Stage III Melanoma
Time is relative

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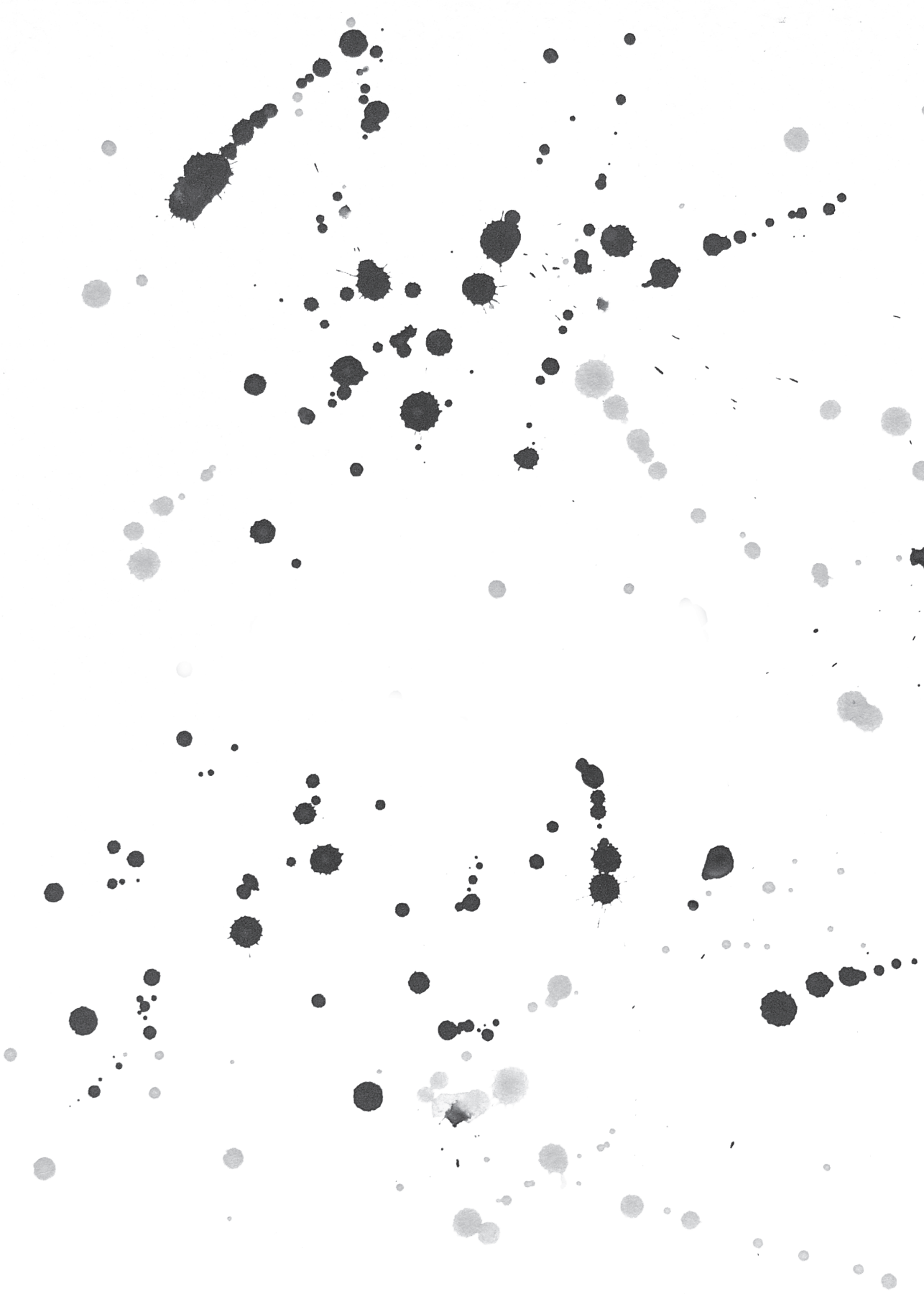
Contents



Chapter 1

Introduction and Outline of this Thesis

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Dirk J. Grünhagen
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Introduction

Epidemiology

Melanoma, while responsible for a minority of all new skin cancers, is certainly the most lethal, accounting for 90% of skin cancer associated deaths[1]. Incidence of this deadly disease has risen in the past decades, both on a global level[2, 3] , and on a national level[4, 5]. In the Netherlands, the number of newly diagnosed melanomas increased from 2,593 in 2005 to 5,926 in 2015[6], and the number of melanoma related deaths increased from 624 to 826 in the same time span. Globally, 230,000 melanomas were diagnosed and 55,489 melanoma related deaths occurred in 2012 [7, 8]. Melanoma incidence and mortality world adjusted standardized rates (WSR) in the Netherlands were the second highest of Western Europe[8]: in 2014 national incidence was 20.6/100,000 WSR, and mortality was 2.5/100,000 WSR[4].

