Nodal metasases and biomarkers in melanoma

Kruijff, Schelto

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Schelto Kruijff

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General introduction and outline of thesis

GENERAL

Melanoma is the most malignant of all skin cancer types. It causes more than 75% of all skin cancer related mortality.[1] Cutaneous melanoma incidence has increased rapidly in the last years and estimates predict a continuing increase.[2] In the Netherlands, the total number of melanoma patients is expected to increase from 2,400 patients in 2000 to 4,800 patients in 2015 and already reached 3,500 patients in 2005.[2] The incidence and mortality of populations have reached either a plateau or maybe a decline in younger age groups while there is an increase in older age groups.[3-7] In Australia, mortality rates seem to have reached a plateau, even though incidence rates are increasing. This may be due to the fact that most new melanomas are thinner which usually do not lead to short term death.[8]

AJCC Staging

Patients with melanoma are staged according to the American Joint Committee on Cancer TNM-staging manual. In 2009 the 7th version was published and a summary is given in table 1 and 2.[9] This updated manual has an evidence-based approach and reflects the improved understanding of the disease.[9] The main histological biomarkers influencing T- staging are Breslow thickness and ulceration. N-staging is influenced by the involvement of regional lymph nodes. In the latest version of the AJCC staging system, micro-metastases were also included which are nowadays detected as a consequence of the routine use of the sentinel node biopsy procedure in the treatment of melanoma. The M-staging describes metastatic disease and differentiates between subcutaneous or extra regional involved lymph nodes, pulmonary metastases and other metastatic disease. For the first time a serum biomarker LDH was added to the AJCC staging system.

TABLE 1 TNM Staging categories for melanoma (American Joint Committee on Cancer 2009)[9]

Tumour	Breslow thickness	Ulceration status/mitoses
Tis	NA	NA
T1	≤ 1,0 mm	T1a: without ulceration and mitoses < 1/mm^2 T1b: with ulceration and/or mitoses ≥ 1/mm^2
T2	1,01-2,0 mm	T2a: without ulceration T2b: with ulceration
T3	2,01-4,0 mm	T3a: without ulceration T3b: with ulceration
T4	> 4,0 mm	T4a: without ulceration T4b: with ulceration
Node	**No. of metastatic nodes**	**Nodal metastatic burden**
N0	0	NA
N1	1	N1a: micrometastasis* N1b: macrometastasis**
N2	2-3	N2a: micrometastasis* N2b: macrometastasis** N2c: In-transitmetastases/satellites without metastatic nodes.
N3	4+ metastatic nodes, or matted nodes, or in-transitmetastases/satellites with metastatic nodes	
Metastases	**Site**	**Serum LDH**
M0	No distant metastasis	NA
M1a	Distant skin, subcutaneous or nodal metastases	Normal
M1b	Lung metastasis	Normal
M1c	All other visceral metastasis	Normal
	Any distant metastasis	Elevated

*Micrometastases are diagnosed after sentinel lymph node biopsy. **Macrometastases are defined as clinically detectable nodal metastases confirmed pathologically. LDH Lactate dehydrogenase. NA Not applicable

Treatment and staging of primary localized melanoma; AJCC stage I/II

Local control is the main component of primary cutaneous melanoma treatment. Therefore, surgical resection is the first choice of treatment. The treatment of a suspect nevus or skin lesion is a diagnostic excision with a 2 to 3 millimetre margin of normal skin. Most important is a disease free margin. According to the Dutch melanoma guidelines and most other international guidelines the therapeutic, surgical excision margins are defined as 0,5 cm for in-situ melanoma, 1 cm for T 1-2 melanoma and 2 cm for T 3-4 melanoma (Table 3).[10-28] In case of unfavourable melanoma characteristics, excision margins of more than 2 centimetres, if not harmful for the patient, are advised in the majority of guidelines.[19,30]

Nodal staging: AJCC stage IIIa en Sentinel node biopsy (SLNB)

The value of sentinel node biopsy (SLNB) in Stage I and II patients has been controversial.[31] In most countries SLNB is performed as a staging procedure in patients with melanomas

TABLE 2 Anatomic stage groupings for melanoma (AJCC 2009)[9]

	Clinical staging*				Pathological staging**		
O	Tis	N0	M0	**O**	Tis	N0	M0
IA	T1a	N0	M0	**IA**	T1a	N0	M0
IB	T1b	N0	M0	**IB**	T1b	N0	M0
	T2a	N0	M0		T2a	N0	M0
IIA	T2b	N0	M0	**IIA**	T2b	N0	M0
	T3a	N0	M0		T3a	N0	M0
IIB	T3b	N0	M0	**IIB**	T3b	N0	M0
	T4a	N0	M0		T4a	N0	M0
IIC	T4b	N0	M0	**IIC**	T4b	N0	M0
III	Any T	N1-3	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/b	N2c	M0
				IIIC	T 1-4b	N1b	M0
					T 1-4b	N2b	M0
					Any T	N3	M0
IV	Any T	Any N	M1	**IV**	Any T	Any N	M1

* 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. **Pathologic staging includes microstaging of the primary melanoma and pathologic information about the regional lymph nodes after partial (ie, sentinel node biopsy) or complete lymphadenectomy. Pathologic stage 0 or stage IA patients are the exception; they do not require pathologic evaluation of their lymph nodes.

over 1 mm Breslow thickness or under 1 mm in case of prognostic unfavourable tumour characteristics. In approximately half of the guidelines SLNB is stated to be a standard, routine procedure which all patients meeting criteria should undergo (Table 3).

The results of the Multicenter Selective Lymph Node Trial (MSLT), the 3rd interim analysis, showed that the SLNB procedure reflects mainly staging information and patients who underwent a SLNB were associated with a longer disease free interval.[31,32] According to the Dutch melanoma guidelines, SLNB is only advised combined with inclusion in clinical trials or for patients who wish to be optimal informed about the stage of their disease.[19]

However, today the technique seems to have undisputedly proven to be a reliable staging method and the fourth interim analysis of the MSLT I study reveals that SLNB indeed leads to improved melanoma specific survival.[33]

TABLE 3 Guidelines for primary treatment and staging primary stage I-II melanoma.

Country	Excision margins (cm)				Initial staging				
	Primary	Tis	T ≤2mm	T >2mm	SLNB *		Imaging		Blood tests
					Tumour	Routine	US	CXR	
Australia & NZ [10]	0.2	0.5	1-2	2	>1mm	-	-	-	-
Austria [11]	0.3-1.0	0.5	1-2	3	>1mm	+	+	+	LDH,CBC
Belgium[12]	0.2	0.5	1***	2***	≥pT1b	-	+	+	-
Canada (BC) [13]	NR	0.5-1	1	2	1-4mm	+	-	0	-
Denmark[24]	0.5	0.5	1-2	2-4	pT1b	+	-	-	-
France[15]	NR	0.5	1-2	2-3	≥pT1b	-	-	-	-
Germany[16]	NR	0.5	1	2	>1mm	+	+	+	LDH,S100,CBC
Ireland[17]	0.1-0.3	0	1-2	2	≥pT1b	+	+**	+**	LDH**
Italy[18]	1.0	0	0	1-2	>1mm	-	-	-	-
Netherlands[19]	0.2	0.5	1	2	>1mm	-	-	-	-
Norway[20]	0.2-0.5	0.5	1	2-3	Trial	-	-	-	-
Poland[21]	0.1-0.2	0.5	1	2	≥1 mm	+	-	-	-
Scotland[22]	0.2	0.2-0.5	1-2	2	≥pT1b	-	-	-	-
South Africa[23]	0.2	0.5	1-2	2	NR	-	-	-	-
Spain[24]	0.1-0.2	0.5	1-2	2-3	≥pT1b	+	+	+	LDH
Sweden[25]	0.2	0.5	1-2	2	≥pT1b	+	-	-	-
Switzerland[16]	NR	0.5	1	2	>1mm	+	-	-	-
United Kingdom[27]	0.2-0.5	0.2-0.5	1-2	2-3	Stage II	-	+**	+**	LDH, CBC**
United States[28]	0.1-0.3	0.5	1-2	2	≥pT1b	+	-	-	-

*All guidelines recommend that completion lymph node dissection (CLND) should follow positive SNLB or otherwise in clinical trial setting. ** Only for stage IIB and higher. *** T≤1 mm and T>1mm. CBC Complete blood cell count CXR Chest X-ray. NR Not reported in guideline. LDH Lactate dehydrogenase. LNB Sentinel lymph node biopsy. US Ultrasound of draining lymph node basins with/without abdomen

Palpable nodes : AJCC Stage IIIb

In AJCC stage III melanoma patients, a therapeutic lymph node dissection is the first choice of treatment. However, this is a group of patients with a remarkable heterogeneity in survival. Recently, Charles Balch published a multivariate analysis of prognostic factors among 2,313 patients with stage III melanoma. The five year overal survival was 63% varying from 67% for patients with nodal micro-metastases to 43% for nodal macrometastases.[34]

Staging studies analyzing patients with palpable lymph nodes, whole-body FDG-PET and/or spiral CT have proved their value with 27% upstaging to stage IV disease. A sensitivity of

79-92% and a specificity of 86-90% to detect distant metastases was found.[35,36] The combined FDG-PET and spiral CT scan leads to a change in the planned dissection in 37% of the patients.[37] Analyzing the heterogeneity of stage III melanoma, the suspicion rises that numerous patients have a form of subclinical dissemination, which can even remain undetected

by standard FDG-PET and CT imaging.[38] Biomarkers could identify these high risk melanoma patients with the goal to treat this group more aggressively and to downsize follow up frequency in low risk melanoma patients.

Metastatic disease (AJCC stage IV)

For stage IV melanoma treatment results remain unsatisfactory. No systemic therapy has demonstrated to affect overall survival, although the recent immunotherapy with Ipilimumab and the introduction of the BRAF pathway inhibitors have shown promising results.[39,40] Treatment of metastatic disease is mainly based on the individual situation of the patient. If disseminated melanoma disease presents itself as a single isolated metastasis, metastasectomy might be the only treatment.[41,42] The failed phase III Canvaxin trial enrolled 496 patients at 80 centers between June 1998 and March 2005. Canvaxin did as well as expected, but the placebo arm did better than predicted for surgery in stage IV melanoma. At 5 years, 40% of patients in both arms of the trial were still alive (unpublished data SSO conference 2011). All had received surgery for stage IV disease before entering the trial, which was testing postsurgical adjuvant use of the vaccine. Although the trial was not designed to test the efficacy of surgery in stage IV disease, the largest multicenter clinical trial undertaken using surgical resection as initial therapy for melanoma metastatic to distant sites prolonged survival and suggested that the more widespread use of surgery is indicated in stage IV melanoma. The role of surgery versus the 'best systemic treatment' for limited stage IV disease is now studied.[43] Radiotherapy can be indicated for local control and reduction of pain in metastatic disease.[44]

Follow-up

The overall prevalence of melanoma is increasing as a result of the rising worldwide incidence of melanoma and the stabilizing mortality rates.[45] This has resulted in a growing number of melanoma patients needing follow-up and consequently more doctors are required to deliver this service. International consensus on the follow-up of melanoma patients does not exist and high-frequency follow-up after treatment for melanoma is still standard practice in many countries around the world.[46] The main purpose of follow-up is early detection of recurrent disease and second primary melanomas that could be treated successfully by surgery or other treatment modalities.[47] However, it is still difficult to predict the behavior of melanoma in the individual patient and overall treatment results of metastatic or recurrent disease are disappointing.[47] No evidence-based optimal follow-up schedule for melanoma patients exists, although most guidelines for management advise a more vigorous follow-up strategy for patients in more advanced stages. Future investigations should be focused on determining patient-tailored follow-up regimens. Therefore, in our centre a prospective, randomized, high quality methodological research has been started in order to develop meaningful applicable guidelines (MELFO).[48]

TABLE 4 Follow-up recommendations per guideline for follow-up in stage I-III primary melanoma

Country	Year	Follow-up schedule Number of visits per year after diagnosis						Routine additional testing Type and number per year			
		AJCC or T-class	1	2	3	4	5	6-10	USLN	CXR	PET/CT	S-100
Australia & New Zealand[10]	2008	I	2	2	2	2	2	1	-	-	-	-
		II	3-4	3-4	3-4	3-4	3-4	1	-	-	-	-
		III	3-4	3-4	3-4	3-4	3-4	1	-	-	-	-
Austria[11]	2010	Tis	1	1	1	1	1	1	-	-	-	-
		T1	2	2	2	2	2	2	1	-	-	-
		T2-3a	2-4	2-4	2-4	2-4	2-4	2-4	2	1-2	1-2	-
		T3b-4b	4	4	4	4	4	4	2	1-2	1-2	-
		III	4-12	4-12	4-12	4-12	4-12	4-12	4	4	4	-
Belgium[12]	2007	I	4	4	2	2	2	1	-	-	-	-
		II	4	4	2	2	2	1	-	-	-	-
		III	4	4	2	2	2	1	-	-	-	-
Canada (British Columbia)[13]	2009	I	2	2	1	1	1	1[a]	-	-	-	-
		II	2-4	2-4	2	2	1	1[a]	-	-	-	-
		III	2-4	2-4	2	2	1	1[a]	-	-	-	-
Denmark[14]	2003	Ia	1	1	1	1	1	1	-	-	-	-
		Ib	4	4	2	2	2	1				
		II	4	4	2	2	2	1	-	-	-	-
		III	4	4	2	2	2	1	-	-	-	-
France[15]	2005	I	2	2	2	2	2	1[a]	-	-	-	-
		IIA-IIB	4	4	4	4	4	1[a]	2-4	-	-	-
		IIC-III	4	4	4	4	4	1[a]	2-4	-	-	-
Germany[16]	2007	I (≤1mm)	2	2	2	2	2	1-2	-	-	-	-
		I-IIB	4	4	4	4	4	1-2	2	-	-	2-4
		IIC-III	4	4	4	4	4	2	2-4	2	-	2-4
Ireland[17]	2006	I (≤1mm)	2	2	-	-	-	-	-	-	-	-
		I-II	2	2	2	2	2	-	-	-	-	-
		III	2	2	2	2	2	1[a]	-	-	-	-
Italy [18][b]	2009	O	1	1	1	1	1	1	-	-	-	-
		I	2	2	2	1	1	1	-	-	-	-
		IIAB-IIIA	3	3	2	2	1	1	1-3	1-2	-	-
		IIC-IIIB	3	3	3	1	1	1	1-3	1	1	
		IIIC	3	3	3	2	2	1	2-3	2	0-3	2-3
Netherlands[19]	2005	Id	4	3	2	2	2	-	-	-	-	-
		II	4	3	2	2	2	1	-	-	-	-
		III	4	3	2	2	2	1	-	-	-	-
Norway[20]	2007	Tis	1	-	-	-	-	-	-	-	-	-
		<1 mm	4	4	4	-	-	-	-	-	-	-
		≥1 mm	4	4	4	2	2	-	-	-	-	-

General contents follow-up visits						Follow-up by which doctor?
History	Scar Exam	ITM Exam	LRLN	ComplSkin	Self-exam	
+	+	+	+	-	+	Doctor preferred by patient
+	+	+	+	-	-	Surgeon in specialized center
+	+	+	+	+	-	Dermatologist
+	+	+	+	+	-	Not specified
+	+	+	+	+	-	Yr 1-5: DMG Member Yr 5-10: GP
+	+	+	+	+	+	Not specified
+	+	+	+	+	-	Dermatologist
+	+	+	+	+	+	Pigmented skin lesion specialist
+	+	+	+	+	-	Surgeon or Dermato-logist
+	+	+	+	-	-	Not specified
+	+	+	+	+	-	Surgeon or Dermato-logist

TABLE 4 *cont.*

Country	Year	Follow-up schedule Number of visits per year after diagnosis							Routine additional testing Type and number per year			
		AJCC or T-class	1	2	3	4	5	6-10	USLN	CXR	PET/CT	S-100
Poland[21]	2009	All stages	3-4	3-4	2	2	2	1	-	-	-	-
Scotland[22]	2007	All stages	Frequency and duration individually based on tumor and patient characteristics.						-	-	-	-
South Africa[23])	2004	All stages	Frequency and duration individually based on tumor characteristics.						-	-	-	-
Spain[24]	2006	0	1	1	1	1	1	1[a]	-	-	-	-
		IA	2	2	2	1	1	1[a]	1	1	-	-
		IB-IIA	2-4	2-4	2-4	1-2	1-2	1[a]	2	2	-	-
		IIB/C-III	2-4	2-4	2-4	1-3	1-3	1[a]	-	-	1-2	-
Sweden[25]	2007	All stages	No follow-up schedule provided by national guideline.[c]						-	-	-	-
Switzerland[26]	2005	<1mm	2	2	2	2	2	1[a]	-	-	-	-
		IB-IIB	4	4	4	2	2	1[a]	1	1	-	-
		IIC-III	4	4	4	2	2	1/2[a]	1	-	1	-
United Kingdom[27]	2002	<1 mm	4	4	4	-	-	-	-	-	-	-
		≥1 mm	4	4	4	2	2	-	-	-	-	-
United States of America[28]	2009	0	1	1	1	1	1	1[d]	-	-	-	-
		IA-IIA	1-4	1-4	1-4	1-4	1-4	1[d]	-	-	-	-
		IIB-IV	2-4	2-4	1-4	1-4	1-4	1[d]	-	-	-	-

[a] Lifelong follow-up
[b] Personal communication from dr. C.R. Rossi, University of Padova, Italy
[c] No follow-up for Breslow <1 mm and melanoma in situ
[d] At least annual skin exam for life
Compl skin Complete skin examination
CXR Chest X-ray

SUMMARY

The incidence of melanoma has increased drastically although mortality rates have stabilized which has resulted in a growing number of melanoma patients needing follow-up. Research should be continued to search for an adequate and optimal follow up regimen. Survival rates of stadium III and IV are still disappointing. Up to today, no systemic therapy has proven survival benefit. New promising treatment strategies with monoclonal antibodies and inhibitors are currently tested and new phase II and III trials designed.[39,40] Biomarkers should be evaluated to optimize the staging system as to strive to a more tailored systemic therapy regimen.

General contents follow-up visits						Follow-up by which doctor?
History	Scar Exam	ITM Exam	LRLN	ComplSkin	Self-exam	
+	+	+	+	+	-	Specialist in oncology center
+	+	+	+	+	+	Appropriate local specialist
+	+	+	+	+	-	Not specified
+	+	+	+	+	-	Dermatologist
+	+	+	+	+	+	Not specified
+	+	+	+	+	-	Not specified
+	+	+	+	+	-	Not specified
+	+	+	-	-	+	Not specified

ITM Exam Examination of in-transit metastasis pathway
LRLN Palpation of loco-regional lymph nodes
PET/CT Positron emission tomography with/without CT scanning
USLN Ultrasonography of draining lymph node basin(s)
DMG Danish Melanoma Group
GP General practitioner

OUTLINE OF THIS THESIS

Improvement in staging allows more precise classification and more accuracy of predicting the likely prognosis and outcomes for individuals with a disease. However, cutaneous melanoma still represents a paradox among all solid tumors. It is the cancer for which the best prognostic markers are available, yet there is very little understanding of their biological significance. It can be expected that the strongest biological markers, such as the ones that were included in the last AJCC staging system, are surrogates of key biological events. The studies in this thesis discuss the use of prognostic markers in the treatment of melanoma and try to create understanding about the correlation between melanoma biomarkers and tumor biology. Also, the potential use of various prognostic markers as LDH, S-100B, SUV and Breslow thickness in melanoma disease is evaluated.

TABLE 5 Proposed optimal follow-up schedule and experimental schedule MELFO study [48]

AJCC Stage	Number of visits per year after diagnosis					
	1	**2**	**3**	**4**	**5**	**6-10**
IA	1					
IB	1	1	1	1	1	
IIA	2	2	1	1	1	1
IIB	3	3	2	1	1	1
IIC	3	3	2	1	1	1
III	4	3	2	2	2	1
IV	Individually tailored to patient's needs					

PART I: DETECTION AND TREATMENT OF NODAL MELANOMA METASTASES

The overall prevalence of melanoma is increasing and therefore patients needing follow-up are increasing. International consensus on the follow-up of melanoma patients has not been achieved and high-frequency follow-up still practiced in many countries around the world. In chapter two the reader is informed about the influence of lymph nodes metastases detected by patients or by physicians at the outpatient department and the influence on survival. We evaluated the role of the method of detection in nodal disease in the prognosis of melanoma patients who underwent therapeutic lymph node dissection (TLND). Understanding the outcomes of self-detection or physician detection is of vital importance for the design of follow-up studies. Numerous studies have also demonstrated that older melanoma patients have a lower survival rate, especially those over 60 years of age.[49-54] The older patient might not detect positive lymph nodes because of a certain physical negligence that occurs with aging. Therefore we also evaluated the difference in self detection between elderly en younger patients and influences on survival.

Ilio-inguinal lymph node dissection for stage III melanoma is often complicated by wound healing disturbances. In the third chapter we conducted an extensive retrospective study to assess the present morbidity of therapeutic or completion lymph node dissection to determine risk factors for short term morbidity and to evaluate recommendations on peri-operative treatment. A retrospective study was performed to investigate the wound healing disturbances after therapeutic ilio-inguinal lymph node dissection.

PART II : PROGNOSTIC MARKERS IN MELANOMA

When compared with other markers and tumor characteristics as ulceration, Clark level and Mitosis index, Breslow thickness is the most important biomarker and predictor for mortality.[9,55] Because of the increasing melanoma incidence in the last decades and predictions

showing a continuing increase in the next years the aim of the study described in the fourth chapter, was to assess trends in melanoma incidence, Breslow thickness (BT), and melanoma survival among young and elderly patients in the Netherlands. Patients diagnosed with melanoma in the Netherlands 1994-2008 were selected from the Netherlands Cancer Registry (NCR) and overall 40,880 patients were included and analyzed.

For stadium IV melanoma it was already known that the tumor marker S-100B in melanoma patients could be used for the detection of recurrences.[56] However, the role of S-100B for patients with palpable lymph node metastases before undergoing therapeutic lymph node dissection, was still unknown. In chapter five the role of S-100B for disease-free survival (DFS) was evaluated and compared with the tumor marker LDH.

In chapter six, S-100B values and Standardized Uptake Value (SUV) in FDG-PET for clinically stage III melanoma patients were analyzed as indicators of early recurrence in stage III disease. Aim was to assess the association between these markers and their relation with survival.

In chapter seven the effect of neoadjuvant treatment with bevacizumab in stage III melanoma is discussed. For patients with palpable nodes, a lack of resources in healthcare has resulted in a waiting list of at least 5 weeks before a lymph node dissection can be performed. A feasibility study was performed to investigate the effect of induction treatment with bevacizumab for Stage III melanoma, while waiting for lymph node dissection.

In chapter eight a review was composed describing the role of S-100B and how it can be used for the different tumor stages in melanoma.

A summary of the work undertaken is given in English and Dutch at the end of this thesis. Finally, in the 'future perspectives' section, new research developments in the field of melanoma markers are discussed.

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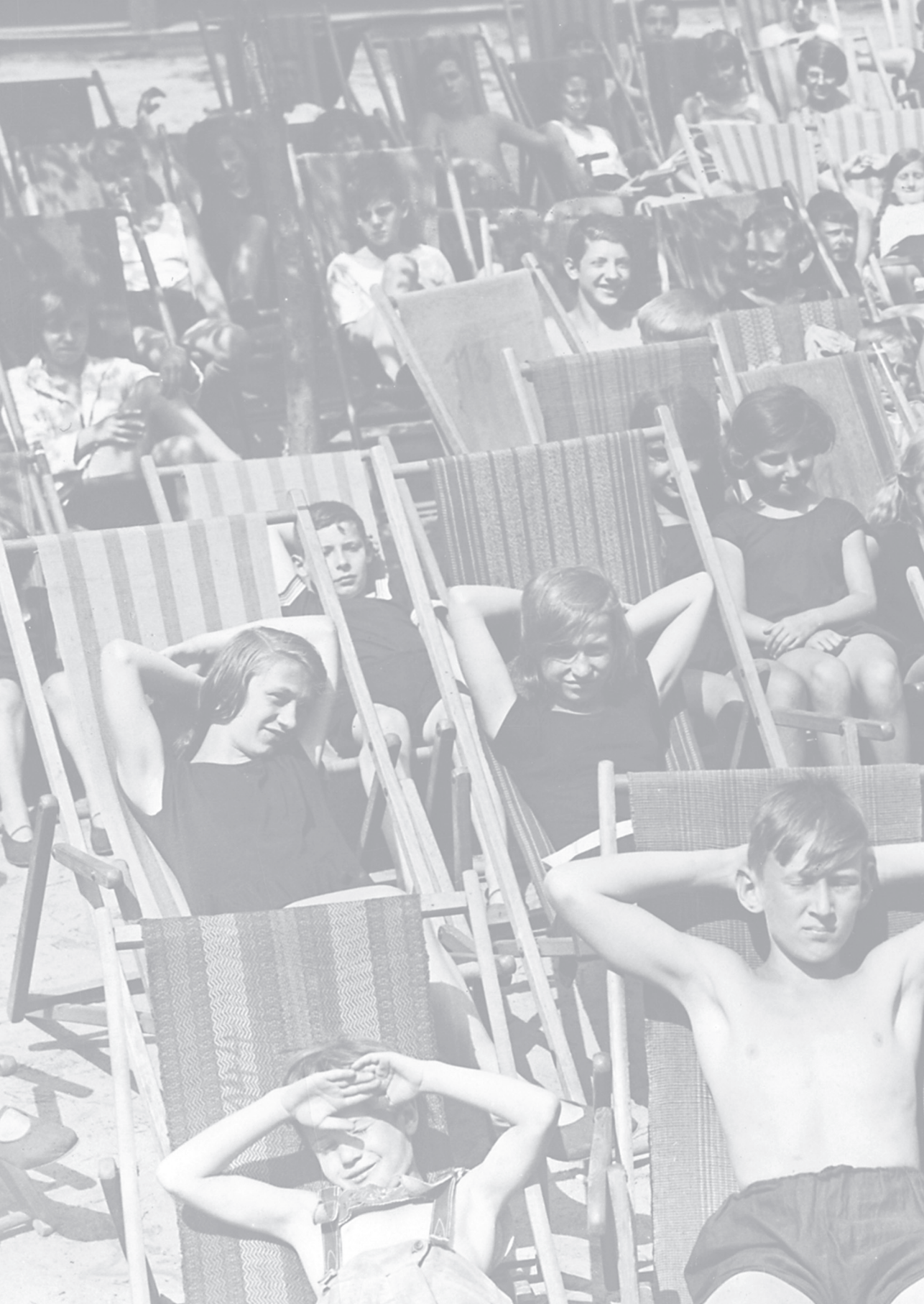

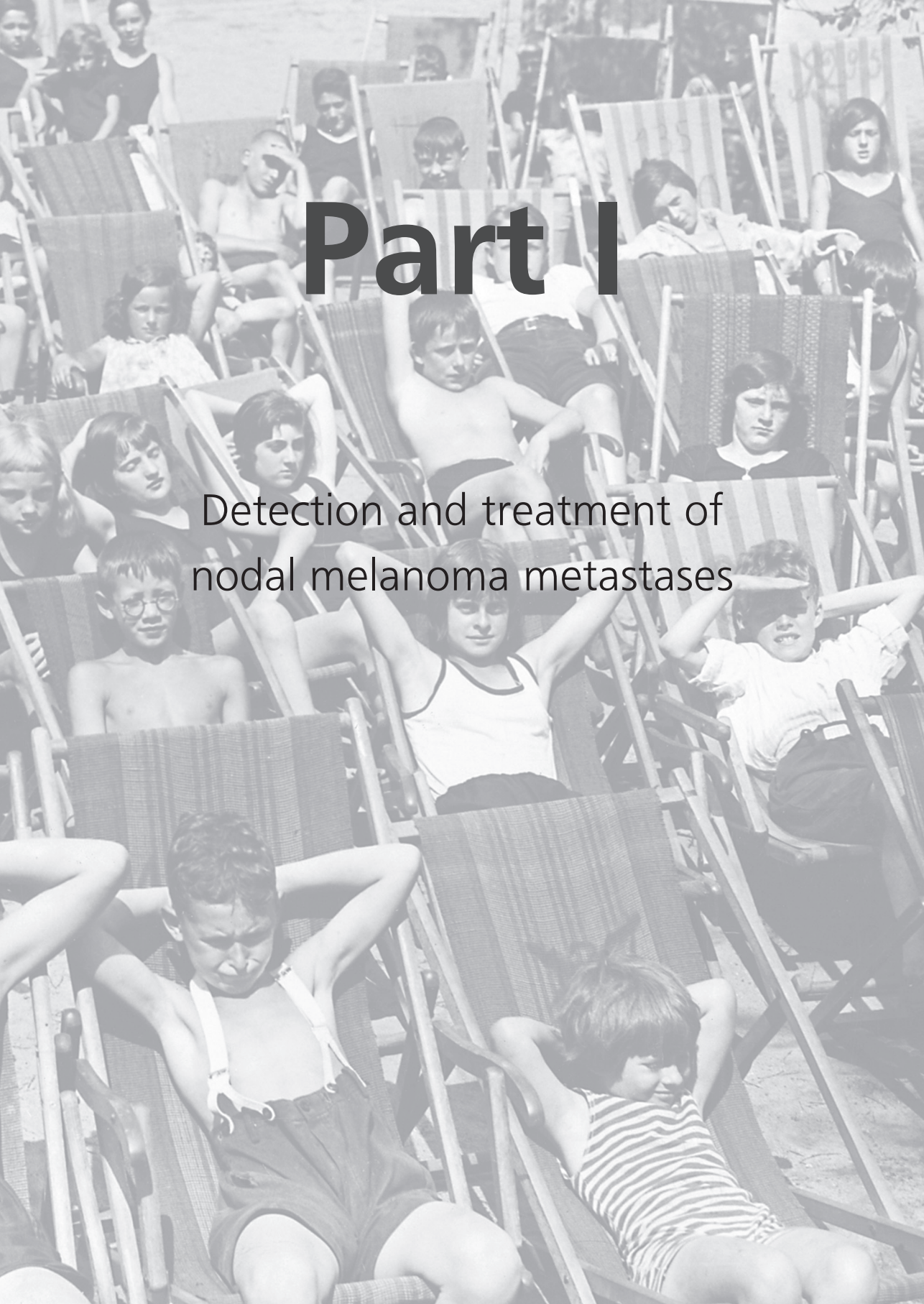

Part I

Detection and treatment of nodal melanoma metastases

2

Detection of melanoma nodal metastases; differences in detection between elderly and younger patients do not affect survival

| S Kruijff, E Bastiaannet, AJH Suurmeijer, HJ Hoekstra | Ann Surg Oncol. 2010;17:3008-14

ABSTRACT

Background: Melanoma lymph nodes metastases may be detected by patients or by physicians. Understanding the outcomes of self- or physician detection is essential for the design of follow-up studies. We evaluated the role of the method of detection in nodal disease in the prognosis of melanoma patients who underwent therapeutic lymph node dissection (TLND).

Methods: All melanoma patients with palpable lymph nodes were included in a prospective database (n=98) and the method of detection was recorded. Detection of lymph node metastases compared with pathological findings in the TLND was assessed by multivariate logistic regression. Disease free survival (DFS) and disease specific survival (DSS) were assessed by univariate and multivariant Cox proportional hazard analysis.

