netherlands.[18] Also in a large study in the United States where 16,598 patients were included, in only half of the patients use of a SLNB was reported.[19] In the latter study SLNB was not only associated with clinicopathologic factors but also with health system factors.

Approximately one out of four patients with Breslow thickness >1.0 mm, cN0 or cNx in our study had metastasis in the regional lymph nodes (SLNB+). These tumor foci are apparently too small to detect clinically and may also be missed by radiological examination due to low sensitivity of high resolution ultrasound.[20,21]

Thus, SLNB provides pathologic status information that would otherwise be missed in approximately half of the patients, according to the present study. This accurate staging will become more important in the future if new (neo-) adjuvant treatment options for non-distant metastatic melanoma patients may become available, In the current study, several patient and melanoma characteristics were associated with receiving a SLNB. Older patients over the age of 60 years received significantly fewer SLNB than younger patients. This was also found in another study where patients over the age of 55 years were less likely to receive SLNB than younger patients.[18] An explanation for this could be that older patients more often have comorbidities which may lead to the decision to refrain from a SLNB.[22,23] Older patients also have more aggressive primary melanoma features as a higher ulceration rate and mitotic index (among others) and age is associated with a higher mortality; in contrast they have a lower SLNB + rate.[24,25] Physicians may therefore be more reluctant to perform SLNB for these patients and potentially feel less urge to perform a diagnostic procedure for a patient that does not have a long life ahead. However we are dealing here with a minimal invasive staging procedure with minimal morbidity and the possibility, in case of a positive sentinel lymph node, of a better regional disease control and a better quality of life.[26-28] Nevertheless, the results of the MSLT-II study have not shown a melanoma-specific survival gain, so for patients with a positive SLNB, shared decision making with high quality information is important to make an informed choice on whether to undergo lymph node dissection or observation.[12,16]

According to the literature, in patients with a melanoma in the head and neck area, SLNB is less often performed as it is technically a more challenging procedure to perform in this area.[29] The results of our study indeed confirm that performance of SLNB in patients with a melanoma located outside the head and neck area was associated with significantly higher odds to receive SLNB. In this study we also observed that female patients have significantly lower odds to receive SLNB compared with male patients, for which no explanation was found. These findings of a lower odds to receive SLNB for female patients, older patients and patients with a melanoma located in the head and neck area, was also previously observed in a study where 4,571 clinically node negative melanoma patients with a Breslow thickness > 4 mm were identified.[30] Furthermore in the Dutch study earlier mentioned, an association with performance of SLNB with a lower SES and diagnosis made in a university hospital was observed.[18] In

current study only the clinicopathologic features of the patients in the regions were compared. Future research should focus on the specific reason why these patients have a lower chance to receive a SLNB. During the studied time period the proportion of patients who received SLNB increased which could indicate that there is a slow growing awareness of the importance of SLNB and increased adherence to the advice in the guideline.

The rate of performance of CLND in SLNB positive patients with Breslow thickness >1.0 mm cN0 or cNx varied from 51.2% to 75.6% in the regions. This confirms the results of another observational study which found that only 328 of the 495 (66%) patients who had positive lymph nodes underwent CLND. In that study there were two factors associated with omitting CLND: older age and melanoma of the lower extremities.[31] Treatment related morbidity due to inguinal CLND is high compared to axillary dissection; wound complications often occur on the short term and on the long term lymphedema is a common complication.[32-35] In the current study, the TLND rate for patients with a clinically suspect sentinel lymph node was higher than for patients with clinically unsuspected lymph nodes and ranged between 58.8% and 91.3%.

Limitations and Strengths

Although the intention was to analyze data from all regions in the Netherlands, the registration rules from 1 out of 9 regions were too different to be used in this study. Nevertheless, the treatment strategies of the remaining 8 regions were compared. Data was used from 28,550 patients diagnosed between 2005 and 2013 in a real life population without patient selection. Specific attention was given to the coding of the variable SLNB and outliers in the regions and over time, leading to the exclusion of one region and earlier incidence years. Some accidental coding errors might however have occurred. Data before 2010 may be less reliable due to registration rules, however time trends did show a similar trend in all regions indicating that there were no large differences over time and between the regions.

In the post-hoc analysis the factors associated with the performance of SLNB were examined. However we were restricted by the variables that were available in the database and were therefore not able to analyze this in detail. We were not able to evaluate the adherence to the guidelines of resection margins as this was not registered in the database. We acknowledge that other patient and melanoma specific factors may also play a role in selecting patients for SLNB which should be subject of future studies.

Conclusion

There is considerable regional practice variation in the surgical loco-regional treatment of non-distant metastatic melanoma patients in the Netherlands. This variation is present for both SLNB and CLND performance. Only half of the patients actually received a SLNB, and consequently many patients are not adequately staged. This practice variation can possibly be explained by the patient and tumor characteristics and the coherent comorbidity. Although compliance with the SLNB staging guidelines is increasing over time, future research should assess factors associated with the omission of SLNB in detail, to improve a better minimal invasive melanoma staging and to assess whether this practice variation leads to unwanted variations in clinical outcome.

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PART II

PREDICTION OF NODAL STATUS IN COMPLETION LYPMH NODE DISSECTION USING THE BIOMARKER S-100B

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THE PREDICTIVE POWER OF SERUM S-100B FOR NON-SENTINEL NODE POSITIVITY IN MELANOMA PATIENTS

ABSTRACT

Background. Completion lymph node dissection (CLND) in sentinel node (SN) positive melanoma patients leads to substantial morbidity and costs, while only approximately 20% have a metastasis in non-sentinel nodes (NSNs). The aim of this study was to investigate if the biomarkers S-100B and Lactate Dehydrogenase (LDH) are associated with NSN positivity, to identify patients in whom CLND could safely be omitted.

Methods. All SN positive patients who underwent CLND at the University Medical Centre Groningen between January 2004 and January 2015 were analysed. Patient and tumor characteristics, and serum S-100B and LDH values measured the day before CLND were statistically tested for their association with NSN positivity.

Results. NSN positivity was found in 20.6% of the 107 patients undergoing CLND. Univariate analysis revealed male gender (p=0.02), melanoma of the lower extremity (p=0.05), Breslow thickness (p=0.004), ulceration (p=0.04), proportion of involved SNs (p=0.045) and S-100B value (p=0.01) to be associated with NSN positivity. LDH level was not significantly associated with positive NSNs (p=0.39). In multivariable analysis, S-100B showed to have the strongest association with NSN positivity, within its reference interval of 0.20µg/l (p=0.02, odds ratio 5.71, confidence interval 1.37-23.87).

Conclusion. In this study, the preoperatively measured S-100B value is the strongest predictor for NSN positivity in patients planned for CLND. Fluctuations of the S-100B level within the reference interval might give important clues about residual tumor load. Although further validation will be needed, this new closer look of S-100B could be of value in patient selection for CLND in the future.

INTRODUCTION

Sentinel lymph node biopsy (SLNB) is recommended in all patients with an American Joint Committee on Cancer (AJCC) stage IB-IIC cutaneous melanoma.[1] After a positive SLNB, positive non-sentinel nodes (NSNs) are found in only approximately 15-20% of the patients undergoing a subsequent completion lymph node dissection (CLND). This means a great number of sentinel node (SN) positive patients might not benefit from this procedure.[2,3] Therefore, the indication for CLND should be considered carefully, as the procedure causes significant morbidity and economic burden.[4] Currently, there is no evidence that CLND improves melanoma-specific survival.[2,3,5-7] Nevertheless, CLND remains the standard of care in SN positive patients, until the final results of the second Multicenter Selective Lymphadenectomy Trial (MSLT-II) will be available, in which CLND versus ultra-sonographic nodal observation is being compared.[8]

Various parameters have been investigated to select patients with a low risk for NSN positivity. An association with NSN positivity is described for male gender,[9] Breslow thickness,[10-12] regression,[9] ulceration,[7] number of positive lymph nodes in SLNB,[7,9] maximum size of metastasis in SN,[3,10-15] invasion depth of metastasis in SNs,[7,16] non-subcapsular location of metastasis in SN,[9,17] extra-nodal extension of metastasis in SN,[7,13] and the presence of perinodal lymphatic invasion.[9] Independently, those parameters lack predictive strength to stratify risk for NSN involvement, so risk scores based on conjunction of the significant factors in multivariable models were developed and validated.[6,9,11] However, these scores still show false negatives and the assessment of histologic parameters of melanoma deposits in SNs is prone to inter-observer variation.[18] Although serum biomarkers could have better reproducibility, their predictive value for the selection of these patients has not been investigated before.

For melanoma, the biomarkers S-100B and Lactate Dehydrogenase (LDH) have been described extensively. LDH was implemented in the AJCC system in 2001 to classify stage IV patients.[19] The melanoma-associated molecule S-100B was found to be a prognostic tumor marker in AJCC stage III and IV disease.[20] Compared to LDH, elevated levels of serum S-100B are stronger associated with recurrence risk and decreased survival in melanoma patients presenting with palpable nodal metastases.[21] More recently, C-Reactive Protein (CRP) was also reported to be a prognostic marker in all stages of cutaneous melanoma.[22]

Hypothetically, biomarkers could increase the accuracy of risk stratification for NSN involvement in SN positive melanoma patients. The aim of present study was to investigate whether levels of preoperatively measured serum S-100B and LDH are associated with NSN positivity in these patients, and to evaluate the potential value of biomarkers in the selection of patients for CLND.

METHODS

All SN positive cutaneous melanoma patients who underwent a CLND between January 2004 and January 2015 were prospectively registered. SLNB was performed in patients presenting with a primary melanoma AJCC stage IB to IIC, except for one AJCC stage IA patient, who had opted for SLNB. The study cohort consisted of patients who underwent wide local excision and SLNB at the University Medical Centre Groningen (UMCG, a melanoma center), as well as patients referred to the UMCG with a positive SN. In case of referral, histopathologic review of the primary tumor and the sentinel lymph nodes was performed.

Histopathologic processing of the SNs consisted of blocking in paraffin and cutting of 4µm sections, with a distance of 250µm between them, at four different levels for routine hematoxylin and eosin staining, with additional immunohistochemistry for S-100B and Melan-A. If metastatic melanoma was found during this procedure, the SLNB was considered positive and CLND was scheduled and performed by an experienced melanoma surgeon. For NSNs harvested during CLND, histopathologic analysis was done by cross-section of each lymph node with subsequent hematoxylin and eosin staining.

Characteristics of the patients, the primary tumors, SLNB, and CLND were collected in a database. The recorded parameters included: age, gender, site of primary melanoma, histologic type, Breslow thickness, Clark level, ulceration, mitotic rate (number of cells in mitosis per mm^2), lymphovascular invasion (the presence of melanoma cells in lymphatic or blood vessels), regression (defined as partial or complete replacement of invasive melanoma by angiofibroplasia with/without associated inflammation and melanophages), total number of harvested SNs, number of involved SNs, proportion of involved SNs, size of the largest metastasis in SN, extra-nodal growth pattern of the metastasis, site of CLND, number of harvested NSNs, and number of involved NSNs. Serum S-100B and LDH values were measured the day prior to CLND.

Biomarker Assay and Reference Cut-off

S-100B levels were calculated on the basis of a calibration curve and checked against internal standards with a known concentration of S-100B. The S-100B cut-off value was determined by analysis of S-100B values in 120 healthy individuals (median 0.07; range 0.01-0.59) according to the Clinical and Laboratory Standards Institute EP28-A3c guideline (formerly C28-A2), resulting in a reference cut-off point of 0.20µg/l at our institution.[23] LDH was analyzed routinely by means of Roche Modular (Hitachi) with an enzymatic activity measurement. Normal values of LDH were considered to be below the reference cut-off of 250U/l.

Statistical Analysis

Characteristics of the patient (age and gender), primary melanoma (site, histologic type, Breslow thickness, Clark level, ulceration, mitotic rate, lymphovascular invasion and regression), harvested SNs (total number of nodes, number of involved nodes, proportion involved SN, size of the largest nodal metastasis, extra-nodal growth pattern), and preoperatively measured S-100B and LDH levels were analyzed for their association with NSN positivity using the Chi-squared test for univariate analyses and logistic regression analysis for the multivariable model (IBM SPSS Statistics version 22).

S-100B and LDH were both analyzed in three different ways: 1) continuous, 2) categorical with the cut-off value for normal level, and 3) categorical within the reference interval, to test whether minimal variation of S-100B is relevant in patients with low tumor burden. The subcategories within the reference interval were determined by dividing the number of patients by the 33- and 66-percentiles, using the corresponding S-100B and LDH values. Because S-100B has a distribution skewed to the left, we log-transformed this variable, which resulted in the most optimal model fit for the linearity assumption in the logistic regression model.

All characteristics associated with NSN positivity on a 10% significance level in univariate analysis were entered in a multivariable model (gender, localization, histology, Breslow thickness, ulceration, lymphovascular invasion, proportion SN involved, size of the SN, and preoperative S-100B) and logistic regression analysis was performed, using a p-value <0.05 to identify significant associations.

RESULTS

A total of 107 SN positive melanoma patients were studied. Clinical features of the study group are presented in Table 1. The majority of patients were men (59.8%, n=64), with a median age of 56 years. Most patients presented with truncal melanoma (50.5%, n=54), followed by melanoma of the lower extremity (31.8%, n=34), upper extremity (14.9%, n=16), and head or neck (2.8%, n=3). The median Breslow thickness was 3.0mm, and ulceration was present in 43.9% (n=47) of the tumors. In 70 patients (65.4%) more than one SN was harvested, with a median of two per patient. In 25 of these patients (35.7%) more than one SN contained metastases, with a median of one SN. Multiple SN metastases were found in 3 of the 6 SLNBs from the neck (50.0%), 11 of the 57 SLNBs from the axilla (19.3%), and 11 of the 44 SLNBs from the groin (25.0%). The median size of the metastases found in the SN was 1.5mm.

A total of 57 axillary (53.3%), 44 groin (41.1%), and 6 neck (5.6%) CLNDs were performed. Positive NSNs were found in 22 of the 107 patients (20.6%). Involvement of more than one NSN was found in 10 patients, with a median of one NSN.

Factors Associated with Positive NSNs in CLND

Univariate analysis revealed the following characteristics to be associated with NSN positivity: male gender (p=0.02), melanoma of the lower extremity (p=0.05), thicker Breslow (p=0.004), ulceration (p=0.04), and proportion of involved SNs (p=0.045). S-100B analyzed as continuous variable showed a significant association with NSN positivity (p=0.01). LDH was not associated with NSN positivity in univariate analysis (p=0.39).

Multivariable analysis included gender, localization of the primary melanoma, histologic type of melanoma, Breslow thickness, ulceration, lymphovascular invasion, proportion of SN involved, size of the SN, and preoperatively measured S-100B level (continuous). Only male gender (p=0.04) and S-100B level as continuous variable within the reference interval (p=0.02) were significantly associated with NSN positivity (Table 1 and 2).

S-100B as Categorical Variable

Using the reference cut-off of 0.20µg/l for S-100B, there was no association with NSN positivity when analyzed in categories above and below the reference cut-off (respectively 0% and 20.8%, p=0.61). However, S-100B did show a

significant association when analyzed in subcategories within the reference interval in univariate analysis (<0.05µg/l; 18.2%, 0.05-0.07µg/l; 5.0%, >0.07µg/l; 41.2%, p=0.001) and in multivariable analysis (OR 4.59, 95% CI 1.37-23.87, p for trend=0.038).
LDH did not show any significant association with NSN positivity, neither when categorized in above and below the reference cut-off of 250U/l, nor when categorized in subcategories within the reference interval (p=0.25 and p=0.31 respectively, Table 2).

Table 3 shows the number of patients with and without positive NSNs, and the accompanying S-100B values. The negative (NPV) and positive (PPV) predictive value were calculated, based on the categorical distribution of S-100B in a "low" (<0.05µg/l) and "high" (>0.07µg/l) subgroup. This resulted in a NPV of 81.8% (65.0-98.6) and a PPV of 41.2% (20.1-62.3).

TABLE 1.

Univariate and multivariable analysis of preoperative characteristics of 107 SN positive patients undergoing CLND, tested for their association with NSN positivity

Characteristic[a]	n (%)	NSN positivity (%)	p-value[b]	Multivariable OR (95% CI)	p-value[b]
Age (years)					
Continuous (median, IQR)	56, 43-67		0.77		
<50	40 (37.4)	8/40 (20.0)			
≥50	67 (62.6)	14/67 (20.9)			
Gender[c]					
Female	43 (40.2)	4/43 (9.3)	**0.02**	1	**0.04**
Male	64 (59.8)	18/64 (28.1)		4.99 (1.05-23.74)	
Site of melanoma[c]					
Lower extremity	34 (31.8)	12/34 (35.3)	**0.05**	1	0.19
Head/neck	3 (2.8)	0/3 (0.0)		-	
Trunk	54 (50.5)	9/54 (16.7)		0.32 (0.07-1.56)	
Upper extremity	16 (14.9)	1/16 (6.3)		0.15 (0.01-1.74)	
Histologic type[c]					
Superficial spreading	70 (66.4)	10/70 (14.3)	0.09	1	0.32
Nodular	31 (29.0)	10/31 (32.3)		3.10 (0.71-13.54)	
Other	6 (5.6)	2/6 (33.3)		2.25 (0.12-42.31)	
Breslow thickness (mm)[c]					
Continuous (median, IQR)	3.0, 1.8-4.3		**0.004**	1.13 (0.81-1.56)	0.47
T1: <1.00	3 (2.8)	0/3 (0.0)			
T2: 1.01-2.00	28 (26.2)	3/28 (10.7)			
T3: 2.01-4.00	44 (41.1)	8/44 (18.2)			
T4: >4.00	32 (29.9)	11/32 (34.4)			
Clark level					
II/III	18 (16.8)	2/18 (11.1)	0.26		
IV	62 (58.9)	13/62 (21.0)			
V	22 (20.6)	7/22 (31.8)			
Unknown	5 (4.7)				
Ulceration[c]					
No	60 (56.1)	8/60 (13.3)	**0.04**	1	0.26
Yes	47 (43.9)	14/47 (23.8)		2.37 (0.53-10.63)	
Mitotic rate (per mm2)					
Continuous (median, IQR)	4, 3-8		0.53		
<5	44 (41.1)	7/44 (15.9)			
≤5	43 (40.2)	9/43 (20.9)			
Unknown	20 (18.7)				

TABLE 1.

