AUS DER UNIVERSITÄTS-HAUTKLINIK TÜBINGEN

Abteilung Dermatologie (Allgemeine Dermatologie und Poliklinik)

Ärztlicher Direktor: Professor Dr. M. Röcken

Risk factors for a positive sentinel lymph node dissection in cutaneous melanoma. Does the surgeon play a role?

Inaugural-Dissertation

zur Erlangung des Doktorgrades der Medizin

der Medizinischen Fakultät

der Eberhard-Karls-Universität

zu Tübingen

vorgelegt von

Julia Angelika Löffler

aus Reutlingen

2010

AUS DER UNIVERSITÄTS-HAUTKLINIK TÜBINGEN

Abteilung Dermatologie (Allgemeine Dermatologie und Poliklinik)

Ärztlicher Direktor: Professor Dr. M. Röcken

Risk factors for a positive sentinel lymph node dissection in cutaneous melanoma. Does the surgeon play a role?

Inaugural-Dissertation

zur Erlangung des Doktorgrades der Medizin

der Medizinischen Fakultät

der Eberhard-Karls-Universität

zu Tübingen

vorgelegt von

Julia Angelika Löffler

aus Reutlingen

2010

Dekan:	Professor Dr. I. B. Autenrieth
1. Berichterstatter:	Professor Dr. M. Möhrle
2. Berichterstatter:	Frau Professor Dr. T. Fehm

Für meine Familie

Table of contents

1. Introduction

A histologically positive SLN is an important prognostic factor for survival and the risk of recurrence [4; 8; 9], the absence of metastases in the SLN implies that the entire lymph node basin is tumour-free [7].

Since 1996, Sentinel lymph node dissection (SLND) has been performed at the Department of Dermatology, University of Tuebingen in Germany, to stage and identify patients with cutaneous melanoma who may benefit from an early, complete lymphadenectomy (CLA) and adjuvant therapy. Originally initiated by Morton et al. [15], the SLND technique offered the possibility to identify patients who harbour lymph node micrometastases by using this minimally invasive procedure, while potentially sparing lower risk patients from undergoing CLA [1]. Because in the majority of cases the first spreading of the tumour takes place to the regional lymph nodes, SLND emerged in the last few years [15; 20]. Today SLND is the nodal staging procedure of choice in patients with clinically non-metastatic cutaneous melanoma [5].

The aim of the present study was to explore the histopathological and clinical risk factors for a positive SLND and to examine the role of individual surgeons and their SLND experience on SLN results.

2. Patients and Methods

2.1 Patients

This study includes 999 consecutive patients (547 male / 452 female) with clinical stage I/II cutaneous melanoma who were prospectively followed up from January 2000 to October 2006 at the Department of Dermatology at the University of Tuebingen. The SLND was generally offered to patients having a melanoma with a thickness ≥ 1.00mm or having a melanoma thinner than 1.00mm with histological regression or ulceration.

In 21 patients with a melanoma < 1.00mm and without regression or ulceration there was a strong demand by the patient and/or the referring physicians to perform SLND. The routine preoperative clinical and technical examinations (ultrasound of the regional lymph nodes, chest x-ray, abdominal ultrasound or computed tomography) didn't disclose any evidence for regional or distant metastases. The patients had given written informed consent to documentation and evaluation of their data stored in the Central Malignant Melanoma Registry of the German Dermatological Society and the Melanoma Registry of the Department of Dermatology at the University of Tuebingen.

2.2 Sentinel Lymph Node Dissection (SLND)

SLND was performed using the so-called triple-technique (lymphoscintigraphy, gamma-probe & blue dye injection), thus the SLN could be distinguished from other lymph nodes of the draining lymphatic basin. The method of SLN identification has been described previously [15]. SLND was performed using tumescent local anaesthesia prior to the injection of patent blue V [3].

Lymphoscintigraphy:

Preoperatively lymphoscintigraphy was performed to detect the draining lymph node basin. Five to 20 hours before the operation, 30-100 MBq Technetium nanocolloids were carefully injected into the dermis in equal amounts in 4 to 6

parts around the localization of the primary tumour, respectively close to the melanoma excisions scar. After several minutes lymphoscintigraphy was conducted, until the first appearance of SLN.

Detection via gamma-probe:

The SLN was localized by a transdermal measurement of radioactivity with a hand-held gamma-probe (C-Trak Automatic. Morgan Hill,Ca).

Preoperative blue dye injection:

Ten minutes before skin incision, 0.5 to 1 ml of isosulfane blue (Patent blue V, Byk Gulden) was injected intradermally around the tumour respectively the previous excision site.