Several attributable factors have been identified such as ultraviolet (UV) radiation and increased sun exposure in fair skin phenotypes, and a history of sunburn (as a child)[9], but still much is unknown about the development and progression of cutaneous melanoma, as not all melanomas are UV-radiation induced[10]. Classification systems have been brought into life to aid in stratifying patients according to their prognosis: which is currently accurately defined in the TNM melanoma staging system by the American Joint Committee on Cancer (AJCC) [11].

(History of) Nodal Staging

For melanomas with a Breslow thickness of >1mm (or <1mm but presence of high risk features such as ulceration, high level of Clark invasion or a high mitotic rate), the risk of nodal metastases is substantial, warranting adequate nodal staging[1, 11-13]. Nodal status is one of the most powerful prognostic factors for primary melanomas with clinically negative nodes[11, 14]. Determination of nodal status is ideally performed based on histopathological proof of melanoma metastases in lymph node tissue, as physical examination and imaging techniques alone are not accurate enough[15-17]. Traditionally, elective lymph node dissections were carried out in order to determine nodal status, acquire adequate locoregional control, and potentially improve survival[3, 18]. While in theory a promising method, trial results turned out to be negative; there was absolutely no survival benefit for patients undergoing elective lymph node dissection compared to patients who received nodal observation only, albeit that some studies suggested benefits for subgroups[19-24]. Meanwhile, the morbidity of an elective lymph node dissection was substantial, consisting of prolonged wound infections, seroma and development of chronic lymph edema. As only around 20% of all patients undergoing elective lymph node dissection have positive nodes, a more elegant way was sought to identify these patients with poor prognosis. This led to the introduction of the lymphatic mapping

technique for sentinel node biopsy by Morton et al[25]. In short, this technique consists of preoperative lymphoscintigraphy with Tc99-colloid, dermal injection of patent blue around the melanoma scar, and perioperative use of a handheld gamma-probe Geiger teller to localize the sentinel node(s)[25, 26]. The concept of the sentinel node was based on the hypothesis that melanoma cells spread from the primary tumor site to the sentinel node prior to reaching adjacent lymph nodes in the same regional lymph node basin. In case of lymphatic metastasis the sentinel node will be affected first, acting as a barrier. If the sentinel node is negative, adjacent lymph nodes are unlikely to be affected[25, 27-29].

SNB and CLND

Since then, it has proven its staging value unequivocally[11, 30, 31], but its therapeutic value is still under debate. To date no prospective trial has demonstrated evidence of a clear survival benefit due to sentinel node biopsy. The largest international multicenter clinical randomized trial investigating wide local excision and sentinel node biopsy versus wide local excision and nodal observation, the MSLT1, did not show a difference in melanoma specific (MSS) survival, primary endpoint of the trial for all included patients[30]. These results support the alternative hypothesis that melanoma spreads simultaneously to lymph nodes and distant sites, rendering the sentinel node as an indicator of metastatic spread instead of a barrier.

Ideally, nodal status is to be determined in a non-invasive manner, especially when regarding the lack of evidence proving a therapeutic effect. Ultrasound of clinically non-suspicious lymph node regions is a procedure which has been investigated extensively, and while proven to be effective in reducing the number of SNBs for breast cancer[32], results for melanoma remain variable at best[16, 33, 34]. Voit et al. showed that in highly dedicated hands a fair sensitivity and specificity could be reached[35, 36], but these results remain to be reproduced in a multicenter setting.

Since the therapeutic value of SNB remains to be proven, any additional value of completion lymphadenectomy is likely to be limited. While results of the MSLT 2 trial[37] randomizing between CLND and nodal observation only after a positive SN are to be awaited, the DeCOG trial by Leiter et al. with a similar trial design, did not show any distant metastasis free survival difference at 3-years follow-up[38]. Full accrual was not reached due to a lower than expected inclusion rate, and the majority of patients had a small SN tumor burden, but the fact that there was absolutely no difference in survival renders the hypothesis that CLND is therapeutic more unlikely.

Then why perform a SNB and CLND at all? To date, the SNB remains the most powerful nodal staging tool for melanoma, which is highly valuable in informing patients and their treating physicians on prognosis[11, 30].