Continued

Characteristic[a]	n (%)	NSN positivity (%)	p-value[b]	Multivariable OR (95% CI)	p-value[b]
Lymphovascular invasion[c]					
No	98 (91.6)	18/98 (18.4)	0.06	1	0.06
Yes	9 (8.4)	4/9 (44.4)		8.45 (0.88-80.84)	
Regression					
No	95 (88.8)	21/95 (22.1)	0.31		
Yes	11 (10.3)	1/11 (9.1)			
Unknown	1 (0.9)				
Number of SN					
Quantitative (median, IQR)	2, 1-3		0.13		
1	36 (33.6)	9/36 (25.0)			
2	36 (33.6)	9/36 (25.0)			
3 or more	35 (32.7)	4/35 (11.4)			
Number of positive SN					
Quantitative (median, IQR)	1, 1-1		0.24		
1	82 (76.6)	14/82 (17.1)			
2	21 (19.6)	7/21 (33.3)			
3 or more	4 (3.7)	1/4 (25.0)			
Proportion involved[c]					
Continuous (median, IQR)	72, 50-100		**0.045**	1.02 (0.99-1.05)	0.20
≤50%	47 (43.9)	6/47 (12.8)			
>50%	60 (56.1)	16/60 (26.7)			
Size of metastasis (mm)[c]					
Continuous (median, IQR)	1.5, 0.6-4.0		0.10	1.01 (0.87-1.18)	0.89
≤0.50	23 (21.5)	1/23 (4.3)			
0.51-2.00	34 (31.8)	8/34 (23.5)			
2.01-10.0	31 (29.0)	9/31 (29.0)			
>10.0	6 (5.6)	2/6 (33.3)			
Unknown	13 (12.1)				
Extranodal growth					
No	105 (98.1)	21/105 (20.0)	0.30		
Yes	2 (1.9)	1/2 (50.0)			

Abbreviations: IQR, interquartile range; SN, sentinel node; NSN, non-sentinel node; CLND, completion lymph node dissection. [a] *Continuous characteristics and quantitative discrete characteristics were tested using logistic regression analysis. Categorical characteristics were tested with Chi squared test.* [b] *All p-values <0.05 are printed in* **bold**. [c] *Associated with NSN positivity on 10% significance level in univariate analysis, entered in multivariable model, tested using logistic regression analysis.*

TABLE 2.

Association of LDH and S-100B (continuous and categorical) with NSN positivity

Characteristic[a]	NSN positivity (%)	p-value[b]	Multivariable OR[c] (95% CI)	p-value[b]
Preoperative LDH (U/l)				
Continuous (median, IQR)	175, 163-193	0.39		
LDH Reference cut-off				
≤250	19/100 (19.0)	0.25		
>250	2/5 (40.0)			
Unknown	2			
LDH categorical				
≤165	4/34 (11.8)	0.31		
166-189	8/38 (21.1)			
≥190	9/33 (27.3)			
Unknown	2			
Preoperative S-100B (µg/l)				
Continuous[d] (median, IQR)	0.06, 0.03-0.09	**0.01**	5.71 (1.37-23.87)	**0.02**
S-100B Reference cut-off				
≤0.20	22/106 (20.8)	0.61		
>0.20	0/1 (0.0)			
S-100B categorical				
<0.05	6/33 (18.2)	**0.001**	1	**0.038**[e]
0.05-0.07	2/40 (5.0)		0.24 (0.02-2.55)	
>0.07	14/34 (41.2)		4.59 (0.84-25.11)	

[a] *Preoperatively measured S-100B and LDH levels were analyzed for their association with NSN positivity using the Chi-squared test for univariate analyses and logistic regression analysis for the multivariable model.* [b] *All p-values <0.05 are printed in* **bold**. [c] *All analyses adjusted for gender, localization, histology, Breslow thickness, ulceration, lymphovascular invasion, proportion SN involved and size of the SN. Tested using logistic regression analysis.* [d] *Log-transformed due to a skewed distribution.* [e] *P-value for trend. Contrast p-value for the second group p=0.24, for the third group p=0.079. Abbreviations: LDH, Lactate Dehydrogenase; IQR, interquartile range.*

TABLE 3. **The distribution of S-100B in three categories, in relation with NSN positivity**

S-100B level / NSN involvement	<0.05 µg/l	0.05-0.07 µg/l	>0.07 µg/l
No (n, %)	27 (81.8%)	38 (95.0%)	20 (58.8%)
Yes (n, %)	6 (18.2%)	2 (5.0%)	14 (41.2%)

Abbreviations: NSN, non-sentinel node; n, number.

DISCUSSION

To our knowledge, this is the first study to investigate the predictive capacity of tumor biomarkers with NSN positivity in melanoma patients. This study reveals the significant association of S-100B (within its reference interval) with NSN positivity, suggesting S-100B might be a valuable parameter for the selection of patients in which CLND can safely be omitted after a positive SLNB.

Factors Associated with NSN positivity

To date, CLND is recommended in case of a positive SN, until the risks of CLND omission are fully explored by the MSLT-II.[8] In anticipation of the MSLT-II results, various studies were performed to identify clinicopathologic factors that predict the risk for NSN positivity. Current literature describes many predictive factors, based on characteristics of the patient, the primary melanoma, or the SN metastasis.

In particular, the size of the SN metastasis seemed a good predictor for this purpose. In 1984 Cascinelli et al. already reported growth pattern and extend of nodal metastases to be the most relevant criteria for prognosis in stage II melanoma.[24] Some authors have suggested that SN metastases smaller than 0.1mm should be considered SN negative (Rotterdam criteria).[3,12,15] On the contrary, other studies report that these very small (<0.1mm) deposits of melanoma in SNs may be associated with adverse clinical outcomes, despite the low risk of additional nodal involvement.[25, 26] The impact on prognosis of CLND omission in patients with minimal SN tumor burden is currently being explored by the European Organization for Research and Treatment of Cancer (EORTC) MiniTub registration study.[27]

Other frequently reported predictors for NSN positivity are: male gender, thicker Breslow, regression, ulceration, satellitosis, neurotropism, angiolymphatic invasion, number of positive nodes in SLNB, maximum size of SN tumor deposits, invasion depth of metastases in SNs (Starz-classification), non-subcapsular location of metastases within SNs (Dewar-classification), extra-nodal extension of metastases in SNs, and the presence of perinodal lymphatic invasion.[3,7,9-11,13-17] Besides S-100B, the present study found a significant association with NSN positivity in univariate analysis for male gender, melanoma on the lower extremity, thicker Breslow, ulceration, and the proportion of involved SNs, in accordance with previously described literature.[7,9-11,13]

These histopathologic and clinical parameters, especially when combined in NSN risk scores based on multivariable analyses, were previously found to enable stratification of risk for NSN positivity in SN positive melanoma patients.[6,9-11] Nevertheless, the question remains which parameter or conjunction of parameters effectuates the most accurate risk stratification for NSN involvement. Considering the results of this study, the biomarker S-100B seems a very promising candidate for this purpose.

S-100B as Predictor for NSN positivity

While the debate on whether or not to perform a CLND triggers further investigation on predictors of NSN positivity, no studies concerning the use of biomarkers to improve patient selection have been published. One major advantage of biomarkers is the absence of inter-observer variation.[18] Previously, our institution stated that the preoperatively measured S-100B level is one of the most important independent predictors of melanoma prognosis in patients undergoing therapeutic lymph node dissection (TLND) for nodal macro-metastases, suggesting the serum level of S-100B to be correlated with nodal tumor load.[20,21] For AJCC stage I and II melanoma, various studies have concluded that neither serum S-100B nor LDH were capable of predicting SN status, because of low sensitivity of these markers with the used cut-off points, based on healthy individuals (S-100B cut-off range 0.12-0.16µg/l).[28,29]

The results of this study reveal that S-100B levels, in stage IB-IIC melanoma patients, show a strong association with NSN positivity, in contrast to LDH. Of all patients, 20.6% had metastatic involvement of NSNs after CLND. Stratification of risk for NSN positivity was not possible using S-100B with the 0.20µg/l reference cut-off of our institution, since the cut-off value was exceeded in only one patient. However, when analyzed as a continuous variable (median 0.06µg/l, interquartile

range 0.03-0.09µg/l), S-100B turned out to have the strongest independent association with NSN positivity, based on the odds ratio (OR 5.71; p=0.02).
In categories within the reference interval, S-100B showed significantly more NSN positivity in patients with values above 0.07µg/l (41.2%, OR 4.59, overall p=0.038, p-value significant for trend), resulting in a sensitivity of 64%, a NPV of 81.8% and a PPV of 41.2%. In other words, a 'low' level indicates the absence of NSN involvement, whereas a relatively 'high' S-100B level does not necessarily prove metastatic tendency. The six NSN positive patients in the lowest category (<0.05µg/l) had no or only slightly elevated S-100B levels during follow-up, even when nodal or distant metastases occurred. Hypothetically, tumor markers do not always predict the amount of tumor load, depending on the differentiation of the primary tumor, or because lack of melanoma cell lysis due to absence of tumor necrosis or immunologic responses. Awaiting the results of the MSLT-II, 'watchful waiting' through clinical and ultra-sonographic nodal observation would be justified in the lowest category, as described in recent literature.[2,5,8]

The Use of S-100B within the Reference Interval

A predictive capacity for S-100B within the reference interval might feel counterintuitive, as one would assume that S-100B values within the reference range based on healthy individuals could hardly reflect melanoma tumor load. Nevertheless, biochemical studies show that the S-100B protein promotes tumor cell proliferation by inhibiting tumor suppression and apoptosis in melanoma by binding to tumor protein p53 (TP53), thereby contributing to disease progression.[30] Following this theory, S-100B could enhance the metastatic tendency of melanoma cells. Thus patients with slightly higher S-100B levels, although within the reference interval, show more aggressive tumor biology and higher risk for NSN involvement. This mechanism with S-100B as driver, can explain the finding that although S-100B is within the reference range, minimal elevation is important when trying to predict NSN status.

The Clinical Applicability of S-100B

Before using the biomarker S-100B for omitting CLND, its predictive capacity and sensitivity should be validated in larger independent patient cohorts. Also, the recently finished MSLT-II trial should demonstrate first whether CLND improves the outcome compared to clinical and ultra-sonographic monitoring of regional node fields, with a TLND only in cases with manifest nodal metastasis.[8] If the future results show no differences in survival, a 'high risk' subgroup could be

identified, based on a relatively high or rising S-100B value, in which direct CLND might improve survival. All SN positive patients with low S-100B values could then be spared for CLND. However, if the results show a clear survival difference, only a very low S-100B value might justify CLND omission and ultrasonographic nodal observation for an identified 'low risk' subgroup. Besides, patients with 'elevated' serum S-100B after a positive SLNB might be either regionally or distantly metastasized, since distant metastases can also elevate this biomarker.[20] Therefore, a FDG PET/CT could be performed first in these patients, to rule out disseminated disease and to assess if there is an indication for systemic treatment, rather than for CLND.

Furthermore, to enable clinical applicability, the accurateness of risk stratification for NSN positivity could be further increased by converting the S-100B value together with other predictive clinicopathologic parameters into a weighted risk score.

Conclusion

This study shows the promising predictive capacity of the biomarker S-100B for NSN positivity in patients planned for CLND. Further validation in larger patient cohorts and in conjunction with other predictive parameters, will be needed to better define the utility of preoperative S-100B levels in their ability to predict NSN positivity and the need for CLND in SN positive melanoma patients. However, this new closer look of serum S-100B within its reference interval, will be of value in patient selection for CLND in the future.

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A PREDICTION TOOL INCORPORATING THE BIOMARKER S-100B FOR PATIENT SELECTION FOR COMPLETION LYMPH NODE DISSECTION IN STAGE III MELANOMA

ABSTRACT

Introduction. Completion lymph node dissection (CLND) in sentinel node (SN)-positive melanoma patients is accompanied with morbidity, while about 80% yield no additional metastases in non-sentinel nodes (NSNs). A prediction tool for NSN involvement could be of assistance in patient selection for CLND. This study investigated which parameters predict NSN-positivity, and whether the biomarker S-100B improves the accuracy of a prediction model.

Methods. Recorded clinicopathologic factors were tested for their association with NSN-positivity in 110 SN-positive patients who underwent CLND. A prediction model was developed with multivariable logistic regression, incorporating all predictive factors. Five models were compared for their predictive power by calculating the Area Under the Curve (AUC). A weighted risk score, 'S-100B Non-Sentinel Node Risk Score' (SN-SNORS), was derived for the model with the highest AUC. Besides, a nomogram was developed as visual representation.

Results. NSN-positivity was present in 24 (21.8%) patients. Sex, ulceration, number of harvested SNs, number of positive SNs, and S-100B value were independently associated with NSN-positivity. The AUC for the model including all these factors was 0.78 (95%CI 0.69-0.88). SN-SNORS was the sum of scores for the five parameters. Scores of ≤ 9.5, 10-11.5, and ≥ 12 were associated with low (0%), intermediate (21.0%) and high (43.2%) risk of NSN involvement.

Conclusions. A prediction tool based on five parameters, including the biomarker S-100B, showed accurate risk stratification for NSN-involvement in SN-positive melanoma patients. If validated in future studies, this tool could help to identify patients with low risk for NSN-involvement.

INTRODUCTION

Sentinel lymph node biopsy (SLNB) is the standard procedure for accurate staging in melanoma patients, with a minimal treatment related morbidity.[1,2] SLNB identifies patients with nodal metastases, who may benefit from immediate completion lymph node dissection (CLND).[3] Despite the current recommendation on performing CLND in all sentinel node (SN)-positive patients, its therapeutic value is highly debated.[4-9] Currently, about 80% of patients yield no additional metastases in non-sentinel nodes (NSNs), and the procedure is accompanied with morbidity and costs.[10,11] The availability of an accurate prediction tool for the identification of patients with a low risk for NSN-involvement, could improve future patient' selection for CLND.

Several prediction tools for survival and prognosis in melanoma have been described and some are used in clinical practice.[12] For SLNB patient selection, the Memorial Sloan Kettering Cancer Center (MSKCC) developed and validated a nomogram for SN-status prediction.[13] Although not yet included in clinical guidelines, prediction models based on independently associated parameters were developed and validated, to enable risk stratification for NSN-positivity.[14,15] Recently, serum S-100B was also found to be independently associated with NSN-involvement in SN-positive melanoma patients.[16] Besides, elevated levels of S-100B appeared to be associated with recurrence risk and worse survival in patients presenting with palpable nodal metastases, suggesting a relation with melanoma tumor burden.[17] Although S-100B has been reported to be a prognostic biomarker in cutaneous melanoma patients since the nineties, no consensus has been achieved on its implementation in clinical follow-up.[18] To date, only German and Swiss national guidelines recommend evaluation of serum S-100B in melanoma follow-up.[19]

The predictive value of S-100B could possibly be used to increase the accuracy of a risk stratifying model for NSN-involvement in SN-positive melanoma patients. The aim of this study was to develop such a prediction model, and to test whether incorporation of S-100B would improve its accuracy. A reproducible prediction tool could be used to optimize the selection of patients at low risk for NSN-involvement, in whom CLND could safely be omitted.

METHODS

Patients and Procedure

At the University Medical Center Groningen (UMCG), SLNB is performed routinely in AJCC stage IB-IIC cutaneous melanoma patients, followed by a subsequent CLND in case of SN-positivity. All SN-positive patients, diagnosed at the UMCG or referred from other hospitals, who underwent a CLND between 2005 and 2015 were prospectively registered. The study was conducted in accordance with the Declaration of Helsinki, and conforms to the guidelines of the central medical ethics committee.

For the SNs, the histologic protocol consisted of blocking in paraffin and cutting of 4µm sections, with 250µm distance, at four different levels in the SN for routine hematoxylin and eosin staining, with additional immunohistochemistry for S-100B and Melan-A. In CLND specimens, all NSNs were sectioned at one level with subsequent hematoxylin and eosin staining.

Clinical features and primary tumor characteristics were recorded. Histologic features assessed for the SNs were the number of harvested SNs, number of involved SNs, proportion of involved SN, size of the largest metastasis in SN, and extra-nodal growth pattern. If more than one SN contained metastases, the highest score for each parameter was recorded. Serum S-100B and LDH values were measured one day before CLND was performed.

S-100B concentrations were determined by performing the S-100B assay (Diasorin) on an ELISA Robot platform (DS2, Dynex Technologies). The reference range was determined according to the Clinical and Laboratory Standards Institute EP28-A3c guideline, resulting in a cut-off value of 0.20µg/l.[20] LDH was analyzed routinely by Roche Modular (Hitachi) with an enzymatic activity measurement. The reference cut-off used for LDH was 250U/l.

Statistical Analysis

Univariable logistic regression analysis was used to investigate the association of clinicopathologic variables with NSN-positivity. All variables were entered in a logistic regression model; backwards stepwise selection was used to build a multivariable model. Log-transformation was used for the skewed distribution of S-100B. Factors associated with NSN-positivity on a 10% significance level were selected in the final model. Extra-nodal growth was excluded in the model, due to the limited number of patients (n=3).