Following a skin incision, the sentinel node or several sentinel nodes were isolated and dissected. Intraoperative identification of the sentinel nodes were facilitated by the greatest radioactivity, which was shown by the gamma probe, and the blue dye of the marked sentinel lymph nodes. All blue nodes and/or nodes whose radioactivity in vivo clearly exceeded the background radioactivity of the lymph node region were removed. Ex vivo the radioactivity was confirmed within the SLN by gamma probe.

2.3 Histopathological Evaluation

In 802 of 976 patients, SLNs were bisected, one half being used for routine pathology and the other half for study purposes. SLNs from the remaining 174 patients were entirely sent to histopathological evaluation.

The excised lymph nodes were fixed in 5% formaldehyde, embedded in paraffin and analyzed by standard histopathology (haematoxilin and eosin staining) and immunohistochemistry. SLNs were cut into 5 sections. Two slices were used for standard H&E staining and three for immunohistochemical studies with Anti-HMB45, Anti-S100 and Anti-MELAN A. In standard H&E staining a distance of approximately 200-400 µm between the sections was followed.

A SLND was defined as positive when tumour cells could either be identified in the H&E-stained specimens or when HMB45 positive solitary cells as well as cell aggregates of S100 positive cells emerged in immunohistochemical investigations.

2.4 Surgeons

In this study we investigated the SLN results of 22 different surgeons in our Department, including the 4 principal surgeons, who had each performed more than 100 SLN procedures. Of the four principal surgeons, surgeon A had performed 124 procedures, surgeon B 321 procedures, surgeon C 171 procedures and surgeon D 162 procedures. The remaining surgeons had each performed between one and 100 SLN procedures. We classified the surgeons in 3 groups. Group 1 had an experience of less than 25 SLN procedures (16 surgeons, accounting for 63 procedures), group 2 from 25 to 100 (2 surgeons, accounting for 106 procedures) and group 3 with more than 100 SLN procedures (4 surgeons, accounting for 778 procedures).

If more than one surgeon was involved in a SLND, we evaluated the most experienced surgeon.

2.5 Statistical Methods

For the statistical evaluation the program JMP 7.0 was used (http://www.jmp.com/). Univariable analysis of dichotomous variables (e.g. sex) were analyzed with the Pearson's chi-square test [12].Variables having more than two values (e.g. histological subtype) were studied using the likelihood ratio test.

In the multivariable analysis a logistic regression analysis (nominal logistic regression) was carried out with a stepwise backward elimination of non-significant variables to detect independent prognostic factors and their

interactions. Statistical significance was tested using the likelihood ratio test. A p-value less than 0.05 was considered as statistically significant.

3. Results

3.1 Clinical and histological risk factors

Between January 2000 and October 2006, SLND was intended in 999 patients with clinical stage I and II cutaneous melanoma. There were 5 patients, in which a lymph node was radioactively labelled but surgery was not performed due to SLN's localization in the deep abdomen or behind the carotid artery. In 7 patients histological examination revealed that the tissue removed was only adipose or connective tissue. In 11 cases a SLN was not detectable intra-operatively or surgery was stopped due to the localization of the marked SLN e.g. adjacent to the facial nerve (Figure 1).

Among the remaining 976 patients SLND was positive in 14.34% (140 patients) and negative in 85.66% (836 patients). The patients' age ranged from 10 years to 89 years (median, 59 years).

Clinical and histological risk factors for metastasized cutaneous melanoma are summarized in table 1.

Sex, age and localization

Men were shown to have a higher risk for a metastasizing melanoma than women. Age was not a significant factor for a positive SLND. Concerning the localization of the primary tumour there was a lower risk for melanoma of the upper extremity (n=12 of 154, 7.79%) than for those located on the head and neck (n=16 of 111, 14.41%), trunk (n=56 of 372, 15.05%) and lower extremity (n=56 of 339, 16.52%).

Tumour thickness

Tumour thickness was highly significant in predicting a positive SLN ($p<0.0001$). Tumour thickness ranged from 0.35mm to 20.00mm. The median tumour thickness was 1.80mm. The distribution of the tumour thickness among the patients is summarized in table 1.

Histological tumour type and further histological features

A highly significant factor for positive SLNs was the histological tumour type (p≤0.0001). The smallest proportion of positive SLNs was observed in LMM (lentigo maligna melanoma) (2.78%; n=1 of 36, tumour thickness 2.75mm, located on the face). The median tumour thickness of LMM was 1.55mm (range from 0.75mm to 4.90mm, mean 1.76mm). In the univariable analysis of LMM versus the other histological types (ALM, NM, SSM) there was a significant benefit in the SLN results (p=0.044) for those patients with a LMM. In the multivariable analysis there was no advantage considering the SLN status in those patients (p=0.067). Patients with an ALM (acral lentiginous melanoma) had a SLN positivity rate of 22.73% (n=20 of 88). The median tumour thickness in all patients with an ALM was 3.00mm.