Results: Nodal metastases were detected by physicians in 45% and by patients in 55% ($p<0.001$). Age was significantly associated with method of detection. Patients ≤ 60 years detected 69% their lymph node metastases as opposed to 32% of patients > 60 years (OR 0.3; $p= 0.007$). However, this was not associated with prognostic findings in TLND, number of positive nodes, tumor size, or extranodal spread. Method of detection or age at the time of nodal metastases was not significantly associated with two year DFS or DSS.

Conclusion: 45% of lymph node metastases in Stage I-II melanoma patients are physician detected. Younger patients detect their own lymph node metastases significantly more often than elderly patients. However, neither the method of detection nor age correlates with DSS. More frequent follow-up would not alter DFS and DSS significantly.

INTRODUCTION

The incidence of melanoma is increasing worldwide. In the Netherlands, the incidence increased from 9.5 to 13.7 per 100,000 in men and from 13.4 to 18.5 per 100,000 in women between 1989-2003. Approximately 90% of patients have stage I or II melanoma at diagnosis and the incidence of patients with a thin melanoma has increased.[1-5] Although the early diagnosis of cutaneous melanoma with small Breslow`s tumour thickness has been responsible for the levelling off of overall melanoma mortality, the incidence at the same time has continued to rise. [6] The number of melanoma patients requiring follow-up surveillance has doubled in the Netherlands.[1] Despite improved public awareness and earlier diagnosis and treatment, mortality from melanoma increased from 2.5 to 3.6/100,000 in men and from 2.0 to 2.4/100,000 in women in the Netherlands between 1989-2003.[1]

Several studies have been performed concerning survival in patient versus physician detected recurrences. Since most of first melanoma recurrences are detected by patients or their partners, the value of high frequency follow-up has often been questioned.[7,8] Over half of patients (55%) with early stage melanoma detect their own recurrence(s).[9-11,14,16-23] In recent decades, several follow-up schedules have been suggested with large variations and without international consensus.[3] Proposals for these schedules are based on risk calculations concerning melanoma recurrence and cost effectiveness. Several authors have recommended a reduction in the intensity of follow-up regimens based on these factors.[9-12] Others claim that a more intensive follow-up will improve outcome because of earlier detection.[13-15]

Of the various follow-up methods performed by physicians, only medical history and physical examination seem to be cost effective.[23] Despite these findings, most melanoma patients are still followed up on a frequent basis, mostly for education, reassurance or inclusion in clinical trials.

Studies concerning the method of nodal metastasis detection have not shown a difference in survival comparing physician- versus patient detected recurrences. Intense follow-up does not seem to contribute to disease free interval or overall survival, as physician detected lesions do not have a better prognosis than those detected by patients themselves.[3,19]

Patients with clinical stage III b melanoma (palpable lymph node metastases) have a 5-year survival of 59%.[24] A better understanding of the method of detection of palpable melanoma metastases (i.e. by patient or physician) and its influence on survival is essential to resolve the above mentioned follow up controversy.

The aim of this study was to evaluate the role of the method of detection, by patient or physician, in melanoma patients with palpable lymph nodes and to analyse the association with pathological findings in therapeutic lymph node dissection (TLND) and the impact of the detection method on disease free and disease specific survival. The method of detection of positive nodes (patient versus physician) might not only have impact on survival but might also influence the number of positive nodes, the tumor size or the presence of extranodal growth in the TLND.

MATERIAL AND METHODS

Patients with primary melanoma were treated with wide local resection (1 or 2 cm) with or without sentinel lymph node biopsy and, if indicated, therapeutic lymph node dissection. Stage I and II melanoma patients were followed after treatment in accordance with the Dutch National Guideline Treatment of Melanoma (www.oncoline.nl). Postoperative follow-up included physical examination every 3 months for the first year, every 4 months for the second year, and every 6 months thereafter. Standard radiographic or serum investigations were not performed.

All patients with clinically and cytological proven lymph node metastases of melanoma (AJCC stage III b) were included in this study. If palpable positive nodes were found during regular follow-up this was recorded as "physician detected nodal metastases". When positive palpable nodes were recorded after a patient-initiated visit, this was recorded as "patient detected nodal metastases". Patients without distant metastases on FDG-PET and CT received a TLND with curative intent and were entered into a single prospective institutional database. The Stage IV upstaged patients were referred for further palliative treatment to their primary physician, medical oncologist or radiation oncologist and excluded for further follow up. Patients, who presented with local recurrence or in transit disease, were not included.

Patients with nodal metastases of ≥ 3 cm and/or ≥ 3 positive lymph nodes and/or extra nodal (EN) disease received adjuvant radiotherapy (20 x 2.4 Gy). Data examined include patient demographics, clinical and histopathologic characteristics of the tumor, date and type of operation, site and date of first (nodal) and second recurrence, method of detection of recurrence (i.e. patient or physician during standard follow-up), and status at last follow-up.

The study was approved by the Medical Ethical Committee of the University Medical Centre Groningen (UMCG).

Statistics

For retrospective analysis, patients were divided by age (less than or equal to 60 versus greater than 60 years of age). Factors associated with method of detection were analysed by univariate and multivariate logistic regression analysis. Four subgroups were generated based on the combinations of young versus elderly and physician versus patient detection to assess for associations with pathological variables. Factors associated with disease free survival (DFS) and disease specific survival (DSS) were assessed by univariate and multivariant Cox proportional hazard analysis. For DFS, any recurrence was recorded as an event; for DSS, death due to melanoma was considered an event. Follow-up was truncated at 4 years.

RESULTS

Patients

In the period 2003-2008, 98 patients 54 males (55.1%) and 44 females (44.9%), median age 57.7 years (range 28.5-86.7) with stage III b melanoma, based on PET and spiral CT, underwent a TLND (Table 1). Median time from primary melanoma treatment to nodal recurrence or nodal recurrence of an unknown primary melanoma was 22.0 (0-315.3) months.

TABLE 1 Patient and tumour characteristics of 98 patients with clinically and cytological proven lymph node metastases of melanoma (AJCC stage IIIb)

		Number	Percentage
Sex	Male	54	55.1
	Female	44	44.9
Age (years)	≤60	61	62.2
	>60	37	37.8
Primary melanoma			
Localisation	Upper extremities	11	11.2
	Head & Neck	8	8.2
	Lower extremities	39	39.8
	Trunk	34	34.7
	Unknown primary	6	6.1
Breslow thickness	≤2.0	43	43.9
	>2.0	49	50.0
	Unknown	6	6.1
Clark level	I-III	33	33.7
	IV-VI	59	60.2
	Unknown	6	6.1
Ulceration	Yes	22	22.5
	No	76	77.5
Lymph node metastases			
Lymph node dissection	Axilla	34	34.7
	Groin	50	51.0
	Neck	14	14.3
Number of nodes removed	<15	48	49.0
	15 or more	50	51.0
Nr of nodes positive	2 or less	53	54.1
	>2	45	45.9
Tumorsize (cm)	≤ 3.0	62	63.3
	>3.0	36	36.7
Extranodal growth	Yes	27	27.5
	No	71	72.5

Detection

Physicians detected 45% of the nodal metastases and patients detected 55% (p= 0.001) (Table 2). Of the 61 patients in the younger age group (≤ 60 years), 19 nodal metastases were physician detected (31%) whereas 42 nodal metastases were patient detected (69%). Of the 37 elderly patients (> 60 years), 12 nodal metastases were patient detected (32%) and 25 nodal metastases physician detected (68%) (p=0.001).

Table 2 also shows the results of univariate and multivariate logistic regression analysis of the association between method of detection and pathological information from the lymph node dissection, sex, age, and nodal bearing area (axilla, groin, neck). Age was significantly associated with method of detection: 69% of patients ≤60 yrs detected their nodal metastases versus 32% of the patients >60 yrs (OR 0.3; p=0.007 in multivariate analysis).

TABLE 2 Detection of lymph node metastases in association with pathological results of lymph node dissection

		% patient-detected	Univariate analyis		Multivariate analysis	
			OR (95%CI)	p-value	OR (95%CI)	p-value
Overall: 44.9% detected by physician and 55.1% by patients (p<0.001)						
Sex	Male	48.2	1.0		1.0	
	Female	63.6	1.9 (0.8-4.3)	0.13	1.8 (0.7-4.6)	0.20
Age	≤60	68.9	1.0		1.0	
	>60	32.4	0.2	0.001	0.3 (0.1-0.7)	0.007
Breslow	≤2.0	67.4	1.0		1.0	
thickness	>2.0	42.9	0.4 (0.2-0.8)		0.5 (0.2-1.3)	
	Unknown primary	66.7	0.9 (0.2-5.9)	0.05	2.1 (0.3-15)	0.22
Lymph node region	Axilla	61.8	1.0			
	Groin	50.0	0.6 (0.3-1.5)			
	Neck	57.1	0.8 (0.2-2.9)	0.56		
Nodes removed (cat)*	<15	58.3	1.0			
	>15 o	52.0	0.7 (0.3-1.7)	0.53		
Nodes positive*	2 or less	47.2	1.0		1.0	
	>2	64.4	2.0 (0.9-4.6)	0.09	1.9 (0.7-4.9)	0.17
Tumorsize (cm)	cont		1.2 (0.9-1.6)	0.09	1.2 (0.9-1.6)	0.17
Extranodal growth	Yes	66.7	1.0		1.0	
	No	50.7	0.5 (0.2-1.3)	0.16	0.7 (0.2-2.0)	0.48

Patients with a thin primary melanoma had a higher ratio of patient-detected positive lymph nodes (p=0.05). This was, however, not statistically significant in multivariate analysis.

Sex, regional nodal basin site , number of nodes removed, number of positive nodes, tumor size and extranodal growth were not associated with method of detection in multivariate analysis.

There were no significant differences in pathological findings when the groups were divided in four groups: patient detected younger age (≤60 yrs), patient detected elderly age (>60 yrs), physician detected younger age (≤60 yrs), and physician detected elderly age (>60 yrs).

Survival

Multivariate analysis showed no significant difference in 2-year DFS for physician versus patient detected nodal recurrences (Table 3). Univariate analyses showed that DFS was associated with the lymph node bearing area, the number of positive nodes, node size, and extranodal growth pattern.

The 2-year DSS in males (53%) was significantly shorter than that in females (75%) (HR 0.3; p=0.004). DSS was significantly reduced in patients with ≥ 2 positive nodes (51%) compared to patients with < 2 positive nodes (72%) (HR 2.2; p=0.03). Multivariate analysis showed no significant differences for DSS related to age, Breslow thickness, ulceration, lymph node bearing area (axilla, groin, neck), number of removed nodes (≤ 15 nodes or >15 nodes), tumor size (≤3cm or >3cm), or extranodal growth (Table 3). Although in univariate analysis a trent in DSS was noted (p=0.08) in favour of the physician detected group, in multivariate analysis, when compared to detection by patients, no significant difference could be found.

TABLE 3 Disease Specific Survival (DSS) and Disease Free survival (DFS) in melanoma patients AJCC stage IIIB in correlation to detection by physician versus detection by patient

		DSS	HR	p- value	HR	p- value	DFS	HR	p-value	HR	p- value
Sex	Male	53.2	1.0	0.02	1.0	0.004	35.2	1.0	0.62		
	Female	75.4	0.4		0.3		36.8	0.9			
Age	≤60	59.7	1.0	0.28			29.7	1.0	0.17		
	>60	66.6	0.7				45.2	0.7			
Breslow	≤2.0	65.8	1.0	0.81			45.4	1.0	0.83		
	>2.0	64.5	0.9				30.4	1.06			
Ulceration	Yes	61.5	1.0	0.61			22.6	1.0	0.21		
	No	62.8	0.8				40.5				
Loc lymph	Axilla	57.2	1.0	0.37			38.8	1.0	0.07	1.0	0.2
	Groin	60.5	0.8				26.0	1.3		1.2	
	Neck	78.6	0.5				66.7	0.4		0.4	
Removed	<15	63.2	1.0	0.9			39.2	1.0	0.78		
	15 or more	61.8	1.1				32.9	1.1			
Positive	2 or less	72.5	1.0	0.02	1.0	0.03	46.7	1.0	0.009	1.0	0.1
	>2	51.4	2.2		2.2		23.8	2.0		1.6	
Tumorsize	≤3.0	69.6	1.0	0.06	1.0	0.39	45.3	1.0	0.01	1.0	0.2
	>3.0	47.9	1.9		1.4		17.3	2.0		1.5	
Extranod	Yes	56.8	1.0	0.52			27.6	1.0	0.03	1.0	0.2
	No	64.8	0.8				39.4	0.6		0.7	
Detection	Physician	67.6	1.0	0.08	1.0	0.14	36.2	1.0	0.2	1.0	0.5
	Patient	58.4	1.8		1.7		36.6	1.4		1.2	

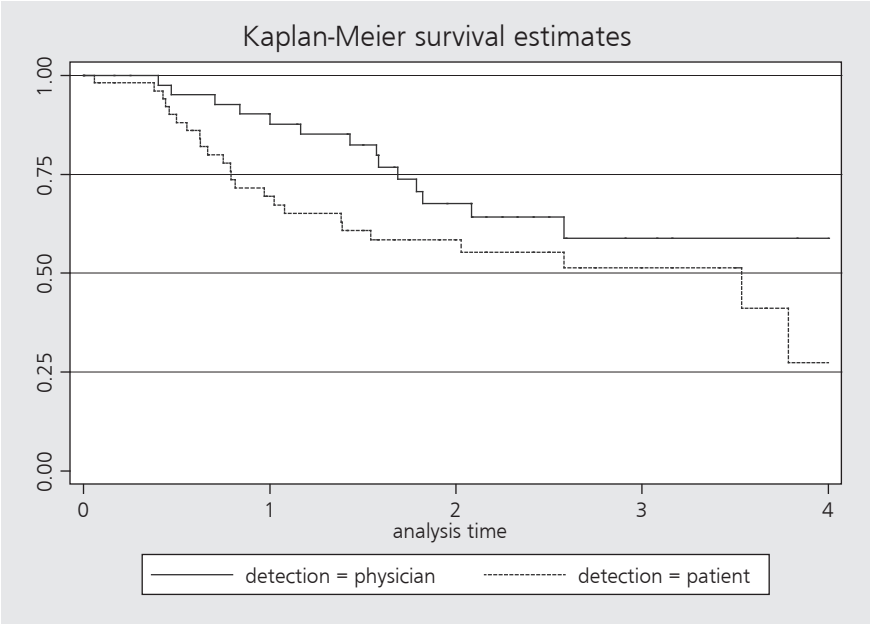

FIGURE 1 KM curve DSS melanoma patients with positive lymph nodes detected by physician versus detected by patient

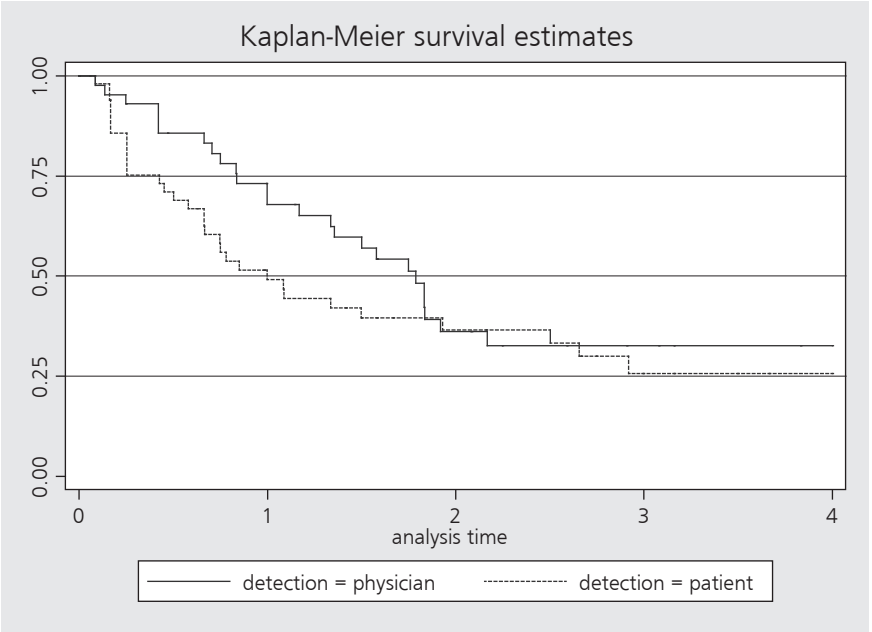

FIGURE 2 KM-curve DFS melanoma patients with positive lymph nodes detected by physician versus detected by patient

DISCUSSION

The most efficacious follow-up scheme for melanoma patients is uncertain. Understanding the impact of the method of detection of nodal recurrence on prognosis is important in determining the most appropriate follow-up. Despite controversy about follow-up in melanoma, our results show that nodal metastases are still detected by physicians in almost 45% of the patients. Young patients detect their own lymph node metastases significantly more often than older patients (68% versus 32%) (p=0.007). However, in patients undergoing close clinical surveillance neither the method of detection (patient versus physician) nor age seems to have a significant influence on two-year DSS or DFS. Furthermore, no correlation could be found between the methods of detection and the number of positive nodes, lymph node size, or extra nodal growth.

Despite this encouraging percentage of almost 45% detected nodes by physicians, this rate does not seem to result in any survival advantage. Earlier follow up studies describe an increasing controversy about the efficacy of an intensive follow-up scheme for melanoma patients as is still organised in a lot of hospitals today.

This is the first study evaluating the method of detection of nodal recurrence in stage III b melanoma and the impact of patient or physician detected nodal metastases on survival.

Patients with clinical stage III b melanoma (palpable lymph node metastases) have a 5-year survival of 59%.[24] The most well recognized causes for this low survival rate is the variation in the number of positive nodes, tumor size, and presence of extranodal growth.

Another cause for low survival rates in this patient category might be under staging. Bastiaannet et al. revealed that staging with FDG-PET of clinically stage III b patients resulted in upstaging of 22% to stage IV. In 19% treatment was changed, usually from surgery to systemic treatment.[26] Patients in our current study were all staged with FDG-PET and CT and therefore can be considered as "true" AJCC stage III b patients.

Survival might also be influenced by follow up and method of detection. We expected physician detected positive lymph node status to correlate with a lower number of positive nodes, smaller tumor size, and absence of extranodal growth in the TLND. This association was not found despite a positive trend in DSS (p= 0.08) in the advantage of physician detected nodes (Table 3).

Follow-up of melanoma has different purposes: to detect first melanoma recurrence, to assess treatment efficacy, and to detect a second primary melanoma (2-6%).[28-30] Also of great importance are patient reassurance and documentation or inclusion for clinical trials. Follow-up schemes are often of high intensity; however, resources in many centres are insufficient to deal with the increasing patient load. Therefore, identifying and integrating evidence concerning the efficacy of follow-up strategies is important.

In 2003 Garbe et al performed a prospective follow-up study in melanoma patients following an intensive follow-up scheme (every 3 months for the first five years and every six months during the sixth to tenth years).[14] The authors claimed that 83% of all recurrences were detected by a physical examination during regular follow-up and only 17% were detected by patients

themselves. However, various AJCC melanoma stages were included in this study. Francken et al performed a large retrospective study and found that only 62% of all types of recurrences were identified by patients themselves; however, three quarters of first melanoma recurrences (FMR) were detected by patients or their partners, of which 11% were due to self examination.[3] In the present study no significant survival difference was found comparing patient - versus physician-detected nodal metastasis.

Additionally, patient age did not seem to have a significant influence on two-year DSS or DFS. In patients older than 60 years, almost 70% of the detected nodes were found by a physician; in contrast, patients at or below 60 years of age detected their own nodes 70% of the time. Earlier literature reports age as an independent prognostic factor, perhaps presenting a surrogate for declining host defence mechanism associated with advancing age.[2] Numerous studies have demonstrated that older patients have a lower survival rate, especially those over 60 years of age.[31-36] The older patient might not detect positive lymph nodes because of a certain physical negligence that occurs with aging. However, our results surprisingly did not identify any survival disadvantage associated with delayed detection of nodal metastases. This might be explained by a small patient population or a follow-up period that is too short. Another explanation could be related to the recent report by Conway et al, who found that lymphatic function declines with age.[37] Hypothetically, in older patients, declining lymphatic function might modify metastatic patterns and slow the process of dissemination.

Overall, it can be concluded that no study to date has proven any disease free survival or overall survival benefit related to intense follow-up surveillance as there are no prospective studies that compare high frequence to no or low frequence follow up.

With the frequency of follow-up which was used in this study, the DFS and DSS for patients capable themselves of detecting a palpable node in the regional nodal basin and those unable to do so, were not significantly different. Otherwise stated, it could be concluded that the above frequency of follow-up is sufficiently close for the detection and treatment of nodal disease. More frequent follow-up probably would not alter the DFS and DSS significantly. Therefore, arguments for more frequent follow-up than the above described scheme are hard to justify as physician detected lesions do not have a better prognosis than those detected by patients.[3,7,8] Whether follow-up with sparser intervals could be designed in a responsible way cannot be answered by this study.

Prospective, randomized, high quality methodological research is required in order to develop meaningful applicable guidelines. Currently no international consensus has been reached concerning the optimal frequency of follow-up visits for melanoma patients. Follow-up should be based on individual patient characteristics; multiple patient factors should be used to design the most appropriate follow-up.[39] A clinical randomized trial (MELFO) is currently underway at the University Medical Centre Groningen to evaluate the safety and cost effectiveness of reduced follow-up surveillance.[40] In this RCT a high frequent follow up scheme will be compared with a less frequent and better differentiated follow up scheme adjusted to melanoma AJCC stage.

In conclusion, more than 55% of lymph node metastases in melanoma are detected by the patient. Younger patients detect their own lymph node metastases significantly more often than elderly patients without any impact on DSS and DFS. The data of our study will ad to the controversy about the value of high frequency follow-up regimens in melanoma. A prospective, randomized, high quality methodological research has been started in order to develop meaningful applicable guidelines (MELFO).

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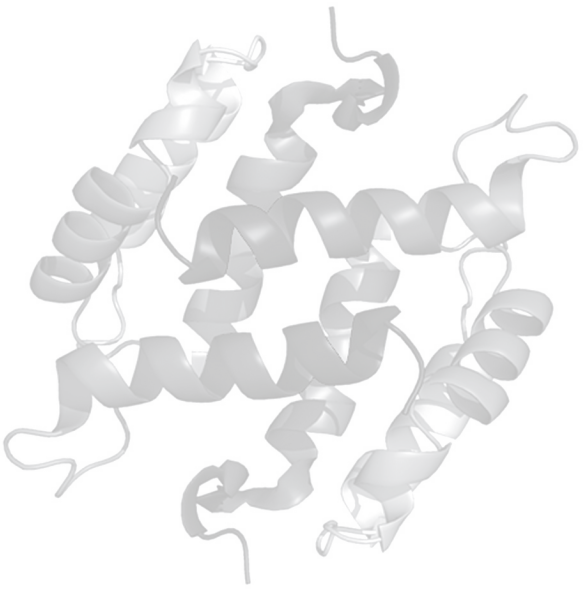

Therapeutic groin dissection for melanoma; Risk factors for short term morbidity

HPAM Poos, S Kruijff, E Bastiaannet, RJ van Ginkel, HJ Hoekstra | Eur J Surg Oncol. 2009;35:877-83

ABSTRACT

Aims: Ilio-inguinal lymph node dissection for stage III melanoma is often complicated by wound healing disturbances. A retrospective study was performed to investigate the wound healing disturbances after therapeutic ilio-inguinal lymph node dissection.

Patients and methods: Between 1989 and 2007, 139 consecutive patients, 73 females (53%) and 66 males (47%), median age 55 (range 20-86) years underwent a therapeutic ilio-inguinal lymph node dissection. Data were recorded on early complications: haematoma, wound infection, wound necrosis and seroma. Univariate and multivariate logistic regression analyses were used to evaluate the influence of a wide range of variables on postoperative complications.

Results: Seventy-two patients had one or more early wound complications (49.7%). These complications comprised haematoma (n=3, 2.1%), wound infection (n=30, 20.7%), wound necrosis (n=25, 17.5%) and seroma (n=31, 21.8%). Wound infections were significantly more common in patients with a body mass index (BMI) of >25 (p=0.019). Wound necrosis developed significantly more often if the Bohler Braun splint was not used postoperatively (p=0.002). The occurrence of one or more early complications was significantly associated with the non-use of a Bohler Braun splint (p=0.026) and age of >55 years (p=0.015).

Conclusions: High BMI was significantly correlated with the occurrence of wound infections. Bed rest with the knee and hip in flexion using a Bohler splint improved wound healing after therapeutic ilio-inguinal lymph node dissection.

INTRODUCTION

The incidence of melanoma is still increasing worldwide. In the Netherlands, the incidence currently amounts to 16.1/100,000, with a mortality-rate of 3.0/100,000. The anatomical localisation of preference in males is the trunk, and in females the leg.[1] In about 90% of the cases, melanoma is diagnosed at tumour stage I or II.[2] Approximately 16-28% of patients with a primary melanoma will develop a tumour recurrence, 20-28% will develop a local recurrence or intransit metastases, 26-60% will develop regional lymph node metastases and 15-50% will develop distant metastases.[3] The value of elective lymph node dissection has been studied to improve disease-free survival and overall survival. Four studies concluded that elective lymph node dissection did not influence the survival of melanoma patients.[4-7] Morton et al. developed the concept of sentinel lymph node biopsy (SLNB) to detect regional lymph node metastases at an early stage.[8] In patients with a primary melanoma of intermediate tumour thickness (1.2-3.5 mm) and a positive SLNB, completion lymph node dissection led to significant improved disease-free survival. An improved overall survival was not found, however.[9] Sentinel lymph node biopsy is a diagnostic procedure that involves a certain degree of morbidity.[10-11] Therefore, studies on the value of preoperative ultrasound examination of the regional lymph nodes are currently being performed.[12] Morbidity after elective and therapeutic ilio-inguinal lymph node dissection can comprise: wound necrosis, wound infection, seroma and/or lymphoedema.[13-23] Efforts have been made to improve the surgical technique and reduce the risk of complications. An ilio-inguinal lymph node dissection can be conducted via one vertical incision crossing the inguinal fold, that removes a skin ellipse (Fig. 1A), or via two separate incisions above and below the inguinal fold (Fig. 1D).[13,24-26] At the University Medical Centre Groningen (UMCG), the first technique, with the excision of a skin ellipse, is used.[13,26]

Previously, an extensive analysis on the complications that can arise after ilio-inguinal lymph node dissection was performed at the UMCG. Morbidity was present, but limited, while the risk of lymphoedema was small.[13] Recent research into the morbidity of sentinel lymph node biopsy in the ilio-inguinal region has shown that nowadays, more complications occur after completion lymph node dissections.[10] This prompted us to conduct a more extensive retrospective study to assess the present morbidity of therapeutic or completion lymph node dissection to determine the risk factors for short term morbidity and to evaluate recommendations on perioperative treatment.

METHODS

Patients

From 1989 to 2007, 139 consecutive patients, 73 females (53%) and 66 males (47%), with a median age of 55 (range 20-86) years underwent 143 ilio-inguinal lymph node dissections for locoregional metastasized melanoma.The melanomas were localised on the trunk (n=11,

7.6%), penis or scrotum (n=4, 2.8%), thigh (n=46, 31.7%), lower leg (n=39, 26.9%) and foot (n=30, 20.7%). Thirteen patients had an unknown primary tumour (9%). The median Breslow thickness was 2.5 (range 0.70-20.0) mm. Four patients underwent a bilateral ilio-inguinal lymph node dissection in two separate surgical procedures. Indications for ilio-inguinal lymph node dissection were histologically or cytologically proven ilio-inguinal lymph node metastases (n=112) or positive sentinel lymph node biopsy (n=31). The clinical data of the patients who underwent these 143 therapeutic lymph node dissections are shown in Table 1.

TABLE 1: Clinical data on 143 therapeutic ilio-inguinal lymph node dissections for stage III melanoma

Variable	No. of patients (%)
Gender	
M	69 (48.3)
F	74 (51.7)
Median age (years)	55 (20-86)
Localisation primary tumor	
Trunk	11 (7.6)
Penis / Scrotum	4 (2.8)
Thigh	46 (31.7)
Lower leg	39 (26.9)
Foot	30 (20.7)
Unknown	13 (9.0)
Breslow thickness	
T1 (<1.00 mm)	11 (7.7)
T2 (1.00-2.00 mm)	33 (23.1)
T3 (2.00-4.00 mm)	42 (29.4)
T4 (>4.00 mm)	39 (27.3)
Unknown	18 (12.6)
Median (mm)	2.5 (0.70 – 20.0)
Indication ilio-inguinal lymph node dissection	
Macrometastases	112 (78.3)
positive SLNB	31 (21.7)
Risk factors	
BMI > 25	76 (53.1)
Smoking	41 (28.7)
Cardiovascular disease	37 (25.9)
Pulmonary disease	8 (5.6)
Diabetes mellitus	5 (3.5)
Comorbidity*	43 (30.1)

* Comorbidity: presence of cardiovascular disease and/or pulmonary disease and/or diabetes mellitus

Surgical technique

First an ellipse shaped incision of the skin cranially, 2 cm medial from the superior anterior iliac spine, to 15 cm below Poupart's ligament was performed. The ellipse was approximately 4-6 cm at its broadest point. Any scar tissue from previous lymph node excisions was included in the skin ellipse (Figure 1A). The superficial dissection contained the subcutaneous fat and lymphatic tissue underneath the skin ellipse, limited by the long adductor muscle medially and the sartorius muscle laterally. The saphenous magna vein was taken along. The femoral artery and the femoral nerve became visible, as well as the femoral vein on the dorsal side of the femoral artery. The lymphatic tissue located at the superior aspect of the femoral canal, medially from the femoral vein is called the Cloquet node. It is thought that this tissue represents the leading lymph node draining into the pelvis from the inguinal basin.[27] Poupart's ligament was transected longitudinally 2 cm lateral from the neurovascular bundle, which exposed the iliac and obturator lymph nodes. Then the iliac lymph node dissection was performed. All the lymph nodes were excised up to the bifurcation of the communicating iliac artery, as well as the lymph nodes in the obturator fossa. Poupart's ligament was closed. The origin of the sartorius muscle was transected at the level of the anterior iliac spine and preserved over a length of about 10 cm (Figure 1B). Then it was reverted medially to cover the neurovascular femoral bundle and fixed to Poupart's ligament (Figure 1C).