Five different multivariable logistic regression models were assessed, and the Area Under the Curve (AUC) was calculated and compared for these five models. The model with the highest AUC was used as final model, and an ROC-curve was constructed. Based on these results, a weighted scoring system, the 'S-100B Non-Sentinel Node Risk Score' (SN-SNORS), was devised. SN-SNORS was assessed for its ability to predict NSN-positivity using the AUC. All statistical analyses were performed, using IBM SPSS statistics version 22 (SPSS Inc, Chicago, IL), with p-values <0.05 considered statistically significant.
Subsequently, a nomogram was developed in R version 3.2.1 (Auckland, New Zealand), using the 'rms' package, based on the sum of scores for the five predictive parameters. First, the data distribution was set to logistic regression. Next, the model was built with the five parameters; estimates from the model and the effects of each predictor on the response variable were calculated and plotted together with the predicted probability from the multivariable model (Figure 2).

RESULTS

A total of 110 AJCC stage IB-IIC melanoma patients with a positive SLNB were analyzed. The median age at diagnosis of the primary melanoma was 55 (range 5-88) years, 60.0% were men, and 50.9% presented with a melanoma located on the trunk. Median Breslow thickness was 3.0 (range 0.4-14.0) mm, and ulceration was present in 44.5%. More than one SN was harvested in 72 patients (65.5%), with a median of two per patient (range 1-5). SNs were harvested from the neck (n=7), axilla (n=56), groin (n=44), and popliteal region (n=3). In 26 patients (23.6%) more than one SN contained metastases, with a median of one (range 1-4). Median size of SN metastases was 1.5 (range 0.09-17.0) mm. Extra-nodal growth was present in 3 patients (12.7%, Table 1).
Subsequent CLND was performed in all patients. Positive NSNs were found in 24 patients (21.8%). In 13 patients one NSN metastasis was found in the CLND specimen, and in 11 patients more than one NSN was involved, with a median of one (range 1-16).

TABLE 1.

Preoperative clinicopathologic factors of 110 SN-positive patients undergoing CLND, tested for their association with NSN-positivity

Characteristic	n (%)	NSN positivity (%)		p-value
Age at diagnosis (years)				
Median, range	55, 5-88			0.41
Sex				
Female	44 (40.0)	5/44	(11.4)	**0.03**
Male	66 (60.0)	19/66	(28.8)	
Site of melanoma				
Lower extremity	35 (31.8)	13/35	(37.1)	**0.04**
Head/neck	3 (2.7)	0/3	(0.0)	
Trunk	56 (50.9)	10/56	(17.9)	
Upper extremity	16 (14.5)	1/16	(6.3)	
Histologic type				
Superficial spreading	72 (65.5)	11/72	(15.3)	0.07
Nodular	32 (29.1)	11/32	(34.4)	
Other	6 (5.5)	2/6	(33.3)	
Breslow thickness (mm)				
Median, range	3.0, 0.4-14.0			**0.004**
T1: <1.00	3 (2.7)	0/3	(0.0)	0.06
T2: 1.01-2.00	29 (26.4)	3/29	(10.3)	
T3: 2.01-4.00	45 (40.9)	9/45	(20.0)	
T4: >4.00	33 (30.0)	12/33	(36.4)	
Ulceration				
No	61 (55.5)	8/61	(13.1)	**0.01**
Yes	49 (44.5)	16/49	(32.7)	
Mitotic rate (per mm^2)				
Median, range	4, 1-23			0.53
<5	44 (40.0)	7/44	(15.9)	0.20
≤5	43 (39.1)	9/43	(20.9)	
Unknown	23 (20.9)			
Lymphovascular invasion				
No	100 (90.9)	19/100	(19.0)	0.07
Yes	9 (8.2)	4/9	(44.4)	
Unknown	1 (0.9)			
Regression				
No	98 (89.1)	23/98	(23.5)	0.48
Yes	11 (10.0)	1/11	(9.1)	
Unknown	1 (0.9)			

TABLE 1.

Continued

Characteristic	n (%)	NSN positivity (%)	p-value
Micro-satellites			
No	102 (92.7%)	22/102 (21.6%)	0.82
Yes	8 (7.3%)	2/8 (21.8%)	
Number of SN			
Median, range	2, 1-5		0.14
1	38 (34.5)	10/38 (26.3)	0.32
2	35 (31.8)	9/35 (25.7)	
3 or more	37 (33.6)	5/37 (13.5)	
Number of positive SN			
Median, range	1, 1-4		0.12
1	84 (76.4)	15/84 (17.9)	0.19
2	21 (19.1)	7/21 (33.3)	
3 or more	5 (4.5)	2/5 (40.0)	
Proportion involved			
Median, range	83, 20-100		**0.02**
≤50%	48 (43.6)	6/48 (12.5)	**0.04**
>50%	62 (56.4)	18/62 (29.0)	
Size of metastasis (mm)			
Median, range	1.5, 0.09-17.0		0.07
≤0.50	23 (20.9)	1/23 (4.3)	0.15
0.51-2.00	36 (32.7)	9/36 (25.0)	
2.01-10.0	32 (29.1)	10/32 (31.3)	
>10.0	6 (5.5)	2/6 (33.3)	
Unknown	13 (11.8)		
Extranodal growth			
No	107 (97.3)	22/107 (20.6)	0.06
Yes	3 (2.7)	2/3 (66.7)	
Preoperative LDH (U/l)			
Median, range	175, 108-389		0.20
Preoperative S-100B (µg/l)			
Median, range[a]	0.06, 0.02-0.23		**0.006**

Abbreviations: SN, sentinel node; NSN, non-sentinel node; CLND, completion lymph node dissection.
Continuous characteristics and quantitative discrete characteristics were tested using logistic regression analysis. Categorical characteristics were tested with Chi squared test. P-values <0.05 are printed in **bold**. [a] *Log-transformed due to a skewed distribution.*

Factors Associated with NSN-positivity

A significant association between NSN-positivity and patient or tumor characteristics was shown for sex (p=0.03), localization (p=0.04), Breslow thickness (p=0.004), ulceration (p=0.01), proportion of SNs involved (p=0.02), and preoperative S-100B level (p=0.006). (Table 1) After entering all variables in a backwards stepwise multivariable model, the following parameters were associated with NSN-positivity on a 10% significance level: sex (OR for male 3.26 (95%CL 1.02-10.46); p=0.047), ulceration (OR for presence 2.61 (95%CI 0.93-7.35); p=0.069), number of harvested SNs (continuous; OR 0.51 (95%CI 0.26-0.99); p=0.048), number of positive SNs (continuous; OR 2.20 (95%CI 0.86-5.62); p=0.100), and preoperatively measured S-100B level (continuous; OR 2.60 (95%CI 1.05-6.45); p=0.039, Table 2).

Prediction Model for NSN-positivity

Five multivariable prediction models were tested and compared, each of which included parameters associated with NSN-status in univariate analysis (p<0.1).

TABLE 2. Multivariable logistic regression model (backwards selection)

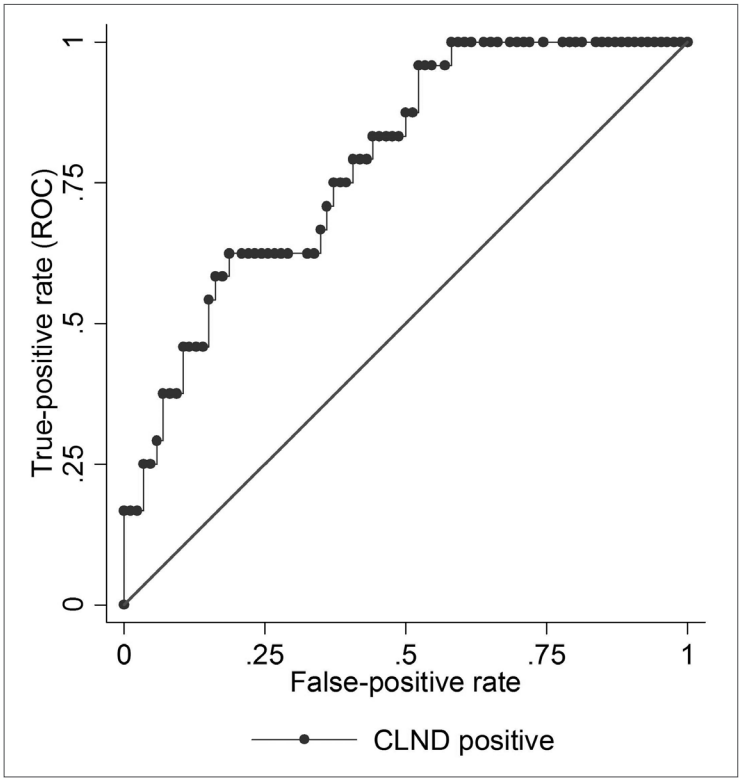
Predictive parameter	OR (95%CI)	p-value
Sex		
Female	1.0 (reference)	
Male	3.26 (1.02-10.46)	0.047
Ulceration		
No	1.0 (reference)	
Yes	2.61 (0.93-7.35)	0.069
Number of SNs harvested		
Continuous	0.51 (0.26-0.99)	0.048
Number of SNs positive		
Continuous	2.20 (0.86-5.62)	0.100
S-100B (µg/l)[a]		
Continuous	2.60 (1.05-6.45)	0.039

Abbreviations: OR, Odds Ratio; SN, sentinel node. [a] *Log-transformed due to a skewed distribution.*

The models differed from one another in the number of parameters, and the incorporation of S-100B or not (Table 3).

The AUC for model 1, with 5 factors, including S-100B, was 0.78 (95%CI 0.69-0.88, Figure 1). For model 2, without S-100B, the AUC was 0.74 (95%CI 0.63-0.85). Model 3, based on 4 factors including S-100B, resulted in an AUC of 0.76 (95%CI 0.66-0.87). Model 4 included 3 factors (AUC 0.73 (95%CI 0.61-0.85)) and model 5 included 2 factors (AUC 0.69 (95%CI 0.56-0.83)). Comparison of the models with regard to NSN-involvement showed a similar predictive ability (p=0.55, p=0.30, p=0.14, and p=0.13 for the models as compared to model 1, Table 3).

FIGURE 1.



ROC curve model 1: sex, ulceration, number of SN harvested, number of positive SN, and S-100B (1000 replications bootstrapping). Area Under the Curve (AUC) = 0.78 (95%CI 0.69-0.88).

TABLE 3.

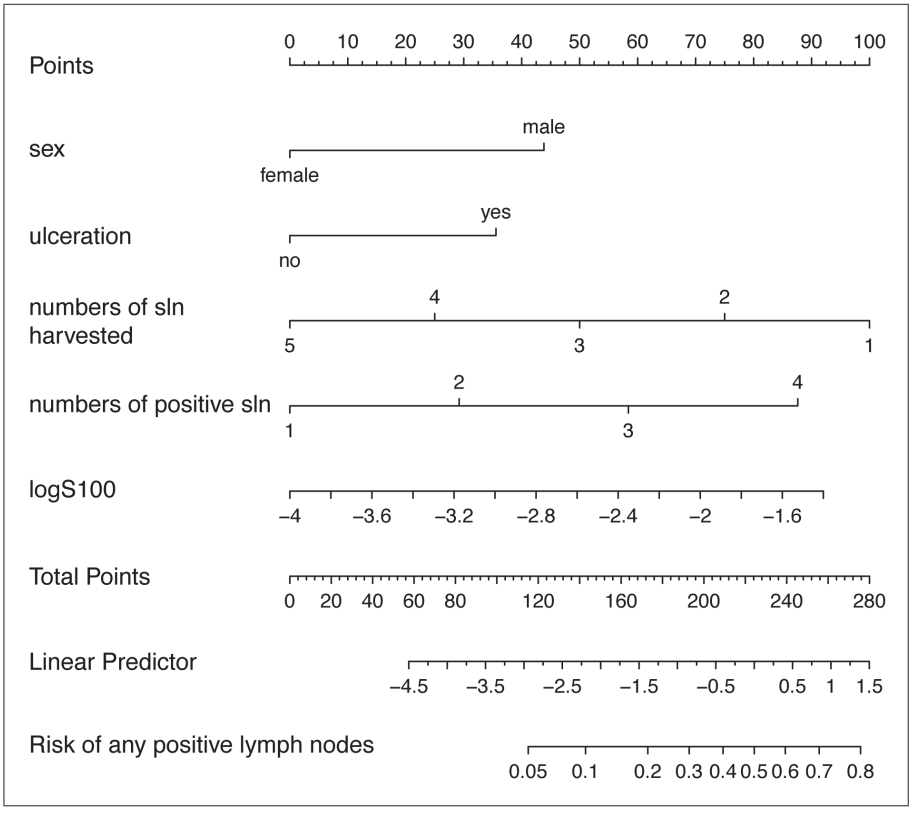
Comparison of five logistic regression models of clinicopathologic factors associated with NSN-positivity

Model	Factors		Multivariable OR (95%CI)	p-value	AUC (95%CI)	p-value AUC
Model 1 - 5 factors Including S-100B	Sex	Male / female	3.26 (1.02-10.46)	0.047	0.78 (0.69-0.88)	Ref.
	Ulceration	Yes / no	2.61 (0.93-7.35)	0.069		
	Nr SN harvested	Continuous	0.51 (0.26-0.99)	0.048		
	Nr SN positive	Continuous	2.20 (0.86-5.62)	0.100		
	S-100B[a]	Continuous	2.60 (1.05-6.45)	0.039		
Model 2 - 4 factors Excluding S-100B	Sex	Male / female	3.05 (0.98-9.48)	0.054	0.74 (0.63-0.85)	0.55
	Ulceration	Yes / no	2.56 (0.93-7.02)	0.069		
	Nr SN harvested	Continuous	0.49 (0.26-0.95)	0.035		
	Nr SN positive	Continuous	2.24 (0.89-5.66)	0.088		
Model 3 - 4 factors	Sex	Male / female	3.56 (1.12-11.25)	0.031	0.76 (0.66-0.87)	0.30
	Ulceration	Yes / no	2.97 (1.08-8.17)	0.035		
	Nr SN harvested	Continuous	0.66 (0.40-1.10)	0.110		
	S-100B[a]	Continuous	2.64 (1.07-6.52)	0.035		
Model 4 - 3 factors	Sex	Male / female	3.13 (1.01-9.65)	0.047	0.73 (0.61-0.85)	0.14
	Ulceration	Yes / no	2.93 (1.08-7.93)	0.035		
	S-100B[a]	Continuous	2.78 (1.12-6.90)	0.027		
Model 5 - 2 factors	Ulceration	Yes / no	3.20 (1.21-8.50)	0.019	0.69 (0.56-0.83)	0.13
	S-100B[a]	Continuous	2.58 (1.08-6.20)	0.034		

Abbreviations: OR, Odds Ratio; AUC, Area Under the Curve; Ref, Reference Category; SN, Sentinel Node. [a] *Log-transformed due to a skewed distribution.*

Based on the findings of Table 3, a scoring system for NSN-positivity, SN-SNORS, was devised for model 1. Each independent associated factor was included in the scoring system with an assigned value based on the odds ratio of the multivariable model. SN-SNORS was defined as the sum of scores for the five predictive parameters. The sum of all values resulted in a score that ranged from 0-16 in all patients. In the present patient cohort, SN-SNORS of 0-9.5, 10-11.5, and ≥12 were associated with low (0%, n=0/36), intermediate (21.0%, n=8/37) and high (43.2%, n=16/37) risk of NSN-involvement, respectively (Table 4).
Using the nomogram in this cohort (Figure 2), 41 patients were defined as 'low risk' (<10%) of which in 2.4% (n=1) a positive NSN was found, 31 patients were associated with intermediate risk (10-25%), of which 25.8% (n=8) had a positive NSN, and 38 patients were 'high risk' (≥25%) for NSN-positivity, of which in 39.5% (n=15) a NSN-metastasis was detected in the CLND specimen.

FIGURE 2.



Nomogram.

TABLE 4.

Final scoring system for stratification of risk of NSN-positivity

SN-SNORS component	SN-SNORS (points)
Sex	
Female	1
Male	3
Ulceration	
No	1
Yes	3
Number of SNs harvested	
1	3
2	2
3	1.5
4-5	1
Number of SNs positive	
1	2
2	3
3	4
4	5
S-100B (µg/l)	
0.0-0.03	1
0.04-0.07	2
0.08-0.12	3
0.13-0.18	4
≥0.19	5

Risk of NSN-involvement	Total SN-SNORS	Patients	CLND+
Low	≤9.5	n=36	0%
Intermediate	10 - 11.5	n=37	21.0%
High	≥12	n=37	43.2%

Abbreviations: SN; Sentinel Node, NSN; Non-Sentinel Node ; SN-SNORS; S-100B Non-Sentinel Node Risk Score.

DISCUSSION

Prediction tools for melanoma survival and prognosis are widely developed, and some are used in everyday clinical practice.[13] This study demonstrates the potential of a prediction model for the presence of NSN metastases in a CLND specimen, and the additional value of the biomarker S-100B for this tool. To our knowledge, this is the first study including a biomarker like S-100B in a risk model for the purpose of predicting NSN-involvement.