Histological ulceration of the primary tumour was a significant risk factor for a positive SLND (p≤0.0001), histological regression and nevus association were not.

Interestingly there was no significant statistic difference whether complete (n=174) or bisected (n= 802) SLNs were evaluated.

3.2 Influence of the different surgeons

One aim of this study was to assess if the outcome of sentinel node biopsy depended on a surgeon's qualification. In 947 SLND we were able to evaluate the surgeon's experience in the SLND procedure (Table 2). Interestingly there was no significant correlation between the practical experience of the surgeons and SLND results (p=0.752). Surgeons who had performed less than 25 SLNDs detected a positive SLN in 14.29% (n=9 of 63), those who had performed SLND between 25 and 100 times had a positivity rate of 11.32% (n=12 of 106) and surgeons with the most experience (>100 SLN procedures) revealed a positive SLN in 13.88% (n=108 of 778).

Among the surgeons there were 4 principal surgeons, who had performed the SLND between 124 and 321 times in the study period. The rate of positive SLND ranged from 9.68% (n=12 of 124) to 16.96% (n=29 of 171) for each surgeon (p=0.30). Furthermore no statistical difference was demonstrated between the SLND results of the 4 principal surgeons and the surgeons who had operated less than 100 times (p=0.40).

Multivariable logistic regression analysis

Logistic regression analysis with stepwise backward elimination of non-significant variables was performed to identify factors correlating significantly with positive SLNs including the following factors in the model: sex, tumour thickness, histological tumour type, ulceration, and tumour localization. Because we noticed a better prognosis for primary melanomas of the upper extremies, we compared this site with head and neck, trunk and lower extremities (Table 3).

Increasing tumour thickness, ulceration and a tumour site on head and neck, trunk or lower extremities were independent significant factors for metastasis to the SLN.

We also evaluated the surgeons' impact in various models showing no effect of the surgeons' role on the SLND.

4. Discussion

4.1 Clinical and histological risk factors

The SLND positivity rate of 14.34% in this series is relatively low compared to the literature (11.9%-29%) [4; 5; 9; 10; 11; 16]. This might be explained by the high proportion of thin and intermediate lesions (≤2.00mm) in this cohort (57.56%) and, in part, by the number of histopathological slices analyzed per lymph node (no serial sections were done). It is known that the rate of SLN positivity does increase with the histopathological work-up of the biopsies.

In the multivariable analysis increasing tumour thickness, ulceration and defined localizations such as head and neck, trunk and lower extremity were identified as risk factors for metastasis to a SLN.

With increasing tumour thickness the proportion of histopathologically positive SLNs rises [4; 8; 9; 10; 15; 20; 24]. In our series tumour thickness was the most important risk factor for a positive SLND. Metastasis to the SLN was identified in only 1.15% of the patients having a tumour thickness ≤ 1.00mm, in 8.6% of those with a tumour thickness of 1.01 to 2.00mm, in 18.06% of the cases with tumour thickness of 2.01 to 4.00mm and in 36.80% of the patients with tumour thickness >4.00mm.

Histological ulceration of the primary tumour was strongly associated with a positive SLND. This finding is corroborated by other investigations when equivalent methods had been used [8; 9; 14].

Localization of the melanoma on the head and neck area, the trunk or the lower extremities was observed with a two-fold odds ratio for a positive SLND. The effect of localization was independent from tumour thickness, ulceration or the histological type.

Remarkably only 1 of 36 LMM patients (2.78%) had a positive SLND (2.75mm; LMM in the face). In the univariable analysis patients with LMM had significantly more negative SLNBs than other tumour types. In the multivariable analysis

only tumour thickness, histological ulceration and localization remained significant. Here, the question rises, wether it is reasonable to perform SLND on patients having a LMM which predominantly is located in the head and neck area. The low probability of a positive SLN in LMM and a moderate benefit of the SLND procedure have to be balanced against the surgical risk of scar formation and facial nerve damage.

Interestingly the presence of histological regression was more frequently associated with a negative SLN (9.32% vs. 15.03%, p=0.097) in our study. This observation seems to be controversial with respect to the general clinical assumption that before the onset of regression the tumour had been even thicker. But it was reported before by Paek et al. [16]. A potential explanation could be a potent immune response of the host against the aggressive tumour thus reducing metastases or other mechanism like oncogene and growth factors [17].