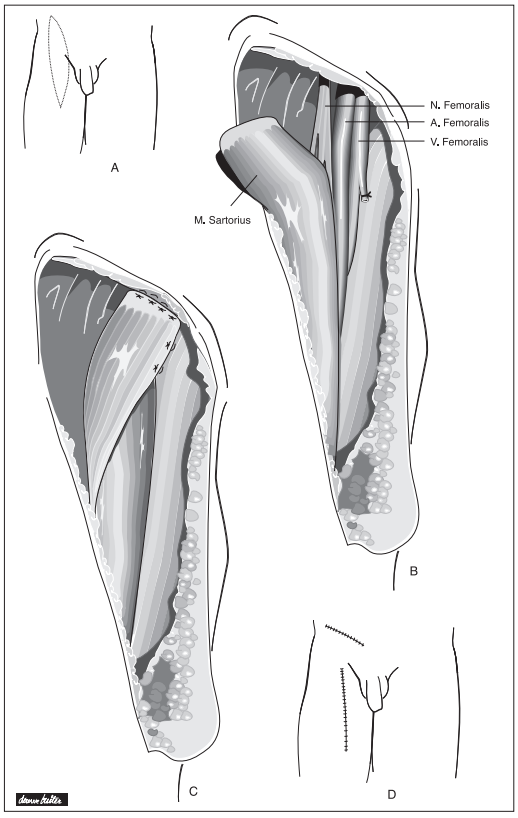

FIGURE 1.
A. Ellipse shaped incision
B. The origin of the sartorius muscle transected and preserved over a length of about 10 cm
C. The sartorius muscle reverted medially to cover the neurovascular femoral bundle and fixed to Poupart's ligament
D. Separate incision above and below the groin

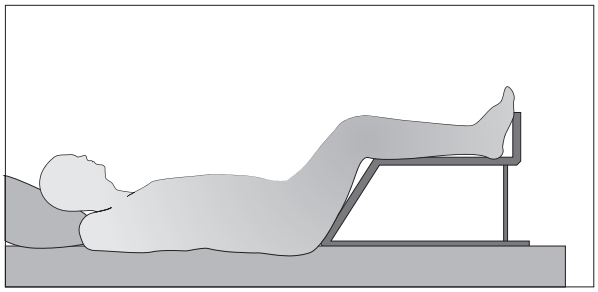

FIGURE 2. Bed rest with the hip and the knee in flexion on a Bohler Braun splint.

Postoperative treatment

During the first 10 days postoperatively, the patient had strict bed rest. From 1989 to 2000, in the majority of patients (n=58, 70.7%) a Bohler Braun splint was used for the first 5 or 10 days to place the patient's hip and knee in flexion in order to reduce the tension along the wound. This concept was thought to support wound healing (Figure 2). A Bohler Braun Splint is often used in orthopaedics to provide stabilization with or without traction for a variety of angulated lower extremity fractures. Since 2000 the distal end of the bed was elevated for the first 5 days with the hip and knee in slight flexion and in only 5 patients (8.2%) a Bohler Braun splint was used after 2000. The use of the Bohler Braun splint was gradually abandoned in the late nineties because the 'old hospitals beds' were then replaced by 'modern hospital beds' with the option to place the hip and the knee in flexion. The wound drains were removed after a minimum of 7 days, and if the production was less than 20 ml per 24 hours. For the first 6 months postoperatively, patients were prescribed a tailor made support stocking that had been made for the affected leg prior to surgery. Perioperatively and during the period of bed rest, all patients received prophylactic low molecular weight heparin (fraxiparine). Antibiotics were not administered routinely during surgery.

Complications

Four types of complications were scored: 1, haematoma that required surgical intervention; 2, wound infection if the wound was culture-positive and/or therapeutic antibiotics were indicated; 3, wound necrosis if the edges of the wound were necrotic, irrespective of whether necrotomy had been performed, and if there was secondary wound healing; 4, seroma that required puncture.

Variables

The following variables were analysed for their possible influence on postoperative morbidity: age (≤55 years vs >55 years), gender, smoking, body mass index (BMI, ≤25 vs >25), comorbidity (diabetes mellitus (DM), cardiovascular disease and chronic obstructive pulmonary disease), surgeon (fellow vs staff surgeon), operative time (150 min vs >150 min), bed rest (Bohler Braun splint vs no Bohler Braun splint), operative period (1989-2000 vs 2001-2007) and surgical indication (macrometastases vs positive SLNB).

TABLE 2: Univariate and multivariate analysis on Infection, Wound necrosis and 1 or more complications

Variable	Wound infection n=30 (%)	Univariate Analysis p-value	Multivariate analysis OR (95%CI) p-value	Wound necrosis n=25 (%)
Age (years)				
≤ 55	12/74 (16.2)		1.57 (0.62-3.94)	11/74 (14.9)
> 55	18/69 (26.1)	0.147	0.339	14/69 (20.3)
Gender				
M	16/69 (23.2)			14/69 (20.3)
F	14/74 (18.9)	0.531		11/74 (14.9)
Smoking				
+	7/41 (17.1)			9/41 (22.0)
-	23/102 (22.6)	0.467		16/102 (15.7)
BMI				
> 25	22/76 (29.0)		3.28 (1.21-8.88)	16/76 (21.1)
≤ 25	6/58 (10.3)	0.009	0.019	7/58 (12.1)
Comorbidity				
+	13/43 (30.2)		1.39 (0.53-3.65)	12/43 (27.9)
-	17/100 (17.0)	0.075	0.500	13/100 (13.0)
Surgeon				
Staff	9/54 (16.7)			8/54 (14.8)
Fellow	21/89 (23.6)	0.324		17/89 (19.1)
Operative time				
≤ 150 min	14/63 (22.2)			12/63 (19.1)
> 150 min	16/80 (20.0)	0.746		13/80 (16.3)
Bed rest				
Bohler Braun splint +	12/63 (19.0)			5/63 (7.9)
Bohler Braun splint –	18/80 (22.5)	0.615		20/80 (25.0)
Operation year				
89-00	14/82 (17.1)		1.51 (0.63-3.62)	13/82 (15.9)
01-07	16/61 (26.2)	0.184	0.353	12/61 (19.7)
Indication				
Macrometastasis	23/112 (20.5)			17/112 (15.2)
Positive SLNB	7/31 (22.6)	0.805		8/31 (25.8)

Statistical analysis

The statistical analyses were performed with SPSS version 14.0.2. Early complications were analysed separately and also as combined variables. Chi square tests and multivariate logistic regression were used to assess factors associated with the early complications. Factors with $p < 0.20$ in the univariate analysis were entered into the multivariate model.

Univariate Analysis p- value	Multivariate analysis OR (95%CI) p-value	1 or more complications n=72 (%)	Univariate Analysis p-value	Multivariate analysis OR (95%CI) p-value
		27/74 (36.5)		2.71 (1.21-6.05)
0.393		45/69 (65.2)	0.001	0.015
		38/69 (55.1)		
0.393		34/74 (45.9)	0.275	
		21/41 (51.2)		
0.372		51/102 (50.0)	0.895	
	1.89 (0.67-5.35)	44/76 (57.9)		2.05 (0.94-4.50)
0.172	0.229	23/58 (39.7)	0.036	0.071
	2.72 (0.99-7.50)	30/43 (69.8)		2.13 (0.86-5.29)
0.031	0.053	42/100 (42.0)	0.002	0.101
		26/54 (48.1)		
0.513		46/89 (51.7)	0.682	
		35/63 (55.6)		
0.662		37/80 (46.3)	0.269	
	6.62 (1.94-22.53)	24/63 (38.1)		3.14 (1.14-8.57)
0.008	0.002	48/80 (60.0)	0.009	0.026
		35/82 (42.7)		1.30 (0.48-3.54)
0.552		37/61 (60.7)	0.034	0.608
	0.43 (0.13-1.38)	57/112 (50.9)		
0.168	0.155	15/31 (48.4)	0.805	

RESULTS

General

The median duration of hospitalisation was 14 (range 7-44) days. There was no mortality due to surgery. Overall perioperative morbidity was 55.2%. General complications were observed in eight cases: two delirium (1%), four urinary tract infections (3%) and two bladder retentions (1%); there were no pulmonary and thrombo-embolic complications.

Early wound complications comprised: haematoma (n=3, 2.1%), wound infection (n=30, 20.7%), wound necrosis (n=25, 17.5%) and seroma (n=31, 21.8%). Seventy-two patients had one or more early wound complications (49.7%).

Multivariate analysis

No significant associations were found between the different variables and the complications comprised haematoma and seroma. Wound infections occurred significantly more frequently in patients with a BMI of >25. Wound necrosis was more common if the Bohler Braun splint was not used postoperatively. The occurrence of one or more postoperative complications was significantly correlated with not applying a Bohler Braun splint and with more advanced age.

Comorbidity was related to a higher risk of wound necrosis, although not significantly proven. An overview of the univariate and multivariate analyses of local complications is presented in Table 2.

DISCUSSION

General

In the present study, a BMI of >25 was correlated with a higher risk of wound infection. Additionally, advanced age (>55 years) was associated with a higher incidence of one or more early complications. And, applying a Bohler Braun splint in the postoperative phase of strict bed rest after ilio-inguinal lymph node dissection reduced the risk of wound necrosis and significantly prevented one or more early wound complications. At least 10 studies have been

TABLE 3: Literature overview of wound complications in melanoma patients who underwent therapeutic or elective groin dissection

Author	Year	No. of patients	Inguinal / Ilio-inguinal	Thera-peutic / Elective	Wound infection %	Wound necrosis %
Baas et al.[13]	1992	151	8 / 143	138/31	9	3**
Beitsch et al.[14]	1992	168	168 / 0	132/45	29	26
Karakousis et al.[17]	1994	205	94 / 111	90/115	16	8
Pearlman et al.[19]	1995	19	0 / 19	19/0	26*	?
Strobbe et al.[22]	1999	52	0 / 52	52/0	17	15
Hughes et al.[16]	2000	132	60 / 72	132/0	13	8
Lawton et al.[18]	2002	56	0 / 56	12 / 44	30	4
Serpell et al.[21]	2003	27	25 / 2	27/0	25	7
Van Akkooi et al. [23]	2007	129	0 / 129	129/0	29	?
Sabel et al.[20]	2007	212	181 / 31	212/0	19*	?
Poos et al.	2008	139	0 / 143	143/0	21	18/7**

* Wound infection with or without partial flap necrosis/wound dehiscence
** Requiring surgical treatment

published about wound healing disturbances after lymph node dissection since the early 1990s.[13-23] Unfortunately, it was not possible to perform a subgroup analysis on the series that compares therapeutic lymph node dissection with elective procedures.[13,14,17,18] There was a wide variation in the incidence of complications: haematoma 0-5%, wound infection 9-30%, wound necrosis 3-26% and seroma 5-46% (Table 3). The rates found in the present study were very similar.

Our institute

However, the results of the present study were much worse than those published by our research group over the period 1970-1984.[13] In the 1970s and the 1980s, our ilio-inguinal lymph node dissection patients were given 10 days of bed rest with a Bohler Braun splint that held their hip and knee in flexion (Figure 2). This might explain the lower number of postoperative complications: wound infection 9%, wound necrosis 3% and seroma 17%. From the 1990s, we reduced the period of bed rest with a Bohler Braun splint to 5 days and in the year 2000, we abandoned the Bohler Braun splint in the postoperative phase. In our study, three factors appeared to significantly influence early complications: a BMI of >25, an age of >55 years and abandoning the use of a Bohler Braun splint. The only factor that changed between 1989 and 2007 was the duration/non-use of the Bohler Braun splint. In the study by Baas et al., in which it was standard practice to use a Bohler Braun splint for the first 10 days postoperatively, the median age was only 44 years compared to 55 years in the present study and only 17.2% had severe obesity (BMI >30) compared to the 53% moderate obesity (BMI >25) in the present study. These findings most likely explain the differences in the complication rates between the two retrospective studies performed at our institute.

Seroma %	Incision type	Bed rest days
17	Vertical	10
14	Vertical	?
5	Vertical	0
?	Separate	1
15	Vertical /Separate	0
23	Separate	?
21	Separate	0
46	Separate	7
20	Separate	3
?	Vertical /Separate	?
22	Vertical	10

Bed rest

The use of a Bohler Braun splint to nurse the hip and the knee in flexion during the period of strict bed rest has not been described in any of the other publications. In the studies by Serpell and van Akkooi, bed rest was prescribed for 7 and 3 days, respectively.[21,23] Karakousis et al. reported to prescribe flexion of the leg during bed rest and at night, but contrastingly, they mobilised patients directly after the operation and this resulted in a low risk of complications.[17] In the other studies, the patients were mobilised as soon as possible with a support stocking.[18,19,22] It has to be noticed that nowadays, it is also possible to elevate the legs with the hip and the knee in flexion by adjusting the position of modern hospital beds.

Patient characteristics

Advanced age (>50 years), obesity and smoking are known to increase the risk of wound complications.[14,28] Age over 55 years was only correlated with the combined variable (one or more early complications), which is probably the result of smaller numbers in the separate analysis. A longer duration of surgery might also contribute to a higher risk of complications.[28] We were unable to confirm the latter in the present study.

Surgical techniques

Different surgical techniques for performing an ilio-inguinal nodal dissection are known. Firstly, the ilio-inguinal node dissection can be performed in two different ways: either one straight incision with a skin ellipse and vertical transection of the inguinal ligament or two separate incisions above and below the groin, which was introduced by Baronofsky (Figure 1D).[13,24,26] The vertical incision is thought to cause minimal damage to the lymphatic vessels and therefore less risk of lymphoedema.[13] In contrast two separate incisions are thought to leave the groin free, which should reduce the risk of wound infection.[24,25] Sabel et al. did not find a difference in complication rates between the two different techniques. However, in their study, two incisions were only used in 15% of the ilio-inguinal node dissections.[20] Pearlman et al. and Sabel et al. did not report wound necrosis as a separate wound complication; van Akkooi et al. did not mention wound necrosis at all. The low incidences of wound necrosis in the studies mentioned before (Table 3) could suggest that using separate incisions may be less invasive.

Lymph node dissection after SLNB

Completion lymph node dissection after a positive SLNB may cause an increased risk of wound infection and postoperative oedema, possibly because two operations are performed on the groin within a very short period.[10]

The study by Sabel et al. described that patients who underwent a completion lymph node dissection after positive SLNB (n=132) had significantly less wound complications and less often lymphoedema than patients who underwent a therapeutic lymph node dissection for palpable lymph node metastases (n=80).[20] However, in this series 181 superficial nodal

dissections were performed and only 31 ilio-inguinal lymph node dissections were carried out. The indication for a deep nodal dissection was based on a positive Cloquet's node or intraoperative suspicion for iliac nodal disease. This might explain the differences with the current study.

Surgical volume

It has been suggested in some studies that experience and/or surgical volume are correlated with decreased morbidity; we were not able to confirm this in the present study.[29] It should be noted that surgical oncology fellows generally performed the operations under the supervision of an experienced surgical oncologist.

Consideration and conclusion

Therapeutic groin dissection for melanoma remains a surgical procedure with a high morbidity. Presently, more complications occur after therapeutic ilio-inguinal lymph node dissection than 20 years ago. To comply with our data there are two explanations for this difference: (1) nowadays patients have a higher BMI and (2) a Bohler Braun splint was not used anymore during strict bed rest for 10 days. The majority of patients had a BMI of >25, taking along a higher risk of pulmonary and thrombo-embolic complications.

For these patients early mobilisation is indicated. It has not been investigated so far whether long term immobilisation after an ilio-inguinal lymph node dissection to reduce the risk of wound healing complications is cost-effective. This study was performed retrospectively and therefore it is possible that there were differences in the reported complications in the medical records. Lymphoedema, as a complication of groin dissection was not included in this study, as the extent of this complication cannot be obtained from medical records, because lymphoedema should be identified clinically by measuring the volume of the leg. In a recent study the limb volume was measured, and slight oedema was found after a performed groin dissection.[10] Considering our results, we advise performing a groin dissection with one vertical ellipse shaped incision and strict postoperative bed rest of 5 days with the hip and the knee in flexion, to reduce tension on the wound and to facilitate healing of the transferred sartorius muscle. This regimen is followed by mobilisation with a tailor made support stocking. However, we are aware that only a randomized controlled trial can determine which technique (vertical vs separate incisions) and which postoperative treatment cause the least morbidity. If the patient has recently undergone SLNB and required a completion lymph node dissection, prophylactic antibiotics are indicated during surgery to reduce the risk of wound infection. Whether perioperative antibiotics in therapeutic groin dissections are valuable, can also only be evaluated in a well designed prospective trial.

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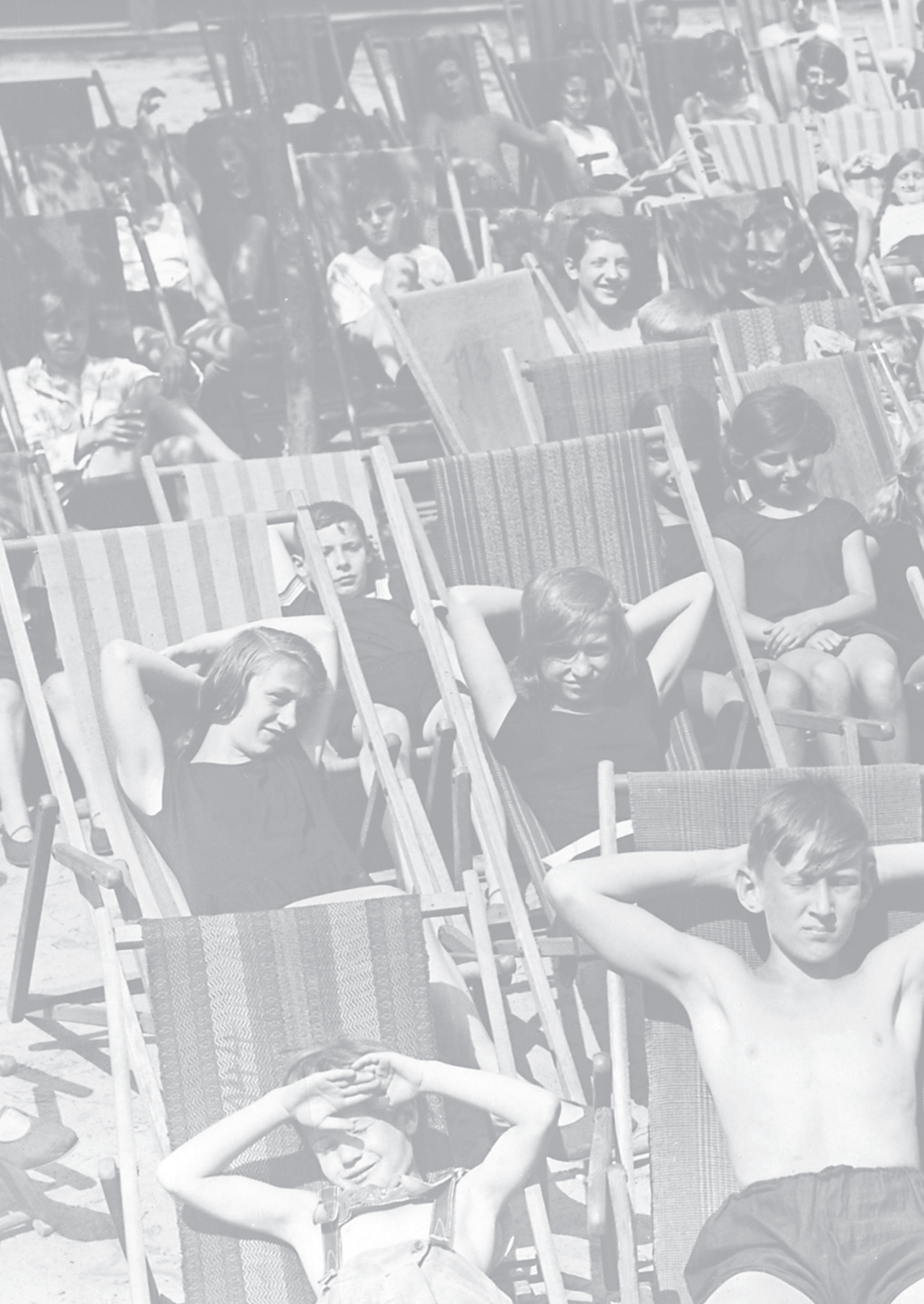
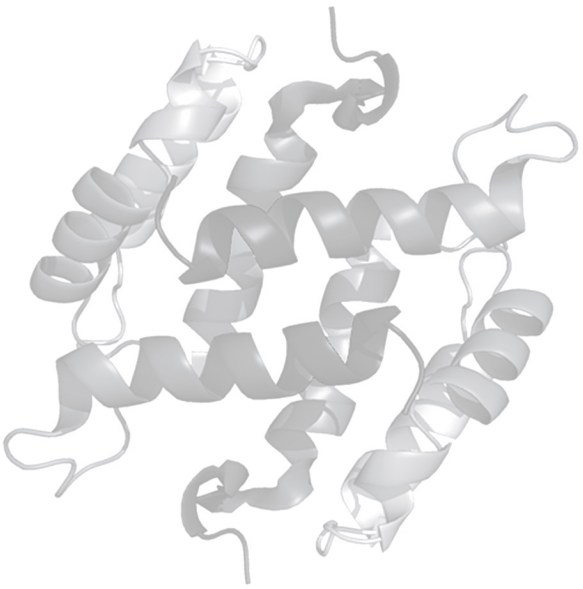


Part II

Prognostic markers in melanoma

4



Breslow thickness in the netherlands: a population-based study of 40,880 patients comparing young and elderly patients

S Kruijff, E Bastiaannet, AB Francken, M Schaapveld, M van der Aa, HJ Hoekstra | Submitted

ABSTRACT

Background: Melanoma incidence has increased rapidly in the last decades, and predictions show a continuing increase in the years to come. The aim of this study was to assess trends in melanoma incidence, Breslow thickness (BT), and melanoma survival among young and elderly patients in the Netherlands.

Methods: Patients diagnosed with invasive melanoma between 1994 and 2008 were selected from the Netherlands Cancer Registry (NCR). Incidence (per 100,000) over time was calculated for young (<65 years) and elderly patients (≥65 years). Distribution of BT for young and elderly males and females was assessed. Regression analysis of the log-transformed BT was used to assess changes over time. Relative survival was calculated as the ratio of observed survival to expected survival.

Findings: Overall 40,880 patients were included (42.3% male, 57.7% female). Melanoma incidence increased more rapidly among the elderly (5.4% Estimated Annual Percent Change (EAPC), p <0.0001) than among younger patients (3.9% EAPC, p <0.0001). The overall BT declined significantly over time (p <0.001). Among younger patients, BT decreased for almost all locations. Among elderly males, BT decreased for melanomas in the head and neck region (p=0.001) and trunk (p <0.001), but did not decrease significantly for the other regions. Among elderly females, BT only decreased for melanomas at the trunk (p=0.01). The relative survival of elderly patients was worse compared with that of younger patients (p <0.001).

Interpretation: Melanoma incidence increases more rapidly for elderly than for younger patients and the decline in Breslow thickness is less prominent among elderly patients than among young patients. Campaigns in the Netherlands must focus more on early melanoma detection in the elderly.

INTRODUCTION

The incidence of cutaneous melanoma has increased in the last decades and estimates predict a continuing increase in the coming years.[1] High incidence rates are found in populations of predominantly European origin, with white populations in Australia and New Zealand having the highest incidence rates and Asian and black populations having the lowest.[2]

In Australia, despite rising incidence rates, mortality rates seem to have reached a plateau, possibly due to the fact that most newly diagnosed melanomas are thinner melanomas, which usually do not lead to death.[3] In the Netherlands, the total number of melanoma patients is expected to increase from 2,400 patients in 2000 to 4,800 patients in 2015, and has already reached 3,500 patients in 2005.[1] In contrast with Australia, in the Netherlands this increase in incidence rate is accompanied by a rising mortality rate. This finding seems to contradict the earlier hypothesis that rising incidence in the Netherlands might be the result of increased awareness only.[4]

Increased exposure to the sun and sunburns during childhood are still the most important risk factors for melanoma. A recent meta-analysis has also shown that significantly increased melanoma risk is associated with sun bed and sun lamp exposure.[5,6]

Various studies have already shown that the melanoma incidence and mortality among elderly individuals (>65 years) is growing rapidly.[7,8] As the geriatric population increases in most industrialized nations (life expectancy almost doubled during the last century), melanoma will become an important health issue for the elderly age group in this century.[9-11]

The most important predictors of mortality, included in the American Joint Committee on Cancer (AJCC) staging, are tumor characteristics such as Breslow thickness (BT), ulceration, mitotic rate, and the presence of metastases.[12,13] However, BT has been shown to be the single most important prognostic factor for survival.[12-14] Therefore, the aim of this study was to assess incidence rates and differences in BT and survival between young and elderly patients with invasive melanoma in the Netherlands between 1994 and 2008.

METHODS

Patients

Patients diagnosed with invasive melanoma between 1994 and 2008 were selected from the Netherlands Cancer Registry (NCR), which covers all patients in the Netherlands. The nationwide Dutch network and registry of histopathology and cytopathology (PALGA) regularly submits reports of all diagnosed malignancies to the cancer registries. The national hospital discharge databank, which receives discharge diagnoses of admitted patients from all Dutch hospitals, completes case ascertainment. After notification, well-trained registry personnel collect data on diagnosis, staging, and treatment from the medical records, including pathology and surgery reports, using the registration and coding manual of the Dutch Association

of Comprehensive Cancer Centers. For the present study, patients with their first primary melanoma were selected. Stage was defined according to the AJCC staging system. The study was approved by the local medical ethics committee.

Statistical analysis

Incidence of invasive melanoma per 100,000 Dutch individuals was calculated for young (younger than 65 years) and elderly (65 years and older) patients in the Netherlands. Because BT measurement was missing for 9.5% of patients, we used multiple imputation (5 imputations) to generate a complete dataset. A model was built that included sex, location, stage, year of diagnosis, age, and status as predictors to assess the patients with missing BT measurements. Median BT was calculated according to sex, age, and localisation, as well as Breslow distribution over time. Because the TNM Classification of Malignant Tumors changed in 2003, stage distribution before and after 2003 were not comparable and therefore a composite stage variable was not used in the analysis. BT was divided according to the AJCC staging system into the following size categories: ≤1.0 mm, 1.0-2.0 mm, 2.0-4.0 mm, and >4.0 mm. Patients with an unknown primary were excluded from the analysis.

BT distributions for young and elderly males and females were assessed over time. Because BT is skewed toward smaller tumours (most melanomas are ≤1.0 mm), BT was log-transformed. A regression analysis was modeled with log(Breslow) to assess the changes over the years of incidence according to age, sex, and location.

Vital status and date of last follow-up were established either directly from the patient's medical record or through linkage of cancer registry data with municipal population registries (follow-up until January 1, 2009), which record information on vital status. Relative survival is the preferred way to describe the prognosis of (elderly) patients with melanoma, as it takes into account the risk of dying from causes other than melanoma. Relative survival was calculated as the ratio of the observed survival among cancer patients to the survival that would have been expected based on the corresponding general population (with respect to age, sex, and year of diagnosis). National lifespan tables were used to estimate expected survival (Ederer II method). Relative Excess Risks of death (RERs) for year of diagnosis were estimated using a Poisson regression model.

RESULTS

This study includes 40,880 patients diagnosed with melanoma between 1994 and 2008. Table 1 shows the characteristics of the melanoma patients in the Netherlands between 1994 and 2008 of which 42% were male and 58% were female. Median age was 54 years (range 0-105 years). The trunk was the most frequently involved melanoma site (36%). From 1994 to 1996, 5,907 new melanoma patients were diagnosed (15%), increasing to 11,023 (27%) in the period from

2006 to 2008. Almost one-half (49%) of newly diagnosed melanoma patients had a BT ≤1.0 mm, while for 12% the BT was >4.0 mm for thick melanomas (Table 1).

TABLE 1 Characteristics of melanoma patients in the Netherlands, 1994-2008

Characteristic		Original data		Multiple imputation
		N	%	%
Sex	Male	17 305	42.3	42.3
	Female	23 575	57.7	57.7
Age	≤40 years	9378	23.0	23.0
	41-54 years	11 506	28.1	28.1
	55-64 years	8217	20.1	20.1
	≥65 years	11 779	28.8	28.8
Location	Head & neck	5612	13.7	13.7
	Trunk	14 693	35.9	35.9
	Upper extremities	8163	20.0	20.0
	Lower extremities	12 246	30.0	30.0
	Other	166	0.4	0.4
Year	1994-1996	5907	14.5	14.5
	1997-1999	6720	16.4	16.4
	2000-2002	7779	19.0	19.0
	2003-2005	9451	23.1	23.1
	2006-2008	11 023	27.0	27.0
Breslow category	≤1.0 mm	19 325	47.3	48.7
	1.0-2.0 mm	8622	21.1	22.7
	2.0-4.0 mm	5598	13.7	16.7
	>4.0 mm	3451	8.4	11.9
	Unknown	3884	9.5	

Melanoma incidence increased more rapidly among elderly patients (≥65 years) than among younger patients (Figure 1). In 1994, melanoma incidence was 189 among elderly patients and 144 among younger patients, and in 2008 these numbers increased to 362 and 241, respectively. The Estimated Annual Percentage Change (EAPC) for young patients was 3.9% (p <0,0001) and for the elderly the EAPC was 5.4% (p <0,0001). Especially since 2002, melanoma incidence appears to be increasing at a faster rate among the elderly than among their younger counterparts.

Figure 2 shows the proportion of patients that were diagnosed with thin, median, and thick melanomas over time. The percentage of young patients with a thick melanoma (Breslow >4.0 mm) has declined over time. In 1994, 16% of males and 10% of females had a thick melanoma; by 2008, these incidences had declined to 9% and 5%, respectively (p <0.001).