The discussion often arises whether or not all SN-positive patients should be exposed to the operative risks of CLND, as there is currently no evidence for CLND to improve melanoma-specific survival, especially when uninvolved nodes are being removed.[4-9,21] Affirmative, a recent randomized trial comparing CLND with observation in SN-positive patients, seems to refute the traditional thought that radical surgery is needed to improve survival in these patients, and the authors even recommend not to perform CLND in patients with metastases of ≤1mm.[8] A prediction tool for NSN-involvement could be the way to decide in which patients observation is the appropriate strategy, and which benefit from extended surgery, regardless the accompanying morbidity.
The incidence of NSN-involvement (18%) in this study is in accordance with the 14-24% reported in literature.[14,15,21-23] The necessity of a routine CLND for SN-positive patients is still under investigation in the EORTC 1208: MiniTub (NCT01942603).[24] However, the recently published MSLT-II results report 5% better disease free survival, and no benefit in overall or melanoma specific survival by performing CLND in unselected SN-positive patients, after a relatively short median follow-up of 43 months.[4] Also, the (underpowered) DeCOG-SLT was not able to show survival benefit of CLND for unselected SN-positive patients.[8] Individual parameters reported to be associated with NSN-positivity include male sex,[15] thicker Breslow,[14,22] regression,[15] ulceration,[5] satellitosis,[5] neurotropism,[5] angiolymphatic invasion,[5] number of positive SNs,[5,15] maximum size of SN-metastases,[6,14,25-27] invasion depth (Starz-classification),[5,28] non-subcapsular location (Dewar-classification),[15,23] extra-nodal growth,[5,25] and presence of perinodal lymphatic invasion.[15] The great variation in reported predictors for NSN-status can be explained by the differences in sample size, study populations, pathological protocols, and statistical methods. Besides, many of these histopathologic parameters are prone to inter-observer variation in pathologic interpretation.[29]

Although not used in daily practice, scores based on multivariable models are found to enable risk stratification for NSN-positivity.[14,15,21,22] A relatively complicated scoring system, based on the number of tumor-involved step sections and centripetal depth of tumor in the SN, was described by Starz et al. in 2001.[30] Reeves et al. stratified risk by a combined size/ulceration score, by assigning 1 point for ulceration in the primary tumor and 1 point for a SN metastasis of more than 2mm.[31] Nevertheless, according to validation studies, this system was prone to a high level of false negative results, which is not desirable when used for selecting 'low-risk patients'.[23,32] Thereafter, Gerschenwald et al. developed a scoring system based on tumor thickness, size of largest SN metastasis, and number of SNs harvested, resulting in low (4.0%), intermediate (22.2%) or high (46.7%) risk of NSN-involvement.[14] Although based on different parameters, a similar risk distribution was found with the present scoring system: 0.0%, 21.0%, and 43.2%. Most recently, Murali et al. proposed a scoring system using a weighted risk score, the Non-Sentinel Node Risk Score (N-SNORE), based on the sum of scores for five parameters: sex, regression, proportion of harvested SNs involved with metastases, perinodal lymphatic invasion (PLI) in SN, and maximum size of largest tumor deposit in SN. A regressed melanoma was suggested to be more advanced, however, regression was not found to be an independent predictor for NSN-positivity in the present study. The N-SNORE has been validated to be a useful tool.[21,33]
Based on the method used for development of the N-SNORE, a risk score and nomogram were developed, including sex, ulceration, number of SN harvested, number of SNs involved with metastases, and S-100B level. Although SN tumor size is described to be a strong predictor for NSN-status, this parameter did not remain significantly associated in this multivariable model.[6,14,27] An advantage of using S-100B in a prediction tool is the absence of inter-observer variation, thereby increasing its reproducibility. Prediction models can be used as web-based calculators, like the MSKCC 'Risk of Sentinel Lymph Node Metastasis nomogram' for the prediction of SN involvement (https://www.mskcc.org/nomograms/melanoma/sentinel-lymph-node-metastasis).[13]

A limitation of this study is that not all previously reported predictive parameters, like PLI, were registered. Even though the currently standard pathologic examination methods for tumor detection in SNs and NSNs were used, there might be errors due to the potential presence of undetected tumor.[34] Extra-nodal growth might be a strong predictor for NSN-involvement,

but due to the small number (n=3) in this study, this parameter was excluded in the model. One of the shortcomings of clinical prognostic tools in melanoma in general, is the absence of validation.[12] Unfortunately, the relatively small number of included patients did not allow internal validation, and due to the specific measurement moment of S-100B (one day before CLND) and the used analyzing method (Diasorin assay on an ELISA Robot platform), no data were available for external validation.

Although biomarkers like LDH, S-100B, YKL-40, Melanoma Inhibitory Activity protein (MIA), and C-Reactive Protein (CRP) are reported as prognostic markers in different stages of melanoma, biomarkers have not been implemented in prediction tools for NSN-involvement before.[17,35-38] For AJCC stage I and II, some studies did report that S-100B was not capable of predicting the SN status, due to low sensitivity with the used cut-off points (0.12-0.16μg/l).[39-41] Very recently, our institution reported the S-100B value to be independently associated with the risk for NSN-positivity, even within the reference interval.[16]
The finding that S-100B increases the accuracy of a prediction model for NSN-positivity can be further supported by the fact that S-100B is reported to be stronger associated with survival than LDH in stage III melanoma, and that elevated S-100B values are associated with decreased disease-free survival.[35,42] Also, the suggestion has been made that the serum S-100B level is correlated with nodal tumor load, and that S-100B could possibly be used as a prognostic marker in the stratification of new adjuvant trials to select stage III melanoma patients for adjuvant systematic treatment.[17]

The recently published final results of the MSLT-II show no difference in overall survival and a slight benefit regarding disease-free survival, suggesting the possible risks of CLND omission are negligible for the whole SN-positive group.[4] If future recommendations regarding CLND will change to a more conservative policy, this scoring system could be used to identify a 'high risk' subgroup in which direct CLND might improve disease free and/or overall survival. Besides, with current and future developments in effective systemic therapies, this 'high risk' subgroup might be selected for adjuvant treatment after CLND or even directly after the positive SLNB. A low score justifies CLND omission and ultra-sonographic nodal observation.

In conclusion, this study shows that various cliniopathologic parameters predict NSN-involvement, and that incorporation of S-100B into the model strengthens the predictive capacity. If validated in future studies, a web-based calculator based on such a scoring system could be a useful and reproducible tool to identify SN-positive melanoma patients with low risk of NSN-involvement, assisting both patient and surgeon in the decision process of performing or omitting CLND. Future studies will need to reveal whether CLND and/or adjuvant systemic treatment can improve the prognosis for SN positive melanoma patients with high risk for NSN involvement.

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PART III

ACCURATE DETERMINATION OF THE BIOMARKER S-100B

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ADIPOCYTES IN VENIPUNCTURES CAUSE FALSELY ELEVATED S-100B SERUM VALUES

TO THE EDITOR

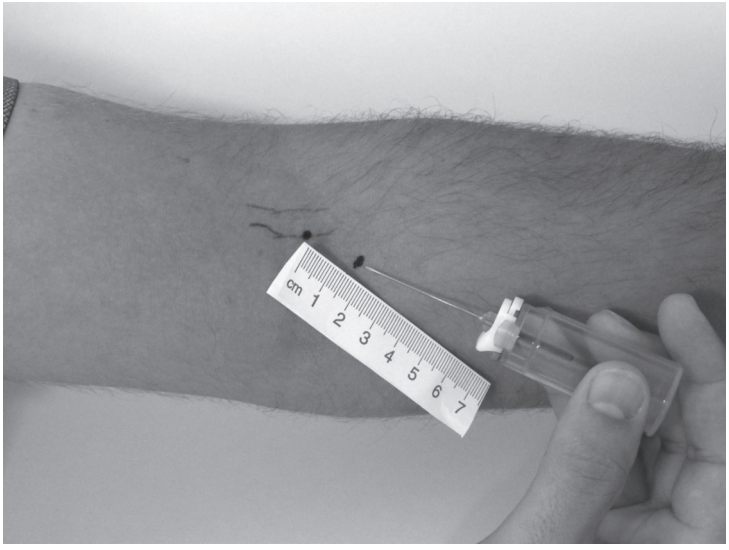
The calcium-binding protein S-100B is increasingly used in melanoma as a serum biomarker to reflect tumor load, but also as a prognostic tool in advanced melanoma.[1,2] In neurology, S-100B in serum and cerebrospinal fluid is predominantly used to detect and quantify brain injury.[3] Previously, multiple studies have also described the presence of S-100B in adipocytes.[4-6]
Determination of the serum S-100B concentration in patients is performed by drawing a blood sample by venipuncture and subsequent analysis of S-100B by immunoassay. With the increased clinical applications and use of S-100B, accurate analysis and interpretation of this biomarker becomes more important, especially in monitoring and predicting prognosis of melanoma patients where minor changes of serum S-100B might have important clinical consequences.[2]
As S-100B is present in adipocytes, the hypothesis was that damaged subcutaneous adipocytes, trapped in the needle before entering the vein during a venipuncture, could contaminate the serum used for S-100B analysis. False positive values of S-100B, caused by adipocytes in a blood sample, have not been reported before. The aim of this study was to investigate 1) the influence of adipocyte contamination in a blood sample on S-100B values, 2) whether difficult venipunctures could result in falsely elevated S-100B values, and 3) the difference in S-100B values of the first and second drawn serum separation tube. For clinical purposes, it seems to be of high importance to prevent contamination with adipocytes, as falsely high S-100B values might lead to potential hazardous over-staging and mismanagement, and potential wrongly informed patients regarding their prognosis.[2,3]

Two subsequent experiments were performed, in accordance with the Declaration of Helsinki and after written approval by the medical ethics review committee of the University Medical Center Groningen (METC ABR NL42601.042.12). Differences between the sample groups were assessed for statistical significance ($p<0.05$), using a one sample T-test for the normally distributed differences and Wilcoxon signed Rank test or Kruskal Wallis for not normally distributed values (IBM SPSS statistics version 22, Chicago, IL, USA).
The first experiment was conducted to determine whether the presence of adipocytes would increase S-100B values in a serum sample. In two healthy men, aged 27 and 28 years, a single blood sample was drawn and divided into

two tubes after centrifugation. Subsequently, subcutaneous adipocytes were obtained from the abdominal subcutis of the same individual using a 40 mm 18 G needle, and mixed with one of each individuals' blood samples. The four samples were stored at -20 °C overnight to induce lysis of the present adipocytes due to freeze thawing before the samples were analyzed, and to mimic the handling and storage conditions in a routine laboratory. After addition of the adipocytes, the samples were analyzed also in dilution to exclude high-dose hook effect. The serum mixed with adipocytes showed extremely high S-100B levels: 73.8 µg/L and 55.1 µg/L, whereas the control tubes (serum only) both had S-100B values <0.01 µg/L.

In the second experiment, after informed consent and completion of a questionnaire, three subsequent serum separation tubes were drawn in 20 individuals by entering the vein after a 1.5 cm subcutaneous route, simulating a difficult venipuncture (**Figure 1**). The study group consisted of 11 female and 9 male volunteers, median age 32 (range 22-63) years and median Body Mass Index (BMI) of 23.6 (range 18.5-29.4) kg/m^2. None of the individuals reported particularities. Blood samples were collected by venipuncture in 8.5 mL Vacutainer tubes (Becton Dickinson, Belliver Industrial Estate, Plymouth, UK).

FIGURE 1.

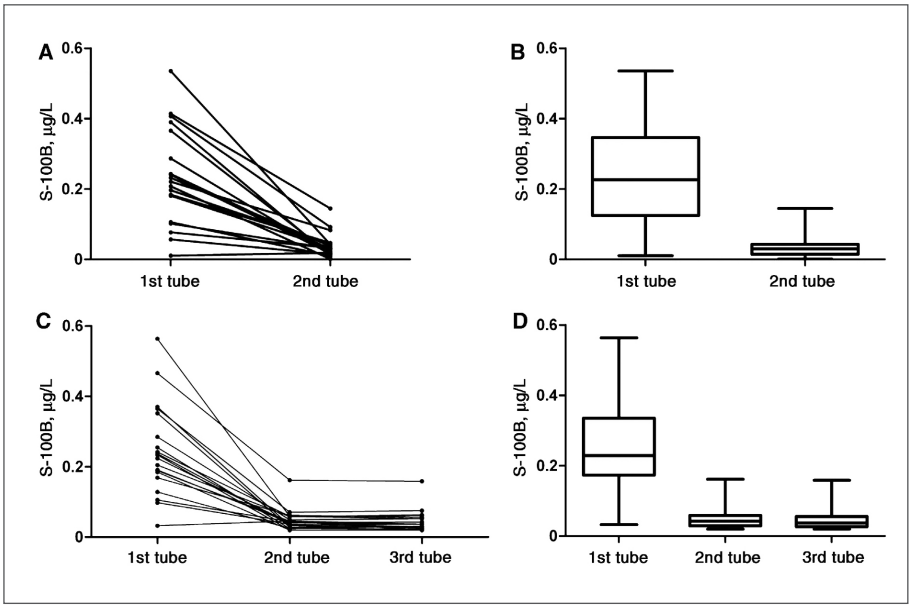


Measuring and marking the 1.5 cm subcutaneous route before venipuncture.

After routine centrifugation, serum was separated from the tubes, aliquoted and stored at -80 °C. After thawing the samples, S-100B concentrations were determined by performing the S-100B assay (Diasorin, Saluggia, Italy) on an ELISA Robot platform (DS2, Dynex Technologies, Magellan Biosciences, Worthing, United Kingdom).
The within run assay variation Coefficient of Variation (CV) of the S-100B automated ELISA was 7.2%, 5.4% and 6.0% at 0.04 µg/L, 0.194 µg/L and 2.121 µg/L respectively. The between run CV of the assay was 11.8%, 13.4% and 5.6% at 0.05 µg/L, 0.209 µg/L and 2.066 µg/L respectively. The Limit of Blank was determined to be 0.0034 µg/L, whereas the Limit of Quantitation (20% CV) was determined to be 0.092 µg/L.

The first two tubes of all individuals were analyzed. The subsequent samples showed median S-100B values of 0.23 µg/L and 0.03 µg/L for the first and second tube respectively, with a significant mean difference of -0.198 µg/L (95% CI: 0.257-0.140, p<0.001) (**Figure 2**). This demonstrates a relatively high contamination effect, considering the reference cut-off of S-100B (0.20 µg/L) used at our institution. Theoretically, smaller quantities of adipocyte contamination associated with shorter subcutaneous tracks in uncomplicated venipunctures could also cause clinically relevant elevations of the S-100B level. S-100B reference values that were previously established by analysis of healthy individuals, which will be the case for most hospital laboratories, should probably be re-established from adipocyte-free venipunctures. This might lead to a lower cut-off point, making the biomarker more sensitive.
According to the literature, in vivo S-100B secretion from adipocytes is decreased by insulin, but increased by glucagon, stress, physical training or fasting.[5,7,8] Some studies reported a correlation between serum S-100B and BMI, whereas others did not find this association.[6,8] In our study, no correlation was found, possibly due to the absence of weight loss or obesity in these apparently healthy volunteers.
A reanalysis was performed after four months, now also including the third drawn sample. This resulted in median S-100B values of 0.23 µg/L, 0.04 µg/L, and 0.04 µg/L for tubes 1, 2 and 3 respectively. The pre-analytical stability of S-100B is previously reported to be very high over a wide range of time periods (within 24 hours) and temperatures.[9] However, the present study found a slight, although significant, elevation of S-100B in the second tube (0.01 µg/L, 95% CI 0.002-0.019, p=0.02) after longer storage time and an extra freeze-thaw cycle, in accordance with previous literature.[10] This elevation of S-100B could be the result of lysis of

FIGURE 2.



The effect of adipocyte contamination on S-100B values measured in three subsequent drawn tubes from 20 individuals, after venipuncture using a 1.5 cm subcutaneous route before entering the vein.

A. *First analysis; significant decrease in S-100B value in 2nd tube (median 0.03 µg/L, SD 0.03, range 0.001-0.15 µg/L) compared to 1st tube (median 0.23 µg/L, SD 0.13, range 0.01-0.54 µg/L), p<0.001.* **B.** *Box plot summarizing the results of Fig. 2.A.* **C.** *Second analysis; significant decrease in S-100B value in 2nd tube (median 0.04 µg/L, SD 0.03, range 0.01-0.16 µg/L) compared to 1st tube (median 0.23 µg/L, SD 0.13, range 0.03-0.56 µg/L), 95% CI: 0.257-0.140, p<0.001. No difference between 2nd tube and 3rd tube (median 0.04 µg/L, SD 0.03, range 0.01-0.16 µg/L).* **D.** *Box plot summarizing the results of Fig. 2.C.*

a larger quantity of adipocytes.[5] Nevertheless, the first tube still contained the highest value of S-100B after four months storage (p<0.001) (Figure 2).

Although adipocytes are the main cell type in subcutal tissue, it contains other molecules that can be measured during serum analysis, like triacylglycerol and free fatty acids.[5] The presented research setup could be used to test which other clinically relevant serum parameters suffer the same serum contamination during venipuncture caused by (sub)cutaneous molecules.
To our knowledge, this is the first study showing contamination of the first drawn blood sample with subcutaneous adipocytes to cause significant elevation of S-100B values in serum analysis. The risk of adipocyte induced elevated S-100B values is higher in difficult venipunctures, but might even be present in easy

venipunctures. Therefore, we recommend to avoid the use of the first drawn blood sample for S-100B analysis, especially when used as a tumor marker in melanoma patients.

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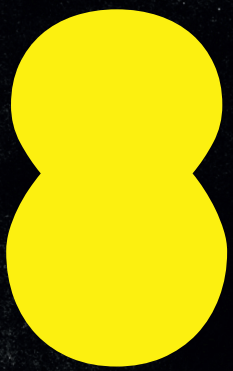


DOUBLE VENIPUNCTURE IS NOT REQUIRED FOR ADEQUATE S-100B DETERMINATION IN MELANOMA PATIENTS

ABSTRACT

Background. S-100B is a useful biomarker in melanoma follow-up. This serum biomarker is also present in adipocytes, therefore subcutaneous adipocytes trapped in the needle before entering the vein during a venipuncture, could contaminate the serum used for S-100B analysis. The aim was to study the possible influence of adipocyte contamination in a blood sample on S-100B levels and to investigate whether difficult venipunctures could result in falsely elevated S-100B values.