In our analysis we didn't incorporate the risk factors mitotic index, lymphocytic infiltration and satellitosis. However, in the literature increasing mitotic rate (especially in younger patients) and angiolymphatic invasion were mentioned as risk factors which were associated with a greater likelihood of positive SLN status [16]. Especially in ALM the presence of microsatellites and a high mitosis rate were independently correlated with survival [18].

We did not use micromorphometric features. The "s-classification" by Starz et al. [22] includes the number of millimetric slices involved by metastasis and depends on the maximum depth of invasion of melanoma cells towards the centre of the lymph node. Thereupon, subgroups of patients were defined who might have a greater benefit from SLND than others [6; 21; 22].

4.2 Influence of the different surgeons

One essential aim of this study was to clarify the surgeon's impact on the result of the SLND. Here, the SLND results of all surgeons were evaluated in a

retrospective manner for 947 patients from January 2000 to October 2006. At the Department of Dermatology of the University of Tuebingen SLND has been the standard procedure since 1996. In former publications a direct correlation between the success in identifying the SLNs and the number of procedures performed by each surgeon was documented [14; 15; 20]. A “learning curve” was denoted and the triple-technique was recommended to be performed only by physicians with suitable training [2; 5; 14; 15; 19; 20; 23]. Morton and colleagues indicated that a learning phase of 30 cases may not be sufficient for lymphatic mapping and SLND, and suggested a minimum of 55 cases to identify the SLN with 95% accuracy [13].

In contrast, our results did not show a significant correlation between the numbers of procedures per surgeon and the SLN positivity (p=0.752). Perhaps these good results of “beginners” may be influenced by training and supervision of less experienced surgeons by an experienced consultant, who guided through the SLN procedures. Ultimately there seems to be no learning curve when “beginners” are supervised.

4.3 Conclusions

The surgeons’ experience did not play a significant role on the result of a SLND. Fortunately the “beginners” were supervised and not “left alone”. There seems to be no learning curve when “beginners” are supervised.

Multivariable analysis demonstrated increasing tumour thickness, ulceration as well as defined localizations of the primary tumour like head and neck, trunk and lower extremity to be independent risk factors for a positive SLND. Based on this study and the literature, a model for calculating the risk of a histological positive SLN could be derived. Such a model might improve the present inclusion criteria for SLND beyond tumour thickness.

5. Summary

Background: Patients with early stage I and II cutaneous malignant melanoma have a good prognosis after surgical excision of the primary tumour. The sentinel lymph node (SLN) status is known to be one of the most important predictive factors. The accuracy of the SLN's detection is attributed to the surgeons' practical experience.

Objectives: The aim of this study was to define risk factors for SLN metastasis in malignant melanomas and to investigate the impact of individual surgeons on the results of SLN dissection (SLND).

Patients and methods: 999 consecutive patients with stage I/II melanoma underwent lymphatic mapping for SLND in the Department of Dermatology, University of Tuebingen, from January 2000 to October 2006. 978 patients had a tumour thickness ≥1.00mm or <1.00mm and regression or ulceration. 21 patients were included having a tumour thickness <1.00mm without ulceration or regression. Clinical, histological and surgical parameters were studied with reference to SLN metastasis using univariable and multivariable analysis. Moreover we evaluated the SLND results in relation to the surgeons and their professional experience in SLND.

Results: 14.34% of the sentinel lymph nodes contained tumour -cells. Using multivariable logistic regression analysis with successive elimination of non-significant variables, significant parameters for SLN metastasis were sex ($p=0.05$), tumour thickness ($p\leq0.0001$), ulceration ($p=0.02$) and defined localizations ($p=0.03$) like head and neck, trunk and lower extremity. SLN results were not different in surgeons who performed less than 100 SLND compared to surgeons with an experience ≥100 SLNB. Positivity of SLNs in relation to the 4 main surgeons ranged from 9.68% to 16.96%, but was neither statistically significant in univariable nor in multivariable analysis.

Conclusions: Increasing tumour thickness, histological tumour type (acral lentiginous melanoma, nodular melanoma), ulceration, tumour localization on head and neck, trunk and lower extremity and male sex were associated with a greater probability of positive SLN status. There was no statistically significant correlation between surgeons and SLN results. The results of the present study support the use of other parameters beyond tumour thickness to select for SLND in patients with malignant melanoma.

6. Summary in German

Der Status des Wächterlymphknotens wird als einer der prognostisch wichtigsten Faktoren im klinischen Stadium I und II des malignen Melanoms der Haut gesehen. Die Genauigkeit des Auffindens des Wächterlymphknotens wird dabei der praktischen Erfahrung des Operateurs zugeschrieben.