A decline was also observed among the elderly population, and especially among elderly females, although it was not as precipitous as among young patients. In 1994, 25% of elderly

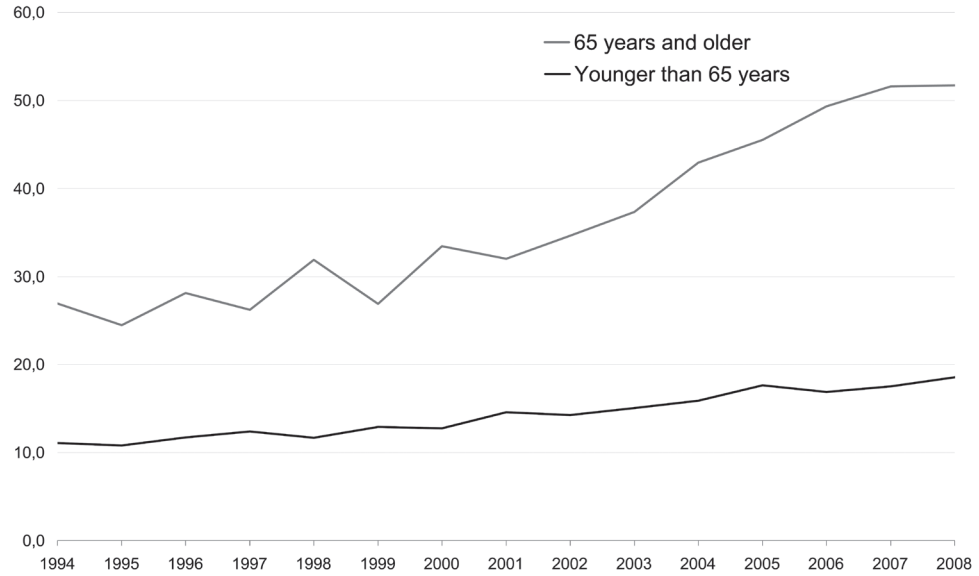


FIGURE 1 Incidence of invasive melanoma among young and elderly patients

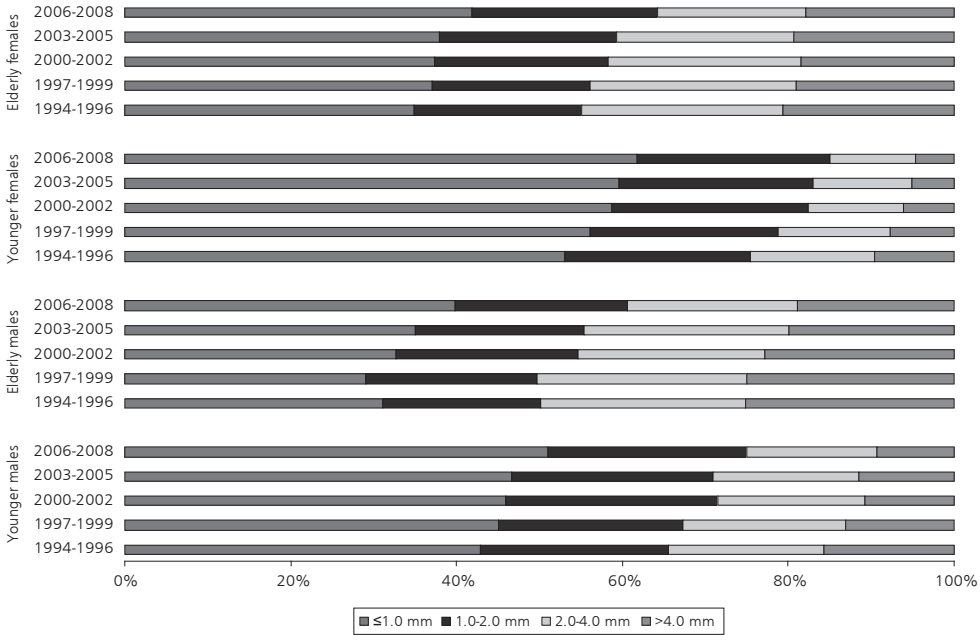


FIGURE 2 Distribution of Breslow thickness over time among young and elderly patients

males and 20% of elderly females had a thick melanoma, compared to, respectively, 19% and 18% in 2008 ($p < 0.001$). Although younger patients generally had thinner melanomas than elderly patients, the proportion of thin melanomas increased in both age groups. In 1994, 43% of young males and 53% of young females had a thin melanoma (Breslow ≤1.0 mm); these numbers

TABLE 2 Changes in Breslow thickness (log transformation) over time according to age, sex, and lesion location

			Regression coefficient (95% CI)		p-value
Young	Males	Head & neck	-0.43	-0.66 to -0.20	<0.001
		Trunk	-0.26	-0.37 to -0.14	<0.001
		Upper extremities	-0.36	-0.56 to -0.16	<0.001
		Lower extremities	-0.16	-0.41 to -0.1	0.2
	Females	Head & neck	-0.21	-0.51 to 0.1	0.2
		Trunk	-0.31	-0.45 to 0.17	<0.001
		Upper extremities	-0.51	-0.70 to -0.32	<0.001
		Lower extremities	-0.33	-0.46 to -0.19	<0.001
Elderly	Males	Head & neck	-0.35	-0.56 to -0.14	0.001
		Trunk	-0.42	-0.60 to -0.25	<0.001
		Upper extremities	-0.20	-0.47 to 0.1	0.2
		Lower extremities	-0.28	-0.61 to 0.1	0.1
	Females	Head & neck	-0.18	-0.44 to 0.1	0.2
		Trunk	-0.36	-0.62 to -0.10	0.01
		Upper extremities	-0.15	-0.37 to 0.06	0.2
		Lower extremities	-0.13	-0.32 to 0.1	0.2
Overall	Males & females	All locations	-0.25	-0.30 to -0.20	<0.001

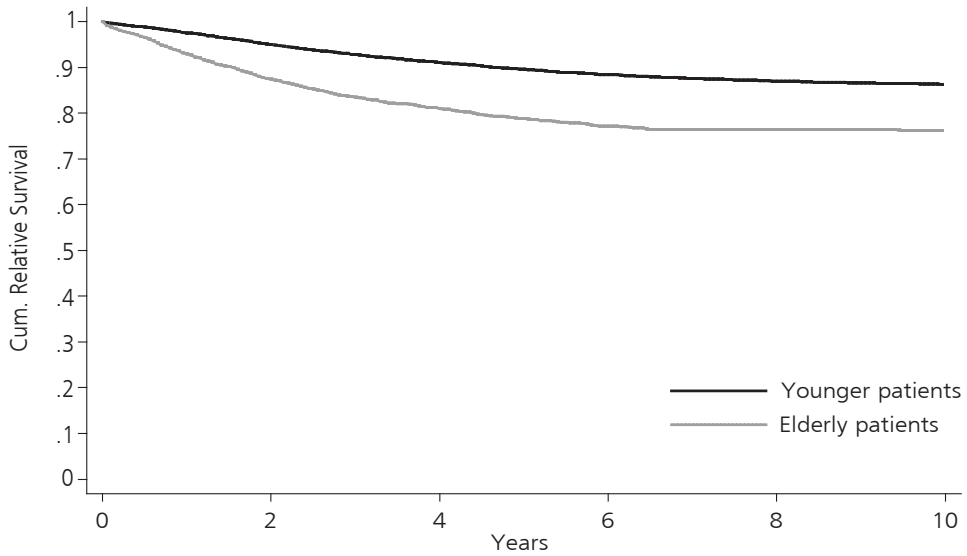


FIGURE 3 Cumulative relative survival among young and elderly melanoma patients

TABLE 3 Relative Excess Risk (RER) for elderly (65 years and older) versus young patients, stratified according to Breslow category and lesion location

Stratification		Adjusted RER* elderly vs. young	p-value (adjusted*)
Breslow thickness	≤1.0 mm	1.9 (1.4-2.6)	<0.001
	1.0-2.0 mm	1.6 (1.3-2.0)	<0.001
	2.0-4.0 mm	1.6 (1.4-1.9)	<0.001
	>4.0 mm	1.7 (1.5-1.9)	<0.001
Location	Head & neck	1.7 (1.5-2.0)	<0.001
	Trunk	1.7 (1.5-1.8)	<0.001
	Upper extremities	1.5 (1.3-1.8)	<0.001
	Lower extremities	2.0 (1.8-2.4)	<0.001

*adjusted for sex, year, N, M, Breslow thickness, and location (when not stratified for that factor

increased to 51% and 62%, respectively, in 2008. In 1994, 31% of elderly males and 34% of elderly females had a thin melanoma, compared with 40% and 42%, respectively, in 2008 (p <0.001).

Table 2 depicts the changes in BT over time according to age, sex, and location. BT significantly decreased over time for head and neck, trunk, and upper extremities among young males (p <0.001), and for trunk, upper extremities, and lower extremities among young females (p <0.001). However, among elderly males a significant decrease in BT over the years was only observed for melanomas in the head and neck (p=0.001) and trunk (p <0.001), and for elderly females a decrease in BT is noted only on the trunk (p=0.01).

Relative survival is worse among elderly patients than among patients younger than 65 years (RER 2.1 for elderly patients; 95% CI 2.0–203; p <0.001). Adjusted for BT, location, sex, year, lymph node status, and distant metastases, RER remained higher among the elderly (RER 1.7; 95% CI 1.6–1.8); p <0.001). The RER for elderly (65 years and older) versus young patients was significantly worse for the elderly in all BT categories (≤1.0 mm, 1.0–2.0 mm, 2.0–4.0 mm, > 4.0 mm) and locations (head and neck, trunk, upper extremities, and lower extremities).

DISCUSSION

Melanoma incidence increases more rapidly among the elderly than among younger individuals. Additionally, rising incidence is accompanied by decreasing Breslow thickness (BT) mainly in younger individuals. The decrease in melanoma Breslow thickness was less prominent among elderly individuals than younger individuals, and elderly individuals are still frequently diagnosed with thick melanomas. For most melanoma locations, BT in the elderly has not been declining. Especially in elderly men, the proportion of thick melanomas has declined only minimally. The larger proportion of thick melanomas only partly explains the worse survival among elderly melanoma patients when compared with the younger

population; even within several strata of thickness, survival remains worse among the elderly than among younger patients.

In general, for males and females young and old, the percentage of thin melanomas increased over the period 1994-2008. This is in accordance with trends in several population-based studies in various other industrialized countries. Lashithiotakis et al, who studied 1980 patients diagnosed with melanoma in southern Germany in the period 1976-2003, observed that median BT decreased steadily during that period ($p < 0.01$).[15] Buettner et al studied all 45,483 melanoma patients diagnosed between 1976 and 2000, and also noted a significant decrease in median BT ($p < 0.0001$).[16]

In our study, the highest proportion of thin melanomas was observed in young females, a finding that is shared by many other studies.[17] Women are likely more alert to skin changes, resulting in thinner melanomas at diagnosis. Various studies have also shown that female patients were more likely to discover their melanoma or melanoma recurrence by themselves, even when adjusted for localisation.[18,19]

A trend towards prognostic ally more favorable melanomas has been described in the United States, Australia, and Europe, and can most likely be attributed to increased awareness in the population leading to earlier detection and treatment.[20,21]

The most prominent finding in our data was that elderly patients present with significantly thicker melanomas, a phenomenon described in both Europe and Australia.[9-11, 22-25] The question arises: why do elderly individuals fail so comprehensively to follow the trend towards early detection of melanoma that has been observed for the younger age group? Elderly patients have more difficulty recognizing or detecting changes in their skin, and therefore delay visits to their general practitioner.[22] Difficulty in detecting melanoma could be related to deteriorating vision, increased isolation because of loss of a partner, decreased flexibility, development of skin lesions in locations that may be difficult to see, and reduced awareness. Another difficulty for elderly patients is often the development of multiple seborrhoeic keratoses that may appear similar to melanoma. Additionally, melanomas are found on the back in 48% of cases in elderly men, which does not facilitate detection.[11]

Despite physical deterioration, it has been demonstrated that older people should be very well able to detect skin changes associated with early melanoma.[25] This finding does suggest that public education campaigns and increased alertness in the context of elderly care might be useful to encourage the detection of skin changes. Although previous educational campaigns have been effective at promoting awareness, they have generally been focused on younger individuals, with a continuous lack of attention directed toward elderly individuals.[11]

Various studies report age as an independent prognostic factor, perhaps presenting a surrogate for declining host defence mechanism associated with advancing age.[9] Treatment outcome is also influenced negatively, because older patients often receive a delayed diagnosis, incorrect staging, suboptimal surgery, and lower standard radio- or chemotherapy, possibly due to a reluctance to treat aggressively because of patients' co-morbidities or disabilities.[9,10] Numerous studies have already demonstrated that elderly patients, especially those over 60 years of age, have a lower disease-specific survival rate.[26-28]

In Australia, skin cancer has been identified as a national health priority, and awareness in the population is at a high level thanks to health-promotion activities.[29] In contrast, some studies demonstrate that melanoma awareness in Europe is not at such a high level, and focus has been mainly on young individuals.[7,22,30] This hypothesis might explain the high percentage of thick melanomas in the elderly, which (given the rising incidence) could have been caused by sun exposure in the era before melanoma became a health priority.

In conclusion, melanoma incidence has increased much more rapidly among the elderly than among younger individuals. Additionally, in contrast to younger patients, in which rising incidence is accompanied by a decline in BT, elderly patients still have the highest proportion of thick melanomas and the decline in BT among the elderly is not present at all melanoma locations. Campaigns in the Netherlands should focus more on early detection of melanoma in the elderly.

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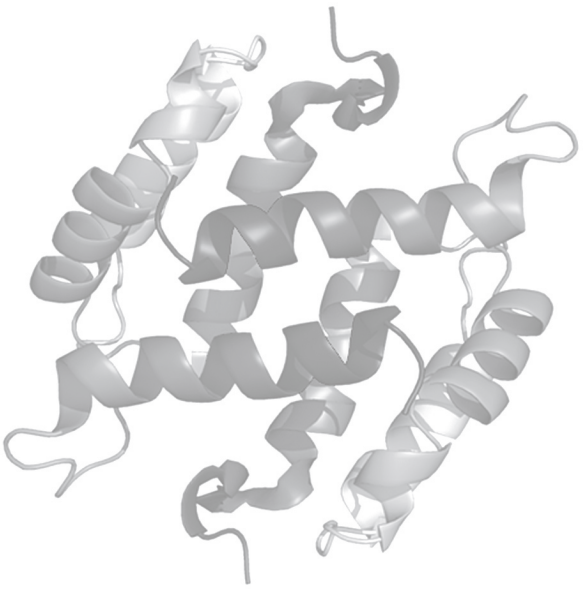

S-100B concentrations predict disease-free survival in stage III melanoma patients

S Kruijff, E Bastiaannet, AC Muller Kobold, RJ van Ginkel, AJH Suurmeijer, HJ Hoekstra | Ann Surg Oncol. 2009;16:3455-62.

ABSTRACT

Background: Elevation of the tumor marker S-100B in melanoma patients is a highly specific indicator of recurrence.

Materials and methods: The role of S-100B in disease free survival (DFS) was evaluated in stage III melanoma patients (staged with fluorodeoxyglucose positron emission tomography [FDG-PET] and computed tomography [CT]) with palpable lymph node metastases who underwent therapeutic lymph node dissection. S-100B and LDH were measured on the day before surgery (d= -1) and on days 1, 2, and 7 postoperatively. Multivariate logistic regression was used to study factors associated with preoperative elevation of S-100B. Univariate (log-rank test) and multivariate (Cox regression) survival analyses were performed to identify factors associated with DFS.

Results: Between 2004 and 2008, 56 patients (median age 57, range 24-93) years, 27 males (48%) and 29 females (52%) entered the study. Preoperative S-100B elevation was found in 27 patients (48%) and elevated LDH in 20 patients (36%). No association was found between these two markers at any time. Multivariate analysis showed that elevated S-100B preoperatively (hazard ratio [HR] 2.7, p=0.03) was associated with DFS. S-100B elevation was associated with increased tumor size (odds ratio [OR] 3.40; p=0.03).

Conclusion: Elevated S-100B preoperatively in patients with optimally staged clinical stage III melanoma is associated with decreased disease-free survival. S100-B could be used as a prognostic marker in the stratification of new adjuvant trials to select stage III melanoma patients for adjuvant systematic treatment.

INTRODUCTION

The incidence of melanoma is still increasing in the Netherlands. Between 1989 and 2003, the incidence increased from 9.5 to 13.7/100,000 in men and from 13.4 to 18.5/100,000 in women.[1] About 90% of the patients had stage I or II melanoma.[2] After surgical treatment, 20-28% of melanoma patients present with locoregional recurrence or regional dermal metastases, 26-60% with regional recurrences, and 15-50% with distant metastases.[3] Despite improved public awareness as well as early diagnosis and treatment, mortality from melanoma increased from 2.5 to 3.6/100,000 in men and from 2.0 to 2.4/100,000 in women in the Netherlands between 1989 and 2003.[4] It is impossible to predict the metastatic behavior of melanoma in individual patients. At present, the Multicentre Selective Lymphadenectomy Trial (MSLT I) is investigating whether sentinel lymph node biopsy reduces the risk of locoregional metastases and improves survival.[4] In patients with clinical stage III melanoma (palpable lymph node metastases), 5-year survival varied between 26 and 67%.[5] Recent staging studies with whole-body fluorodeoxyglucose positron emission tomography (FDG-PET) and/ or spiral computed tomography (CT) in patients with clinical stage III melanoma have shown that about one quarter of these patients had distant metastases.[6,7] Furthermore, the uptake of FDG in the lymph node metastases in patients with clinical stage III melanoma proved to be of prognostic value.[8] FDG-PET or spiral CT improved the staging of clinical stage III melanoma and led to better selection of patients for therapeutic lymph node dissection.[6,7] Serum lactate dehydrogenase (LDH) had high specificity for melanoma, but low sensitivity. It was an independent prognostic factor in stage IV melanoma.[9-12] Increased S-100B concentrations were first detected in melanoma patients in 1980.[13] Later, S-100B was found to be a serological tumor marker for melanoma and is mostly increased in stage III and IV.[14,15] S-100B is a 21 kilodalton (kDa) protein that was first isolated from the central nervous system in vertebrates. The name is derived from its 100% solvency in saturated ammonium sulfate with neutral pH. It has calcium-binding properties and as a dimer, consists of two isomers a and b. All possible combinations can occur (S-100aa, S-100ab, S-100bb).[15] The S-100B protein is of neuroectodermal and mesodermal origin and is expressed in various parts of the body. S-100B is chiefly found in glial cells and Schwann cells.[16] Serum S-100B can be measured with an immunoradiometric assay (Sangtec 100 IRMA/RIA assay) or with an immunoluminometric assay (LIA Mat Sangtec 100). This study addressed the question whether the perioperative measurement of the tumor markers S-100B has prognostic value in FDG-PET and spiral CT staged patients with stage III melanoma who are selected for therapeutic lymph node dissection.

MATERIALS EN METHODS

Patients

Melanoma patients, earlier staged AJCC I and II, now presenting with clinically and cytologically proven regional nodal metastases of melanoma (AJCC stage IIIb and IIIc) were staged with whole-body FDG-PET (Siemens ECAT EXACT HR + scanner) and with 64-slice spiral CT (Siemens Somatom Sensation). Sentinel node positive patients and patients with regional dermal metastases were excluded for this study. The patients whose test results were negative for distant metastases were eligible for therapeutic lymph node dissection with curative intent. Nodal metastases of ≥3 cm and/or C 3 positive lymph nodes and/or extra nodal growth received adjuvant radiotherapy (20 9 2.4 Gy).[17,18] LDH and S-100B were measured preoperative (day -1) and postoperative days 1, 2, and 7. The study was approved by the Medical Ethical Committee of the UMCG.

Methods

LDH was analyzed routinely by means of Roche Modular (Hitachi) with an enzymatic activity measurement; normal values of LDH were considered to be > 250 U/L.

Concentrations of S-100B were measured using Sangtec 100 immunoassay on the Advantage (Nichols). The S-100B half-life is estimated to be 30 min.[19] Approximately 10 times the half-life after lymph node dissection, S-100B values should reduce to the minimum possible. When tumor cells are still present the release of S-100B will continue or may even increase. Therefore, S-100B levels were measured on days -1, 1, 2, and 7. Levels were calculated on the basis of a calibration curve and checked against internal standards with a known concentration of S-100B. In this assay, a cut-off point of 0.15 μg/L was set. The reference values for the S-100B assay (Liason Sangtec 100) were established by analysis of S-100B values of 120 healthy men and women according to the CLSI C28A2 guideline. Concentrations within this range were considered to be normal, whereas concentration above this range were considered to be elevated. A postoperative increase of S-100B was defined as any increase on the postoperative days 1, 2, or 7 (day 1 as baseline value, elevation on day 2 compared with day 1, and/ or elevation on day 7 compared with day 2).

Statistics

Data were analyzed with STATA version 10.0. Logistic regression analysis was used to assess factors associated with elevated S-100B concentrations. Factors associated with disease-free survival (DFS) were analyzed with standard univariate (log-rank test) and multivariate (Cox proportions hazard) survival methods.

TABLE 1: Characteristics of 56 AJCC stage III melanoma patients

Variable	No. of patients (%)
Gender	
M	27 (48.2)
F	29 (51.8)
Median age (years)	57.0
Localisation primary tumour	
Trunk	24 (42.8%)
Head & neck	3 (5.3%)
Upper extremities	7 (12.5%)
Lower extremities	22 (55.8%)
Unknown primary	0
Breslow thickness primary tumour	
T1 (<1.00 mm)	7 (12.5%)
T2 (1.00-2.00 mm)	20 (35.7%)
T3 (2.00-4.00 mm)	21 (37.5%)
T4 (>4.00 mm)	8 (14.2%)
Clark level primary tumour	
1-3	24 (42.6%)
4-5	31 (55.4%)
unknown	1 (1.8%)
Localisation lymph nodes	
neck	6 (10.7%)
axilla	20 (35.7%)
groin	30 (53.5%)
No. of nodes removed	
< 15	27 (48.2%)
≥ 15	29 (52.8%)
No. of positive nodes	
< 2	36 (64.3%)
≥ 2	20 (35.7%)
Extranodal growth	
Yes	24 (42.6%)
No	42 (56.4%)
Tumour size	
< 3 cm	28 (50%)
≥ 3 cm	28 (50%)
Ulceration primary tumour	
Yes	13 (23.2%)
No	43 (76.8%)
Comorbidity	
Yes	16 (28.6%)
No	40 (71.4%)

RESULTS

Patients

In the period from January 2004 to January 2008, 56 patients, 27 males (48%) and 29 females (52%), median age 57 (range 24-93) years underwent therapeutic lymph node dissection (Table 1). Preoperatively, 27 patients had elevated S-100B concentrations (48%), while 20 patients had increased LDH levels (36%). The range on day -1, 1, 2, and 7 for S-100B was 0.03–23.3, 0.02-1.5, 0.03-0.82,0.01-0.22 and for LDH 109-428, 106-816, 108-335, 115-802. No associations were found between S-100B and LDH levels on days -1, 1, 2, and 7 (p=0.45, p=0.87, p=0.84, and p=0.90, respectively). The S-100B concentration normalized postoperatively in 22 of the 27 patients (81.4%) and the LDH in 18 of the 20 patients (90%).

Preoperative S-100B elevation was associated with increased lymph node size (odds ratio [OR] 3.4, p=0.03),however not significantly with extranodal growth (p=0.8), patients (43%), of which 17 patients were diagnosed with distant metastasis and 7 patients with a regional recurrence. As shown in Table 3, DFS was not associated with sex, localization of the lymph node, or the size of the lymph nodes. Two-year DFS in patients with elevated preoperative S-100B concentrations was 34% versus 61% for patients without elevated preoperative S-100B concentrations (hazard ratio [HR] 2.6, p=0.03). In the patients whose S-100B increased postoperatively, 2-year DFS was 30% versus 51% for patients without a postoperative S-100B increase (HR 2.0, p=0.1) (Figure 1). In multivariate analysis (model with positive lymph nodes, extranodal growth, preoperative and postoperative S-100B and substage IIIb or IIIc), extranodal growth (HR 0.4, p=0.05), and elevated preoperative S-100B concentrations (HR 2.6, p=0.03) were significantly associated with decreased DFS. Patients with increasing postoperative S-100B serum levels showed a trend toward statistical significance when correlated to DFS (HR 2.6, p=0.07).

TABLE 2: Logistic regression analysis on elevated S100-b in stage III melanoma patients

Variable	Elevated S-100b %	OR	p-value
Sex			
Male	51.9	Ref	
Female	44.8	0.75 (0.26-2.16)	0.599
Age			
< 55 years	44.0	Ref	
≥ 55 years	51.6	1.36 (0.47-3.91)	0.571
Positive lymph nodes			
≤ 2	44.4	Ref	
> 2	55.0	1.52 (0.51-4.59)	0.450
Extranodal growth			
Yes	52.2	Ref	
No	48.4	0.86 (0.29-.2.53)	0.783
Tumour size			
< 3	35.7	Ref	
≥ 3	65.4	3.40 (1.11-10.4)	0.032

TABLE 3: Disease-free survival (DFS) of AJCC stage III melanoma patients

Variable		%	2 year DFS (%)	Univariate analysis		Multivariate analysis	
				HR (95% CI)	p-value	HR (95% CI)	p-value
Sex	Male	48.2	45.1	Ref			
	Female	51.8	47.2	1.16 (0.52-2.59)	0.713		
Age (yrs)	< 55	44.6	46.7	Ref			
	≥ 55	55.4	46.7	1.10 (0.49-2.48)	0.821		
Co-morbidity	Yes	28.6	32.3	Ref			
	No	71.4	54.9	0.82 (0.36-1.89)	0.649		
Clark level	1-3	44.6	46.1	Ref			
	4-5	55.4	45.9	0.81 (0.36-1.81)	0.608		
Breslow thickness	< 2	48.2	52.7	Ref			
	≥ 2	51.8	40.7	1.30 (0.58-2.91)	0.519		
Localisation LN	Neck	10.7	83.3	Ref			
	Axilla	35.7	50.9	3.29 (0.41-26.4)			
	Groin	53.6	35.8	4.94 (0.65-37.5)	0.237		
No. of LNs	< 15	48.2	50.8	Ref			
	≥ 15	51.8	43.8	0.93 (0.41-2.11)	0.869		
Positive LNs	≤ 2	64.3	63.5	Ref		Ref	
	> 2	35.7	22.5	3.32 (1.47-7.50)	0.004	1.98 (0.81-4.87)	0.135
Ulceration	Yes	76.8	30.8	Ref			
	No	23.2	50.8	1.59 (0.66-3.85)	0.303		
Extranodal growth	Yes	42.6	28.4	Ref		Ref	
	No	57.4	57.9	0.37 (0.16-0.84)	0.018	0.32 (0.13-0.81)	0.015
LN size (cm)	< 3	49.0	53.2	Ref			
	≥ 3	51.0	34.5	1.45 (0.65-3.25)	0.365		
Pre-operative S100-b	Normal	51.8	60.6	Ref		Ref	
	Elevated	48.2	34.3	2.56 (1.09-6.00)	0.030	2.69 (1.13-6.44)	0.026
Peri-operative S100-b	No increase	82.1	51.3	Ref		Ref	
	Increase	17.9	30.0	2.00 (0.83-4.81)	0.124	3.04 (1.14-8.13)	0.027

LN=Lymph node

DISCUSSION

This is the first study that demonstrates a potential prognostic value of preoperative S-100B concentrations in patients who underwent a therapeutic lymph node dissection after preoperative staging with FDG-PET and CT. According to the literature, the proportions of patients with elevated S-100B concentrations were 0-9% in stage I/II, 5-98% in stage III, and 40-100% in stage IV melanoma (Table 4). The highest S-100B concentrations were found in patients with liver and/or skeletal metastases.[20,21] Serum S-100B concentrations are therefore correlated with the clinical stage of the disease. The aim of this study was to evaluate the

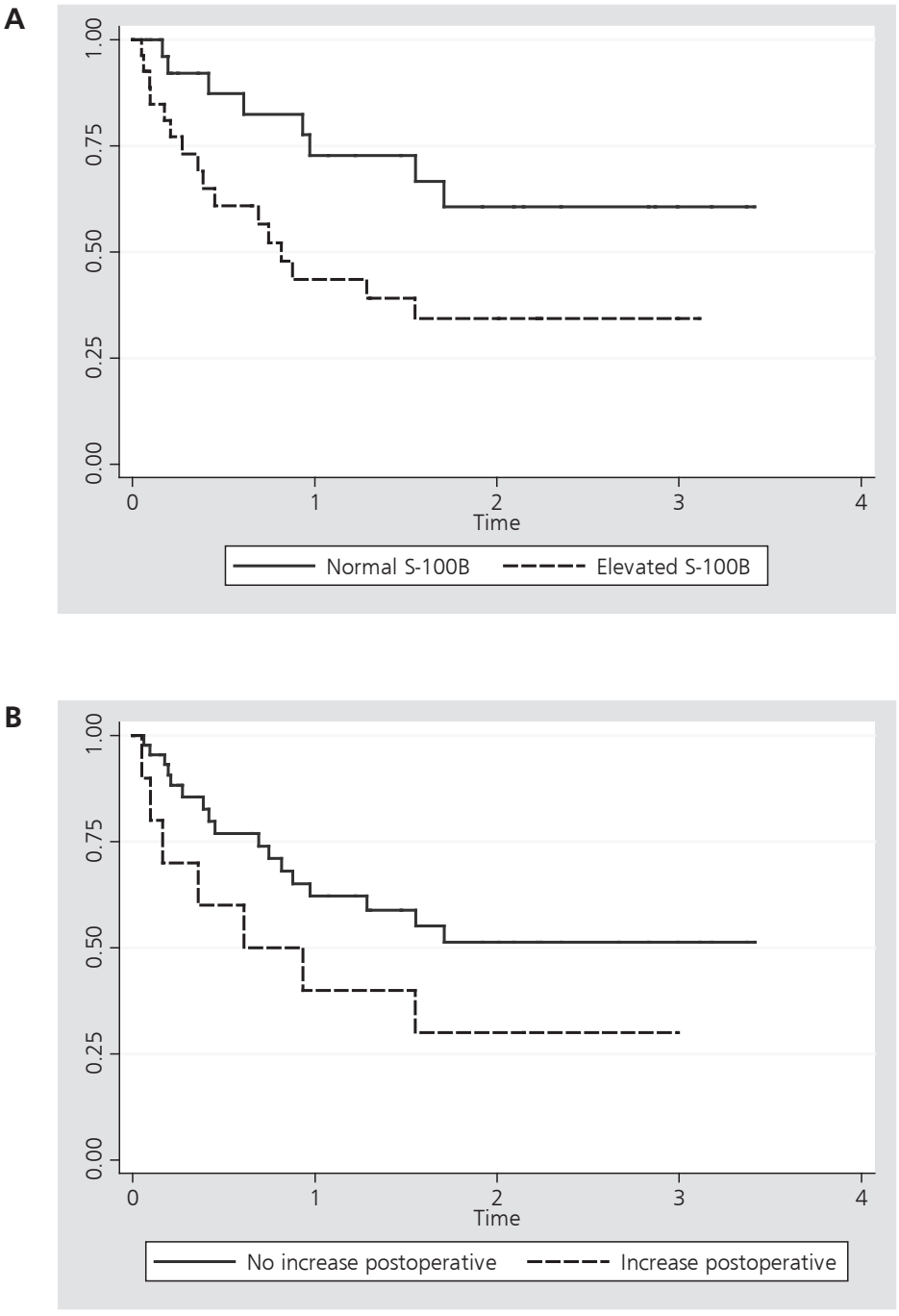

FIGURE 1 Kaplan-Meier curve for preoperative S-100B and disease-free survival of AJCC stage III melanoma patients. **A** Elevated preoperative S-100B (34% 2-year DFS) versus not elevated (61% 2-year DFS) levels are associated with a decreased disease-free survival (p=0.03). **B** Increasing postoperative S-100B levels (30% 2-year DFS) versus not increasing (51% 2-year DFS) (p=0.1)

TABLE 4: Review literature S100-B values above cut-off point in melanoma patients

Reference	N (patients)	Assay	Cut- off Value	Stage I/II	Stage III	Stage IV
Guo et al. 1995[12]	126	IRMA	0.15	1%	9%	74%
Miliotes et al. 1996[34]	67	IRMA	0.05	47.8%	-	-
Abraha et al. 1997[35]	97	IRMA	0.2	9%	82%	-
Bosserhof et al. 1997[36]	112	IRMA	0.15	0%	62%	-
Buer et al 1997[21]	99	IRMA	3.00	-	-	22.2%
Henze et al. 1997[26]	73	IRMA	0.3	4%	21%	79%
Tofani et al. 1997[37]	53	IRMA	0.5	0%	55%	-
Schultz et al. 1998[38]	84	IRMA	0.3	0%	31%	69%
Bonfrer et al 1998[39]	251	LIA	0.16	I/II:1%	-	III/IV: 79%
Seregni et al. 1998[40]	438	IRMA	0.2	I:4.2%,II:5.3%	38.5%	-
Hauschild et al. 1999[41]	412	IRMA	0.2	2%	19%	68%
Curry et al 1999[19]	147	IRMA/LIA	0.2 / 0.12	-	23%/47.5%	-
Berking et al. 1999[42]	352	LIA	0.2	7%	8%	48%
Jackel et al. 1999[14]	276	LIA	0.12	6%	5%	48%
Kaskel et al. 1999[43]	570	LIA	0.11	9%	94%	-
Brouard et al. 2000[44]	122	LIA	0.09	0%	54%	84%
Vuoristo et al 2000[45]	50	LIA	0.12	-	-	64.0%
Jury et al. 2000[46]	214	LIA	0.2	7%	29%	92%
Mohammed et al. 2001[18]	68	LIA	0.2	-	-	73.5%
Martenson et al. 2001[17]	727	LIA	0.10	12%	-	III/IV: 72%
Juergenson et al 2001[47]	50	LIA	0.12	-	-	I/II/III/IV:50%
Rebmann et al 2002[48]	183	LIA	0.12	-	-	I/II/III/IV: 21%
Banfalvi et al. 2002[27]	59	LIA	0.12	40%	25%	59%
Garbe et al 2003[29]	296	LIA	0.12			
Banfalvi et al. 2003[28]	478	LIA	0.18	I:29%, II: 19%	32%	48%
Andres et al 2004[49]	85	LIA	0.15	-	I/II/III:15%	89%
Ugurel et al 2005[50]	300	LIA	0.12	-	-	I/II/III/IV: 20%
Smitt et al 2005[13]	145	LIA	0.16	-	-	78%
Schmidt et al 2005[51]	85	LIA	0.15	-	-	59%
Domingo-et al 2007[52]	97	LIA	0.15	-	II/III: 21%	-
Cao et al 2007[53]	42	LIA	0.20	-	-	50%
Tarhini et al 2008[33]	670	LIA	0.15	-	IIb/III: 13%	-
Kruijff et al 2009	56	LIA	0.15	-	48%	-

(LIA: Lumino imunometric Assay, IRMA: Immuno Radiometric Assay)

prognostic value of preoperative and postoperative concentrations of the tumor marker S-100B in clinically stage III melanoma patients who underwent a so-called “curative lymph node dissection” after preoperative staging with whole-body FDG-PET and spiral CT. Our results showed that preoperative elevated concentrations of S-100B was statistically significantly associated with a decreased DFS. It is difficult to compare the studies investigating the

value of S-100B in patients with melanoma, because various assays (IRMA or LIA-mat) and cut-off points and groups of patients with different stages were used (Table 4).[8] Garbe et al. also found a relation between S-100B and disease-free survival in stage II and III melanoma patients. Postoperative elevated S-100B preceded detection of developing metastases by imaging techniques.[2] According to Martenson et al. and Curry et al., elevated S-100B in stage III melanoma after lymph node dissection may form an independent prognostic factor.[20,22] Four studies demonstrated that elevated S-100B was associated with shortened (disease-free) survival.[20,23-25] In stage IV patients with normal S-100B levels, survival was better when compared to those with elevated S-100B levels.[15,26] Increasing concentrations of S-100B in stage IV patients who were receiving systemic treatment were associated with disease progression.[14,27-29] In a study on 1007 melanoma patients, S-100B was not only correlated with the clinical stage of the disease, but it also appeared to be an independent prognostic marker in stages II and III. The 5-year survival of patients with S-100B values of< 0.10 μg/L was 91%, compared with 51% when the values were ≥0.10 μg/L.[20] In two other studies, similar patterns were found.[30,31]

Two studies comparing 5-S-cysteinyldopa, LDH and S-100B as prognostic markers found that S-100B had the highest specificity.[30,31] Several studies have therefore recommended the use of S-100B as a tumor marker to monitor the course of disease in stage III melanoma patients and to evaluate the effect of therapy in stage IV patients. Successful treatment with lymph node dissection, chemotherapy, or immunotherapy was associated with decreased S-100B concentrations, whereas increased concentrations were an expression of disease progression.[14,27,28] The prognostic value of the preoperative and postoperative course of S-100B concentrations in "true stage III" melanoma patients has not been studied before. In advance, we hypothesized that a significant decrease in S-100B concentration after therapeutic lymph node dissection would correlate with better DFS. In this way we assumed S-100B could be used as a quality-control marker for dissection. In our study, 27 patients (48.2%) had elevated S-100B preoperatively, and indeed in 22 of them (81.4%) the concentrations normalized postoperatively. However, 12 patients of these 22 (55%) developed tumor recurrence during a median follow-up of 1.18 years. Multivariate analyses showed that preoperative S-100B elevation was an important predictor of decreased DFS (p=0.03).