Methods. A dummy tube was drawn before performing the routine venipuncture, during regular follow-up in all AJCC stage III melanoma patients, with no evidence of disease. The dummy tube was anonymously coded, while the second tube was registered in patients' medical results. S-100B levels between the two samples were compared.

Results. A total of 294 serum samples were collected from 147 AJCC stage III melanoma patients. The mean difference between the 1st and 2nd tube was 0.003 (range -0.08-0.15) µg/L (p=0.077), with a decrease in the 2nd tube. Compared to the secondly drawn tube, the S-100B level was higher in the dummy tube in 33.3% of the samples, equal in 36.8% of the samples, and lower in 29.9% of the samples.

Conclusions. No significant difference between the two subsequently drawn tubes was found. Based on current results, there is no evidence for the necessity of implementing a dummy-tube-system for accurate S-100B determination in melanoma patients.

INTRODUCTION

S-100B is a serum biomarker used in both clinical and experimental setting. This calcium-binding protein, with a weight of 21 kilo Dalton, is physiologically present in glial and Schwann cells, and in neurology it is used to detect and quantify brain damage.[1-3] In the 1980's, this biomarker was also found to be present in human melanoma cells and melanocytes, and used to detect melanocytic tumors in pathology.[4,5] Intracellular S-100B concentrations are usually high in disseminated melanoma (American Joint Committee on Cancer, AJCC, stage IV), and serum levels may be elevated.[6,7] The potentially aggressive and unpredictable character of melanoma strengthens the clinical desire to detect the first signs for disease progression as early as possible.[8] In the follow-up of melanoma patients, serum S-100B is increasingly used as tumor marker. Mostly complementary to Lactate Dehydrogenase (LDH), to estimate tumor load, evaluate response to treatment, and as a prognostic tumor marker in advanced melanoma.[7,9-11] To this date however, there is a wide variety in the use of biomarkers in melanoma worldwide.[12] Melanoma studies that have tried to use S-100B for recurrence detection and prediction of sentinel-node positivity encountered problems due to the low sensitivity in these melanoma patients with minimal tumor load.[13,14] Another frequently encountered problem with biomarkers is the undesirable presence of false positive as well as false-negative results.[15] For instance, patients' Body Mass Index (BMI) and different comorbidities are associated with influencing the serum S-100B values.[15,16] False-positive S-100B values may lead to unnecessary anxiety in melanoma patients, potential over-staging and mismanagement, and lead to increased healthcare costs.

Multiple studies reported adipocytes to contain high levels of S-100B, which is secreted in response to epinephrine, glucagon or weight normalization after chronic starvation.[17-23] Determination of serum S-100B values in melanoma patients is performed by drawing a blood sample through a venipuncture and subsequent analysis of S-100B by immunoassay. Accurate analysis of this biomarker is important, as minor changes in serum S-100B levels might have clinical consequences, such as surgery or additional diagnostic tests.[24]
Recently, S-100B values were reported to be falsely elevated when mixed with subcutaneous cells, suggesting adipocytes trapped in a venipuncture could affect the S-100B level, leading to falsely elevated serum values.[25] The risk for

this adipocyte contamination might especially be higher in difficult venipunctures after several attempts. Hypothetically, adipocyte contamination only affects the first tube, as the needle will be flushed after drawing the first sample. The aim of this study was to test whether subcutaneous adipocytes also cause falsely elevated S-100B values in blood samples of melanoma patients in regular venipunctures, and to study clinicopathological factors that influence serum S-100B levels.

METHODS

Study Design and Patients

At the University Medical Center Groningen (UMCG) follow-up with regular intervals for all AJCC stage III and IV melanoma patients with currently no evidence of disease (NED) consists of medical history, physical examination and determination of the S-100B. To determine whether contamination by adipocytes would increase S-100B values in a serum sample after a regularly performed venipuncture in AJCC stage III and IV melanoma patients, two subsequent tubes were drawn by experienced laboratory assistants for S-100B analysis in all patients. Patients with local or distant metastases were excluded. Blood samples were collected by venipuncture in 8.5 mL Vacutainer tubes (Becton Dickinson, Belliver Industrial Estate, Plymouth, UK). A 'dummy' tube was drawn first, after which the 'regular' tube was drawn during the same puncture. To prevent any potential indistinctness regarding the test results, the first (dummy) tube was coded, while the second tube was registered under the patients' data. After routine centrifugation, serum was separated from the tubes. Both tubes were equally analyzed in the same laboratory. The study was conducted in accordance with the Declaration of Helsinki, and conforms to the guidelines of the central medical ethics committee (METc2015.215).

Characteristics of the patients, the primary tumors, Sentinel Lymph Node Biopsy (SLNB) and Completion Lymph Node Dissection (CLND) were collected in a database. The recorded parameters included: age, sex, Body Mass Index (BMI), time since diagnosis, current AJCC stage, comorbidities, site of primary melanoma, histologic type, Breslow thickness, ulceration, mitotic rate (number of cells in mitosis per mm^2), SN metastasis, and CLND metastases. S-100B levels of the dummy tubes and the second tubes were registered, as well as whether a difficult venipuncture or puncture with a subcutaneous route of more than 1cm was reported on the case record form.

S-100B Analysis

S-100B concentrations were routinely determined by performing the S-100B assay (Diasorin, Saluggia, Italy) on an ELISA Robot platform (DS2, Dynex Technologies, Magellan Biosciences, Worthing, United Kingdom), according to manufacturer's protocol. The intra-assay Coefficient of Variation (CV) of the S-100B assay is 7% at levels of 0.04 µg/L (0.0028 µg/L). The reference interval was determined by analysis of S-100B values in 120 healthy individuals (median 0.07; range 0.01-0.59) and calculating the 95% confidence interval according to the Clinical and Laboratory Standards Institute EP28-A3c guideline (formerly C28-A2), resulting in a reference cut-off value for the healthy population of 0.20µg/l.[26]

Statistical Analysis

Sample size analysis for a two-sided test was performed on the difference in S-100B value between the first and second drawn serum samples, with a power β=0.80 and α=0.05. The purpose was to test the nil-hypothesis: no difference in S-100B value between the two subsequently drawn samples. A sample size of 84 serum samples in each group (total n=168) was required to prove a difference between the first and second drawn tube of at least 0.05 µg/l.

Statistical analyses were performed using IBM SPSS statistics version 22 (Chicago, IL, USA). Descriptive statistics were used for data presentation. Differences between the sample groups were assessed for statistical significance ($p<0.05$) using a paired T-test or Pearson Correlation for the normally distributed differences and Kruskal-Wallis for not normally distributed values. Possible factors of influence on S-100B level in the second tube were tested by univariate and multivariate linear regression analysis.

RESULTS

Patients' Characteristics

A total of 294 serum samples were collected from 147 AJCC stage III and IV melanoma patients during follow-up (June 2015 - June 2016). Median age of the patients was 57 (range 26-86) years, 51% was female, and median BMI was 26.5 (range 18.1-54.5) kg/m^2. Median Breslow tumor thickness was 1.94 (range 0.60-27.0) mm, and median time since diagnosis 56 (range 1-400) months. At time of S-100B determination, 39.4% was stage IIIA, 29.9% IIIB, 23.1% IIIC, and 7.4%

TABLE 1.

Clinicopathologic factors of the 147 AJCC stage III melanoma patients

Characteristic	n	%
Age (years)		
Median, range	57, 26-86	
<60	84	57.1%
≥60	63	42.9%
Sex		
Female	75	51.0%
Male	72	49.0%
BMI (kg/m²)		
Median, range	26.5, 18.1-54.5	
Time since diagnosis (months)		
Median, range	56, 1-400	
Current AJCC stage		
IIIA	58	39.4%
IIIB	44	29.9%
IIIC	34	23.1%
IV, NED	11	7.4%
Comorbidities		
None	81	55.1%
Cardiovascular	28	19.0%
Pulmonic	10	6.8%
Neurological	5	3.4%
Other malignancy	10	6.8%
Other	13	8.8%
Histologic type		
Superficial spreading	95	64.6%
Nodular	31	21.1%
Other	9	6.1%
Unknown	12	8.2%

TABLE 1.

Continued

Characteristic	n	%
Breslow thickness (mm)		
Median, range	1.94, 0.6-27.0	
Ulceration		
No	91	61.9%
Yes	38	25.9%
Not reported	18	12.2%
Mitosis		
No	8	5.4%
Yes	123	83.7%
Not reported	16	20.9%
Positive SLNB		
No	16	10.9%
Yes	82	55.8%
Not performed	49	33.4%
Positive CLND/TLND		
No	45	41.7%
Yes	63	58.3%

Abbreviations: BMI, body mass index; AJCC, American Joint Committee on Cancer; NED, no evidence of disease; SLNB, sentinel lymph node biopsy; NSN, non-sentinel node; CLND, completion lymph node dissection; TLND, therapeutic lymph node dissection.

stage IV, all with currently no evidence of disease (NED). Comorbidities were present in 44.9% of patients, of which 19% cardiovascular, 6.8% pulmonic, 6.8% other malignancy in the past, 3.4% neurological, and 8.8% other comorbidities (arthritis, kidney disease, hyperparathyroidism, morbid obesity, lichen sclerosus, gout, Table 1). In 2.0% (n=3) of the samples a difficult venipuncture was reported, and for 8.1% (n=12) a subcutaneous route of more than 1cm. Based on these small numbers, no significant relation was found between a reported difficult venipuncture and the S-100B level in the first drawn (dummy) tube, nor the difference in S-100B levels in the two subsequently drawn tubes.

Evaluation of Adipocyte Contamination by Analyzing the Difference of S-100B Levels in Two Subsequently Drawn Tubes

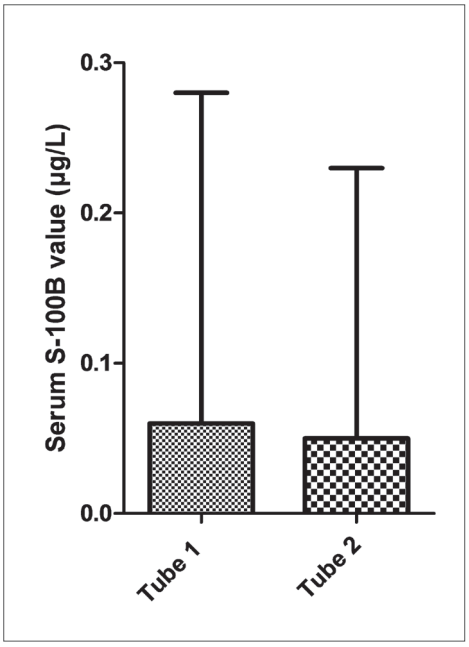
The two subsequently drawn serum samples showed median S-100B values of 0.05 (range <0.02-0.28) µg/L for the 1st (dummy) tube, and 0.04 (range <0.02-0.23) µg/L for the 2nd (regular) tube (Figure 1). The mean difference between the 1st and 2nd tube was 0.003 (range -0.08-0.15) µg/L (95% CI: 0.0004-0.0070, p=0.077), showing a trend for slightly lower S-100B levels in the 2nd tube. The absolute S-100B level measured in the dummy tube was higher in 33.3% (n=49) of the samples, equal in 36.8% (n=54) of the samples, and lower than in the secondly drawn tube in 29.9% (n=44) of the samples. As shown by the scatterplot in Figure 2, a bilateral (measurement) variance of 0.04 µg/L is seen (Figure 2). The positive outliers >0.04 µg/L, 4.1% (n=6 patients) of the samples, had a mean age of 58 years, 33% were female, with a mean BMI of 29.7 kg/m^2, and 50% had cardiovascular comorbidities. Overall, no significant relation was found between age, sex, BMI, or comorbidity and the difference in S-100B level between the two tubes (Table 2).

Determination of Patient Factors Associated with S-100B Serum Level in the Second Tube

By performing univariate linear regression analysis, a significant association between S-100B level, and patient or tumor characteristics was found for BMI (p=0.005) and the presence of another malignancy (p=0.005). A higher BMI was associated with a relatively higher serum S-100B level (Table 3). The presence of another malignancy (such as meningioma, endometrial cancer, breast cancer, non-Hodgkin, and colon cancer) resulted in a slightly lower S-100B level (0.02 µg/L). Patients with comorbidities of cardiovascular, pulmonal, or neurological origin showed no significant difference in S-100B levels (Table 4).

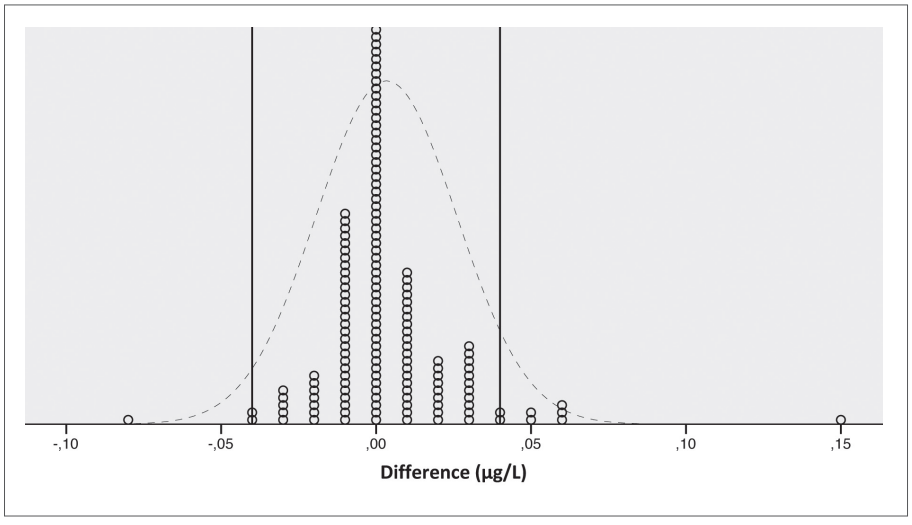
All variables that were associated with the S-100B level in the 2nd tube with a p-value <0.15 in univariate analysis were entered in a multivariable linear regression model. In this model (containing sex, BMI, ulceration, other malignant comorbidity) the following clinical factors were associated with a the S-100B level on a 5% significance level: sex (B=-0.021 for male sex (95%CI: -0.035- -0.006); p=0.006), BMI (B=0.002 for higher BMI (95%CI: 0.000-0.003); p=0.011), other malignancy (B=-0.026 for presence (95%CI: -0.052- -0.001); p=0.041, Table 5).

FIGURE 1.



Mean serum S-100B value in tube 1 (dummy tube) and tube 2.

FIGURE 2.



Difference of serum S-100B values between the two subsequently drawn tubes (tube 1-tube 2), resulting in a bilateral measurement variance of 0.04 µg/L.

TABLE 2.

Difference in S-100B levels between dummy and subsequently drawn tube, and the influence of clinical factors on S-100B level difference between the tubes

	First tube (dummy)	Second tube (regular)	Mean difference (tube 1-tube 2)	p-value
S-100B (µg/l) Continuous	0.05 µg/l	0.04 µg/l	0.003 µg/l	
(median, range)	(0.02-0.28)	(0.02-0.23)	(-0.08-0.15)	0.077^
Age (years) Continuous				
<60	0.050 µg/l~	0.045 µg/l~	0.005µg/l	0.70
≥60	0.040 µg/l~	0.040 µg/l~	0.002µg/l	
Sex				
Female	0.040 µg/l~	0.040 µg/l~	0.003µg/l	0.86
Male	0.050 µg/l~	0.050 µg/l~	0.004 µg/l	
BMI (kg/m²) Continuous				
<25	0.030 µg/l~	0.035 µg/l~	-0.002 µg/l	0.19
25-30	0.060 µg/l~	0.050 µg/l~	0.006 µg/l	
>30	0.050 µg/l~	0.050 µg/l~	0.007 µg/l	
Comorbidity				
None	0.050 µg/l~	0.050 µg/l~	0.00 µg/l	-
Cardiovascular	0.055 µg/l~	0.050 µg/l~	0.01 µg/l	0.37
Pulmonal	0.045 µg/l~	0.040 µg/l~	0.01 µg/l	0.60
Neurological	0.050 µg/l~	0.030 µg/l~	0.00 µg/l	0.66
Other malignancy	0.025 µg/l~	0.020 µg/l~	0.00 µg/l	0.64

Abbreviations: BMI, body mass index. Age and BMI were tested as continuous variables. ~Median values. ^Paired T-test for difference between first and second tube. Pearson Correlation or Kruskal-Wallis test for influence of clinical factors on S-100B level difference.

TABLE 3.

Association of clinical factors associated with S-100B level in second tube

Characteristic	Second tube (median, µg/l)	p-value
Age (years, continuous)		
<60	0.45 µg/l	0.93
≥60	0.04 µg/l	
Sex		
Female	0.04 µg/l	0.11
Male	0.05 µg/l	
BMI (kg/m², continuous)		
<25	0.04 µg/l	**0.005**
25-30	0.05 µg/l	
>30	0.05 µg/l	
Current AJCC stage		
IIIA	0.04 µg/l	0.16
IIIB	0.06 µg/l	
IIIC	0.04 µg/l	
IV (NED)	0.05 µg/l	
Histologic type		
Superficial spreading	0.04 µg/l	0.26
Nodular	0.04 µg/l	
Other	0.04 µg/l	
Breslow thickness (mm, continuous)		
<1	0.05 µg/l	0.65
1-2	0.05 µg/l	
>2	0.04 µg/l	
Ulceration		
No	0.05 µg/l	0.13
Yes	0.04 µg/l	

→ *Continued next page*

TABLE 3.