Ziel dieser Studie war darzustellen, welche Risikofaktoren zur Metastasierung in die Wächterlymphknoten beim malignen Melanom der Haut beitragen. Als weiterer Schwerpunkt wurde untersucht, welche Auswirkungen die einzelnen Operateure auf das Ergebnis der Wächterlymphknotenbiopsie haben.

Bei 999 aufeinander folgenden Patienten der Universitäts-Hautklinik Tübingen mit einem malignen Melanom der Haut Stadium I und II wurde eine Darstellung der Lymphgefäße vorgenommen. Klinische, histologische und chirurgische Parameter wurden bezüglich einer Metastasierung in die Wächterlymphknoten untersucht. Dafür wurden univariate und multivariate Analysen durchgeführt.

Tumorzellen waren in 14.34% aller Wächterlymphknoten enthalten. Zunehmende Tumordicke, der histologische Tumortyp (akrolentiginöses Melanom, noduläres Melanom), Ulzeration, Tumorlokalisation an Kopf, Hals, Stamm und unterer Extremität sowie männliches Geschlecht waren in der univariaten Analyse verbunden mit einer größeren Wahrscheinlichkeit eines positiven Wächterlymphknotens. In der multivariaten Auswertung waren Tumordicke, Ulzeration und die Lokalisation (andere versus obere Extremität) unabhängige Risikofaktoren für eine Metastasierung in den Wächterlymphknoten. Die Ergebnisse der Wächterlymphknotenbiopsie unterschieden sich nicht hinsichtlich der Erfahrung der Operateure. So erreichten diejenigen, welche bisher weniger als 100 Biopsien durchgeführt hatten, ähnliche Ergebnisse wie erfahrenere Chirurgen mit 100 oder mehr Wächterlymphknotenbiopsien.

Schlussendlich konnten wir keinen signifikanten Zusammenhang zwischen den einzelnen Operateuren und den Ergebnissen der Wächterlymphknotenbiopsie feststellen. Neben Tumordicke spielen noch andere Faktoren wie die Ulzeration und Lokalisation im Hinblick auf die Auswahl der Patienten für eine Wächterlymphknotenbiopsie eine Rolle.