Although the results indicated that therapeutic lymph node dissection did have a temporary influence on the postoperative course of S-100B, a more important predictor for shortened DFS seemed preoperative S-100B elevation. For AJCC stage III, postoperative increasing S-100B serum were associated with a decreased DFS. However, when adjusted for stage III substages, probably because of small numbers, only a trend toward statistical significance was observed. S-100B is located in the cytoplasm of melanoma cells. Elevated serum S100-B occurs as the result of loss of melanoma cell integrity.[19] Therefore, the hypothesis that elevation of S-100B should be interpreted as a process of subclinical microscopic metastatic disease seems to be suggested by these data. Preoperative elevated S-100B could be an expression of early dissemination not detected by standard imaging tests.[33] However, the tumor marker is not suitable for screening purposes or for the early detection of recurrence in the follow-up of

melanoma patients.[34] The prognostic biochemical marker S-100B may be of value in the design of future trials on adjuvant systemic chemotherapy or immunotherapy. At present, LDH is the most prominent serum parameter in stage IV melanoma, and it has been included in the new AJCC staging system.[5] Our study on optimally staged clinical stage III melanoma patients has now shown that preoperative S-100B elevation is associated with significantly poorer survival. These results are supported by studies from M.D. Anderson Cancer Center, Barcelona and a recent meta-analysis and a pooled analysis of ECOG studies in high-risk surgically resected melanoma by Kirkwood.[33,35-37] These studies showed that direct postoperative (baseline) S-100B is indeed a prognostic marker for disease-free survival (DFS) and overall survival (OS). Our results seem in accordance with these data, but more importantly reveal that S-100B is a prognostic marker for DFS when determined before dissection. In our study, the 2-year DFS is slightly better than in the recently published ECOG, which is explained by the fact that the patients were staged with FDG-PET and spiral CT.[37] A more refined individualized risk assessment for adjuvant treatment is possible with serum marker S-100B. Therefore, S-100B could be included in future stratification research for stage III melanoma. In summary, preoperative elevation of S-100B levels in patients with true (clinical) stage III melanoma is associated with decreased disease-free survival and might have a prognostic clinical value in high-risk stage III melanoma patients. Therefore S-100B could be used to select patients for adjuvant systematic treatment in the stratification for new adjuvant therapeutic trials as well as to provide information for stage III melanoma patients who want to be informed about disease prognosis.

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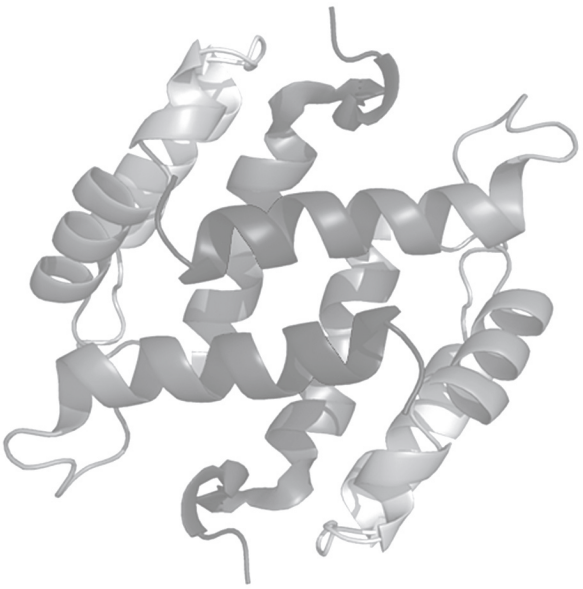

The value of pre-operative S-100B and SUV in clinically Stage III melanoma patients undergoing therapeutic lymph node dissection

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ABSTRACT

Introduction: High preoperative serum S-100B values and Standardized Uptake Values (SUV) of Fluorodeoxyglucose (FDG) in PET for clinically stage III melanoma patients could be indicators of recurrence after surgical treatment. Aim was to assess the correlation and the prognostic value of these markers.

Methods: All melanoma patients with palpable nodal metastases, without distant metastases, were included from February 2004 to December 2007. Preoperative SUV and S-100B was determined. The correlation between SUV and S-100B and and their relations with DFS and DSS were calculated by Cox Proportional Hazard Analysis.

Results: 62 Patients, median age 56.9 years, were included in the study. An elevated S-100B was found in 31 patients (50%) and elevated SUV in 24 patients (38.7%). No relation was found between S-100B and SUV. DFS was reduced (31.1%) for patients with an elevated S-100B (HR 3.1; p=0.02) in comparison to a normal S-100B (44.6%). The DFS was 42.0% for patients with a SUV below the cut-off point and 29.0% for patients with an elevated SUV (HR 1.1; p=0.8). DSS was 60.7% in a normal S-100B and 44.7% for patients with an elevated S-100B (HR 2.2; p=0.07). DSS was 59.1% for patients with a normal SUV and 43.5% for patients with elevated SUV (HR 1.1; p=0.8).

Conclusion: S-100B and SUV in stage III melanoma are not correlated and each have different associations with various histopathological factors. S-100B, in contrast with SUV, is associated with nodal tumor load, and when elevated, predicts a shorter DFS.

INTRODUCTION

Melanoma causes more than 75% of all deaths related to skin cancer and its incidence has increased dramatically worldwide, especially in Caucasian populations.[1] During the period 1998-2007 the Dutch incidence increased from 13.2 to 20.0 newly diagnosed melanoma patients per 100,000 inhabitants.[2] The past 12 years, melanoma incidence rates have increased rapidly and are expected to keep on rising in the future. The absolute total number of new cases in the Netherlands is estimated to be well over 4,800 in 2015, compared to approximately 2400 cases in 2000.[3]

Despite being diagnosed in an earlier phase of disease, which may be a result of increased awareness and better surveillance, melanoma patients presenting with palpable nodal metastases today still have a poor 5-year survival of 59% and 40% for stage IIIB and IIIC respectively.[4] Staging studies with whole-body FDG-PET and/or spiral CT in patients with stage IIIB melanoma have shown that about 27% of patients are upstaged and treatment was changed for one of five patients.[5] Recently Balch et al analyzed 2,313 patients with AJCC stage III disease and for the complete cohort 5-year overall survival was 63%. However, when focusing on more specific patient groups, a tremendous heterogeneity in 5-year survival rates was observed (23%-87% 5-year survival).[6]

More than 60 years ago, Hill published about increased Lactate Dehydrogenase (LDH) as a prognostic serum marker in melanoma patients.[7] The last decades various biomarkers besides LDH have been studied, such as Melanoma Inhibitory Activity (MIA), S-100B and Standardized Uptake Value (SUV) in FDG-PET.[8,9] Weighing the evidence, LDH has high specificity for melanoma and literature demonstrates this marker to be elevated in advanced disease, predominantly in case of dissemination to the liver. However, today the most extensively studied melanoma biomarker is S-100B. This 21 kDa protein was first isolated from the central nervous system in vertebrates.[10] A preoperatively elevated serum S-100B in FDG-PET and CT evaluated patients with palpable nodal metastases is associated with a significantly worse survival.[10-13] Another prognostic marker was studied by assessment of the degree of Fluorodeoxyglucose (FDG) accumulation during Positron Emission Tomography in a melanoma metastasis; the Standardized Uptake Value (SUV).[14] Bastiaannet et al proved that the uptake of FDG in the lymph node metastases in melanoma patients with clinical stage III melanoma is of prognostic value.[15]

High values of S-100B and FDG Standardized Uptake Value (FDG-SUV) measured preoperatively in stage III melanoma patients could both be highly specific indicators of early recurrence after surgical treatment. Possibly, S-100B values and FDG-SUV form a measurable reflection of the presence of a subclinical process of dissemination. Therefore, the aim of this study was to assess the correlation between both markers and to study their association with Disease Free Survival (DFS) and Disease Specific Survival (DSS).

MATERIAL AND METHODS

Patients

Melanoma patients, previously staged as AJCC I or II, now presenting with palpable and pathologically proven lymph node metastases (AJCC stage IIIB or IIIC) were prospectively included in this study from February 2004 to December 2007. Patients were staged with whole-body FDG-PET and spiral CT. In case of negative test results for distant metastases, patients were eligible for therapeutic lymph node dissection (TLND) and were included in this study. Sentinel lymph node positive patients were excluded from this study. Patients received adjuvant radiotherapy (20 x 2.4 Gy) in case of a nodal metastasis of ≥3 cm in diameter, ≥3 tumor positive lymph nodes or the presence of extranodal growth.[16,17]

Patient characteristics and follow up

Age, sex, treatment strategy of primary melanoma, date of primary melanoma diagnosis, characteristics of the primary melanoma (Breslow thickness, localization, ulceration status, Clark level) and characteristics of the lymph node metastases (localization, number of lymph nodes removed, number of tumor positive nodes, presence of extranodal growth, lymph node size and AJCC stage III B/C) were recorded, as well as the date of recurrence or death. Any form of melanoma recurrence after TLND was scored as recurrent disease.

Follow-up strategy was the same for all patients. Three monthly history and physical examination in the first year after TLND, 4 monthly in year 2, 6 monthly in years 3 to 5 and annual visits and chest radiographs for the subsequent five years.

PET protocol

FDG-PET and CT were performed preoperatively in a random order. FDG was produced on site.[18] Before FDG injection, patients were instructed to fast for at least 6 hours and drink 1 liter of water. After intravenous injection of FDG (Range 220 to 690 MBq), whole body PET-imaging was performed; two/three dimensional mode, emission scans 5 minutes per bed position, starting 90 minutes after injection of FDG. Patients were scanned from scalp to feet, using a Siemens ECAT EXACT HR + scanner (Siemens/CTI, Knoxville, TN). FDG-PET readings were performed by attending staff nuclear medicine physicians.

CT protocol

In the CT protocol a 64-slice spiral CT (Siemens Somatom Sensation) was performed of neck, chest and abdomen. Before starting the procedure oral (800 ml) and intravenous contrast agents were administered to the patient using standard imaging protocols. CT images were interpreted by attending staff radiologists.

Standardized Uptake Value (SUV)

The SUV depends on the amount of injected radioactivity, the patient's weight and the calibration factor of the camera. The value is calculated according to the following formula: SUV mean= radioactivity concentration in tissue (Bq/Kg)/ (injected dose [Bq] / patient weight [Kg]). Three dimensional regions of interest were placed semi-automatically over the tumor on multiple slices using a software program and a threshold of 70 % of the maximum pixel value within the tumor. In case of multiple metastases in the lymph node basin, the lesion with the most intense uptake was analyzed. For SUV the median of 8.4 was used as cut-off value in absence of a standardized cut-off value. Currently no cut-off point is described in literature although initiatives have been undertaken to create a new protocol using standardized uptake values.[19]

S-100B

Serum S-100B levels were calculated on the basis of a calibration curve and checked against internal standards with a known concentration of S-100B. In this assay, a cut-off point of 0.15 µg/l was set. The reference values for the S-100B assay (Liason Sangtec 100) were established by analysis of S-100B values of 120 healthy men and women according to the CLSI C28A2 guideline. Concentrations below the cut-off point were considered to be normal, whereas concentration above this point were considered to be elevated. S-100B levels were measured one day before operation.

Statistics

The association between elevated S-100B and elevated SUV was tested with a linear regression model and a scatter plot. Factors correlated with DFS and DSS were analyzed with univariate (Log rank test) and multivariable (Cox Proportional Hazard) tests. Factors associated with elevated S-100B concentrations and elevated SUV were tested using univariate logistic regression. Finally, a stratified survival analysis for DFS and DSS was performed to assess the strata in which the marker S-100B has the highest prognostic value. Data were analyzed with STATA version 10.0. A difference was considered significant when $p \leq 0.05$.

RESULTS

Patient characteristics

Overall, 62 patients (30 males and 32 females), with a median age of 56.9 (range 24.7-93.2) years, were included in the study. The majority of lymph node metastases were found in the groin (48.3%), followed by the axilla (35%) and the neck (16.7%). Size of the lymph node metastases was less than 3.0 cm in 45.2% and 3.0 cm or bigger in 54.8% of patients. An extranodal growth pattern was found in 37.1% of cases. Patient and tumor characteristics are summarized in Table 1.

TABLE 1: Characteristics of 62 AJCC stage III melanoma patients unthergoing lymph node dissection

		N	%
Sex	Male	30	48.4
	Female	32	51.6
Age (yrs)	≤45	16	25.8
	46-64	28	45.2
	≥65	18	29.0
Breslow thickness	≤2.0	30	48.4
	>2.0	32	51.6
Clark Level	I-III	17	27.4
	IV-V		58.1
		36	
	Unknown	9	14.5
Ulceration	No	51	82.3
	Yes	11	17.7
Region	Neck	10	16.7
	Axilla	21	35.0
	Groin	29	48.3
Number removed	<15	27	43.6
	≥15	35	56.4
Positive nodes	<2	26	41.9
	≥2	36	58.1
Lymph node size	<3.0	28	45.2
	≥3.0	34	54.8
Extranodal growth	No	39	62.9
	Yes	23	37.1

S-100B and SUV

Thirty-one patients (50%) had a normal preoperative S-100B level (< 0.15 μg/l) and thirty-one patients (50%) had an elevated S-100B level (≥ 0.15 μg/l). Thirty-eight patients (61.3%) had a low preoperative SUV (<8.4) and twenty-four patients (38.7%) had a high preoperative SUV (≥8.4). No association was found between preoperative S-100B and SUV (p=0.7) in a linear regression model.

Disease free survival (DFS)

For DFS, recurrent disease was interpreted as any form of melanoma recurrence. However, all patients who returned with recurrent disease after TLND had distant metastases and none of the patients presented with local or regional recurrences.

TABLE 2: Univariate and multivariate analysis on Disease-Free Survival (DFS) for SUV and S100-B with any recurrence as event.

Characteristic		3-years DFS	Univariate HR	Univariate p-value
Breslow	≤2.0	44.9	Ref	0.09
Thickness	>2.0	30.0	1.7 (0.9-3.3)	
Melanoma	No	43.2	Ref	0.01
Ulceration	Yes	11.4	2.5 (1.2-5.3)	
Lymph node positive	<2	52.7	Ref	0.02
	≥2	26.8	2.3 (1.2-4.6)	
Extranodal growth	No	46.5	Ref	0.01
	Yes	22.4	2.3 (1.2-4.5)	
Region	Neck	72.9	Ref	0.1
	Axilla	24.5	4.9 (1.1-1.7)	
	Groin	25.9	4.8 (1.1-0.7)	
S-100B	Low	44.6	Ref	0.2
	High	31.1	1.5 (0.8-2.9)	
SUV	Low	42.0	Ref	0.1
	High	29.0	1.7 (0.9-3.2)	

All variables with p≤0.2 were entered in the multivariable model; the following variables were not entered: sex (p=0.4), age (p=0.9), clark level (p=0.7) localization of the primary tumor (p=0.9), number of lymph nodes removed (p=0.3) and tumor size of the lymph nodes metastasis (p=0.6). **SUV and S100 were entered separately in the model.

As shown in Table 2 and Figure 1 DFS was 31.1% for a elevated S-100B and 44.6% for S-100B below cut-off point (univariate: HR=1.5; p= 0.2). The DFS was 29% for a elevated SUV and 42% for a SUV below cut-off point (univariate: HR=1.7; p= 0.1).

In multivariate analysis only variables with p ≤0.2 were entered in the multivariable model. SUV and S-100B were entered separately. In multivariate analyses the DFS was significantly correlated with S-100B (HR 3.1; p=0.02). There was no correlation of DFS found with SUV (HR 1.1; p=0.8). Primary melanoma ulceration (p=0.008 and p=0.01) and ≥ 2 lymph nodes positive (p=0.02 and p=0.03) were both associated with significant reduced DFS.

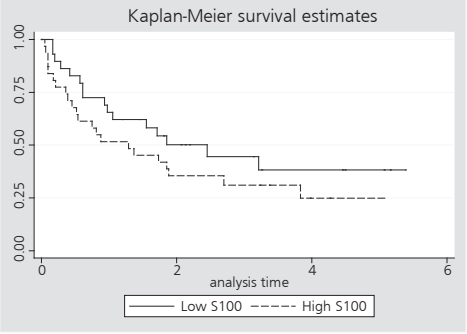
Disease Specific Survival (DSS)

As shown in Table 3 and Figure 1 DSS was 44.7% for an elevated S-100B and 60.7% for a S-100B below the cut-off point (univariate: HR=2.0; p=0.06). The DSS was 43.5% for a elevated SUV and 59.1% for a SUV below cut-off point (univariate: HR=1.7; p=0.1).

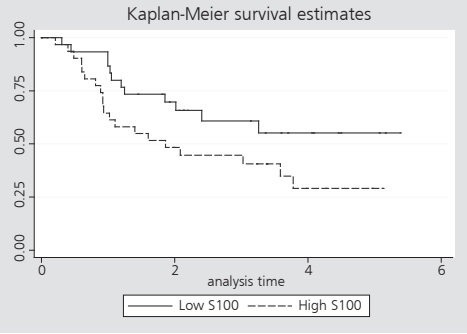
In multivariate analysis an elevated S-100B showed a trend for DSS (HR= 2.4; p=0.06), but an elevated SUV was not correlated with DSS at all (HR=1.0;P=0.9). Primary melanoma ulceration was also correlated with DSS (p=0.05 and p=0.06).

	Multivariable HR	Multivariable p-value	Multivariable HR	Multivariable p-value
	Ref	0.4	Ref	0.7
	1.4 (0.6-3.0)		1.2 (0.5-2.5)	
	Ref	0.008	Ref	0.01
	2.8 (1.3-6.2)		2.7 (1.2-5.9)	
	Ref	0.02	Ref	0.03
	2.7 (1.2-5.8)		2.5 (1.1-5.4)	
	Ref	0.07	Ref	0.2
	2.1 (0.9-4.6)		1.7 (0.8-3.9)	
	Ref	0.1		
	3.2 (0.7-15.3)			
	4.6 (1.1-20.0)			
	Ref	0.02		
	3.1 (1.2-7.8)			
			Ref	0.8
			1.1 (0.5-2.4)	

DFS for S-100B, p=0.02



DSS for S-100B, p= 0.07



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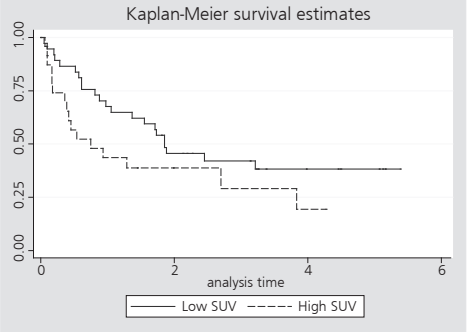

DSS for SUV, p= 0.8

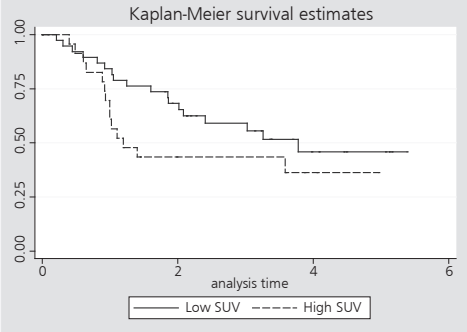

TABLE 3: Univariate and multivariate analysis on Disease-Specific Survival (DSS) for SUV and S-100B with death due to melanoma as event.

Characteristic		3-years DSS	Univariate HR	Univariate p-value
Sex	Male	39.7	Ref	0.2
	Female	64.2	0.6 (0.3-1.3)	
Breslow	< 2.0	63.0	Ref	0.2
thickness	≥ 2.0	42.8	1.6 (0.8-3.3)	
Melanoma	No	58.9	Ref	0.04
Ulceration	Yes	27.3	2.2 (1.0-4.7)	
Lymph node positive	<2	51.9	Ref	0.6
	≥2	44.5	1.2 (0.6-2.5)	
Region	Neck	66.7	Ref	0.2
	Axilla	29.9	2.4 (0.8-7.3)	
	Groin	51.9	1.3 (0.4-3.9)	
S-100B	Low	60.7	Ref	0.06
	High	44.7	2.0 (1.0-4.0)	
SUV	Low	59.1	Ref	0.1
	High	43.5	1.7 (0.8-3.4)	

All variables with p ≤0.2 were entered in the multivariable model; the following variables were not entered: age (p=0.8), clark level (p=0.3) localization of the primary tumor (p=0.3), number of lymph nodes removed (p=0.3), number of nodes positive (p=0.5), tumor size of the lymph nodes metastasis (p=0.7) and extranodal growth (p=0.3). **SUV and S100 were entered separately in the model

TABLE 4a: Correlations of different patient and tumor characteristics for prognostic markers S-100B and SUV

		Associated with high S-100B	Associated with high SUV
Sex	Male	0.9	0.8
	Female		
Ulceration primary melanoma	No	0.9	0.04
	Yes		
Tumorsize lymph node metastasis	<3.0	0.04	0.01
	≥3.0		
Extranodal growth	No	0.8	0.03
	Yes		

Marker association with histopathological factors

Table 4a reflects the association between histopathological factors and an elevated S-100B or SUV. For FDG-SUV primary melanoma ulceration (p=0.04), size of the lymph node metastases (p=0.01) and extranodal growth (p=0.03) were associated. For S-100B, a large tumor size in the lymph node metastasis (p=0.04) was associated with elevated S-100B levels.

	Multivariable HR	Multivariable p-value	Multivariable HR	Multivariable p-value
	Ref	0.1	Ref	0.1
	0.5 (0.2-1.1)		0.5 (0.2-1.2)	
	Ref	0.1	Ref	0.2
	21 (0.9-5.0)		1.8 (0.8-4.4)	
	Ref	0.05	Ref	0.06
	2.3 (1.0-5.1)		2.3 (0.9-5.4)	
	Ref	0.2	Ref	0.3
	1.7 (0.7-3.9)		1.5 (0.7-3.5)	
	Ref	0.8	Ref	0.4
	1.4 (0.4-4.9)		2.0 (0.6-6.9)	
	1.2 (0.4-3.8)		1.0 (0.3-3.3)	
	Ref	0.06		
	2.4 (0.9-5.9)			
			Ref	0.9
			1.0 (0.4-2.6)	

Stratified analysis of the prognostic values S-100B

Table 4b reflects the prognostic value of S-100B for DFS in both males (p=0.03) and females (p=0.01), for patients aged 46-64 yrs (p=0.03), patients with groin metastases (p= 0.02) and with ≥2 positive lymph nodes (p=0.02) or a tumor size <3.0 cm (p=0.04) and patients with extranodal growth (p=0.01).

For DSS, S-100B was of prognostic value especially in elderly patients (≥ 65 years) (p=0.05), in patients with groin metastases (p=0.01) and with size of the lymph node metastases <3.0 cm (p=0.02). A trend towards significance was seen in patients with presence of ≥2 positive lymph nodes (p=0.05).

DISCUSSION

General

No relation was found between the prognostic markers S-100B and SUV in melanoma patients with palpable lymph nodes. A preoperative elevated S-100B predicts a reduced DFS in stage III melanoma and is a prognostic marker in case of high lymph node tumour burden. An elevated SUV, according to the present data, has no correlation with survival. Therefore, evaluating these data, S-100B could be used as a prognostic marker in the stratification for new adjuvant trials, in order to better stage and select clinically AJCC stage III melanoma patients for systemic treatment.

TABLE 4b: Prognostic value of S-100B stratified for different groups

		DFS*	DSS**
		HR (95%CI); p-value	HR(95%CI); p-value
Sex	Male	4.6 (1.1-19.1); p=0.03	2.4 (0.7-8.4); p=0.2
	Female	6.0 (1.5-24.8); p=0.01	2.6 (0.7-9.5); p=0.1
Age (years)	≤45	0.8 (0.1-7.2); p=0.8	3.0 (0.1-75); p=0.5
	46-64	9.1 (1.6-52.1);p=0.03	1.0 (0.2-3.9); p=0.9
	≥65	2.9 (0.2-42.1);p=0.2	22.9 (1.0-520); p=0.05
Localization lymph node metastases	Neck	p=0.9***	p=0.8***
	Axilla	0.4 (0.1-2.7);p=0.3	0.7 (0.1-5.4); p=0.7
	Groin	3.4 (1.2-10.0);p=0.02	5.2 (1.5-18.9); p=0.01
Lymph nodes positive	<2	4.2 (0.9-20.3) ;p=0.07	3.4 (0.7-15.7) ; p=0.1
	≥2	3.6 (1.3-10.0) ; p=0.02	2.7 (1.0-7.6) ; p=0.05
Tumorsize lymph node metastasis	<3.0	4.7 (1.1-21.0); p=0.04	7.4 (1.4-38.3); p=0.02
	≥3.0	2.6 (0.8-8.0); p=0.1	1.7 (0.5-5.2); p=0.4
Extranodal growth	No	2.3 (0.8-6.3); p=0.1	2.6 (0.9-7.8); p=0.1
	Yes	6.0 (1.5-24.3); p=0.01	2.9 (0.8-10.0); p=0.1

*DFS adjusted for ulceration, Breslow, number of positive nodes, localization and extranodal growth
**DSS adjusted for sex, ulceration, Breslow, number of positive nodes and localization.
***no estimation of the HR due to small numbers.

Prognostic markers

This is the first study, to our knowledge, evaluating both prognostic markers S-100B and SUV in melanoma patients before undergoing a therapeutic lymph node dissection. After a lymph node dissection, most melanoma patients die within three years as a consequence of distant metastases. Prediction of survival in AJCC stage III has traditionally been based on the number and size of lymph nodes, the presence of extranodal growth and ulceration of the primary tumor.[20] In the search for effective systemic therapy in stage III melanoma, the development of prognostic markers and staging will be of increasing interest in order to improve patient-tailored adjuvant therapy.

Staging

Accurate staging before treatment is essential for all melanoma patients. However, for the evaluation of early regional lymphatic dissemination in AJCC stage I and II melanoma, determining S-100B or performing a PET/CT scan is unsuitable.[21] Fortunately, nowadays the technique of lymphatic mapping and sentinel lymph node biopsy (SLNB) can be used as a standard method of selecting patients with micro-metastases. The technique has undisputedly proven to be a reliable staging method and the fourth interim analysis of the MSLT I study reveals that SLNB indeed leads to improved melanoma specific survival.[22,23] In staging studies analyzing patients with palpable lymph nodes (AJCC stage III), whole-body FDG-PET and/or spiral CT have proved their value with 27% upstaging to stage IV disease. A sensitivity

of 79-92% and a specificity of 86-90% to detect distant metastases was found.[5,24] The combined FDG PET/CT leads to a change in the planned dissection in 37% of the patients.[25] Acknowledging the heterogeneity of stage III melanoma, the suspicion rises that numerous patients have a form of subclinical dissemination, which can even remain undetected by standard FDG-PET and CT imaging.[6]

SUV and S-100B not correlated

SUV can be easily calculated after a FDG-PET in the diagnostic work-up and might recognize, when elevated, high risk stage IIIB patients. By assessing the degree of FDG accumulation SUV was recorded earlier as a prognostic marker by other researchers.[15] If treatment response can be predicted by measurement of increased glucose metabolism, this would be of particular value in choosing an adequate patient-tailored adjuvant therapy.