Continued

Characteristic	Second tube (median, µg/l)	p-value
Mitosis		
No	0.05 µg/l	0.38
Yes	0.04 µg/l	
Positive SLNB		
No	0.04 µg/l	0.49
Yes	0.05 µg/l	
Positive CLND/TLND		
No	0.05 µg/l	0.87
Yes	0.04 µg/l	

Abbreviations: BMI, body mass index; AJCC, American Joint Committee on Cancer; NED, No Evidence of Disease; SLNB, sentinel lymph node biopsy; NSN, non-sentinel node; CLND, completion lymph node dissection; TLND, therapeutic lymph node dissection. Continuous variables are also displayed in categories, but were tested as continuous. P-values <0.05 are printed in **bold**.

TABLE 4.

Association of comorbidities with S-100B level (µg/l) in second (clinically used) tube

Comorbidity	n	S-100B second tube (µg/l) (median, range)	p-value
Cardiovascular	28	0.05 (0.0-0.14)	0.36
Pulmonal	10	0.04 (0.0-0.11)	0.32
Neurological	5	0.03 (0.03-0.07)	0.74
Other malignancy	10	0.02 (0.0-0.05)	**0.005**
Other[a]	13	0.07 (0.0-0.23)	-

Linear regression analysis. [a] *Other comorbidities: arthritis, kidney disease, hyperparathyreoidism, morbid obesity, lichen sclerosus, gout. This category was excluded for analysis. P-values <0.05 are printed in* **bold**.

TABLE 5.

Multivariate linear regression model (enter methods) of clinical factors associated with S-100B level in second tube

Characteristic	B	95%CI	p-value
Sex (Male ref)	-0.021	-0.035- -0.006	**0.006**
BMI (kg/m²)	0.002	0.000- 0.003	**0.011**
Ulceration	0.001	-0.015- 0.016	0.950
Other malignancy	-0.026	-0.052- -0.001	**0.041**

Abbreviations: BMI, body mass index. All factors with p<0.15 were entered in the multivariate analysis. P-values <0.05 are printed in **bold**.

DISCUSSION

This study was conducted to test the clinical impact of the previously described phenomenon of adipocyte contamination in the determination of serum S-100B levels.[25] The results of this study show that in individual patients, adipocyte contamination can result in falsely elevated S-100B levels, as six patients showed a S-100B level in the dummy tube that was ≥0.05 µg/l higher than in the second tube, which exceeds the analytical variation. However, the overall clinical impact of this finding in the follow-up measurement of melanoma patients seems to remain low. Unfortunately, this study was not able to identify factors that are able to predict falsely elevated S-100B levels.

The biomarker S-100B is increasingly used and has important clinical value in screening, monitoring and predicting prognosis of melanoma patients.[6,24,27] In some national guidelines (Germany, Switzerland) routine measurement of serum S-100B values is recommended in melanoma.[12,28] However, its ability to predict disease progression is still limited not only due to false-negative results in patients with low tumor-burden, but also by false-positive results.[29] Therefore, accurate determination and interpretation of serum S-100B seems to be of high importance, especially in melanoma patients, where even minor changes of serum S-100B might have important clinical consequences. Diagnostic errors, like false positive results, might lead to unnecessary anxious patients, potential hazardous over-staging and treatment, or even malpractice, accompanied with emotional and psychological trauma.

Previously, a significant difference of -0.198 µg/L was found between the first and second subsequently drawn serum tubes in 20 healthy volunteers in which a difficult venipuncture was simulated by entering the vein after a 1.5 cm subcutaneous route, suggesting a substantial contamination effect. The present study found no significant difference in S-100B levels between the two subsequently drawn tubes from 294 regular venipunctures. With a p-value of 0.077 and a mean difference of 0.003 µg/L, a trend could be considered, with slightly higher S-100B values in the first drawn dummy tubes. However, this overall difference seems not clinically relevant. Despite this possible trend, the intra-assay variance should be taken into account as well, with a variance of 0.0028 µg/L (7%) for the S-100B assay. According to the scatterplot of differences (**Figure 2**), six patients seem to have an exceptionally high difference in S-100B levels. With a mean BMI of 29.7 kg/m^2, these are likely to represent the patients in whom adipocyte contamination did result in higher S-100B levels in the first (dummy) tube. The fact that this is such a small percentage of all patients, suggests that almost all venipunctures have been uncomplicated and without a subcutaneous route, possibly due to experienced laboratory assistants, when compared to the previously published data. Unfortunately, the risk for falsely elevated S-100B levels was not associated to whether or not the laboratory assistant reported a difficult puncture in this study. In addition, no subgroup of patients could be identified in whom the appearance of adipocyte contamination was significantly higher (**Table 2**), although a higher BMI seemed to be of some influence on the difference between tubes.
False-positive laboratory test results of S-100B caused by adipocytes in a blood sample have not been reported in literature before, but multiple studies have described the presence of S-100B in adipocytes.[18,30-34] Although it has been proven before that adipocytes contain S-100B, and that the amount of subcutaneous tissue might influence the serum results, no significant decrease (or increase) of S-100B levels in the second drawn tube was found in this study, when tested for all the samples.

According to the literature, the biology and the subsequent serum level of S-100 proteins is complex and multifactorial. The proteins are found to be tumorigenic by cell proliferation, metastasis, angiogenesis and immune evasion.[16] Besides being expressed by melanocytes in melanoma, S-100B is expressed by glial and Schwann cells, and also found to be present in adipocytes.[1-3] In vivo S-100B secretion from adipocytes is described to be decreased by insulin, but

increased by glucagon, stress, physical training or fasting.[18,21,22,35,36] Some studies reported a correlation between serum S-100B and BMI, whereas others did not find this association.[15,17,36] Physiological S-100B levels in humans are found to be associated with adipose tissue mass. Therefore, elevated S-100B levels can even be found in apparently healthy individuals, especially in those with a high Body Mass Index (BMI).[17] In line with these previous studies, the current study found a significant relation between the patients' BMI and S-100B level in the first and secondly drawn tube (p=0.005), but not with the difference between the two tubes (p=0.19). In the present study, only one patient suffered of morbid obesity (BMI 54.5 kg/m^2), presenting with a relatively high S-100B level of 0.08 µg/l. In two patients S-100B values higher than the reference cut-off of 0.20 µg/L was found in both tubes, these patients had a BMI >33 kg/m^2, and no radiological evidence of recurrent disease.

Besides BMI, recent literature found false-positive serum S-100B levels to be associated with several comorbid diseases, such as cardiovascular disease, obesity, liver cirrhosis, inflammatory disease, and neurological disease.[15,16] Contradictory, although not significant, the current study found patients with comorbidities to have lower S-100B levels than patients with no comorbidities. Again, no significant relation with the difference between the tubes was found. Even more puzzling, having a history of another malignancy (meningioma, endometrial cancer, breast cancer, non-Hodgkin, and colon cancer) seemed associated with a significant lower S-100B level, compared to the other comorbidities. Although this finding might be explained by a contradictory expression profile of serum S-100B in different types of human cancer, involving either up-regulation or down-regulation, the number of patients with other present cancers is too small to draw any conclusion on this.[16] In general, S-100 proteins are found to act as damage-associated-molecular-pattern molecules (DAMP), which are released in reaction to cell stress or damage, and are able to activate the immune system.[37] Although excluded for analysis due to the heterogenic character of this group, in patients with 'other' comorbidities, such as arthritis, kidney disease, hyperparathyroidism, morbid obesity, lichen sclerosus, and gout, relatively higher S-100B levels were found. The patient with gout had a S-100B level of 0.23µg/l, which might be explained by the inflammatory character of this disease.

In conclusion, this study did not find a significant clinical impact of adipocyte contamination influencing serum S-100B values in regular follow-up serum

samples. Although the risk of a falsely elevated S-100B value due to addition of adipocytes might still be present in difficult venipunctures, for apparent uncomplicated venipunctures the effect of adipocyte contamination seems to be negligible. When used as a biomarker for melanoma patients, S-100B can safely be determined by venipuncture using a single tube. In case of strongly deviating or elevated S-100B values, however, careful interpretation is important and the clinician should consider the presence of erroneous results due to adipocyte contamination. When false positivity is considered, a second tube could subsequently be drawn for accurate S-100B determination.

Disclosure
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9

SUMMARY & SAMENVATTING

SUMMARY

With a continuously rising incidence of cutaneous melanoma worldwide, mainly due to the increasing prevalence of thin melanomas, the number of patients in clinical follow-up will keep on rising as well.[1,2] In an era of economic cuts, this growing number of patients can become a great burden for health care institutions. The incredibly unpredictable character of cutaneous melanoma makes it difficult to manage and to standardize follow-up regimes.

This thesis focusses on different aspects of melanoma follow-up. An evidence based reduced follow-up schedule was introduced, in order to safely reduce the burden of follow-up for patients as well as health care systems. Patient's preferences regarding information and education were questioned, and regional differences in standardly performing the generally recommended sentinel lymph node biopsy throughout the Netherlands were highlighted. Besides, factors associated with an increased risk of lymph node metastases were identified, from which a prediction tool for adequate patient selection for completion lymph node dissection was distracted. With S-100B being the most specific biomarker for melanoma at this moment,[3] the accuracy of S-100B was tested by implementing a dummy-tube system in melanoma patients.

PART I
FOLLOW-UP IN AJCC STAGE I–II CUTANEOUS MELANOMA

Chapter 2 presents the first results of a multicenter randomized clinical trial, the MELFO-study, proposing a reduced follow-up schedule for AJCC stage IB-II melanoma patients. This study is currently still under investigation, until 5-years of follow-up in all patients has been fulfilled (finished in 2019). According to the results after 1 year follow-up, stage-adjusted follow-up surveillance does not negatively affect patients' mental well-being, nor the detection rate of recurrences when compared to the currently recommended high-intensity surveillance. All costs taken into account, the reduced schedule also seems economically favorable. In line with previous literature, approximately 75% of recurrences are self-detected by patients or relatives.[4] This emphasizes

the importance of adequate patient education at diagnosis in all surveillance programs. In **Chapter 3** patients' preferred method for receiving information regarding melanoma and education on self-inspection of the skin and regional lymph nodes was investigated. AJCC stage I-II melanoma patients were asked to watch instructional self-inspection videos on YouTube, after which a web-based questionnaire was distributed and returned. Melanoma patients' knowledge on melanoma and their own tumor characteristics appeared to be insufficient, showing the importance of patient-tailored information provision. The majority of patients wish to receive information in multiple manners, with oral information from the treating physician being the preferred source, followed by e-Health videos, and lastly in writing. Providing that the quality of e-Health videos is guaranteed, these may additionally contribute to patients' knowledge on melanoma development and prevention, and encourage adequate self-inspection of skin and regional lymph nodes.

With the status of the sentinel lymph node being one of the most important predictors of prognosis and tumor recurrence for melanoma, the sentinel lymph node biopsy (SLNB) is a widely accepted diagnostic procedure.5 Although National guidelines recommend to consider performing a SLNB in all AJCC stage IB-IIC cutaneous melanoma patients,[6,7] **Chapter 4** demonstrates the presence of considerable practice variation in routinely performing a SLNB in different melanoma centers throughout the Netherlands. This population based retrospective study found that only half of these patients underwent a SLNB. Possible explanations for this finding are certain patient characteristics (e.g. age, vitality), patients' and specialists' preference, and accompanied comorbidities. Whether this practice variation leads to unfavorable variations in clinical outcome should be addressed in future studies.

PART II

PREDICTION OF NODAL STATUS IN COMPLETION LYMPH NODE DISSECTION

The necessity of performing a completion lymph node dissection (CLND) in all sentinel node positive patients has been under discussion for several years, and still is.[8] In only 20% of these patients additional lymph node metastases are found, while this procedure is accompanied with significant morbidity and

costs.[9] Nevertheless, due to lack of solid evidence, performing a subsequent CLND in these patients is usually still recommended. In **Chapter 5**, different clinic-pathological characteristics associated with finding additional positive lymph nodes in the CLND specimen are described. Male gender, melanoma of the lower extremity, thicker Breslow thickness, ulceration, and proportion of involved sentinel lymph nodes were found to be associated with non-sentinel node (NSN) positivity. The preoperatively measured serum S-100B value, even within the reference interval, appeared to have the most promising predictive capacity for NSN-positivity. Serum Lactate Dehydrogenase (LDH) was not significantly associated. Further investigations will have to confirm the predictive power of S-100B. Based on the findings of the previous study, a potential prediction tool for additional positive lymph nodes in sentinel node positive patients is proposed in **Chapter 6**. This prediction tool based on five parameters, including S-100B, showed accurate risk stratification for NSN-involvement in SN-positive patients. The aim of developing such a tool, was to enable adequate patient selection for additional completion lymph node dissection. If validated in future studies, a web-based calculator based on such a scoring system could be a useful and reproducible tool to identify so-called 'low-risk' SN-positive melanoma patients, in whom a CLND might safely be omitted. Also, in the light of shared decision making, it could be helpful for patient and surgeon in the decision process of performing or omitting a subsequent CLND.

PART III
DETERMINATION OF THE SERUM BIOMARKER S-100B

The serum biomarker S-100B appears to be a useful marker in melanoma patients.[10] As previous studies of this thesis suggest, minor changes in S-100B values, even within the reference interval, can be of clinical relevance. Therefore falsely elevated measurements should be prevented, possibly leading to hazardous over-staging, unnecessary diagnostic tests and over-treatment.11 The calcium-binding protein S-100B is not only present in melanocytes, but also in glial and Schwann cells, and in adipocytes.[12-14] With S-100B being present in adipocytes, the hypothesis whether subcutaneous tissue could elevate serum S-100B levels was confirmed in **Chapter 7**. Falsely elevated S-100B levels were measured after performing a traumatic venipuncture with 1.5cm subcutaneous

route in 20 healthy volunteers. To test this theory of falsely elevated S-100B values by adipocyte contamination after a difficult venipuncture in AJCC stage II-IV melanoma patients with currently no evidence of disease, a prospective study is described in **Chapter 8**. By implementing a dummy tube system to flush away potential adipocytes in the needle during the venipuncture, the S-100B levels of the two subsequently drawn serum tubes were compared. Although a suggestion is made that Body Mass Index might be of influence on the S-100B level in general, no significant differences were found between the values measured in the two tubes. The adipocyte contamination in regular venipunctures does not seem to be of clinical relevance, therefore performing a double venipuncture is unnecessary in melanoma patients.

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SAMENVATTING

Met een wereldwijde stijgende incidentie van het melanoom van de huid, voornamelijk als gevolg van de toename van dunne tumoren, zal ook het aantal patiënten toenemen dat dienst te worden vervolgd.[1,2] In een tijdperk van economische bezuinigingen kan het groeiende patiënten-aantal voor zorgverleners en zorginstellingen een financiële last worden. Het onvoorspelbare karakter van het melanoom maakt het bovendien moeilijk om follow-up protocollen te ontwikkelen en te standaardiseren.
Dit proefschrift richt zich op verschillende aspecten van follow-up van patiënten met een melanoom. Er werd een evidence-based follow-up schema geïntroduceerd om het aantal controlebezoeken voor zowel patiënten als zorginstellingen veilig te kunnen verminderen. Patiënten werden ondervraagd over hun voorkeur van informatieverstrekking en voorlichting over de ziekte. Daarbij werden regionale verschillen in het standaard uitvoeren van de in Nederland aanbevolen schildwachtklierbiopsie benadrukt. Daarnaast werden verschillende factoren onderzocht die verband houden met een verhoogd risico op lymfkliermetastasen. Op basis daarvan werd een voorspellend instrument, ofwel een nomogram, ontwikkeld voor een adequate selectie van patiënten die baat zouden kunnen hebben bij een aanvullende lymfeklierdissectie. Omdat S-100B op dit moment de meest specifieke tumormerkstof voor het melanoom is,[3] werd de nauwkeurigheid van de serumbepalingen van S-100B getest door in de follow-up een dummy-buissysteem toe te passen.

DEEL I
FOLLOW-UP VAN AJCC STADIUM I-II MELANOOMPATIENTEN

Hoofdstuk 2 geeft de eerste resultaten weer van een multicentrische en gerandomiseerde klinische studie, de MELFO-studie, waarin een beperkt follow-up schema voor patiënten met AJCC stadium IB-II melanoom wordt vergeleken met het intensievere follow-up schema zoals in de Nederlandse richtlijn geadviseerd wordt. Deze studie zal worden voortgezet tot bij alle patiënten een follow-up periode van 5 jaar is volmaakt (afgerond in 2019). Op basis van de resultaten na 1 jaar follow-up leidt het minder intensieve

controleschema, aangepast aan AJCC stadium, tot een vergelijkbaar mentaal welzijn van patiënten en aantal gedetecteerde recidieven, vergeleken met het controleschema dat wordt aanbevolen in de Nederlandse richtlijn.[4] Daarnaast levert het verkorte schema een economisch voordeel op. In overeenstemming met eerdere literatuur wordt ongeveer 75% van de recidieven door patiënten zelf of door familieleden ontdekt.[5] Dit benadrukt het belang van adequate voorlichting en uitleg over zelfonderzoek op het moment van het stellen van de diagnose melanoom, ongeacht de intensiteit van follow-up. In **Hoofdstuk 3** werd de voorkeur van patiënten voor het ontvangen van informatie over melanoom en voorlichting over zelfonderzoek van de huid en regionale lymfeklieren onderzocht. Patiënten met een AJCC stadium I-II melanoom werd gevraagd om instructievideo's te bekijken op YouTube, waarna een vragenlijst werd ingevuld. De kennis over het melanoom in het algemeen en de kenmerken van hun behandelde tumor bleek ontoereikend, wat het belang aantoont van informatievoorziening 'op maat'. Het merendeel van de patiënten wenste informatie op verschillende manieren te ontvangen, waarbij mondelinge informatie van de behandelend arts de voorkeur had, gevolgd door YouTube video's. Schriftelijke informatie bleek het minst favoriet. Op voorwaarde dat de kwaliteit van deze zogenaamde 'e-Health video's' gegarandeerd is, kunnen deze bijdragen aan de kennis van patiënten over het ontstaan en preventie van melanoom en leiden tot adequate zelfinspectie van huid en regionale lymfeklieren.