7. Figures and Tables

Figure 1: *999 patients subdivided into 5 groups (flow chart)*

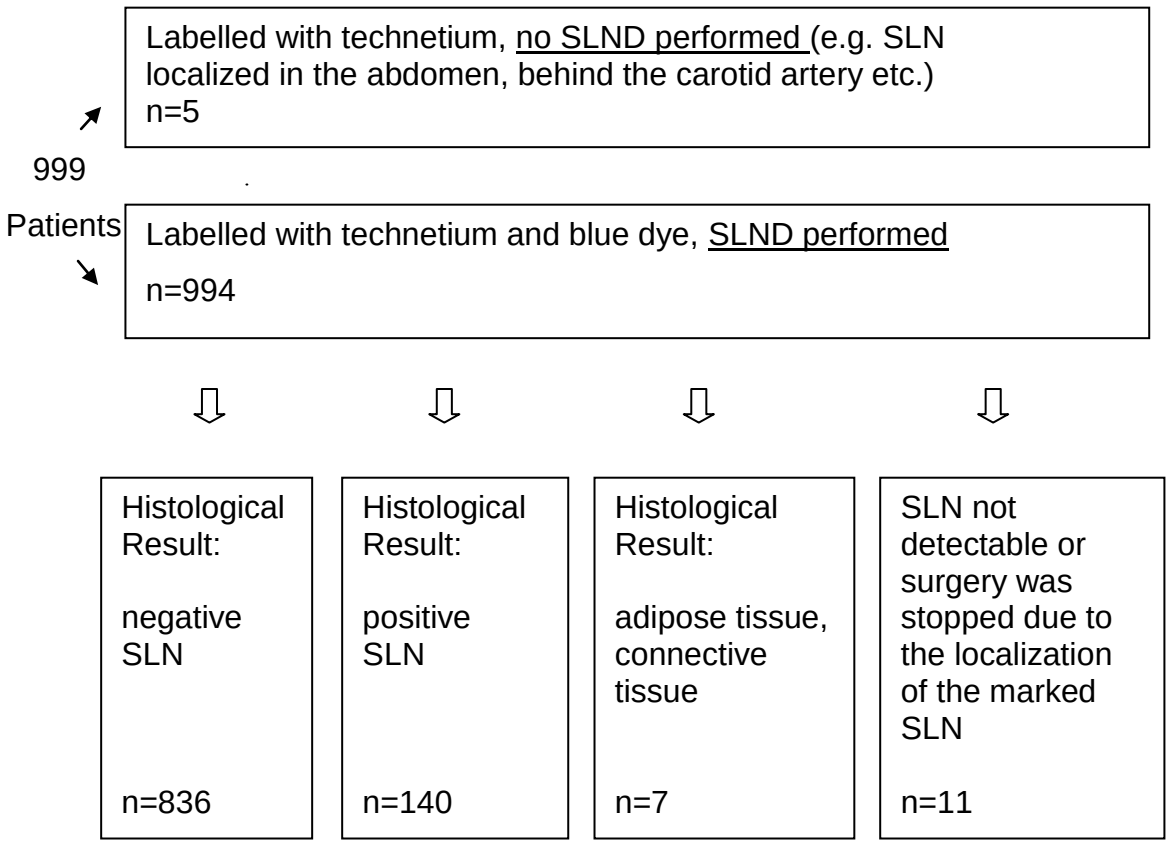

*Table 1***:** *Clinical and histological risk factors for metastasized malignant melanoma. Univariable analysis*

Variable	SLND pos.		SLND neg.		SLND total	
	n	%	n	%	n	p-value
	140	14.34	836	85.66	976	
Patients‘ sex						
Male	89	16.57	448	83.43	537	
Female	51	11.62	388	88.38	439	0.028
Age						
Min. (years)	15		10		10	
Max. (years)	89		87		89	
Median (years)	54.5		59		59.00	0.152
Localization						
Head/Neck	16	14.41	95	85.59	111	
Trunk	56	15.05	316	84.95	372	
Upper extremities	12	7.79	142	92.21	154	
Lower extremities	56	16.52	283	83.48	339	0.053
Tumour thickness (mm)						
Min.	0.90		0.35		0.35	
Max.	15.00		20.00		20.00	
Median	2.95		1.70		1.80	<0.0001
Tumour thickness (mm)						
< 0.75mm	0	0.00	14	100.0	14	
0.75 - 1,00mm	1	1.37	72	98.63	73	
1,01 - 2,00mm	41	8.61	435	91.39	476	
2,01 - 4,00mm	52	18.06	236	81.94	288	
> 4,00 mm	46	36.80	79	63.20	125	< 0.0001
Histological Tumour Type						
LMM	1	2.78	35	97.22	36	
other	139	14.79	801	85.21	940	0.044

Table 1 (continued.): Clinical and histological risk factors for metastasized cutaneous melanoma. Univariable analysis

Variable	SLND pos.		SLND neg.		SLND total	
	n	%	n	%	n	p-value
	140	14.34	836	85.66	976	
Histological Tumour Type						
SSM	55	10.60	464	89.40	519	
NM	48	21.24	178	78.76	226	
LMM	1	2.78	35	97.22	36	
ALM	20	22.73	68	77.27	88	
other	16	14.95	91	85.05	107	<0.0001
Histological Ulceration						
Ulceration	66	24.44	204	75.56	270	
No Ulceration	74	10.48	632	89.52	706	<0.0001
Histological Regression						
Regression	11	9.32	107	90.68	118	
No Regression	129	15.03	729	84.97	858	0.097

Table 2: *Surgical experiences in SLND*

	SLND pos.		SLND neg.		SLND Total	
	n	%	n	%	n	p-value
	140	14.34	836	85.66	976	
Missing values	11		18		29	
Data on surgeons available	129	13.62	818	86.38	947	
Surgeon's experience						
<25 SLND	9	14.29	54	85.71	63	
25-100 SLND	12	11.32	94	88.68	106	
>100 SLND	108	13.88	670	86.12	778	0.752
Surgeons >100 SLND	108	13.88	670	86.12	778	
Surgeons ≤100 SLND	21	12.43	148	87.57	169	0.617
Principal Surgeons						
Surgeon 1	12	9.68	112	90.32	124	
Surgeon 2	42	13.08	279	86.92	321	
Surgeon 3	29	16.96	142	83.04	171	
Surgeon 4	25	15.43	137	84.57	162	
all other Surgeons	21	12.43	148	87.57	169	0.400

Table 3: *Multivariable analysis of risk factors for a positive sentinel node*

Risk factor	Odds ratio	Lower CI	Upper CI	p-value
(log) Tumour thickness (in mm) per unit of magnitude	18.45	8.82	39.40	<0.0001
Histological ulceration yes vs. no	1.55	1.03	2.32	0.038
Other regions vs. upper extremity	2.10	1.15	4.15	0.014

8. References

1 **Albertini, J. J., Cruse, C. W., Rapaport, D., Wells, K., Ross, M., DeConti, R., Berman, C. G., Jared, K., Messina, J., Lyman, G., Glass, F., Fenske, N., Reintgen, D. S.** "Intraoperative radio-lympho-scintigraphy improves sentinel lymph node identification for patients with melanoma." Ann Surg 1996;**223**(2):217-224.