After decades of conducting randomized clinical trials, effective immunotherapies such as ipilimumab (CTLA4 inhibitor)[39-41] and nivolumab/pembrolizumab (anti-PD1)[42-44]

finally have become a game changer for advanced melanoma (i.e. irresectable stage III and stage IV patients). Their efficacy in prolonging recurrence free survival and potentially even melanoma specific survival is shown) [39-41, 43-45]. This is of great value to stage III patients too; as these therapies have the potential to be effective in adjuvant setting as well[46]. Past adjuvant trials mainly were negative; for instance the EORTC 18991 trial with (peg-) interferon-alpha versus placebo, which only showed a potential reduced hazard ratio for ulcerated primaries[47] and the Sunbelt Melanoma Trial with high-dose interferon alpha-2b with no survival differences[48]. Primary results from the EORTC 18071 (adjuvant ipilimumab versus placebo) trial[49] are promising, showing 11% overall survival benefit for patients treated with ipilimumab at a dose of 10mg/kg, and also a reduced hazard ratio for recurrence free survival, in particular for microscopic stage III (i.e. SN positive patients)[50]. This has put adjuvant melanoma treatment into a whole new perspective. Since trials like these require adequate nodal staging with SNB and CLND in case of a positive SN as a major inclusion criterion [49, 51], SNB and CLND remain worthwhile for now.

Therapeutic LND

Clinically evident lymph node metastases require a whole different approach, as regional control and quality of life become equally important to staging. Regional control is often best reached with radical surgery, but the extent of surgery is not always well defined. Lymph node dissections in the groin area are of particular interest with regards to this aspect; as these are typically divided into superficial (inguinal + femoral lymph nodes) and combined superficial and deep (deep inguinal, iliac, obturator lymph nodes) dissections[52]. There is clear evidence that pelvic lymph node metastases (iliac or obturator level) are associated with poor survival[53-56]. As a groin dissection is a morbid procedure, associated with wound infections and development of chronic lymph edema[57-59], proper patient selection is necessary. Historic cohorts show contradicting results on the potential association between extent of surgery and survival [53, 60-62]. One of the possible options to minimize the number of patients whom undergo a combined groin dissection and have negative pelvic nodes is to follow a two-step approach. Identification of patients with a low suspicion of pelvic lymphadenopathy based on preoperative CT- or PET-CT imaging, may prevent these patients from undergoing iliac lymphadenectomy if the pathology results of a superficial groin dissection are in line with these findings. While this approach does not diminish the number of patients at risk for morbidity to zero, this may reduce the number of negative pelvic dissections.

Ultimately, as is the case for patients undergoing CLND, therapeutic lymph node dissections may be reserved as a final resource in case of failed adjuvant therapy. In short, now is the time to reassess the need for an aggressive surgical approach, and perhaps to start to convince ourselves that "less is more".

Outline of This Thesis

Part I – Nodal Staging

Part I of this thesis investigates whether nodal staging of the clinically node negative melanoma patient can be performed using less invasive alternatives, and whether the indication for nodal staging in thin melanomas is justified. In **Chapter 2** the national thin (pT1) melanoma population is examined according to substaging: both the 6th AJCC staging edition and the 7th edition to see whether "high risk" thin melanomas are identified and stratified more accurately. Secondly the effects of the implementation of the 2nd version of the Dutch Melanoma Guideline are visualized including the recommendation to consider a sentinel node biopsy (and thus nodal staging) for pT1b melanomas. In **Chapter 3** ultrasound morphology criteria of the sentinel node, more specific the "echo free island", are discussed, developed to improve accuracy of ultrasound and fine needle aspiration cytology (FNCAC) techniques in clinically node negative melanomas. The "echo free island" may serve as an early sign of micrometastasis in the sentinel node, which should raise suspicion in the ultrasonographist. In **Chapter 4** the long-term results of ultrasound and FNAC of the sentinel node are presented. The Berlin ultrasound morphology criteria, primarily designed to assess the sentinel node for presence of micrometastases, warranting FNAC for confirmation, might be of prognostic value. **Chapter 5** gives an overview of the current evidence on ultrasound of the sentinel node in melanoma, presents the results of a pilot study and presents the study protocol of the GULF trial; Gamma probe and ultrasound guided FNAC of the sentinel node, designed as a potential minimally invasive alternative for the surgical sentinel node biopsy.

Alteration of the staging techniques is one way to minimalize morbidity; another is to reassess the indication for nodal staging.

Part II – Timing of Surgery

Part II focuses on the timing of nodal staging. Time is of the essence in current oncology practice, but the maximum referral times and wait-list times posed by national melanoma guidelines are not based on solid evidence. In a highly stressed referral system, a delicate balance between urgent referrals and minimization of long wait-lists is needed, as otherwise the value of a true high urgency referral is lost, which potentially negatively affects prognosis of patients who do need treatment as soon as possible. In **Chapter 6** the timing of sentinel node biopsy is investigated in sentinel node positive patients, as they are most likely to benefit from early removal of positive lymph nodes. **Chapter 7** includes both sentinel node positive and negative patients, as timing of sentinel node biopsy may affect the sentinel node positivity rate as well as survival outcome. In **Chapter 8** the timing of completion lymphadenectomy is investigated, as removal of any ad-

ditional positive lymph nodes may potentially be the last therapeutic procedure. Should it be performed within a certain amount of time? Is there a window of opportunity?