Met de status van de schildwachtklier als één van de belangrijkste voorspellende factoren voor prognose en recidief, is de schildwachtklierbiopsie (SWK) een algemeen aanvaarde diagnostische ingreep.[6] Alhoewel diverse nationale richtlijnen aanbevelen een SWK te overwegen bij alle AJCC stadium IB-IIC melanomen,[4,7] beschrijft **Hoofdstuk 4** een aanzienlijke variatie tussen verschillende melanoomcentra in Nederland bij het routinematig uitvoeren van een SWK. Deze retrospectieve studie wijst uit dat slechts de helft van de patiënten met melanoom een SWK onderging. Mogelijke verklaringen hiervoor zijn bepaalde patiëntgebonden factoren (bijvoorbeeld leeftijd, vitaliteit), voorkeur van patiënten en specialisten en bijkomende co-morbiditeit. Of deze variatie in de praktijk tot ongunstige klinische uitkomsten zal leiden, moet blijken uit toekomstige onderzoeken.

DEEL II
VOORSPELLEN VAN LYMFEKLIERMETASTASEN BIJ AANVULLENDE LYMFEKLIERDISSECTIE

De noodzaak van het verrichten van een aanvullende lymfeklierdissectie bij alle patiënten met een positieve schildwachtklier is al enkele jaren onderwerp van discussie.[8] In slechts 20% van deze patiënten worden er nog extra lymfekliermetastasen gevonden terwijl deze procedure gepaard gaat met aanzienlijke morbiditeit en kosten.[9] Bij gebrek aan bewijs om ervan af te kunnen zien, wordt het uitvoeren van een aanvullende klierdissectie tot op heden nog steeds aanbevolen. In **Hoofdstuk 5** worden verschillende factoren beschreven die geassocieerd zijn met het vinden van extra tumorpositieve lymfeklieren in het klierdissectiepreparaat. Mannelijk geslacht, melanoom op de onderste extremiteiten, hogere Breslow-dikte, ulceratie en aantal betrokken schildwachtklieren bleken gerelateerd te zijn aan extra gevonden lymfekliermetastasen. De preoperatief in het bloed gemeten tumormerkstof S-100B bleek na een SWK, zelfs binnen het referentie-interval, de meest veelbelovende voorspellende factor te zijn voor het hebben van extra lymfekliermetastasen in het preparaat van de aanvullende klierdissectie. De serumspiegel van lactaatdehydrogenase (LDH) was hier niet significant mee geassocieerd. Verder onderzoek zal de voorspellende waarde van S-100B moeten bevestigen.

Op basis van deze bevindingen is een voorspellend instrument ontwikkeld, waarmee mogelijk het risico op additionele pathologische lymfeklieren bepaald kan worden bij patiënten met een positieve schildwachtklier (**Hoofdstuk 6**). Dit op vijf parameters gebaseerde nomogram, waaronder de serumspiegel van S-100B, toonde een nauwkeurige risico-inschatting voor het hebben van additionele lymfeklier-betrokkenheid bij schilwachtklier-positieve patiënten. Het doel van het ontwikkelen van een dergelijk hulpmiddel was om adequaat patiënten te kunnen selecteren voor het ondergaan van een aanvullende lymfeklierdissectie. Na validatie in toekomstige studies zou een zogenaamde web-calculator, gebaseerd op een dergelijk scoresysteem, een nuttig en reproduceerbaar hulpmiddel kunnen zijn om 'laag risico' schildwachtklier-positieve melanoompatiënten te identificeren bij wie een aanvullende lymfeklierdissectie veilig achterwege zou kunnen blijven. Daarnaast kan de informatie - in het kader van gedeelde besluitvorming - nuttig zijn voor patiënt en chirurg om te besluiten wel of juist niet een klierdissectie uit te voeren.

DEEL III
BEPALING VAN SERUM BIOMARKER S-100B

De in het serum bepaalde S-100B-spiegel lijkt een nuttige tumormerkstof te zijn bij melanoompatiënten.[10] Zoals eerdere onderzoeken van dit proefschrift suggereren, kunnen subtiele veranderingen in S-100B-waarden, zelfs binnen het referentie-interval, klinisch relevant zijn. Het is van belang dat foutief verhoogde metingen worden voorkomen omdat ze mogelijk leiden tot over-stadiering, onnodig aanvullende onderzoek en potentieel schadelijke overbehandeling.[11] Het calciumbindende eiwit S-100B is echter niet alleen aanwezig in melanocyten, maar ook in glia- en Schwanncellen en in adipocyten (vetcellen).[12-14] Gezien de aanwezigheid van S-100B in adipocyten is in **Hoofdstuk 7** de hypothese getoetst en bevestigd dat manipuleren aan het subcutane vetweefsel serumwaarden van S-100B kan verhogen. Foutief verhoogde S-100B-waarden werden gemeten na het verrichten van een traumatische venepunctie met een subcutane route van 1,5cm bij 20 gezonde vrijwilligers. De klinische relevantie van deze foutief verhoogde S-100B-waarden door adipocyten-besmetting na een moeizame venepunctie, wordt in een prospectieve studie voor reguliere venepuncties in de follow-up van melanoompatiënten getest en beschreven in **Hoofdstuk 8**. Met behulp van een dummybuis-systeem, waarbij mogelijke adipocyten worden weggespoeld die bij bloedafname in de naald achterblijven, werden de serumspiegels van S-100B van de twee achtereenvolgens afgenomen buizen (via één venepunctie) vergeleken. Hoewel de suggestie wordt gewekt dat de BMI (Body Mass Index) in het algemeen van invloed is op de serumspiegels van S-100B, werden er geen significante verschillen gevonden tussen de gemeten waarden in de twee buizen. Contaminatie met adipocyten bij reguliere venepuncties lijkt daarom klinisch niet relevant te zijn. Om die reden hoeft er in de follow-up van melanoom patiënten niet standaard een dummybuis te worden gebruikt.

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10 FUTURE PERSPECTIVES

INCIDENCE, PREVENTION & FOLLOW-UP

The incidence of melanoma is continuously rising in most countries, mainly due to the increasing prevalence of thin melanomas.[1,2] In the United States melanoma represents only 2% of all skin cancer cases, but accounts for 80% of skin cancer deaths. In 2018, an estimated 91.270 new melanoma cases will be diagnosed, with an estimated mortality of 9.320.[3] The incidence in the Netherlands is still slowly rising, with a total of 6.734 new melanoma cases registered in 2017.[4] The current stabilization of the incidence in Australia and North-America is a possible result of screening programs and increased public awareness with regard to prevention measures and self-inspection.[5]

Prevention programs mainly focus on reducing exposure to ultraviolet radiation (UVR), such as skin burn in children and the use of tanning beds. For instance, commercial solariums are banned in Australia since 2015.[6] Also, a community wide 'SunSmart' program has been implemented in Australia, introducing the SunSmart Schools Accreditation Program (SSAP) for primary schools that follow a comprehensive sun protection program, recommended by the World Health Organization (WHO).[7-9] Similar programs, such as 'Sunbeatables' developed by the MD Anderson Cancer Center in the United States, and 'Slim met de zon' by the Dutch Cancer Society in the Netherlands, are used in schools to educate children, parents and teachers about the dangers and prevention of sunburn.[10,11] In North-America, the US Preventive Services Task Force (USPSTF) evaluated the effect of years of behavioral counseling for prevention of skin cancer. They found a small increase in sun protection behavior in adults, therefore recommending counseling to minimize UVR exposure in people from 6 months of age to 24 years.[12] In Germany, a photo-aging mobile app (Sunface) was introduced with the aim of reducing melanoma by prevention, and seems to be effective in changing fair-skinned adolescents' behavior.[13] Although evidence is currently limited, future research will have to prove that minimizing exposure to UVR will reduce the risk of developing melanoma.[14] As far as screening for melanoma, only about half of the national guidelines of 34 different countries recommend screening based on clinical risk assessment, especially in 'high-risk' populations.[15] The use of risk-stratification tools for developing a melanoma is not yet implemented in current guidelines, as further validation will be needed first.[16]

Worldwide, there is a great variety in follow-up regimens for melanoma patients, especially AJCC stage I-II.[17-20] This variety expresses in follow-up intensity and duration, clinical setting, skin surveillance regimens and the use of laboratory or radiology diagnostics. National guidelines of Australia, Canada, Germany, the United Kingdom, the United States of America and Switzerland recommend performing diagnostics ranging from chest X-ray, lymph node or abdominal ultrasonography, to FDG-PET/CT.[17,21] However, chest X-ray surveillance for instance, has not been proven to be beneficial due to low sensitivity and specificity.[22] To this date, additional diagnostic tests are not (yet) standardly recommended in the follow-up of stage I-II patients in the Netherlands.[23]

Although the incidence of melanoma is still rising, the number of melanoma-related deaths is falling on average 1.2% each year (2006-2015), leading to an increasing prevalence and number of melanoma patients in clinical follow-up.[24] For stage I and II melanoma, recent data present 5-year melanoma-specific survival rates of 82-99% and 10-year melanoma-specific survival rates of 75-98%.[25] To anticipate on the health-care burden accompanied with this increase, less intense surveillance schedules would be beneficial. Besides, frequent clinic visits are often anxiety associated. By all means, there is a need for guidelines with an evidence-based follow-up frequency.[26] The Melanoma Follow-up (MELFO)-study was designed to determine whether a reduced, stage-adjusted follow-up schedule adversely affects melanoma patients' mental well-being and the detection of 1st recurrences or second primary melanomas, and whether it decreases yearly costs per patient. Awaiting the final results after 5-year follow-up in all patients (expected in 2019), the first results plead for a safe reduction of follow-up frequency, with no delay in recurrence detection and no negative effect on patients' mental well-being. Besides, it is economically favorable. After finalizing the MELFO-study, statistics on disease-free survival and overall survival will have to confirm this safe reduction in surveillance intensity.

PATIENT EDUCATION

Self-screening or skin self-examination is recommended in most national guidelines for early detection of a primary melanoma, emphasizing the importance of adequate education on the development and prevention of melanoma in general.[15] Regardless of the follow-up frequency, recurrences and

second primary melanomas are mostly patient-detected instead of physician-detected.[27] Consequently, patient education on melanoma and on performing self-inspection of the skin and regional lymph nodes is of great importance at time of diagnosis and during follow-up.[26] Although patients seem to prefer being educated face-to-face by their physician, this information can be supported by the use of instructional e-Health videos on YouTube.[28-30] Internet-based resources and social media have been gaining popularity with regard to medical education in the last decade.[31,32] However, information provided online should be centralized and government regulated to warrant reliability and quality.
In this era of rapidly developing technologies, the use of smartphone applications for patient education and skin monitoring could possibly be a valuable addition in the near future.[33] The upcoming so-called tele-dermatology application, a combination of dermoscopy and digital photography, might improve early diagnosis of melanoma.[34] One major concern, however, is the low sensitivity (7-87%) of these automated smartphone apps.[35] Even though it sounds promising, the safety and efficacy of these medical applications will have to be further investigated in future studies. To this date, diagnostic evidence is scare, therefore healthcare providers should be cautious in recommending the use of these applications.[36]

SENTINEL LYMPH NODE BIOPSY & COMPLETION LYMPH NODE DISSECTION

Although the Sentinel Lymph Node Biopsy (SLNB) is known to be of prognostic value, no therapeutic benefit has yet been established. The MSLT-I trial found no overall survival benefit after performing a SLNB, yet a subgroup analysis for Sentinel Node (SN)-positive patients with intermediate thickness melanoma showed a longer melanoma-specific survival after SLNB followed by direct Completion Lymph Node Dissection (CLND).[37] Even within the Netherlands practice variation is found as SLNB is performed in only about 50% of all stage IB-IIC melanoma patients, although this is recommended in the national guideline.[23,38] In the updated American Society of Clinical Oncology (ASCO) - Society of Surgical Oncology (SSO) guideline (February 2018), the recommendations on performing a SLNB are more

nuanced. SLNB may be considered for thin (T1b) melanomas, is recommended for T2-3 melanomas, and may be recommended for T4 melanomas. All decisions should be made after thorough explanation and discussion with the patient.[39] No non-surgical substitute for SLNB, by means of nodal ultrasonography or FDG-PET/CT, has yet been found.[40,41]

To this date, performing a subsequent CLND was recommended after SLNB in case a metastasis was present in the sentinel node. However, additional nodal involvement was found in only 20% of the CLND specimen, resulting in about 80% potential overtreatment. According to the recently published results of the MSLT-II, there seems to be no benefit in survival rates by performing a direct CLND in all SN-positive patients, compared to nodal observation.[42] Therefore, the indication for performing a CLND in all SN-positive patients seems questionable. This might even raise the question whether or not it is still necessary to perform the SLNB procedure in all patients. However, future research will have to point out whether a selected category of 'high-risk' patients might benefit of a subsequent CLND, therefore remaining an indication for SLNB. For 'low-risk' patients, based on information detected by SLNB, nodal observation with ultrasonography seems justified according to the recently updated US guideline.[39] All in all, SLNB seems to be an indispensable procedure, not only for its prognostic value, but also for further follow-up management and treatment. Besides, this prognostic information is still necessary for inclusion in adjuvant trials, as non-invasive prognostic parameters are not yet available. Based on the MSLT-I database, in-basin recurrences are found, especially after nodal dissection in the observation group.[43] Further research is necessary to prove that nodal observation with lymph node dissection only in case of clinically present metastases, is safe with regard to the development of in-basin recurrences at long term. Fluorescence-guided surgery using a near-infrared fluorescent tracer is a promising technique currently under investigation.[44] This may be applicable in the future for detection of nodal of local melanoma recurrence, and assist oncologic surgeons toward more radical resections.

The SLNB-discussion could take another turn if adjuvant systemic treatment options become available for sentinel node positive patients. Several trials regarding adjuvant therapy for AJCC stage III patients are currently running. For example, administering adjuvant ipilimumab after complete resection of stage III melanoma appears to increase recurrence-free survival with 9 months.[45] Stage III patients with N3 status (≥4 involved lymph nodes) and extracapsular

extension are found to be associated with poor prognosis, therefore especially these patients might benefit from adjuvant treatment.[46] The SLNB procedure will then become not only of prognostic relevance, but also of therapeutic value. 'High-risk' patients that might benefit from adjuvant therapy could be identified and selected. Future studies will need to reveal whether CLND and/or adjuvant systemic treatment can improve prognosis for SN-positive melanoma patients with high risk for NSN-involvement or distant metastases.

PREDICTION TOOLS

Despite excessive research, the unpredictable character of cutaneous melanoma makes it difficult to develop prognostic tools. In the context of the currently ongoing 'CLND-discussion', prediction tools could be of assistance in this upcoming era of more selective approach to CLND. Another application for risk-calculation tools would be in the light of adjuvant treatments. Without performing a CLND, high-risk patients that might benefit from adjuvant treatment could then be selected by use of such a nomogram.

Multiple risk factors for poor prognosis have been described in literature. To date, Breslow thickness, ulceration and sentinel lymph node status seem to be the strongest predictors for survival in melanoma patients.[25,47] Recently, smoking has been associated with an elevated risk of sentinel node metastasis, a prognostic factor that might be of value in a prediction tool as well.[48] The American Joint Committee on Cancer (AJCC) introduced a new 8th edition of the staging system at the end of 2017, removing mitotic rate as a T1 subcategory. Another change is the redistribution of stage III into four sub-stages (stage IIIA-IIID), requiring a different interpretation and applicability of data in future research.[25] However, tumor mitotic rate remains to be an important prognostic parameter due to its association with an increased risk of SN-involvement. Therefore potentially useful for the development of future, more individualized prognostic tools.[49,50]

Several prediction tools for melanoma progression have been proposed, but validation in independent patient cohorts is needed before routine clinical implementation is possible.[51] Breslow thickness, sex, localization, ulceration, sentinel node tumor burden, and number of harvested SNs have been the most incorporated parameters this far.[52-55] However, biomarkers like S-100B are expected to play a more prominent role in future prediction tools. Ultimately, a prognostic tool based on individual genetic profiling could be valuable for the identification of high-risk tumors.[56]

BIOMARKERS IN MELANOMA

Biomarkers could be of assistance in selecting 'high-risk' patients that might benefit from CLND or (neo)-adjuvant treatment, in particular when nodal observation will become the standard of care in SN-positive patients. The protein S-100B is found to be a strong prognostic marker in stage III and IV melanoma.[57,58] For stage I-II melanoma patients, there is no evidence yet for routine imaging, like FDG-PET/CT, or determination of serum biomarkers such as S-100B. In clinical stage III melanoma patients, PET-surveillance tends to enable asymptomatic detection of recurrent disease in high-risk subgroups.[59,60] However, frequent PET-surveillance will have great financial impact on healthcare costs, unfavorable in times where hospitals, governments and insurance companies have to economize instead. Although S-100B is currently used mostly (in combination with LDH) in stage IV melanoma patients to evaluate treatment response or disease progression, it might also be able to detect recurrence in asymptomatic stage III patients.[61,62] The University Medical Center Groningen is currently investigating the sensitivity of S-100B for detecting local or distant metastasis before clinical symptoms present in the 'S-100B Watch' (results expected in 2019). S-100B is determined every 3 months during regular surveillance, and in case of a significant rise or elevation in two subsequent S-100B values, a FDG-PET/CT is performed. By doing so, healthcare institutions are able to save on expenses by performing an expensive PET-CT only in specific high-risk patients. Future results will have to prove S-100B has the same or better detection capacity as PET-surveillance for asymptomatic disease progression. In stage IV patients, the correlation of S-100B expression in different metastatic sites, measured on FDG-PET/CT, is currently under investigation at the UMCG as well.