2 **Amersi, F., Morton, D. L.** "The role of sentinel lymph node biopsy in the management of melanoma." Adv Surg 2007;**41**:241-256.

3 **Breuninger, H., Hobbach, P. S., Schimek, F.** "Ropivacaine: an important anesthetic agent for slow infusion and other forms of tumescent anesthesia." Dermatol Surg 1999;**25**(10):799-802.

4 **Cafiero, F., Peressini, A., Percivale, P. L., Rainero, M. L., Faggioni, M., Gipponi, M., Queirolo, P., Nicolo, G., Bertoglio, S.** "Selective lymph node dissection in patients with intermediate thickness melanoma: our experience." Anticancer Res 2000;**20**(1B):497-500.

5 **Cascinelli, N., Belli, F., Santinami, M., Fait, V., Testori, A., Ruka, W., Cavaliere, R., Mozzillo, N., Rossi, C. R., MacKie, R. M., Nieweg, O., Pace, M., Kirov, K.** "Sentinel lymph node biopsy in cutaneous melanoma: the WHO Melanoma Program experience." Ann Surg Oncol 2000;**7**(6):469-474.

6 **Debarbieux, S., Duru, G., Dalle, S., Beatrix, O., Balme, B., Thomas, L.** "Sentinel lymph node biopsy in melanoma: a micromorphometric study relating to prognosis and completion lymph node dissection." Br J Dermatol 2007;**157**(1):58-67.

7 **Gershenwald, J. E., Berman, R. S., Porter, G., Mansfield, P. F., Lee, J. E., Ross, M. I.** "Regional nodal basin control is not compromised by previous sentinel lymph node biopsy in patients with melanoma." Ann Surg Oncol 2000;**7**(3):226-231.

8 **Gershenwald, J. E., Mansfield, P. F., Lee, J. E., Ross, M. I.** "Role for lymphatic mapping and sentinel lymph node biopsy in patients with thick (> or = 4 mm) primary melanoma." Ann Surg Oncol 2000;**7**(2):160-165.

9 **Gershenwald, J. E., Thompson, W., Mansfield, P. F., Lee, J. E., Colome, M. I., Tseng, C. H., Lee, J. J., Balch, C. M., Reintgen, D. S., Ross, M. I.** "Multi-institutional melanoma lymphatic mapping experience: the prognostic value of sentinel lymph node status in 612 stage I or II melanoma patients." J Clin Oncol 1999;**17**(3):976-983.

10 **Harlow, S. P., Krag, D. N., Ashikaga, T., Weaver, D. L., Meijer, S. J., Loggie, B. W., Tanabe, K. K., Whitworth, P., Jr., Kuhn, J., Kusminsky, R., Carp, N. Z., Gadd, M., Rawlings, M., Jr., Slingluff, C. L., Jr.** "Gamma probe guided biopsy of the sentinel node in malignant melanoma: a multicentre study." Melanoma Res 2001;**11**(1):45-55.

11 **Kruper, L. L., Spitz, F. R., Czerniecki, B. J., Fraker, D. L., Blackwood-Chirchir, A., Ming, M. E., Elder, D. E., Elenitsas, R., Guerry, D., Gimotty, P. A.** "Predicting sentinel node status in AJCC stage I/II primary cutaneous melanoma." Cancer 2006;**107**(10):2436-2445.

12 **Lydersen, S., Fagerland, M. W., Laake, P.** "Recommended tests for association in 2 x 2 tables." Stat Med 2009;**28**(7):1159-1175.

13 **Morton, D. L., Cochran, A. J., Thompson, J. F., Elashoff, R., Essner, R., Glass, E. C., Mozzillo, N., Nieweg, O. E., Roses, D. F., Hoekstra, H. J., Karakousis, C. P., Reintgen, D. S., Coventry, B. J., Wang, H. J.** "Sentinel node biopsy for early-stage melanoma: accuracy and morbidity in MSLT-I, an international multicenter trial." Ann Surg 2005;**242**(3):302-311; discussion 311-313.

14 **Morton, D. L., Wen, D. R., Foshag, L. J., Essner, R., Cochran, A.** "Intraoperative lymphatic mapping and selective cervical lymphadenectomy for early-stage melanomas of the head and neck." J Clin Oncol 1993;**11**(9):1751-1756.