Earlier, several other studies have recommended the use of S-100B. Successful treatment with lymph node dissection, chemotherapy or immunotherapy was associated with decreased S-100B concentrations whereas increased concentrations were an expression of disease progression.[26] Also, elevated S-100B was thought to be an expression of early dissemination and an independent prognostic marker of risk for mortality.[11-13]

Our results show that the tumour markers SUV and S-100B are not related. Both tumour markers are correlated with tumor size in the lymph node and SUV is also correlated with extranodal growth and primary ulceration (Table 4a).

Consideration

Interpreting these results leads to the conclusion that serum S-100B elevation seems correlated with lymphatic dissemination and nodal tumor burden. Elevation of S-100B might detect a process of haematogenic and lymfogenic dissemination in melanoma patients. Because S-100B is located in the cytoplasm of melanoma cells, elevated serum S-100B probably occurs as a result of loss of melanoma cell integrity.[13] Earlier evidence concerning FDG uptake suggests that the intensity of uptake does not correlate with lymph node tumor burden, but more with biological aggressiveness and is associated with cell viability and particularly cell proliferative activity.[28]

In the current study SUV did not prove its role as prognostic marker despite earlier findings by Bastiaannet et al. [15] However, the cohorts differed significantly in localization of the primary melanoma, number of positive nodes and extra nodal growth. Furthermore, earlier Bastiaannet et al. used a cut-off point of 6.14 and in the current study 8.4. The cut-off point for SUV is data driven and therefore depends on the patient group. In literature no consensus for a cut-off point for SUV has been formed yet.[19] In general, SUV values are often subject to different sources of variability, such as the timing of PET acquisition after tracer injection, patient size, region of interest definition, partial volume and image reconstruction.[29,30]

CONCLUSION

Preoperative elevation of S-100B is a prognostic marker for AJCC stage III melanoma patients. S-100B, although not significantly correlated with DSS (p=0.06), is a prognostic marker for DFS. The results of the current study, although with a relatively small group, again confirm the value of preoperative S-100B in stage III melanoma.[11] Furthermore, Standardised Uptake Value (SUV) is not associated with S-100B and the marker does not reconfirm its status as independent prognostic factor.

For the future we predominantly recommend the use of S-100B in combination with the established prognostic parameters for better stratification in new adjuvant therapeutic trials. Pre-operative S-100B levels provide information for stage III melanoma patients who want to be optimally informed about their disease prognosis. Ongoing research for prognostic biomarkers in larger prospective, randomized, high-quality methodological studies is essential in establishing treatment better tailored to the characteristics of individual tumor biology in stage III melanoma.

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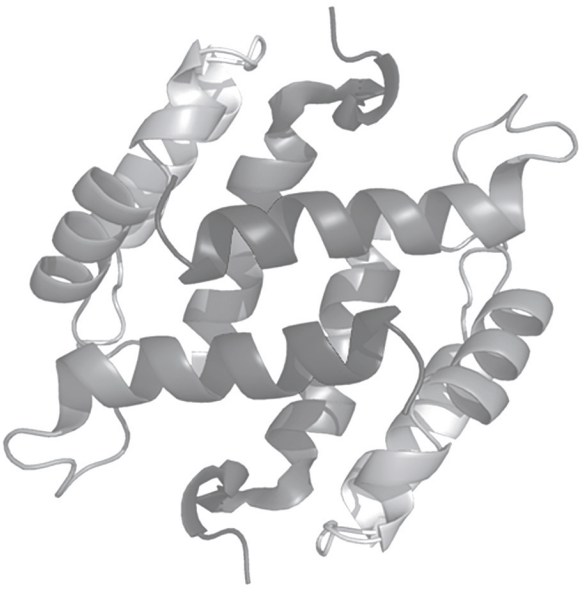

The use of S-100B to evaluate therapy effects during bevacizumab induction treatment in AJCC stage III melanoma

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ABSTRACT

Aim: To investigate the feasibility of using bevacizumab to improve the survival of AJCC stage III melanoma patients, we investigated how a single bevacizumab treatment affected nodal disease and a panel of biomarkers in clinically FDG-PET/CT staged, stage III melanoma patients, prior to therapeutic lymph node dissection (TLND).

Methods: Four weeks before TLND, nine patients (median age, 50 (range 28.8-62.1); two male, seven female) with palpable lymph node metastases received 7.5 mg/kg bevacizumab. Before and after this treatment, all patients were assessed by measurements of the maximum standardized uptake value (SUVmax) by FDG-PET scan, and serum S-100B and LDH. After TLND, the dissection specimen was analyzed for the number of removed lymph nodes, the amount of metastatic lymph nodes, and tumor necrosis.

Results: Median follow-up was 15.5 (2.2-32.9) months. Histopathological analysis revealed tumor necrosis in six patients, of which, five had an S-100B decline and one had an unchanged S-100B level after bevacizumab. The other three patients showed an S-100B increase and no necrosis. Tumor necrosis was correlated with S-100B decrease ($p = 0.048$). No association was found between necrosis and the markers SUVmax and LDH. No wound healing disturbances were encountered.

Conclusion: Tumor necrosis in dissection specimens was associated with declining S-100B levels, while elevated S-100B was only found in cases with no necrosis. Bevacizumab might be useful in treating AJCC stage III melanoma patients prior to TLND, and S-100B appears to be a useful marker for assessment of treatment effects.

INTRODUCTION

Melanoma is an aggressive and highly metastatic disease, which can be fatal with a rapid systemic dissemination. Approximately one third of all melanoma patients will experience disease recurrence.[1,2] While almost all organs can be involved, the most frequent target sites are the liver, bone, and the brain. Treatment results for advanced melanoma remain unsatisfactory. No systemic therapy has been demonstrated to affect overall survival, although the recent studies of immunotherapy with ipilimumab and the introduction of BRAF pathway inhibitors have shown promising results.[3,4,5] For melanoma patients with nodal disease, therapeutic lymph node dissection (TLND), with or without adjuvant radiation, remains the only curative therapy, with 5-year survival rates of 78%, 59%, and 40%, respectively, for patients with AJCC stage IIIA, IIIB, and IIIC disease.[6,7] As a consequence of shortages in health care resources, the growing elderly population in the Western world, and the increasing incidence of cancer, the wait time for surgery at some cancer centers has lengthened to an average of four to five weeks. This period before TLND offers a unique opportunity to test novel induction treatments before surgery.

Tumor angiogenesis is a continuous process that allows cancer cells to grow by supplying the tumor with nutrients and oxygen, disposing of metabolic waste products, and providing a route for metastatic spread.[8,9] Vascular endothelial growth factor A (VEGF-A) is a key growth factor involved in the development and maintenance of tumor angiogenesis.[10] Bevacizumab, a fully humanized monoclonal antibody, binds to all VEGF isoforms with high affinity, thereby blocking ligand-receptor signaling.[11] It is currently used in patients with metastatic colon cancer, non-small-cell lung cancer, and renal cell cancer.[12-14] Bevacizumab was previously evaluated in a randomized phase II trial (BEAM trial) in metastatic melanoma, which compared the effects of the combination of carboplatin and paclitexel, with and without bevacizumab. The addition of bevacizumab had a significant impact on progression-free survival, and some impact on overall survival, although this effect was not significant.[15]

The time spent waiting for a TLND for regional metastatic disease could be used more effectively if an induction therapy could be safely administered to reduce tumor load before surgery. S-100B and SUV are known to be of prognostic value in stage III melanoma; elevated S-100B and SUV in stage III melanoma patients can be specific indicators of disease progression.[16-22] Therefore, we hypothesized that monitoring S-100B and SUV before and after a single bevacizumab treatment might provide a 'measurable reflection' of the response to this angiogenetic treatment.

Here, we investigated the feasibility of using serum biomarkers, S-100B and LDH, and the Standardized Uptake Value (SUV) from FDG-PET to evaluate effects of an induction treatment with a single dose bevacizumab in stage IIIB/C melanoma patients, prior to TLND. We assessed the perioperative changes in biomarker levels following bevacizumab treatment, as well as the induction of tumor necrosis based on final histopathology of the resected lymph nodes.

PATIENTS AND METHODS

Patients

All consecutive melanoma patients presenting with palpable and cytology-proven lymph node metastases (AJCC IIIB/C) at the UMCG between January and July 2008 were evaluated with FDG-PET and spiral CT in addition to the routine staging, which included a complete medical history, physical examination, and blood chemistry profile. If the FDG-PET and CT were negative for distant metastases, patients were offered participation in the bevacizumab trial bridging the waiting time between the diagnosis and the TLND. Eligible patients were ≥18 years of age with AJCC stage IIIb melanoma and a WHO performance status of 0-2. Exclusion criteria included a history of radiotherapy on the involved lymph node basin, major surgery within 28 days of start of the study, administration of any investigational drug within 30 days before start of the study, and clinical evidence of brain metastases. Patients who presented with a positive sentinel lymph node biopsy, local recurrence, and/or in-transit disease were also excluded from this study. Examined data included patient demographics, primary tumor characteristics, date and type of operation, and status at last follow-up. Patients with nodal metastases of ≥3 cm and/or ≥3 positive lymph nodes and/or extranodal (EN) disease received adjuvant radiotherapy (20 × 2.4 Gy).

This study was approved by the local medical ethics committee and written informed consent was obtained from all participants. The study was registered under trial number NTR1941 and was organized as a corollary study in concurrence with another feasibility study investigating the presence of VEGF in melanoma lesions by VEGF-SPECT with ^{111}In-bevacizumab.[23]

Study design

On day 0, all selected patients were evaluated with a FDG-PET scan and on day 7, they received a single dose of 7.5 mg/kg bevacizumab intravenously. On day 40, the patients underwent a second FDG-PET scan, after which TLND was performed on day 42 (five weeks after bevacizumab). S-100B and LDH levels were measured on days 0, 41, and 43 (first visit to outpatient clinic, one day preoperatively, and one day postoperatively). After lymph node dissection, the pathological results were analyzed.

S-100B

Concentrations of serum S-100B were measured using the Liason Sangtec 100 immunoassay on the Advantage (Nichols). The half-life of S-100B is estimated to be about 30 minutes.[24] Levels were calculated using a calibration curve and checked against internal standards with known concentrations of S-100B. In this assay, concentrations were considered normal if they were below a cut-off point of 0.15 μg/L. Concentrations above this range were considered to be elevated. For all patients, the change in S-100B concentration from day 0 to day 41 was determined.

LDH

LDH was routinely analyzed by enzymatic activity measurement with a Roche Modular analyzer (Hitachi). LDH values <250 U/L were considered to be normal.

Standardized uptake value (SUV)

To assess the degree of FDG accumulation before and after bevacizumab treatment, the maximum standardized uptake value (SUVmax) was calculated from a FDG-PET scan. The SUV depends on the amount of injected radioactivity, the patient's weight, and the calibration factor of the camera, and it is calculated according to the following formula: SUVmax = radioactivity concentration in tissue (Bq/Kg)/(injected dose [Bq]/patient weight [Kg]). If multiple metastases were present in the lymph node basin, the lesion with the most intense uptake was chosen. The cut-off point for SUV is data driven and therefore depends on the patient group; there is no consensus in the literature regarding a cut-off point for SUV.[25-27] In the present study, any decrease/increase of SUVmax was recorded simply as the level of decrease/increase.

Surgery

The therapeutic axillary lymph node dissection included a level III resection with resection of the pectoralis minor muscle. The groin lymph node dissection included a superficial (inguinal) and deep (iliac-obturatory) groin dissection. The latter procedure was performed as extensively described by Baas et al.[28] Perioperative surgical complications, the number of harvested lymph nodes, number of metastatic lymph nodes, maximum metastasis diameter, and degree of tumor necrosis were scored. The pathological results after lymph node dissection were analyzed by a pathologist.

Statistics

Fischer's exact test was used to assess associations between the SUV, S-100B and LDH levels, and pathology data.

RESULTS

Patient characteristics

Table 1 shows the characteristics of the nine AJCC stage III melanoma patients, which included two males and seven females, with a median age of 50 (range 28.8-62.1) years. The median follow-up time was 15.5 (range 2.2-32.9) months. In these nine patients, metastatic lymph nodes were detected with spiral CT and FDG-PET imaging. After TLND, the resection specimens displayed a median of 16 nodes (range 8-29). No perioperative or wound healing disturbances were encountered.

TABLE 1 Characteristics of the primary melanoma and localization recurrence.

		Number	%
Sex	Male	2	22.2
	Female	7	77.8
Age yrs (median range)		50.0 (28.8-62.1)	
Localization of primary melanoma	Trunk	5	55.6
	Upper extremities Lower extremities	0	0
	Unknown	3	33.3
Clark level	II	1	11.1
	III	2	22.2
	IV	5	55.6
	Unknown primary	1	11.1
Breslow thickness	≤1.0	4	44.4
	>1.0	4	44.4
	Unknown primary	1	11.1
Mitotic index	≤5	4	44.4
	≥6	4	44.4
	Unknown primary	1	11.2
Ulceration	Yes	3	33.3
	No	5	55.6
	Unknown primary	1	11.1
Localization of lymph node metastases	Axilla	5	55.6
	Groin	4	44.4

TABLE 2 Association between pathology data and the changes in SUV value, S-100B, and LDH.

		SUV		S-100B		LDH	
		Increase	Decrease	Increase	Decrease	Increase	Decrease
Size	≤4.0 cm	3 (75%)	1 (20%)	2 (50%)	2 (40%)	1 (50%)	3 (43%)
	>4.0 cm	1 (25%)	4 (80%)	2 (50%)	3 (60%)	1 (50%)	4 (57%)
Necrosis	Yes	3 (75%)	3 (60%)	1 (25%)*	5 (100%)*	0 (0%)	6 (86%)
	No	1 (25%)	2 (40%)	3 (75%)	0 (0%)	2 (100%)	1 (14%)
Ratio positive / removed nodes	≤0.5	2 (50%)	2 (40%)	2 (50%)	2 (40%)	1 (50%)	3 (43%)
	>0.5	2 (50%)	3 (60%)	2 (50%)	3 (60%)	1 (50%)	4 (57%)
Extranodal growth	No	2 (50%)	3 (60%)	3 (75%)	2 (40%)	1 (50%)	4 (57%)
	Yes	2 (50%)	2 (40%)	1 (25%)	3 (60%)	1 (50%)	3 (43%)
Total		4 (44%)	5 (56%)	4 (44%)	5 (56%)	2 (22%)	7 (78%)

*$p < 0.05$ (Fisher's exact test)

SUV and S-100B and LDH

In five of the nine patients, the SUVmax decreased after bevacizumab treatment. In one patient, SUVmax was not influenced, and in the remaining three patients, the SUVmax increased. Between day 0 and day 41, an LDH decrease was noted in seven patients and an elevation in two patients.

Pathology

The pathologic analysis reported tumor necrosis in six TLND specimens after bevacizumab treatment. In five of these six patients, a decrease in S-100B after bevacizumab was documented on day 41; in the remaining one patient, S-100B was unchanged but was comparatively decreased on day 43. Tumor necrosis was significantly associated with declining S-100B levels ($p = 0.048$). These data are shown in Table 2 and Figure 1. No association was found between the markers SUVmax and S-100B ($p = 0.6$) or LDH, nor was a correlation found between LDH or SUVmax and necrosis.

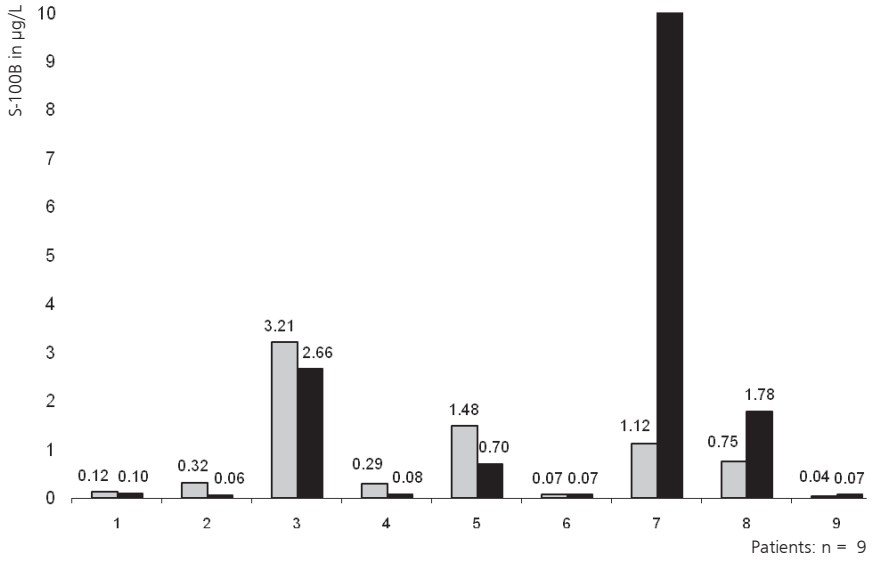

FIGURE 1 S-100B before and after bevacizumab.

DISCUSSION

This is the first study to assess the use of biomarkers to monitor the response following an induction treatment with bevacizumab, a VEGF-specific antibody, prior to TLND in AJCC stage III melanoma patients with nodal disease. Our data suggest that neo-adjuvant bevacizumab may be useful for preoperative tumor load reduction before TLND in AJCC stage IIIB/C melanoma patients.

Neo-adjuvant treatment with bevacizumab might have induced tumor necrosis in the metastatic lymph nodes in six of the nine patients. In all patients with necrosis in the dissection specimen, a significant decline of serum S-100B levels was observed. In contrast, in patients whose S-100B levels continued to rise following treatment, no necrosis was found in the dissection preparations. No relationship was found between SUV and S-100B or LDH and S-100B or any of the other pathological data.

The data from this feasibility study should only be considered preliminary and it is clear that no firm conclusions can be drawn from such a small group of samples. However, the observations certainly indicate that further investigation is warranted. The data from the present study might indicate that a single neo-adjuvant bevacizumab treatment induced tumor necrosis in the tumor bearing lymph nodes. Even more importantly, a decline of S-100B was observed in all patients who exhibited tumor necrosis after induction therapy.

Unfortunately, there are no data available revealing how often necrosis is discovered after lymph node dissection without prior administration of any induction therapy. However, the relatively high necrosis rate within our small sample of patients and the related S-100B decline might suggest a drug-induced tumor necrosis in the lymph nodes. The associated significant decrease of tumor load might explain the S-100B decline in all six patients.

To date, the most extensively studied biomarker in melanoma is S-100B, a 21-kDa protein that was first isolated from the central nervous system in vertebrates. S-100B is located in the cytoplasm and nucleus in cells with a neuro-ectodermal origin, e.g. melanocytes. S-100B has various intra-cellular functions, mainly in cytoskeleton integrity, cell cycle regulation, and apoptosis. Elevated serum S-100B occurs as the result of loss of melanoma cell integrity.[24] The mechanism by which this protein is released into circulation remains uncertain, but it is probably caused by cell damage or apoptosis. The hypothesis that elevation of S-100B should be interpreted as indication of ongoing metastatic disease has been suggested by earlier data.[16] Proportions of patients with elevated S-100B concentrations are 0-9% in stage I/II, 5-98% in stage III, and 40-100% in stage IV melanoma.[29] For clinically FDG-PET and CT-staged stage III patients, a pre-operatively elevated S-100B level is correlated with decreased survival.[16-19] In stage IV, improved survival is observed for patients with low S-100B levels; increased concentrations of S-100B during systemic treatment are associated with disease progression.[30-34] Recently, Bouwhuis et al. revealed that an elevation of S-100B levels throughout serial serum measurements in stage III is of very significant prognostic value, which is even stronger when combined with disease stage and number of positive lymph nodes.[35] However, not only should elevated S-100B be seen as a prognostic factor for survival and a mere reflection of disease progression alone; the presence of the protein S-100B itself induces disease progression as well. S-100B interacts with p53 and thereby down-regulates its function as a tumor suppressor protein by preventing induction of apoptosis of potential melanoma cells.[36,37]

Taking these results into account, an induction therapy before lymph node dissection might interrupt the melanoma proliferation cycle in two ways: first, by reducing tumor load and second, by temporarily suppressing the serum S-100B concentrations and thus preventing down regulation of tumor suppressor protein p53. Bevacizumab as an induction therapy might

temporarily suppress metastatic proliferation and eventually, in combination with surgery, improve outcome.

Anti-angiogenic therapy is a promising strategy in the treatment of cancer. Angiogenesis, or remodeling of an existing network of blood vessels, performs a essential role in diverse pathophysiological processes.[38,39] Because the neo-vascularization process that supports tumor growth may be similar to that involved in physiological wound healing, a delay in wound repair has been a concern. This may be one of the reasons that bevacizumab has not been widely used as induction therapy before major surgical cancer treatment.[40] None of the patients in our study showed any would healing disturbances during the course of the study.

The currently in-progress AVAST-M trial is the only known randomized trial evaluating the VEGF inhibitor, bevacizumab, for adjuvant therapy following resection of AJCC stage IIB, IIC, and III cutaneous melanoma. Many randomized trials of adjuvant therapy have been performed in patients after resection of melanoma, but to date, no treatment has convincingly improved overall survival. The AVAST-M trial is a cancer research UK funded, NCRN trial in which patients with previously resected melanoma at high risk of disease recurrence will be randomized to receive either standard observation or bevacizumab administration for 1 year.[41]

In one of the few examples in which induction therapy effects were monitored by biomarkers, Willet et al. performed a phase I-II trial to test the efficacy of adding bevacizumab to standard chemoradiotherapy as an induction therapy before rectal surgery. The phase II clinical trial involved administration of neoadjuvant bevacizumab, in combination with 5-fluorouracil (5-FU) and radiation therapy. The response of one patient's tumor to this regimen with bevacizumab was dramatic, with no residual cancer in the surgical specimen. More surprisingly, the plasma CEA dropped substantially from a pretreatment value of 122.8 ng/ml to 63.6 ng/ml at 12 days after the first bevacizumab infusion, and declined to 4.9 ng/ml at 1 month after completion of neoadjuvant therapy before surgery.[42]

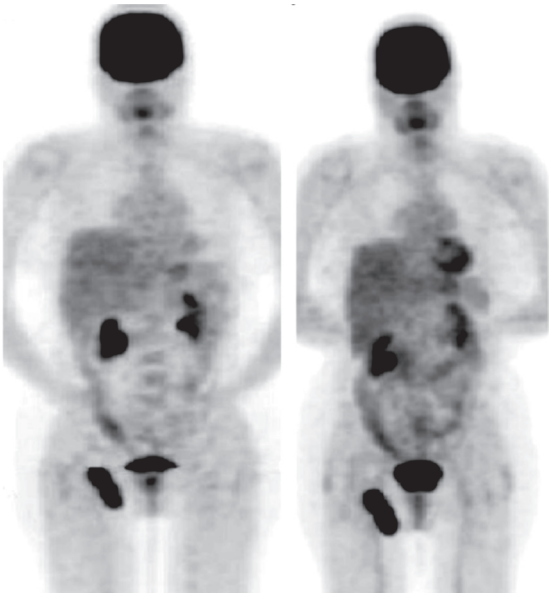

FIGURE 2 Patient (61 years, female), melanoma lower extremities (Breslow 2.1 mm, ulceration). Inguinal lymph nodes metastases with a SUVmax of 9.2 first scan and 10.5 second scan

In addition to S-100B, we also monitored SUVmax and LDH levels in the patients in this study, but did not find any significant correlation between these values and S-100B or pathology findings. In an earlier study, no relation was found between the prognostic markers S-100B and SUV in melanoma patients with palpable lymph nodes.[43] Evidence concerning FDG uptake suggests that the intensity of uptake does not correlate with lymph node tumor burden, but more with biological aggressiveness.[44] Serum lactate dehydrogenase (LDH) has high specificity for melanoma, but low sensitivity; it is an independent prognostic factor in stage IV melanoma.[16] Taking these facts into consideration, it is not surprising that we observed a lack of correlation of these markers with tumor response in this study.

In conclusion, this feasibility study has shown that induction treatment with bevacizumab in stage III melanoma four weeks prior to surgery may be related to induction of tumor necrosis without any observed incidence of wound healing disturbances. More importantly, when tumor necrosis was found in the dissection preparation, a 100% decline of S-100B was noted. Correspondingly, absence of tumor necrosis was correlated with ongoing S-100B elevation. The ability to target therapy towards well-selected subgroups of patients with the use of biomarkers, such as S-100B, could increase the likelihood of benefit and might improve therapeutic outcomes for future melanoma treatments.[45] Induction treatment with bevacizumab followed by therapeutic lymph node dissection could be a potentially successful combined treatment and might improve locoregional control, and therefore disease-free survival, for lymphogenic disseminated melanoma patients.

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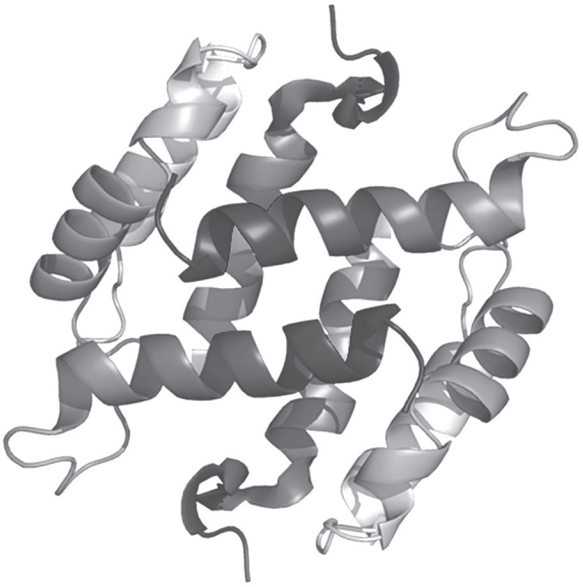

The current status of S-100B as a biomarker in melanoma

S Kruijff, HJ Hoekstra | Submitted

INTRODUCTION

The incidence rate of cutaneous malignant melanoma has increased over the past decades and causes the majority of all skin cancer mortalities.[1] From 1989 to 2008, an annual 4.1% increase in thin melanomas (≤1.0 mm) was reported in The Netherlands, suggesting a true melanoma epidemic.[1] The incidence in the Netherlands is expected to continue to rise from 2,400 new melanoma patients in the year 2000 to a level of 4,800 patients in 2015.[2] Melanoma is increasingly detected earlier, which might explain the growing incidence of thin melanomas.[1,3] When a primary cutaneous melanoma is diagnosed, a curative result can be achieved by means of a simple primary excision of the melanoma with a 1- or 2-centimeter margin according to the Breslow thickness.[4] Disseminated melanoma, on the contrary, is still difficult to treat and almost impossible to cure. The most important prognostic factors for mortality in melanoma remain Breslow thickness, ulceration, mitosis, and the presence of metastases, although the Breslow thickness continues to be the strongest prognostic marker for survival.[5]

About 90% of all melanoma patients have a primary melanoma (stage I or II).[5] Still, after primary excision, there is a 20-28% risk of loco regional recurrence or in-transit metastases, 26-60% risk of regional metastases, and 15-50% risk of distant metastases.[6] Despite campaigns and earlier detection, melanoma mortality increased in the Netherlands from 2.5 to 3.6 per 100,000 in males and from 2.0 to 2.4 per 100,000 in females between 1989-2003.[7] Survival rates for lymphogenic disseminated melanoma (AJCC stage III) remain unsatisfactory. Melanoma is an aggressive and highly metastatic disease, which can be fatal, with a rapid systemic dissemination. Approximately one third of all melanoma patients will eventually experience disease recurrence. While almost all organs can be involved, the most frequent target sites are the liver, bone, and brain. Today, for melanoma patients with palpable lymph nodes, a lymph node dissection is still the only available curative treatment with a 5-year survival of 40-78%.[5] Patients with distant metastases are almost incurable, with a mean survival of only 8-9 months and a 2-year survival of 18-41%.[5]

TUMOUR MARKERS AND SYSTEMIC THERAPY

Currently, no systemic therapy has demonstrated an overall survival benefit, although recent studies of immunotherapy with ipilimumab and the introduction of BRAF pathway inhibitors have shown promising results.[8-10]

Tumour markers in general can be used for several purposes; for screening, as diagnostic instruments, for staging, as a prognostic tool, to detect a recurrence, and finally as a quality control assessment after therapy. Tumour markers are particularly suitable for assessing treatment results or to detect early recurrences.[11]

The ability to target the new types of therapy described above towards well-selected subgroups of patients with the use of biomarkers such as S-100B could increase the likelihood of benefit, and might improve therapeutic outcomes for future melanoma treatments. Additionally, monitoring S-100B after systemic therapy could provide a 'measurable reflection' of the response to treatment; therefore it might be used as a quality assessment.[12]

LDH and S-100B

More than 60 years ago, Hill reported that lactate dehydrogenase (LDH) could be a prognostic marker for melanoma patients.[13] LDH as a tumour marker is very useful for detecting distant metastases, but has a very low sensitivity and seems to be correlated with other malignancies as well.[14] Nevertheless, LDH is still the most prominent tumour marker in melanoma today, and is used in the American Joint Committee on Cancer (AJCC) staging system for stage IV.[5]

S-100B is a 21 kilo Dalton protein that was first isolated by Moore from the central nervous system of vertebrates.[15] S-100B is chiefly found in glial- and Schwann cells. The name is derived from the protein's 100% solvency in saturated ammonium sulphate at neutral pH.[15] S-100B has calcium-binding properties and, as a dimer, consists of two isomers, a and b. All possible combinations can occur (S-100aa, S-100ab, and S-100bb).[16] The S-100B protein is of neuroectodermal and mesodermal origin, and is expressed in various parts of the body.[17]

S100B is found diffusely in the cell cytoplasm, and a substantial fraction is bound to membranes. Moreover, there is strong evidence suggesting that it is involved in cytoskeletal regulation, and has a possible role in cell cycle progression.[17] However, the mechanism by which this protein is shed into the blood by malignant melanocytes is unknown.

S-100B concentrations related to tumour stage

An increasing number of studies found serum S-100B protein levels to be a useful serum marker in melanoma, particularly in the late stages of the disease. In the past decade, serum S-100B is increasingly analyzed during melanoma treatment in various AJCC melanoma stages. The mean serum concentration of S-100B protein is significantly related to clinical melanoma stage, with the lowest levels in stages I and II (0-9%), elevated levels in stage III (5-98%), and high levels in stage IV (40-100%).[18] The highest S-100B concentrations are found in patients with bone or liver metastases.[19]

The evident correlation, which was found with both tumour burden and survival, suggests that S-100B in melanoma patients' blood is not a result of over expression or increased release, but a result of a loss of cell integrity and proteolytic degradation associated with apoptosis and cell death.[17]

Biomarkers are increasingly important in staging and defining prognosis in patients with metastasized melanoma. In the future, biomarkers could be increasingly used to target therapy towards well-selected subgroups of patients to increase the benefit of and improve therapeutic outcomes for future melanoma treatments.

S-100B is a promising diagnostic marker, which is correlated with most stages of melanoma.[18] Therefore, the current status of the applicability of S-100B as a tumour marker in the treatment of melanoma is discussed for the various melanoma AJCC tumour stages.