Although promising, serum S-100B might not be sensitive enough by itself to base therapeutic decisions on. For instance, a combination of S-100B and Melanoma Inhibitory Activity protein (MIA) resulted in a higher sensitivity for predicting recurrence or disease progression.[63] Other biomarkers associated with prognostic information in melanoma are YKL-40, C-Reactive Protein (CRP) and microRNAs (miRNAs).[64-67] Future research will have to reveal which biomarker or combination of biomarkers will be the most sensitive in different stages of melanoma.

SYSTEMIC TREATMENT

For AJCC stage IV melanoma patients, revived hope exists in an era where new systemic therapies develop faster than some melanoma metastases do, resulting in improved survival rates.[68] To date, ipilimumab (anti-CTLA-4 antibody), nivolumab and pembrolizumab (anti-PD1 antibodies), dabrafenib and vemurafenib (BRAF-inhibitors), and trametinib and cobimetinib (MEK inhibitors) are US FDA approved for treatment of advanced melanoma.[26] Immunotherapy and targeted therapy are developing rapidly for metastatic melanoma, and may also be used as adjuvant or even neo-adjuvant therapy in regional metastasized patients.[46,68,69] Therefore, the urge to detect a recurrence earlier by more intense follow-up schedules may grow. However, the interesting question that arises, is whether early detection and potential treatment improves survival, or only increases the so-called lead time bias and psychosocial distress, possibly resulting in a reduced quality of life.[18] Besides, treatment-related morbidity, like toxicity and side-effects, accompanied by the use of systemic agents should not be underestimated. Would it be preferable for quality of life improvement and economic benefit to handle more of a 'wait-and-see' policy with less intense surveillance schedules, and only start systemic treatment in case of clinical metastases? This is an interesting discussion which is expected to continue until the day curative treatment is discovered for metastatic melanoma. Albeit the development of systemic treatments for melanoma is expanding, prognosis of advanced melanoma remains poor and there will always be a role for surgical treatment for local disease control.

One thing is certain: future surveillance recommendations should be evidence based, adjusted in the light of new systemic treatments and applied in a more patient-tailored manner, based on a patient's personal preference and individual risk of recurrence. Besides, to decrease the incidence of melanoma, more attention is needed for educational programs regarding sun exposure, and the prohibition of tanning beds should be imposed by the Dutch government.

The behavior of melanoma remains incredibly unpredictable.

'ONE SIZE FITS NONE'

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APPENDICES

LIST OF PUBLICATIONS

AUTHORS & AFFILIATIONS

DANKWOORD

CURRICULUM VITAE

PUBLICATIONS

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PRESENTATIONS

Adipocytes can cause falsely elevated serum S-100B levels in difficult venipunctures. 4th European MD / PhD Conference 2015, Groningen, the Netherlands. 2015 June.

Melanoom follow-up in een nieuw tijdperk. Het melanoom anno 2016, regionaal symposium Integraal Kankercentrum Nederland, Zwolle, the Netherlands. 2016 Jan.

Prospective Randomized Clinical Trial for the Evaluation of a Stage Adjusted Reduced Follow-up Schedule in Cutaneous Melanoma Patients - Results after One Year. SSO's 69th Annual Cancer Symposium, Boston, United States. 2016 Mar.

The Additional Value of S-100B in a Risk Stratifying Model for the Prediction of Non-Sentinel Node Positivity in Melanoma Patients. SSO's 69th Annual Cancer Symposium, Boston, United States. 2016 Mar. (poster)

Prospectief gerandomiseerde studie voor de evaluatie van een gereduceerd follow-up schema bij stadium I-II melanoompatiënten: de MELFO-studie. NVvH Chirurgendagen 2016, Veldhoven, the Netherlands. 2016 May.

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DANKWOORD

'Dag Sammie, domme domme Sammie, kijkt niet om zich heen, doet alles alleen en vindt de wereld heel gemeen. Hoog Sammie, kijk omhoog Sammie...'
(Ramses Shaffy - Sammie)

Dit lied, dat te pas en te onpas ingezet wordt bij mijn entree, gaat duidelijk over een andere Sammie. Het schrijven van dit proefschrift was me alleen NOOIT gelukt! Wie had überhaupt gedacht dat ik zou promoveren? Hier een greep uit alle mensen die me direct of indirect hebben bijgestaan tijdens dit promotietraject.

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Josette Hoekstra-Weebers, copromotor. De echtgenote van, maar zeker niet de minste. Officier in de orde van Oranje Nassau, zo verdiend! Beste Josette, waar zijn we allemaal aan begonnen? Kwalitatief onderzoek met vragenlijsten, jouw specialiteit, mijne duidelijk niet. Na weken zwoegen in het IKNL, konden we eindelijk gaan analyseren. Wat heb je me vaak, veel en goed geholpen met schrijven. 'Actief schrijven, Sammie!' Ik neem het mee. Wat was ik blij als de tekst niet meer helemaal rood was. Maar wat hebben we ook gelachen! Spontane acties bij jullie thuis, actief zaken doen en ondertussen HJH uitleg geven over het bereiden van de curry. Waar Harald en ik soms wat kort door de bocht waren, wist jij ons weer op de weg te krijgen. Zonder jou had ik dit proefschrift niet zo kunnen neerzetten, dank daarvoor!

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Muziek. **Bob Marley**, mijn inspiratie voor dit onderzoek; melanoma took his life.. **Opgezwolle**; Rico & Sticks, onbewuste inspiratie voor de kaft, grijsgedraaid tijdens het onderzoek. **Tribe Called Quest, Outkast, Postmen, the Pharcyde..** De hele gang kent nu de nodige klassiekers.

Tellthecockitwasdelicious. **Ilsalien Bakker, Susanne Stokmans, Arne de Niet**. Een echte kookclub is het nooit geworden, we hadden beter 'Tellthecockdatweuitetengaan' kunnen heten. Ils, Dr. Bakker, roomie-collega-vriendin-reisbuddy. Als ik jou niet over melanomen had horen praten op Curaçao, was deze dag nooit tot stand gekomen. Curaçao, Jamaica, Trinidad & Tobago, Aruba, Gambia, Senegal, Sri Lanka, IJsland, Amerika, Canada; de halve wereld heeft ons al gezien, nu de rest nog! Stokkie, twee Amsterdammers in het hart van Groningen, daar moesten die nuchtere Groningers even aan wennen.. Vanaf dag 1 terug van Curaçao sleurde je me naar elke borrel of activiteit, met een onuitputtelijk enthousiasme wat niemand ooit kan evenaren. Hou vol, ooit sta jij hier ook! Arnie, probleemkind als ik, altijd balanceren op de grens en toch de meest trouwe jongen die ik ken. Golden boy, staat altijd voor me klaar (met Rendang of gore Donuts). Lekkahh G!

Dorpsgekken. **Rob de Vries, Eric Deckers**. Paal en Perk. Hartsvrienden. Nimmer nuchter, altijd droeloe. 'Je hebt al gekozen'. Rob, Ronald, Bambi. Gelukkig organiseerde ik de Cup toen jij semi-arts was. Inmiddels samen bij Groen-Geel, straks samen in opleiding. Gekke huisvriend, laminaat-/tegelzetter en natuurlijk robdefotograaf, dank voor het portret achterin. Eric, Deck, Gino, schl... Soms weet je het niet en alles ken je nalopen, maar toch weet ik waar ik je vinden kan. MELFO-mate for life, doe alles maar even zoals ik. 'Damudeniedoen, Otisweerzover'. Woordspeelkoning in skinny-jeans met snelle planga. 'Kijk nou om je heen, je hebt m'n nummer al!'

Rebelluhhh. **Otis Vrielink en Rinne Peters**. You've got my back. Professor directeur Peters, beetje braaf doen met je brilletje, maar ondertussen.. Wat heb ik me bescheurd toen ik jou in moest werken op de flebopoli. Nooit geweten dat Erica bestond, maar jij woont erOP. En hoe? Ik wil op z'n minst dat één kip Sam gaat heten. Otie, rebel no1, braafste meisje van de klas, tot ze onrecht ruikt. Ippon, BAM. Als rouwdouwers off-piste achter de baas aan, koprollend op ons snowboard. Geen

wintersport voor jou dit jaar, wat moet ik nu? Je ruilt me in voor een 2-tal! Nieuw avontuur, doodeng, maar wij zijn bij je! Als ze niet huilen, wil ik misschien ook wel een keertje oppassen..

Chirurgen van Treant. Allen dank voor jullie vertrouwen en begeleiding. **Tjeerd Boelstra** en **Marloes van den Brand**, jullie staan erin! **Annelies Kemper, Stephan van der Hagen, Frank Kloppenberg** en **Leonie Smit**; dank voor alle speciale (opleidings)momenten. Opleiders **Michiel van den** (her)**Berg** en **Rutger Hissink**, als 'kind van de duivel' ben ik blij dat jullie toch altijd achter me staan. **Harmen Zwaving**, mentor en vriend in goede en mindere tijden. Eén koprol en het pact was gesloten. Jij weet van elke situatie een les te maken en me op de rit te houden als ik té lollig of té serieus ben. Je kan niet altijd 6 gooien, gelukkig is er 'Z&D Consultancy'. Dank voor de nodige duw in de rug bij het afmaken van dit proefschrift.

Dr. Storosum. Geen man, niet lid geweest, geen vader die chirurg is, maar nu wél gepromoveerd! Aan de rest ga ik nog werken... Dank voor de (sceptische) motivatie!

Dushi Korsou. **Rosa, Eva, Jan, Greetje, Geerke, Jan Willem, Jorne, Suus, Mahsa, Diaz, Rugina, Tiarah**. Een internationale vriendschap die altijd stand heeft gehouden. Heerlijk om te zien dat iedereen zich heeft weten te specialiseren, toch een goede basis, dat Curaçao. Snel weer reünie?! **David Nellensteijn**, dank voor je steun. 'Denk aan je selectie-criteria..'

Ex-collega's Spaarne. **Jantine (JPdeB), Jorien, Wytze, Wiebe, Heuff, de Korte, van der Elst**. Dank voor het overtuigen dat promoveren heus wel leuk is en het geloof in mijn opleidbaarheid tot chirurg. 'Jij komt er wel, maar niet hier'. Klopt! Gelukkig zie ik jullie nog vaak genoeg!

Groen Geel Dames 3; van aanvoerder naar bijna altijd afwezig. En toch altijd welkom. Vanaf nu zal ik me weer maximaal inzetten om kampioen te worden! Wedstrijd na een nachtdienst zal nog wel onder de opties vallen, gelukkig speel ik dan als Marcello.. **Jint, Marjan, Kyra**; dankzij jullie vlucht ik niet meer elk weekend naar Amsterdam. Groen! Geel!

Hakketak GIRLS. **Rosa, Nora, Willemijn, Nannie, Maloe, Zinzi**. Altijd weer een feest om bij jullie te zijn! Dankbaar dat jullie mij 'geadopteerd' hebben en altijd maar blijven uitnodigen in Damsko, ondanks dienst en afstand. Kom snel weer naar Grunchi voor EIERBALLEN! **Sniz**, uit onverwachte hoek ontstond een superhechte vriendschap, alsof we elkaar al jaren kenden. Bloedbroeder met dezelfde verslaving; NIKES! Het liefst Jordans, nachten op Ebay voor hetzelfde paar.. Curaçao, Colombia, Berlijn; wat wordt de volgende trip?

De BASIS. **Alette, Caroline, Hilde, Laura, Marèn, Mariette, Marleen, Rosa, Soraya, Welmoed, Yomi**. Al is het soms totaal onduidelijk voor jullie wat ik allemaal aan het doen ben in het Hoge Noorden, de interesse en steun is er altijd. Eén jaar werd twee jaar, twee jaar werd 8 jaar en wie weet hoe lang daarna nog.. Gelukkig is

de basis sterk en heb ik onbeperkt belminuten. Ondertussen hoop ik nog op een Shinkansen tussen Amsterdam en Groningen..

Familie-Ubuntu. **Ma Rinia, Steve, Poekie, Lotte, Clayton, Ashley**. 'No spang, niet stressen Samantha, lekker eten Samantha, goed slapen Samantha..' Jullie helpen me ontspannen in drukke tijden en zorgen dat mijn bere bigi blijft. Suriname was te gek! **Dennis** & **Jessica**; zonder jullie woonden we nu nog steeds in een bouwval. Nu is het huis nagenoeg af en heb ik ook nog aan dit proefschrift kunnen werken. Aboeng!!

Family. **Michael, Adrienne, Babette, Annabel, Maartje**. Dank voor jullie onvoorwaardelijke trots en enthousiasme! **Oma Mary**, zó jammer dat je hier niet bij bent. Onze tien-verschillen skills kwamen voor deze druk wel goed van pas.. **Heidi, Chuck, Sean, Natasha, Neva, Jessie, Jeremiah**. Distance is a b*tch, but family always sticks together! Heidster, thanks for being my favorite auntie! Too bad you live at the other side of the world, but distance never separated us. We travel the world together! Proud of the way you take life. Love you!

Mariëlla Molleman. Mam, je houdt hier niet van, maar ik moet toch íets opschrijven. Nuchter, intelligent, ontwikkeld en soms een beetje té stellig. Nog steeds kan ik zoveel van je leren en zou ik niet weten wat ik zonder je zou moeten. Dank voor al je (tekstuele) hulp en mentale steun. Dat uurtje reistijd zorgt in ieder geval voor genoeg telefoontjes. Doe een beetje rustig, je moet nog even mee..

Douglas & Astrid Damude. Altijd bereikbaar voor spoedoverleg en enorme hulp geweest bij de verbouwing. Pap, dat eigenzinnige is gelukkig niet erfelijk geweest... Maar heeft me wel gebracht waar ik nu ben. Work hard - play hard. Je kunt alles bereiken door hard te werken, zelfs vanuit het buitenland. Vroeger begreep ik nooit waarom dat moest. Nu loert de zon (wel goed insmeren!). Ik hoop natuurlijk dat jullie in NL blijven, zo niet, dan graag een huis met logeerkamer!

Gaby Damude. Grote broer waar ik nog altijd tegenop kijk. We schelen 10 jaar en toch zijn we er altijd voor elkaar. Als je me (voorzichtig) in elkaar sloeg, huilde ik als een baby. Ben ik een grote meid door geworden.. Ook al snap je niet veel van deze hele promovatie, je vraagt altijd hoe het gaat en luistert naar alle saaie verhalen. Ik ben supertrots op wat je allemaal doet en bereikt heb, je bent een 'legend' onder de DJ's. Samen met **Sènami** heb je me het tofste nichtje en neefje gegeven; **Ifèlayo** en **Ayodéji**, jullie zijn de BOM!

Reflino Nijbroek. Ubuntu. Ook al zeg ik het nooit, want dat is niet cool, zonder jou had ik het niet volgehouden. Jij hebt me in weten te burgeren in Groningen, waar ik nooit geweest was en eigenlijk snel weer weg wilde. 'Minder takkie, meer ballie!' Oftewel: minder praten, meer doen. Hoe vaak je mij wel niet uit bed getrapt hebt om weer aan de slag te gaan. Hoe vaak ik niet thuis kwam eten, omdat ik "nog even iets af moest maken". En toch sta je altijd klaar en zorg je dat ik niet omval. Nu kunnen we eindelijk leuke dingen gaan doen!

CURRICULUM VITAE

Samantha (Sammie) Damude werd geboren op 16 maart 1986 in Amsterdam. Op haar derde verhuisde zij met haar Canadese vader Douglas Damude, Nederlandse moeder Mariëlla Molleman en haar broer Gaby naar Bloemendaal. Na de lagere school ging zij naar het Stedelijk Gymnasium Haarlem, waar zij in 2004 haar diploma behaalde.

Een wereldreis was het plan, studeren werd een feit. In 2004 ging zij Theaterwetenschap studeren aan de Universiteit van Amsterdam (in 2005 propedeuse behaald) en volgde daarnaast in de weekenden het Oriëntatiejaar aan de Gerrit Rietveld Academie. Na dit creatieve jaar besloot zij toch voor een concreter vak te kiezen. In 2005 begon zij met de studie Geneeskunde aan de Universiteit van Amsterdam. In 2009 vertrok zij naar Canada voor een wetenschappelijke stage naar de ziekte van Parkinson op de Afdeling Neurologie in het Toronto Western Hospital, onder leiding van dr. S.H. Fox. Tijdens haar coschappen in het Academisch Medisch Centrum en ziekenhuizen in de regio (o.a. Flevoziekenhuis Almere) ontdekte zij haar passie voor de chirurgie. Haar keuze-coschap volgde ze op de Afdeling Chirurgie in het Onandjokwe Lutheran Hospital in Namibië en het oudste-coschap op de Afdeling Chirurgie in het Slotervaartziekenhuis te Amsterdam.

Na het behalen van haar artsexamen in 2012 werkte zij als ANIOS chirurgie in het Spaarne Ziekenhuis te Hoofddorp (heden Spaarne Gasthuis), opleider dr. G.J.M. Akkersdijk. Na dit jaar kreeg zij de kans om de uitdaging aan te gaan als ANIOS snijdende specialismen in het St. Elisabeth Hospitaal te Curaçao, onder leiding van dr. D.R. Nellensteijn. Vanaf Curaçao legde zij contact met Prof. dr. H.J. Hoekstra in het Universitair Medisch Centrum Groningen (UMCG). Eind 2014 kon zij daar starten met een promotietraject bij de Chirurgische Oncologie, resulterend in dit proefschrift.

Per 1 september 2016 is zij gestart met de opleiding chirurgie. Na het eerste opleidingsjaar in het UMCG (opleider Dr. R.J. van Ginkel), vervolgt zij nu haar opleiding in Treant Zorggroep te Emmen, Stadskanaal en Hoogeveen (opleider Dr. M. van den Berg).