15 **Morton, D. L., Wen, D. R., Wong, J. H., Economou, J. S., Cagle, L. A., Storm, F. K., Foshag, L. J., Cochran, A. J.** "Technical details of intraoperative lymphatic mapping for early stage melanoma." Arch Surg 1992;**127**(4):392-399.

16 **Paek, S. C., Griffith, K. A., Johnson, T. M., Sondak, V. K., Wong, S. L., Chang, A. E., Cimmino, V. M., Lowe, L., Bradford, C. R., Rees, R. S., Sabel, M. S.** "The impact of factors beyond Breslow depth on predicting sentinel lymph node positivity in melanoma." Cancer 2007;**109**(1):100-108.

17 **Paredes, B. E.** "Regression in malignant melanoma. Definition, etiopathogenesis, morphology and differential diagnosis." Pathologe 2007;**28**(6):453-463.

18 **Phan, A., Touzet, S., Dalle, S., Ronger-Savle, S., Balme, B., Thomas, L.** "Acral lentiginous melanoma: histopathological prognostic features of 121 cases." Br J Dermatol 2007;**157**(2):311-318.

19 **Ross, G. L., Shoaib, T., Scott, J., Soutar, D. S., Gray, H. W., MacKie, R.** "The learning curve for sentinel node biopsy in malignant melanoma." Br J Plast Surg 2002;**55**(4):298-301.

20 **Ross, M. I., Reintgen, D. S.** "Role of lymphatic mapping and sentinel node biopsy in the detection of melanoma nodal metastases." Eur J Cancer 1998;**34 Suppl 3**:S7-11.

21 **Starz, H., Balda, B. R.** "Benefit of sentinel lymphadenectomy for patients with nonulcerated cutaneous melanomas in the Breslow range between 0.76 and 1 mm: a follow-up study of 148 patients." Int J Cancer 2007;**121**(3):689-693.

22 **Starz, H., Siedlecki, K., Balda, B. R.** "Sentinel lymphonodectomy and s-classification: a successful strategy for better prediction and improvement of outcome of melanoma." Ann Surg Oncol 2004;**11**(3 Suppl):162S-168S.

23 **Testori, A., Bartolomei, M., Grana, C., Mezzetti, M., Chinol, M., Mazzarol, G., Lazzari, I., Paganelli, G., Geraghty, J. G., Andreoni, B., Veronesi, U.** "Sentinel node localization in primary melanoma: learning curve and results." Melanoma Res 1999;**9**(6):587-593.

24 **Ulmer, A., Fischer, J. R., Schanz, S., Sotlar, K., Breuninger, H., Dietz, K., Fierlbeck, G., Klein, C. A.** "Detection of melanoma cells displaying multiple genomic changes in histopathologically negative sentinel lymph nodes." Clin Cancer Res 2005;**11**(15):5425-5432.

9. Acknowledgements

- First of all I want to express my deepest gratitude to Prof. Dr. Matthias Moehrle for his exhaustless assistance and encouragement that this work could become true.
- Prof. Dr. M. Roecken, head of the Department of Dermatology in Tuebingen.
- Prof. Dr. Claus Garbe, head of the dermatological oncology at the University of Tuebingen, who has essentially contributed that the Melanoma registry of Tuebingen could be established and allows this statistical work of melanoma patients.
- I thank Prof. Dr. Klaus Dietz, head of the Institute for medical biometrics, for his very useful advices in statistical issues and reliable answers and solutions in statistical questions.
- I appreciate the thoughtful cooperation of the “Follow-up- team” of the melanoma registry of the Department of Dermatology at the University of Tuebingen. Not to forget I want to thank the porters at the reception desk for their kind issue and return of keys and phone calls.
- For proof-reading the manuscript I show gratitude to Dr. Gordon Hunter.
- Last but not least I want to thank my partner Conrad for his support and good advice in technical aspects.

10. Curriculum Vitae

Personal information

Name	Löffler, Julia Angelika
Date of birth	01.02.1983
Place of birth	Reutlingen
Parents	Gabriele Löffler née Copray and Dr. Thomas Löffler

Education

1988-1992	Primary school (Ersberggrundschule, Nürtingen)
1992-2002	Grammar school (Max-Planck-Gymnasium, Nürtingen)
30.06.2002	School-leaving examination (Abitur)
2002-2004	apprenticeship as nurse (Universitätsklinikum Tübingen)

Higher education

2004-2006	Study of medicine in Ulm
30.03.2006	First state examination (1. Abschnitt der ärztlichen Prüfung)
2006-2010	Study of medicine in Tuebingen
20.05.2010	Second state examination (2. Abschnitt der ärztlichen Prüfung)