S-100B in AJCC stage I and II

Primary melanoma is accompanied by an average 10-year survival in 93-97% of patients with T1aN0M0 melanoma. In the case of poor prognostic factors like thick melanoma (Breslow > 4 mm) with ulceration (T4bNoMo, AJCC stage IIC), the expected 10 year survival decreases to 39%.[4] The detection of micro-metastases by means of a sentinel node biopsy (SLNB) has a great influence on prognosis.[4] Recently, the results of SLNB followed by immediate lymph adenectomy, when compared with nodal observation and delayed lymph adenectomy for nodal recurrence, provided a significant 10-year survival advantage of 60.9% versus 41.8% ($p = 0.01$).[20]

Less invasive diagnostics like FDG-PET/CT or the determination of S-100B in AJCC stage I and II seem inadequate for detecting micro metastases. FDG-PET/CT has a 5% sensitivity and a negative predictive value of 78% for appropriate prediction of lymph node status.[21] Neither S-100B nor LDH are suitable for predicting sentinel node status.[22] Therefore, there is no place for assessing serum LDH or S-100B nor the use of FDG-PET CT in AJCC stage I and II melanoma.[21-23]

S-100B in AJCC stage III melanoma

Melanoma patients with palpable nodes, AJCC stage IIIb, have an average 5 year survival of 39-70%.[4] A therapeutic lymph node dissection, with or without postoperative radiotherapy, is the only available possible curative treatment.[24,25] Survival of this stage of melanoma is related to the number of lymph nodes with metastases, and the size of the malignant nodes.[4]

Balch et al. analyzed 2313 patients with AJCC stage III disease, and the 5-year overall survival was 63% for the cohort. However, when focusing on more specific patient groups, a tremendous heterogeneity in 5-year survival rates was observed (23-87% 5-year survival).[26] Preoperative elevated S-100B in patients with palpable nodes in optimally-staged (i.e. PET/CT) clinical stage III melanoma is associated with decreased disease-free survival (Figure 1).[14, 26-29] S-100B seems to be a strong prognostic marker for lymphogenic metastasized melanoma.[14, 26-29] S-100B is located in the cytoplasm of melanoma cells, and elevated S-100B probably occurs as the result of lost integrity of the melanoma cells.[15,17] Therefore,

elevation of S-100B should be interpreted as a process of subclinical microscopic metastatic disease, and preoperative elevated S-100B could be an expression of early dissemination not detected by today's most advanced imaging techniques.[14, 26-29]

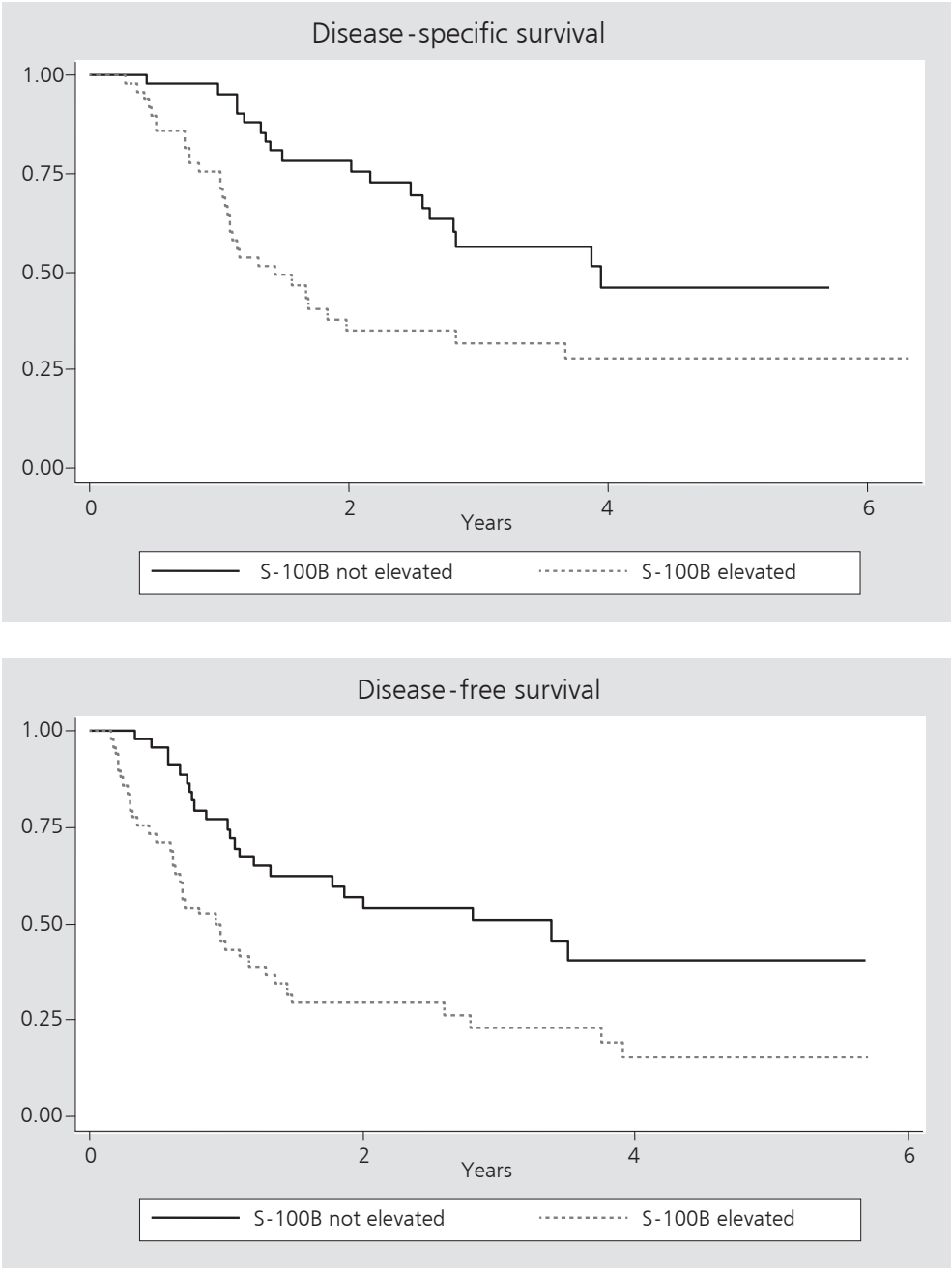

FIGURE 1. Disease-specific survival and disease-free survival in patients with elevated S-100B and melanoma AJCC stage IIIB disease.[29]

Recently at this centre, a phase 1 study was published in which S-100B was evaluated to measure therapy effects after bevacizumab-induction treatment in AJCC stage III melanoma in advance of lymph node dissection.[11] This feasibility study showed that induction treatment with bevacizumab 4 weeks prior to surgery may be related to induction of tumour necrosis, but more importantly, when tumour necrosis was found in the dissection preparation, a 100% decline in S-100B was noted.[12] Correspondingly, the absence of tumour necrosis was correlated with ongoing S-100B elevation. The ability to target therapy towards well-selected subgroups of patients with the use of biomarkers, such as S-100B, could increase the likelihood of benefits, and might improve therapeutic outcomes for future melanoma treatments and strategies.[11,14]

S-100B in AJCC stage IV

Patients with distant metastases (AJCC stage IV) have a short 2-year survival of 18-40%.[5]

For a select group of patients with 1 to 3 metastases, who could be operated on with curative intent, a 5-year survival of 40% can be achieved.[5] Treatment results for advanced melanoma have been very unsatisfactory. Patients with stage IV melanoma have traditionally been managed with various systemic treatments; however, overall survival using this approach has been disappointing. Most melanoma patients in this staging group receive palliative systemic treatment or palliative radiotherapy. In melanoma patients with generalized metastases, S-100B is elevated in 40-100% of cases and represents tumour load.[14,18] When S-100B is not elevated earlier in stage IV melanoma, survival was significantly longer.[30] On the contrary, continuing elevation of S-100B during treatment with systemic chemotherapy or immunotherapy is a sign of progressive disseminating disease.[30,31] An effective therapy measured by the RECIST criteria is often correlated with declining S-100B concentrations.[32]

For decades, the progressive treatment of metastasized melanoma has been disappointing with no survival benefit. No systemic therapy has been shown to affect overall survival. Currently however, two promising treatment concepts have arisen. Immunotherapy with ipilimumab, and the introduction of B-Raf proto-oncogene serine/threonine-protein kinase (BRAF) pathway inhibitors have shown spectacular results.[8-10] First, on March 25, 2011, the Food and Drug Administration approved the cytotoxic T-lymphocyte antigen-4 monoclonal antibody ipilimumab (Yervoy), making it the first agent indicated for non- resectable or metastatic melanoma in more than a decade.[7] Second, since the identification in 2002 of a mutation in the BRAF gene in a subset of melanoma patients, research focused on developing inhibitors of the mutated BRAF protein as a therapeutic target in disseminated melanoma.[9,10] Recently, Phase 1 and 2 clinical trials of the BRAF kinase inhibitor vemurafenib (PLX4032) showed response rates of more than 50% in patients with metastatic melanoma with the BRAF V600E mutation.[10] On August 17, the FDA approved vemurafenib (Zelboraf) as a treatment for late-stage melanoma, since it was shown to extend patients' survival. Now that an effective (adjuvant) therapy for loco-regional metastatic and disseminated melanoma seems to have been discovered, the role of S100-B in the treatment of melanoma disease may change dramatically.[33]

DISCUSSION

Various studies have reported on the use of S-100B as a tumour marker during melanoma treatment. Effective therapy by means of a lymph node dissection, systemic therapy, or immunotherapy is associated with declining S-100B levels, whereas elevated S-100B concentrations correlated with disease progression.[14,27-29] The prognostic biochemical marker, S-100B may be of value in the design of future trials on adjuvant systemic chemotherapy or immunotherapy.

Presently, LDH is still the most prominent serum parameter in stage IV melanoma, and is still part of the AJCC staging system.[4] For optimally-staged clinical stage III melanoma patients, it is clear that preoperative S-100B elevation is associated with significantly poorer survival.[14,29] These results are also supported by an ECOG study in high-risk surgically-resected melanoma by Kirkwood.[27] In this study, postoperative (baseline) S-100B was indeed a prognostic marker for disease-free survival and overall survival in stage III melanoma patients. Recently, Bouwhuis et al. showed that elevated S-100B levels in serial serum measurements in stage III melanoma is of very significant prognostic value, which was even stronger than disease stage and number of positive lymph nodes.[28]

Therefore, S-100B could be used for patient selection for adjuvant systematic treatment in the stratification for new adjuvant therapeutic trials as well as to provide information for stage III melanoma patients who want to be informed about disease prognosis.

Also, in the near future, serum S-100B can also be used to assess response to induction therapy with systemic treatment for nodal disease.[12] The ability to target therapy towards well-selected patient subgroups with the use of biomarkers such as S-100B could increase the likelihood of treatment benefits, and might improve therapeutic outcomes for future melanoma treatments.[12] Not only should elevated S-100B be seen as a prognostic factor for survival and a reflection of disease progression, the presence of the S-100B protein itself induces disease progression as well. S-100B interacts with p53, thereby down-regulating its function as a tumour suppressor protein by preventing induction of apoptosis of potential melanoma cells.[34,35] Taking these results into account, an induction therapy might slow down melanoma proliferation in stage III in two ways; first, by inducing tumour necrosis and second, by temporarily suppressing serum S-100B concentrations, thus preventing down regulation of the tumour suppressor protein p53.

CONCLUSION

S-100B can be used to perform a individualized risk assessment before administering adjuvant treatment on the one hand, and to measure response to induction therapy for nodal disease on the other.[12,14] Despite these prognostic values, S-100B is not suitable for routine screening purposes in stage I-III melanoma patients, or for the early detection of recurrences during follow-up of melanoma patients. The results of these measurements do not yet translate into

an adequate therapeutic intervention or survival benefit because of limited treatment options in advanced melanoma. However, now that an effective (adjuvant) therapy for loco-regional metastatic and disseminated melanoma seems to have been discovered, the routine use of biomarkers like S-100B may change significantly in the future.[33]

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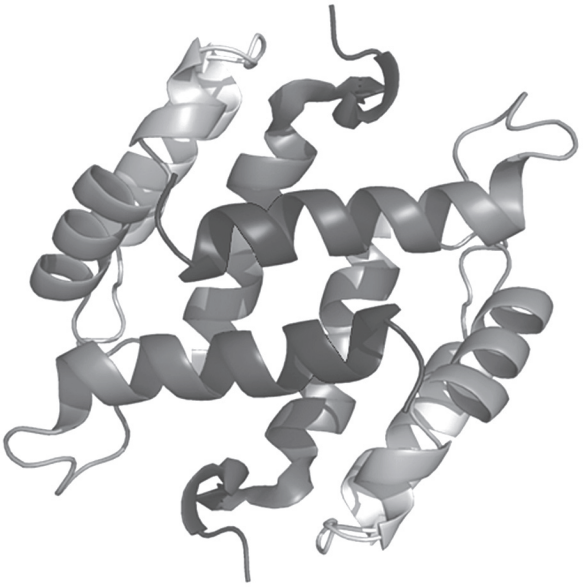

General conclusion and future perspectives

As described in this thesis the protein S-100B is a strong prognostic marker in stage III and IV melanoma and is currently the best studied biomarker in melanoma disease. It has the potential to identify high risk stage III melanoma patients who may benefit from adjuvant systematic treatment in the future. The following paragraph describes aspects of currently ongoing research and future research in the development of follow up, elderly, biomarkers in stage III disease and future strategies of metastatic melanoma treatment.

FOLLOW-UP

There is no international consensus on the follow-up of melanoma patients and in many countries high-frequency follow-up after treatment for melanoma is still standard care.[1]

The main purpose of follow-up is early detection of recurrent disease and second primary melanomas that could be treated successfully by surgery or other treatment modalities. However, it is still difficult to predict the behavior of melanoma in the individual patient although three quarters of the melanoma recurrences are still patient detected.[2] Generally, overall treatment results of metastatic or recurrent disease have been disappointing.

In the first chapter in which we evaluated the role of the method of detection in nodal disease for the prognosis, almost 50% of lymph node metastases in Stage I-II melanoma patients are patient detected.[2] Besides, the method of detection of lymph node recurrence by patient or physician didn`t have any impact on survival. The data of this study have added to the controversy about the value of high frequency follow-up regimens in melanoma.[3,4] A prospective, randomized, high quality methodological research has been started in order to develop meaningful applicable guidelines (MELFO).[5,6,7] This study, currently conducted in the Netherlands and the UK, randomizes stage IB–IIC patients between the follow-up schedule proposed by the national guideline and an evidence-based schedule with a reduced follow-up

frequency.[5] With its primary end point being health-related quality of life, these results should clarify if reduced follow-up frequency has any effect on patient well-being. Although melanoma can recur more than 10 years after diagnosis, this concerns a very small percentage of patients. We conclude that it is not necessary to keep patients in life-long follow-up.[6,7] During follow-up, doctors are recommended to emphasize educating the patient in detecting recurrences and second primary melanoma, in order to promote early detection. Self-examination of nodes basins and skin changes seems suitable for many patients, but could be improved for optimal benefit, for example, by using the internet.[8, 9]

To date, there is no evidence for routine imaging studies, for example PET scanning, or routine measurement of any available serum biomarkers, such as S-100B and LDH, in follow-up for melanoma. Only clinical stage III melanoma patients should be staged with whole-body FDG-PET, PET-CT or spiral-CT as well as using the serum biomarker S-100B.[10,11] Regional treatment, curative or palliative, should be based on the extent of the disease. A randomized clinical trial showed that adjuvant radiotherapy improved regional control in melanoma patients at high risk of regional relapse after lymphadenectomy without affecting survival.[12]

ELDERLY

For the elderly patient various studies have already shown that the incidence and mortality of melanoma in the older population (>65 yrs) is growing rapidly.[13-17] As the geriatric population increases melanoma will become an important health issue for the elderly age group in this century.[18-21] As was described in the third chapter the incidence for the elderly increased much more rapidly than for the younger melanoma patients. Besides, in contrast to younger patients, elderly patients are still diagnosed with the thickest melanomas. Especially in elderly men the percentage thick melanoma's declined only minimally the last decades. This finding probably explains a worse relative survival for the elderly melanoma patient when compared to the younger population. Literature reveals that awareness in Europe is not at such a high level despite an increasing incidence and focus has been mainly on the young patient.[15,21,22] This might explain the high percentage of thick melanoma's in the elderly who, looking at the rising incidence, seem to have started to pay the costs for earlier sun abuse in the era before melanoma became a health priority. For the future, campaigns in the Netherlands should focus on early detection of melanoma in general and especially on early detection in the elderly.

BIOMARKERS IN MELANOMA

LDH

Although LDH has a low sensitivity and therefore doesn`t seem to be the adequate parameter in staging and follow-up for the earlier stages of melanoma (AJCC stage I, II and III), it is an

independent prognostic factor in disseminated disease. (AJCC stage IV) Almost 60 years ago Hill et al. already reported on increased LDH serum parameters in melanoma patients.[23] In the 1970s, LDH was reported to be an indicator for liver metastases with a specificity of 95% and a sensitivity of 83% in AJCC stage II patients and, respectively, 87 and 57% in stage III patients.[24] Even stage I disease patients with elevated serum LDH seemed to have a significant decreased survival.[25] Weighing today's evidence, LDH has high specificity for melanoma and literature demonstrates that LDH is especially elevated in advanced disease, predominantly when disease has progressed to the liver.[26] Deichmann et al. claimed LDH to be the only significant marker for disease progression when analyzing the marker in combination with S-100B and MIA in stage IV disease.[27] LDH can indeed be used as a prognostic marker for detecting distant metastases (AJCC stage IV) but has a poor sensitivity and is not melanoma specific. Despite these facts, today LDH is still part of the AJCC staging manual for patients with distant metastases (stage IV M1c).[28]

S- 100B

Increased S-100B levels were first detected in melanoma patients in 1980.[29] S-100B is a 21-kDa protein that was first isolated from the CNS in vertebrates. Later, S-100B was found to be a serological tumor marker for melanoma and seemed to be mostly increased in stage III and IV.[30,31] According to the literature to date, the proportions of patients with elevated S-100B concentrations are 0-9% in stage I/II, 5-98% in stage III and 40-100% in stage IV.[11] The highest S-100B concentrations were found in patients with liver and bone metastases.[32,33] It can, therefore, be concluded that S-100B concentrations are correlated with the clinical stage of the disease. Several studies have recommended the use of S-100B as a tumor marker to monitor the course of the disease in stage III melanoma or to evaluate the effect of therapy in stage IV disease. Successful treatment with lymph node dissection, chemotherapy or immunotherapy was associated with decreased S-100B concentrations, whereas increased concentrations were an expression of disease progression.[11,34,35] Today, for AJCC stage III melanoma patients, a therapeutic lymph node dissection is still the first choice of treatment. The lymfogenic disseminated AJCC stage III melanoma patient group is known to be a be a patient group with a remarkable heterogeneity which was published by Balch in a multivariate analysis of prognostic factors among 2,313 patients with stage III melanoma. The five year overall survival was 63% varying from 67% for patients with nodal micro-metastases to 43% for nodal macrometastases.[36] Recently it was also reported that S-100B can be used as a prognostic marker for disease-free survival and overall survival directly after therapeutic lymph node dissection in stage III melanoma.[37-40] Data from our centre were not only in concordance with the above described findings, but also revealed that S-100B can be used as a prognostic marker for disease-free survival when determined before dissection after staged with whole-body FDG-PET, PET-CT or spiral-CT. Multivariate analysis showed that preoperative elevated S-100B in PET, PET-CT or spiral-CT negative patients in stage III melanoma was an important predictor for a significantly worse survival.[11] The hypothesis that elevating S-100B should be interpreted

as a process of active hematogenic and lymfogenic dissemination seems to have been confirmed by these data. Thus, clinical stage III melanoma patients should be staged with whole-body FDG-PET, PET-CT or spiral-CT as well as the use of serum biomarker S-100B.[11] Further regional treatment, curative or palliative, should be based on the extent of the disease.

In the future, S-100B can thus be used to perform a more refined individualized risk assessment before administering adjuvant treatment. Above all, it provides information to stage III melanoma patients who want to be optimally informed about their disease prognosis. Despite the described prognostic values, this biomarker is not suitable for routine screening purposes in stage I-III melanoma patients or or for the early detection of recurrence in the follow-up of melanoma patients; that is, early detection of recurrences in follow-up as the results of these measurements do not yet translate into an adequate therapeutic intervention nor survival benefit due to limited treatment options in advanced melanoma.

COMPLETE LYMPH NODE DISSECTION FOR REGIONAL NODAL METASTASIS

The primary management of lymph nodes involved with metastatic melanoma is regional lymfadenectomy. Axilary or inguinal node complete lymph node dissection (CLND) is performed after occult metastases are found by sentinel lymph node biopsy, or after a clinically apparent regional lymph node metastasis. CLND completely removes all lymph-node-bearing tissue in a nodal basin. Lymph node dissections are known for a considerable morbidity. Whereas a reduction through technical modifications seems to be difficult, efforts to limit the procedures to defined patient subgroups may prove to be a more promising .

SLNB has helped spare the majority of melanoma patients, who are node-negative, from the morbidity of ELND.

Whether or not CLND in patients with positive sentinel lymph nodes increases overall melanoma specific survival remains unclear. The procedure continues to be controversial. No randomized prospective studies have yet determined the survival advantage of CLND. Most national guidelines recommend that all patients with stage III melanoma still have a CLND.[41,42]

Underway is the second MSLT (MSLT-II), a randomized trial that will accrue 4,500 patients from more than 30 countries.[43] Results of MSLT-II will indicate whether removal of the SN alone is adequate nodal surgery in certain patients with regional metastatic melanoma and this trial is therefore expected to clarify this issue.[43]

STAGE IV DISEASE

Surgery in patients with stage IV metastatic melanoma

Treatment results for advanced melanoma have been very unsatisfactory. Patients with stage IV melanoma have traditionally been managed with various systemic treatments; however, overall survival with this approach has been disappointing. Surprisingly, findings of many retrospective, single-institution, and multicentre studies suggest that participants treated with complete metastasectomy for stage IV metastases have an enhanced overall 5-year survival.[38] Systemic treatment alone for patients with stage IV melanoma have not yet conferred the survival advantage of a complete metastasectomy.[44]

One of the strong believers of metastasectomy in patients with stage IV melanoma, Donald Morton, launched an international trial.[45,46] To be eligible for the study, all participants had to undergo complete metastasectomy for stage IV metastases with tumor free surgical margins. After surgery, patients were randomized to either adjuvant immunotherapy with onamelatucel-L or placebo. Although the main aim of the trial-to assess onamelatucel-L as an adjuvant treatment after surgery-did not show a survival benefit, two important conclusions were made. First, correct selection of patients is vital. Only people with three or fewer visceral sites of stage IV disease who could be rendered clinically and radio graphically free of disease were eligible for this trial. Second, uniformity of resection can be accomplished. In an international, randomized controlled trial surgeons from around the world undertook similar complete metastasectomy procedures for various anatomical sites and achieved tumor-free surgical margins. This similarity in resections led to an 40% 5-year survival for the entire study cohort.[46] Such results for 5-year survival have not yet been achieved in any study of chemotherapy or biological treatment for patients with stage IV melanoma.[45,46] Considering the evidence, the conclusion has to be drawn that complete surgical resection of metastatic disease to stage IV sites-including skin, soft tissue, distant lymph nodes, lungs, or other non-CNS visceral regions-offers the best chance for prolonged survival. Therefore, it seems the time has come to reposition surgery as the first option for properly selected patients with stage IV disease, as long as complete metastasectomy can be undertaken and there is no better alternative systemic treatment.[47]

Of course the use of this aggressive surgical approach should be tempered with the knowledge that incomplete resections put patients at increased risk and without any proven survival benefit these should be reserved only for palliation of symptoms. Thus, the first step for people with newly diagnosed stage IV disease should be assessment of resectability by a skilled surgical oncologist.[44]

Adjuvant therapy

Adjuvant systemic chemotherapy or immunotherapy with various nonspecific immune-stimulatory agents in the treatment of high-risk melanoma is not indicated outside the context of a clinical trial.[48] However, adjuvant immunotherapy with high intermediate- and low-dose

IFN-a was extensively studied during the last decade. No systemic therapy has demonstrated to affect overall survival. Currently however, two promising treatment concepts are being explored. The recent immunotherapy with ipilimumab and the introduction of the BRAF pathway inhibitors have shown spectacular results. First, on March 25, 2011, the Food and Drug Administration approved cytotoxic T-lymphocyte antigen-4 monoclonal antibody (Ipilimumab), making it the first agent indicated for unresectable or metastatic melanoma in more than a decade.[49,50] Second, since the identification in 2002 of a mutation in the B-Raf proto-oncogene serine/threonine-protein kinase (BRAF) gene in melanoma, research is focused on developing inhibitors of the mutated BRAF protein as a therapeutic target in disseminated melanoma.[51] Recently, Phase 1 and 2 clinical trials of the BRAF kinase inhibitor vemurafenib (PLX4032) have shown response rates of more than 50% in patients with metastatic melanoma with the BRAF V600E mutation. At 6 months, overall survival was 84% in the vemurafenib group and 64% in the dacarbazine group. In the interim analysis for overall survival and final analysis for progression-free survival, vemurafenib was associated with a relative reduction of 63% in the risk of death and of 74% in the risk of either death or disease progression, as compared with dacarbazine ($p<0.001$ for both comparisons).[52]

Maybe, since an effective (adjuvant) therapy for loco-regional metastatic and disseminated melanoma seems to be discovered, the entire discussion with respect to follow-up intensity and routine imaging and use of biomarkers will be significantly altered.[53]

The use of biomakers to evaluate therapy effects

S-100B has the potential to identify high risk stage III melanoma patients who may benefit from adjuvant systematic treatment. As described in the sixth chapter, in the near future, with the aid of the biomarkers serum S-100B and LDH and Standardized Uptake Value (SUV) in FDG-PET, a response to induction therapy with systemic treatment might be observed for nodal disease. S-100B and SUV are known to be of prognostic value in stage III melanoma and elevated S-100B and SUV in stage III melanoma patients can be specific indicators of disease progression.[33-40,54,55] Recently Bouwhuis et al revealed that an elevation of S-100B levels in serial serum measurements in stage III is a very significant prognostic value, which is even stronger when compared with disease stage and number of positive lymph nodes.[56] However, not only should elevated S-100B be seen as a prognostic factor for survival and a mere reflection of disease progression alone, the presence of the protein S-100B itself induces disease progression as well. S-100B interacts with p53 and thereby down-regulates its function as a tumor suppressor protein by preventing induction of apoptosis of potential melanoma cells.[57,58] Taking these results into account, an induction therapy might slow down melanoma proliferation in stage III in two ways. First, by inducing tumor necrosis and second by temporarily suppressing the serum S-100B concentrations and thus preventing down regulation of tumor suppressor protein p53. The ability to target therapy towards well selected subgroups of patients with help of biomarkers as S-100B in melanoma could increase the likelihood of benefit and might improve therapeutic outcome in the future.[59]

MELANOMA CLINICS

The challenge for medicine in general and for treating melanoma is to create the right kind of focus in the health care delivery process. This can be achieved by creating special treatment centers in hospitals; units that excel in delivering one type of care.[60] For melanoma this implicates a need for creating integrated multidisciplinary practice units within focused melanoma clinics.[61] So in the coming years these dedicated multidisciplinary clinics are the next step in melanoma patient care. It is a crucial step towards higher treatment efficiency and efficacy; by increasing quality of care and reducing costs, higher Phase I, II and III trial participation and optimizing follow-up. The ultimate goal of melanoma clinics is to improve the survival of patients diagnosed with melanoma. Consequently, the University Medical Center Groningen has recently started a melanoma clinic in which dedicated nurse practitioners participate in the routine follow-up of melanoma patients and clinical trials to reduce the workload of specialists and improve patient satisfaction.[62] Additionally a part of melanoma care may possibly be transferred to GPs.[62,63] Individual patients prognostic models for stage I, II and III were recently developed, made available online and can be used for patient-tailoring initial management and subsequent follow-up.[64,65]

RISING COSTS

The cost of cancer care is on the rise, from $104 billion in 2006 to over $173 billion in 2020. The rise in cost is driven by both the increasing cost in therapy and the extent of care.[66,67]

Efficacy of these new drugs often do not outweigh their cost-effectiveness, resulting in an increasing cost curve that is not sustainable. The new melonama systemic therapies will definitely not improve this situation. For example, earlier this year, the Food and Drug Administration (FDA) approved the recent developed immunotherapy with ipilimumab which cost about $120,000 per treatment. The definite price of Vemurafenib is not yet defined but will be likely to cost tens of thousands of dollars per year.

Medical oncologists directly or indirectly control or influence the majority of cancer care costs, including the use and choice of drugs, the types of supportive care, the frequency of imaging, and the number and extent of hospitalizations. Smith and Hillner recentley suggested suggesting five changes in medical oncologists behavior and five changes in their attitudes and practice that would bend the cancer-cost curve downward.[68]

In conclusion, some areas of oncology - for example, clinical trials and curative as well as proven adjuvant treatments - should become off limits when it comes to primary cost considerations. This will probably be extraordinarily difficult as patients facing death have a very different and important perspective on risk.[69]

CONCLUSION

Melanoma is the most aggressive form of skin cancer and its incidence is rising worldwide. While early stages of melanoma can be curatively treated by surgical excision, advanced stages have been uniquely resistant to current therapies. However, we now recognize that melanomas are far more variable at a molecular level than they appear under the microscope.

Therefore, melanoma is to be included in The Cancer Genome Atlas project and this will, in the future, provide more insight into molecular-based profiling studies in melanoma patients.[70] Therefore, rather than treating melanoma as a single disease, eventually molecular biology will unravel the mystery by which melanoma has been surrounded purely by stratifying tumors into molecular subtypes and treating each with the most appropriate therapies.

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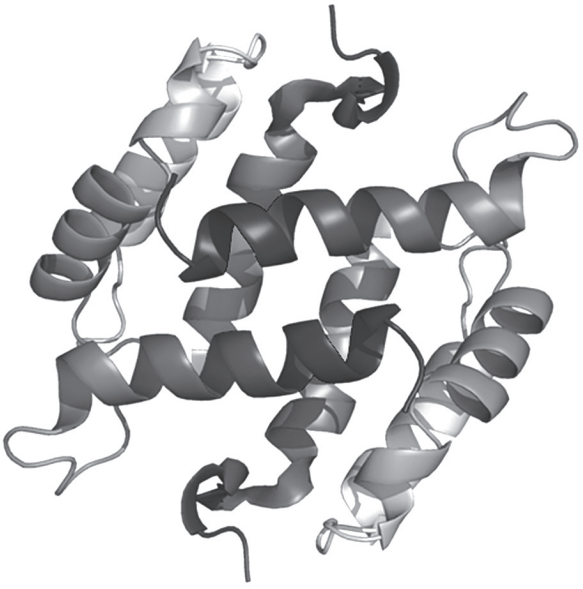

FIGURE 1 : Structure of the protein S-100B

Summary

Melanoma causes more than 75% of all deaths related to skin cancer and melanoma incidence rates have increased rapidly worldwide and are expected to keep on rising in the future. The absolute total number of new cases in the Netherlands is estimated to be well over 4,800 in 2015.[1] Despite being diagnosed in an earlier phase of disease as a result of increased awareness and better surveillance, melanoma patients presenting with palpable nodal metastases today still have a poor 5-year survival (59% and 40% for stage IIIB and IIIC respectively) but a tremendous heterogeneity in 5-year survival rates is observed (23%-87% 5-year survival).[2,3] Biomarkers measured preoperatively in stage III melanoma patients could both be highly specific indicators of early recurrence after surgical treatment and could optimize and refine the staging of melanoma patients with palpable nodes.[4]

In this thesis the main focus on stage III melanoma and how to identify stage III melanoma patients who may benefit from adjuvant systematic treatment with the aid of biomarkers as

S-100B (figure 1) and FDG- SUV in order to improve, in the long run, survival.

PART I : DETECTION AND TREATMENT OF NODAL MELANOMA METASTASES

In chapter two the role of the method of detection in nodal disease in the prognosis of melanoma patients who underwent therapeutic lymph node dissection (TLND) was evaluated. International consensus on the follow-up of melanoma patients has not been achieved and high-frequency follow-up still practiced in many countries around the world. It was found that the method of detection of lymph node recurrence in melanoma patients by patient or physician has no impact on survival. 45% of lymph node metastases in Stage I-II melanoma patients are physician detected. Younger patients detect their own lymph node metastases significantly more often than elderly patients. However, neither the method of detection nor

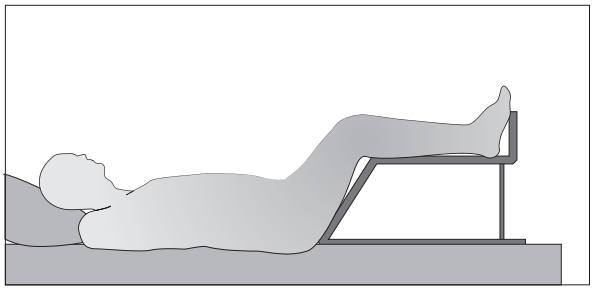

FIGURE 2: Bed rest and flexion of the hip and knee in flexion using a Bohler splint

age correlates with DSS. More frequent follow-up would not alter DFS and DSS significantly. The data of this study will add to the controversy about the value of high frequency follow-up regimens in melanoma. A prospective, randomized, high quality methodological research has been started in order to develop meaningful applicable guidelines (MELFO). In the third chapter we conducted an extensive retrospective study to assess the present morbidity of therapeutic or completion lymph node dissection to determine risk factors for short term morbidity and to evaluate recommendations on peri-operative treatment. Therapeutic groin dissection for melanoma remains a surgical procedure with a high morbidity. Presently, more complications occur after therapeutic ilio-inguinal lymph node dissection than 20 years ago. Analysing our data it was found that a high BMI was significantly correlated with the occurrence of wound infections. Bed rest and flexion of the hip and knee in flexion using a Bohler splint (figure 2) improved wound healing after therapeutic ilio-inguinal lymph node dissection.

PART II : PROGNOSTIC MARKERS IN MELANOMA

When compared with other markers and tumor characteristics as ulceration, Clark level and Mitosis index, Breslow thickness is the most important biomarker and predictor for mortality. In chapter four, patients diagnosed with invasive melanoma between 1994 and 2008 were selected from the Netherlands Cancer Registry (NCR). Incidence (per 100,000) over time was calculated for young (<65 years) and elderly patients (≥65 years). Distribution of Breslow thickness for young and elderly males and females was assessed. Regression analysis of the log-transformed Breslow thickness was used to assess changes over time. Relative survival was calculated as the ratio of observed survival to expected survival.

We found that melanoma incidence increased more rapidly among the elderly (5.4% Estimated Annual Percent Change (EAPC), $p < 0.0001$) than among younger patients (3.9% EAPC, $p < 0.0001$) (figure 3) and the overall Breslow thickness declined significantly over time ($p < 0.001$). However, the decline in Breslow thickness is less prominent among elderly patients than among young patients with a relative survival of elderly patients that was worse compared with that of younger patients ($p < 0.001$). Campaigns in the Netherlands must focus more on early melanoma detection in the elderly.

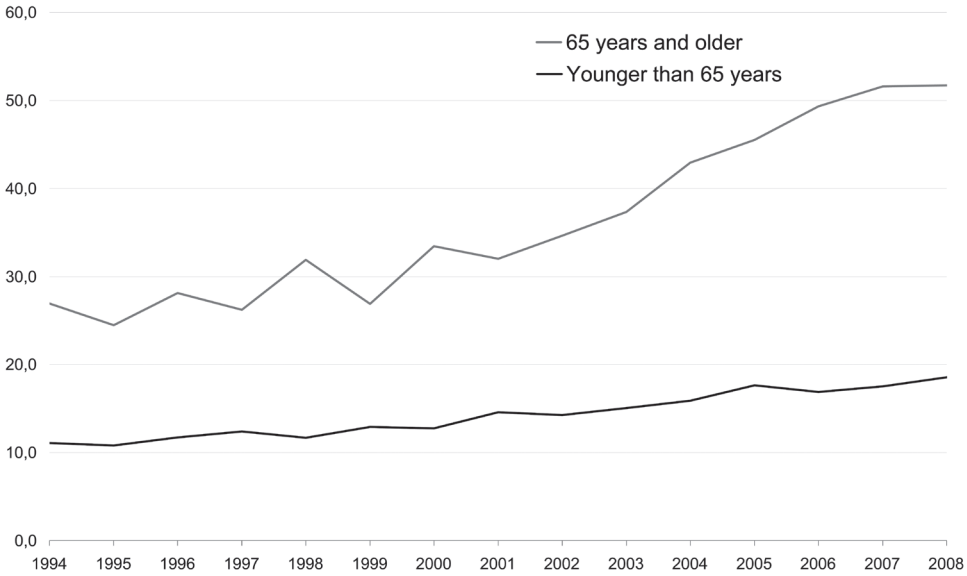

FIGURE 3 Incidence of invasive melanoma among young and elderly patients

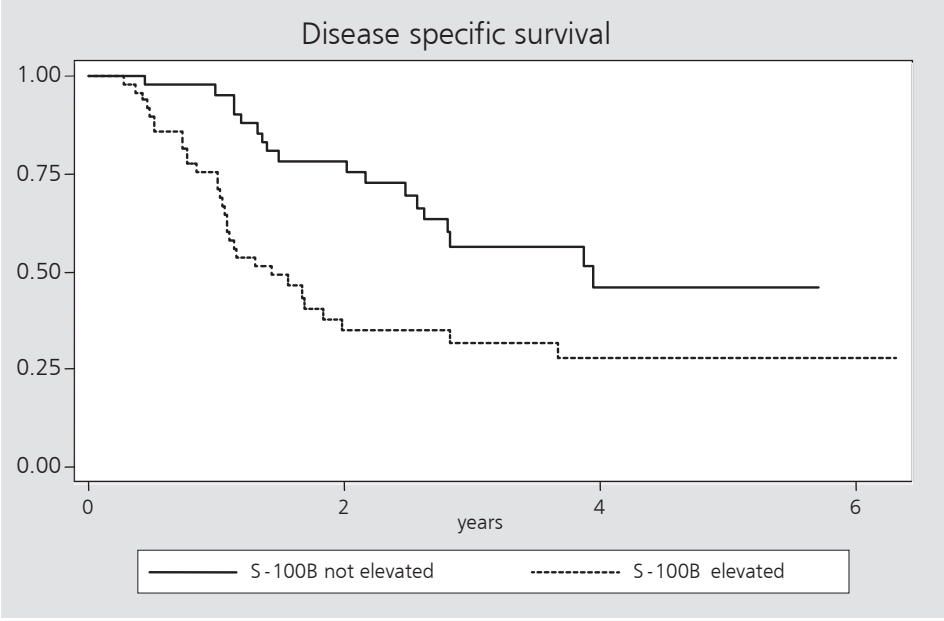

FIGURE 4 : Disease specific survival (DSS) correlated to S-100B in AJCC stadium IIIB.

Up to today the role of S-100B for patients with palpable lymph node metastases before undergoing therapeutic lymph node dissection was still unknown. In chapter five the role of S-100B for disease-free survival (DFS) was evaluated and compared with the tumor marker

LDH. Preoperative S-100B elevation in patients with optimally staged clinical stage III melanoma was correlated with shortened disease-free survival (figure 4).

The new serum tumour marker S-100B has the potential to identify stage III melanoma patients who may benefit from adjuvant systematic treatment. In the stratification of new adjuvant therapeutic trials in patients with loco regional recurrence stage III melanoma, we would recommend the incorporation of S-100B in the stratification instead of the more commonly used LDH tumour marker. Pre-operative S-100B levels provide information for stage III melanoma patients who want to be optimal informed with respect to their disease prognosis.

In chapter six S-100B values and Standardized Uptake Value (SUV) in FDG-PET for clinically stage III melanoma patients were analysed as indicators of early recurrence in stage III disease. S-100B and SUV in stage III melanoma seemed to have no correlation and both markers have different associations with various histo-pathological factors. However, was conclud in chapter five, S-100B, in contrast with SUV, is associated with nodal tumor load, and when elevated, predicts a shorter DFS.

In chapter seven to investigate the feasibility of using bevacizumab to improve the survival of AJCC stage III melanoma patients, we analyzed how a single bevacizumab treatment affected nodal disease and a panel of biomarkers in clinically FDG-PET/CT staged, stage III melanoma patients, prior to therapeutic lymph node dissection (TLND). Four weeks before TLND, nine patients with palpable lymph node metastases received 7.5 mg/kg bevacizumab. Before and after this treatment, all patients were assessed by measurements of the maximum standardized uptake value (SUVmax) by FDG-PET scan, and serum S-100B and LDH. After TLND, the dissection specimen was analyzed for the number of removed lymph nodes, the amount of metastatic lymph nodes, and tumor necrosis. Tumor necrosis in dissection specimens was associated with declining S-100B levels, while elevated S-100B was only found in cases with no necrosis. Bevacizumab might be useful in treating AJCC stage III melanoma patients prior to TLND, and S-100B appears to be a useful marker for assessment of treatment effects. Future research, preferably in a randomized controlled trial using serum S-100B for response monitoring, should determine the effect of (neoadjuvant) bevacizumab, and adjuvant Ipilimumab and/or BRAF pathway inhibitors as a new combined treatment strategy of systemic treatment with TLND to improve disease free and overall survival in clinically stage III melanoma patients.

The current status of S-100B in treatment of melanoma is described in chapter eight. A review was composed describing the role of S-100B for the different tumor stages in melanoma and how the protein could be used by a practicing surgical oncologist. S-100B was already known as a marker in stage IV palliative melanoma treatment, however in the future it could be used for high risk stage III melanoma to perform better staging. S-100B can be used to perform a individualized risk assessment before administering adjuvant treatment on the one hand and on the other hand to measure response to induction therapy for nodal disease.[4,5] Despite these described prognostic values, S-100B is not suitable for routine screening purposes in stage I–III melanoma patients or or for the early detection of recurrence in the follow-up of melanoma patients; that is, early detection of recurrences in follow-up do

not yet translate into an adequate therapeutic intervention nor survival benefit due to limited treatment options in advanced melanoma. However, maybe, since an effective (adjuvant) therapy for loco-regional metastatic and disseminated melanoma seems to be discovered, the routine use of biomarkers as S-100B seems about to alter dramatically in the future.[6-9]

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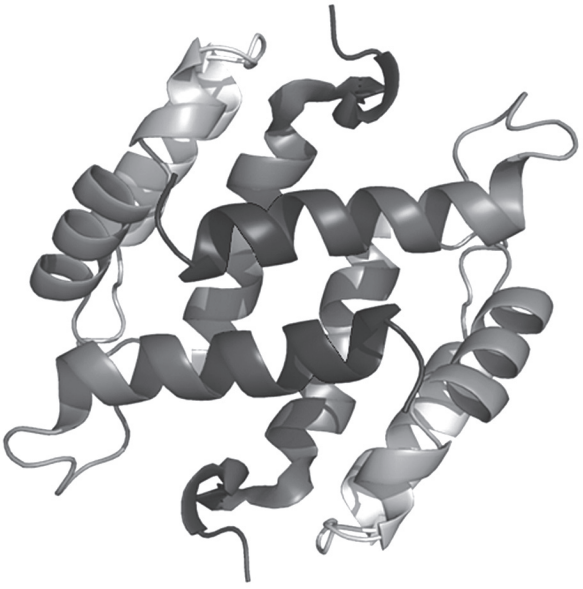


FIGUUR 1 : Structuur van het eiwit S-100B

Samenvatting

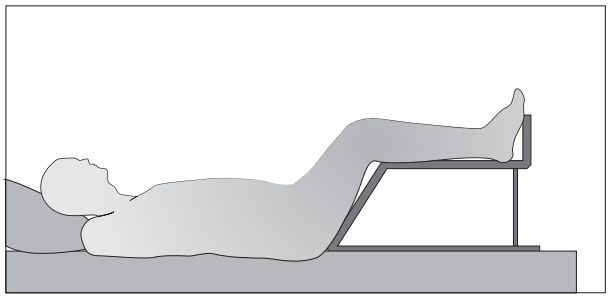
Het melanoom veroorzaakt meer dan 75% van alle huid kanker gerelateerde mortaliteit en de incidentie van deze maligne huidaandoening heeft wereldwijd een grote vlucht genomen. De verwachting is dat die opwaartse trend zich zal voortzetten in de toekomst en dat het absolute aantal nieuwe patiënten dat zich in 2015 zal presenteren met een melanoom in Nederland rond de 4800 is.[1] Ondanks dat melanomen eerder vastgesteld lijken te worden met een toename aan dunnere melanomen, mogelijk als een gevolg van een toegenomen patiënt bewustzijn en toezicht, hebben patiënten die zich presenteren met palpabele klieren (AJCC stadium III) nog altijd een zeer matige prognose. Opvallend is met name de heterogeniteit in 5 jaar overleving uiteenlopend van 23% tot 87%.[2,3] Voor deze patiënten zou daarom een strategie gehanteerd kunnen worden waarbij in eerste instantie biomarkers bepaald worden. Enerzijds met het doel in een vroege fase een recidief te kunnen vaststellen maar met name om het stadierings proces te verfijnen voor deze heterogene groep.[4] Dit proefschrift richt de aandacht op de melanoom patiënt met lymfogene metastasen en op de mogelijkheid om met behulp van biomarkers zoals S-100B (figuur 1) die specifieke hoog risico patiënt te identificeren die in de toekomst overlevingsvoordeel zou kunnen hebben van systemische therapie.

DEEL I : HET VASTSTELLEN EN BEHANDELEN VAN LYMFKLIER METASTASEN BIJ HET MELANOOM

In hoofdstuk 2 werd de prognostische invloed van de wijze van vaststellen van lymfklier metastasen (door de dokter of door de patiënt zelf) bij patiënten die later een lymfklier dissectie ondergingen, geëvalueerd. Er bestaat nog steeds geen algemene internationale consensus over de follow-up van melanoom patiënten en in verscheidene landen over de gehele wereld wordt nog altijd een hoog frequente follow-up strategie toegepast. Vastgesteld werd dat de wijze van vaststellen van palpabele klieren dan wel door een dokter tijdens de follow-up dan wel door een

patiënt zelf geen invloed had op de overleving van deze patiënten groep. 45 % van de lymfklier metastasen in stadium I en II melanoom patiënten worden door een dokter vastgesteld tijdens follow-up. Jonge patiënten ontdekken hun lymfklier metastasen significant sneller dan oudere patiënten. Echter, noch de wijze van vaststellen noch de leeftijd van de patiënt lijken gecorreleerd met ziekte specifieke overleving. Het intensiveren van de follow-up frequentie zou de ziekte specifieke- of ziekte vrije-overleving niet beïnvloeden. De data van deze studie dragen bij aan de controverse die bestaat over de waarde van het uitvoeren van hoog frequente follow-up bij het melanoom. Een prospectieve gerandomiseerde methodologische hoog kwalitatieve studie is derhalve reeds gestart met als doel uiteindelijke waardevolle goed toepasbare follow-up richtlijnen te creëren (MELFO).

In het derde hoofdstuk voerden we een zeer uitgebreide retrospectieve studie uit om de huidige morbiditeit van therapeutische ilio-inguinale liesklier dissecties verricht in ons



FIGUUR 2: Flecteren van de heup door middel van een Bohler splint

centrum te evalueren. Het doel was de risicofactoren voor korte termijn morbiditeit in kaart te brengen en daarmee aanbevelingen te doen voor het peri-operatieve beleid. Een therapeutische liesklierdissectie is een chirurgische procedure met een hoge morbiditeit. Vandaag de dag lijken meer complicaties op te treden na een therapeutische ilio-inguinale lies klierdissectie dan 20 jaar geleden. Uitgebreide analyse van de data uit dit centrum leverde onder meer de conclusie op dat bij patiënten met een hoog body mass index (BMI) een significante correlatie bestond met het optreden van wondinfecties. Daarnaast ging bij die patiënten bij wie postoperatieve bedrust en het flecteren van de heup door middel van het gebruik van een Bohler splint was gehandhaafd (figuur 2) de wond genezing gepaard met minder problemen.

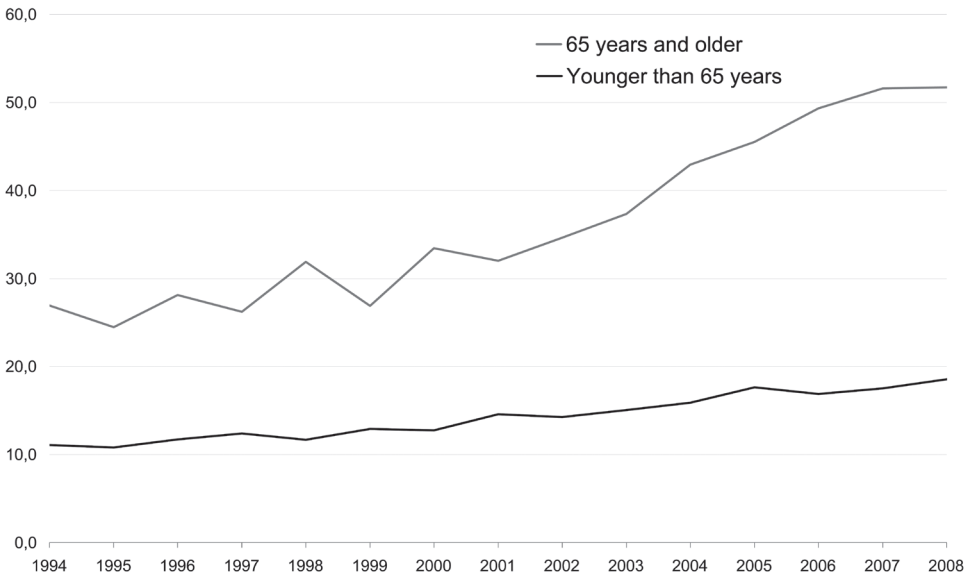
DEEL II : PROGNOSTISCHE MARKERS VOOR HET MELANOOM

Wanneer de Breslow dikte vergeleken wordt met andere markers en tumor kenmerken als ulceratie, Clark niveau en Mitose index , blijft de Breslow dikte van het melanoom de meest belangrijke biomarker en voorspeller voor mortaliteit. In het vierde hoofdstuk werden alle patiënten bij wie tussen 1994 en 2008 de diagnose melanoom gesteld werd, geselecteerd vanuit de Nederlandse integrale kanker centra (IKC). De incidentie (per 100 000) over de tijd werd

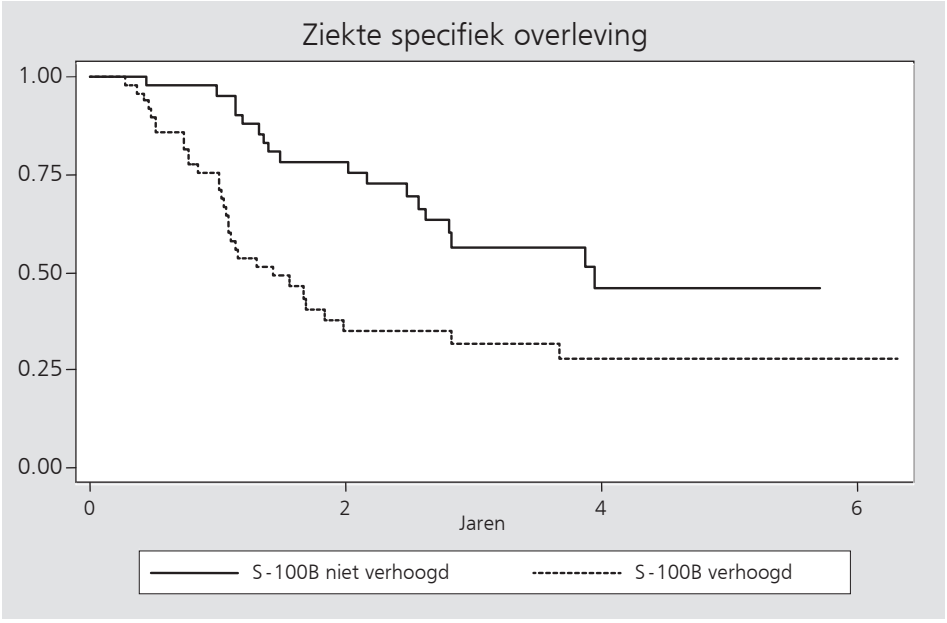
uitgerekend voor de jongere (<65 jaar) en oudere patiënten (≥65 jaar). De verdeling van Breslow dikte van jongere en oudere mannen en vrouwen werd in kaart gebracht. Er werd een regressie analyse gebruikt om de veranderingen van de Breslow dikte over de tijd in beeld te brengen. De relatieve overleving brachten we in kaart als de ratio van de geobserveerde overleving en de verwachte overleving. De resultaten lieten zien dat de incidentie van het melanoom veel sneller steeg onder ouderen y (5.4% Estimated Annual Percent Change (EAPC), p <0.0001) dan onder jongere patiënten (3.9% EAPC, p <0.0001) (figuur 3). Weliswaar daalde als verwacht de Breslow dikte over de tijd (p<0.001) maar deze daling was opvallend minder onder ouderen dan onder jongere melanoom patiënten met een relatieve overleving die eveneens slechter was voor de oudere patiënt (p<0.001). Het lijkt daarom toenemend van belang in Nederland meer aandacht te gaan vestigen op het vroeger vaststellen van het melanoom bij oudere patiënten.

In het vijfde hoofdstuk werd de rol van het eiwit S-100B en ziekte vrije overleving geëvalueerd en vergeleken met de klassiek gebruikte tumor marker LDH die nu nog deel uitmaakt van het AJCC stadierings systeem. Tot op heden was de rol van S-100B voor patiënten met lymfogeen gemetastaseerde ziekte, voordat zij een klierdissectie ondergingen, nog volledig onbekend. Uit onze resultaten bleek dat een preoperatief verhoogd S-100B bij AJCC bij stadium III patiënten die eerder optimaal geanalyseerd waren door middel van FDG PET en CT scan, significant gecorreleerd was met een verkorte ziekte vrije overleving (figuur 4).

De nieuwe tumor marker S-100B heeft de potentie om hoog risico stadium III patiënten te identificeren die zouden kunnen profiteren van adjuvante therapie. Bij het selecteren van patiënten met een loco-regionaal lymfogeen recidief voor nieuwe therapeutische trials, zouden we het toevoegen van S-100B aan het AJCC stadierings systeem sterk willen aanbevelen in plaats van de klassieke tumor marker LDH. In stadium III melanoom geeft het preoperatief



FIGUUR 3 De incidentie van het melanoom onder jonge en oudere patiënten



FIGUUR 4 : Ziekte specifieke overleving (DSS) gecorreleerd met S-100B in AJCC stadium IIIB.

bepalen van serum S-100B informatie aan melanoom patiënten die optimaal geïnformeerd willen worden over hun prognose.

In het zesde hoofdstuk werd zowel serum S-100B als de marker Standardized Uptake Value (SUV) geanalyseerd bij de klinisch stadium III melanoom patiënt. S-100B en SUV leken geen correlatie te hebben met elkaar en beide markers bleken beiden tevens verschillende relaties met histo- pathologische factoren te hebben. Duidelijk was, zoals ook al eerder geconcludeerd in hoofdstuk vijf, dat S-100B evident gecorreleerd was met de lymfogene tumor massa bij patiënten en wanneer verhoogd een goede voorspeller voor een verkorte ziekte vrije overleving. De Standardized Uptake Value (SUV) bleek geen relatie te hebben met ziekte vrije overleving.

In hoofdstuk zeven werd een pilot studie verricht om de mogelijkheid te onderzoeken voor het gebruik van bevacizumab bij stadium III patiënten om de algehele overleving te verbeteren.

We analyseerden hoe een eenmalige toediening van bevacizumab de behandeling van lymfogene ziekte en het verloop van de biomarkers S-100B en LDH kon beïnvloeden voorafgaand aan een therapeutische lymfklierdissectie. Vier weken voor de klierdissectie kregen negen stadium III patiënten 7.5 mg/kg bevacizumab toegediend waarbij hiervoor en erna bij alle negen patiënten de maximale standardized uptake value (SUVmax) en S-100B en LDH werd afgenomen. Na de dissectie werd het klier preparaat pathologisch geanalyseerd op het aantal verwijderde klieren, de hoeveelheid metastatische lymfklieren en de hoeveelheid tumornecrose. De hoeveelheid tumor necrose in het dissectie preparaat bleek gecorreleerd aan een dalend S-100B daar waar een doorstijgend S-100B alleen werd gezien wanneer de tumornecrose ontbrak. Concluderend zou bevacizumab dus van waarde kunnen zijn bij de behandeling van AJCC stadium III melanoom patiënten voorafgaand aan een klierdissectie, en daarnaast zou

S-100B een waardevolle marker kunnen zijn voor het evalueren van het behandelresultaat. Toekomstig onderzoek, bij voorkeur in de vorm van een gerandomiseerde trial waarbij serum S-100B toegepast wordt om behandelrespons te meten, zal moeten gaan uitmaken of het effect van (neoadjuvante) bevacizumab, eventueel in samenwerking met de veelbelovende adjuvante middelen als Ipilimumab en/of BRAF remmers als nieuwe gecombineerde strategie, de ziekte vrije en algemene overleving zal verbeteren in stadium III melanoom patiënten.

In hoofdstuk 8 werd de huidige status van het gebruik van het eiwit S-100B beschreven bij de behandeling van het melanoom in een overzichtsartikel. Hier wordt de rol van het S-100B eiwit beschreven volgens de verschillende tumor stadia van het AJCC classificatie model en hoe het gebruikt zou kunnen bij de behandeling van het melanoom nu en in de toekomst.

S-100B was reeds bekend als een marker bij de palliatieve behandeling van stadium IV melanoom maar lijkt dus in de toekomst ook gebruikt te kunnen gaan worden bij de behandeling van hoog risico stadium III melanoom om beter te kunnen stadieren.

Daarnaast kan het eitwit gebruikt worden om een betere individuele risico analyse te maken voordat adjuvante behandeling toegediend wordt alsmede voor het meten van response op een inductie behandeling voorafgaand aan een klierdissectie bij lymfogene metastasen.[4,5]

Ondanks deze prognostische kwaliteiten, lijkt S-100B niet geschikt voor routine screenings doeleinden in stadium I en II patiënten of voor het gebruik bij vroeg opsporing van recidieven in de follow up; dat wil zeggen, vroege opsporing van recidieven tijdens de follow-up vertaalt zich nog niet in overlevingsvoordeel doordat er nog te weinig behandelopties zijn voor het gemetastaseerd melanoom. Echter, misschien, doordat er een serie aan veel belovende effectieve adjuvante middelen voor loco-regionaal en gegeneraliseerd gemetastaseerd melanoom op de markt zijn gekomen, zou het gebruik van biomarkers als S-100B in de nabije toekomst drastisch kunnen gaan veranderen.[6-9]

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Een proefschrift is een proeve van bekwaamheid in de wetenschap. Mijn proeve van bekwaamheid beschouw ik als een zeer leerzame periode waarin mij bijgebracht is hoe een onderzoek op te zetten, te analyseren, op te schrijven en vervolgens te publiceren. Ik zie dit proefschrift als een begin; een begin van het bedrijven van wetenschap met een zekere kwaliteit.
De totstandkoming van dit werk heb ik beschouwd als een opleiding. Het was voor mij een voorrecht deze opleiding te kunnen combineren met mijn klinisch chirurgische opleiding. Het was namelijk een project van mijzelf, waar alleen ik eindverantwoordelijk voor was. Ik heb het als prettig ervaren om naast de dagelijkse patiëntenzorg aan dit project te werken. Want patiëntenzorg is prachtig werk - maar nooit af. Een artikel is op een dag wel af. En het voelt goed voor een mens om iets af te ronden.
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Enschede , 29 augustus 2011
Schelto Kruijff

Mijn
d a n k
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Curriculum vitae

Schelto Kruijff werd geboren op 18 mei 1977 in Rijpwetering, als laatste uit een gezin van drie kinderen. Vanaf zijn zevende jaar ging hij in het naburige Leiderdorp (heen en weer 14 kilometer fietsen) naar zijn lagere school. Zijn eindexamen VWO behaalde hij in 1996 hij aan het Stedelijk Gymnasium in Leiden. Daarna volgde een wereldreis van een jaar.

In 1997 startte Schelto in Groningen met de studie Biologie, maar toen bleek dat hij het ontleden van haaien interessanter vond dan het determineren van planten, stapte hij over naar de Geneeskunde.

Tijdens zijn studietijd in Groningen was Schelto actief op de studentenvereniging aan de Grote Markt 27, wat leidde tot een jaar onderbreking om deel uit te maken van het bestuur van die vereniging. Ook de studiegebonden activiteiten, zoals het leverteam en de stichting Johannes Borgesius, werden met groot plezier uitgevoerd.

Tijdens de co-schappen in Deventer, waar Schelto twee jaar in een boerderij in de bossen achter Diepenveen woonde, raakte hij voorgoed verslingerd aan de Heelkunde. Na het beëindigen van zijn studie werkte Schelto vier maanden in het Nkhoma mission Hospital in Malawi samen met Herman Firma (tropenarts en chirurg). Tijdens deze indrukwekkende periode ontwikkelde Schelto een visie op zijn toekomst als dokter. Vanuit Malawi stuurde hij zijn sollicitatieformulieren voor de Heelkunde op.

Via een Agnio-schap Heelkunde in het MST in Enschede werd Schelto aangenomen voor de opleiding Heelkunde per mei 2006 in het UMCG onder leiding van Prof. dr. H.J. ten Duis. Aldaar begon hij aan zijn oncologisch onderzoek over het eiwit S-100B en de melanoompatiënt met lymfogeen gemetastaseerd melanoom onder begeleiding van Prof. dr. H.J. Hoekstra. Nauwelijks met het onderzoek aangevangen, vertrok hij in 2008 naar het MST. Daar vervolgde hij zijn opleiding onder leiding van Dr. W.J. Mastboom en legde hij zich toenemend toe op zijn promotie.

In Enschede raakte Schelto klinisch steeds meer geïnteresseerd in de chirurgische oncologie en kreeg hij de kans zich hierin verder te ontwikkelen. Daarnaast was Schelto tijdens zijn opleiding bestuurlijk actief in de Jonge orde en het bestuur van de Vagh. Schaatsen is een passie en hij verreed de alternatieve Elfstedentocht op de Weissensee dan ook drie keer.

Schelto zal zijn opleiding voltooien in juli 2012. Daarna zal hij met zijn gezin vertrekken naar Sydney vanwege een fellowship in de endocriene chirurgie.

Schelto is getrouwd met Annelies van Walsem, die werkzaam is in het onderwijs. Zij hebben drie kinderen: Otto, Alexa en Eelco.

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