Part III – Extent of Groin Dissections

The final part of this thesis focuses on the extent of surgery, in particular the extent of groin lymph node dissection. There is clear evidence that presence of pelvic lymph node metastases (iliac or obturator level) is associated with poor survival. By correctly identifying these patients prior to iliac lymphadenectomy, which is a morbid procedure, patients without pelvic lymph node metastases can be spared the pelvic part of a groin dissection. **Chapter 9** presents a possible two-step approach for melanoma patients with palpable groin metastases and no suspicious preoperative imaging (CT or PET/CT) who are scheduled for superficial groin dissection (inguinal lymph nodes only) or combined superficial and deep groin dissection (inguinal and iliac lymph nodes).

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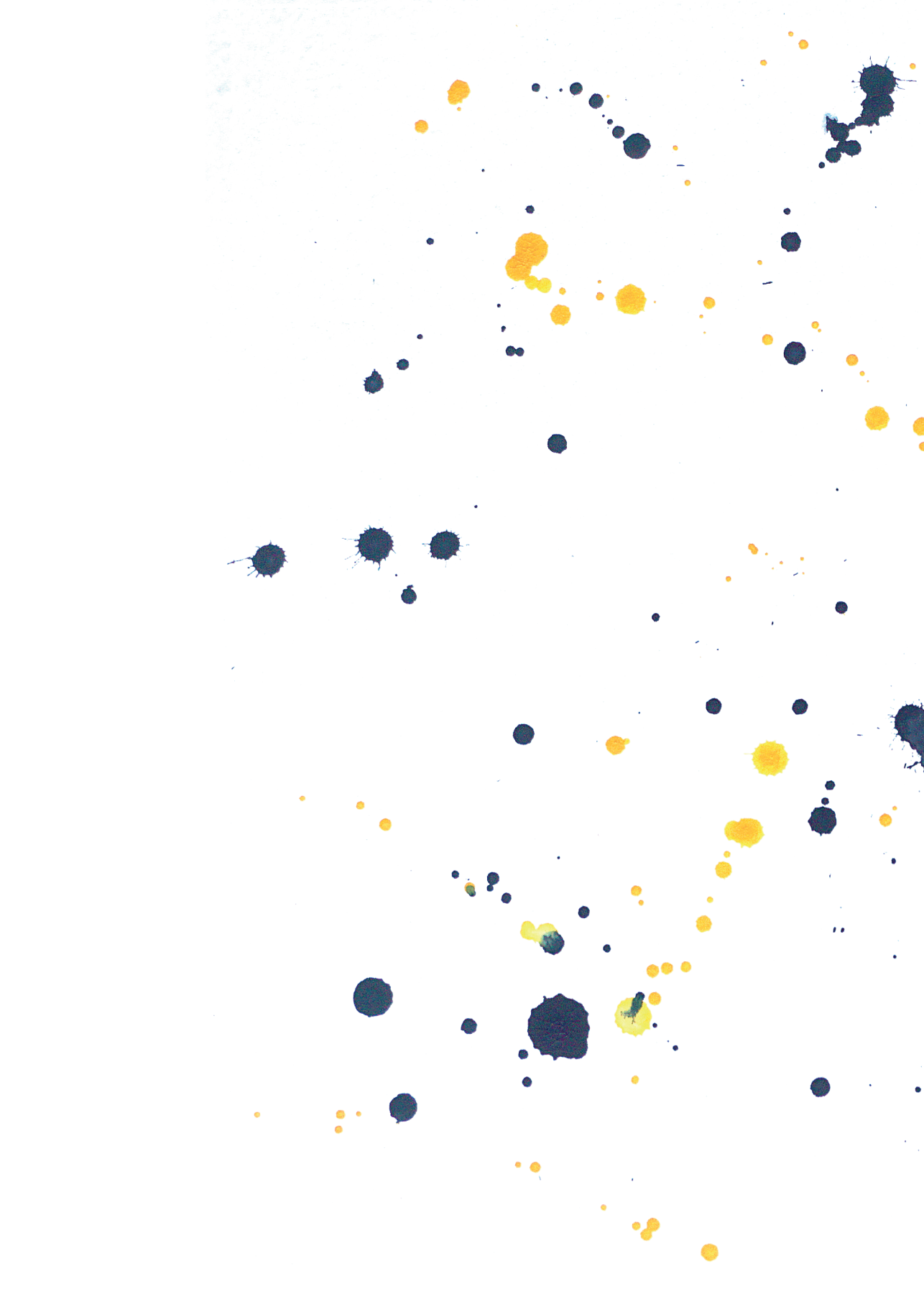
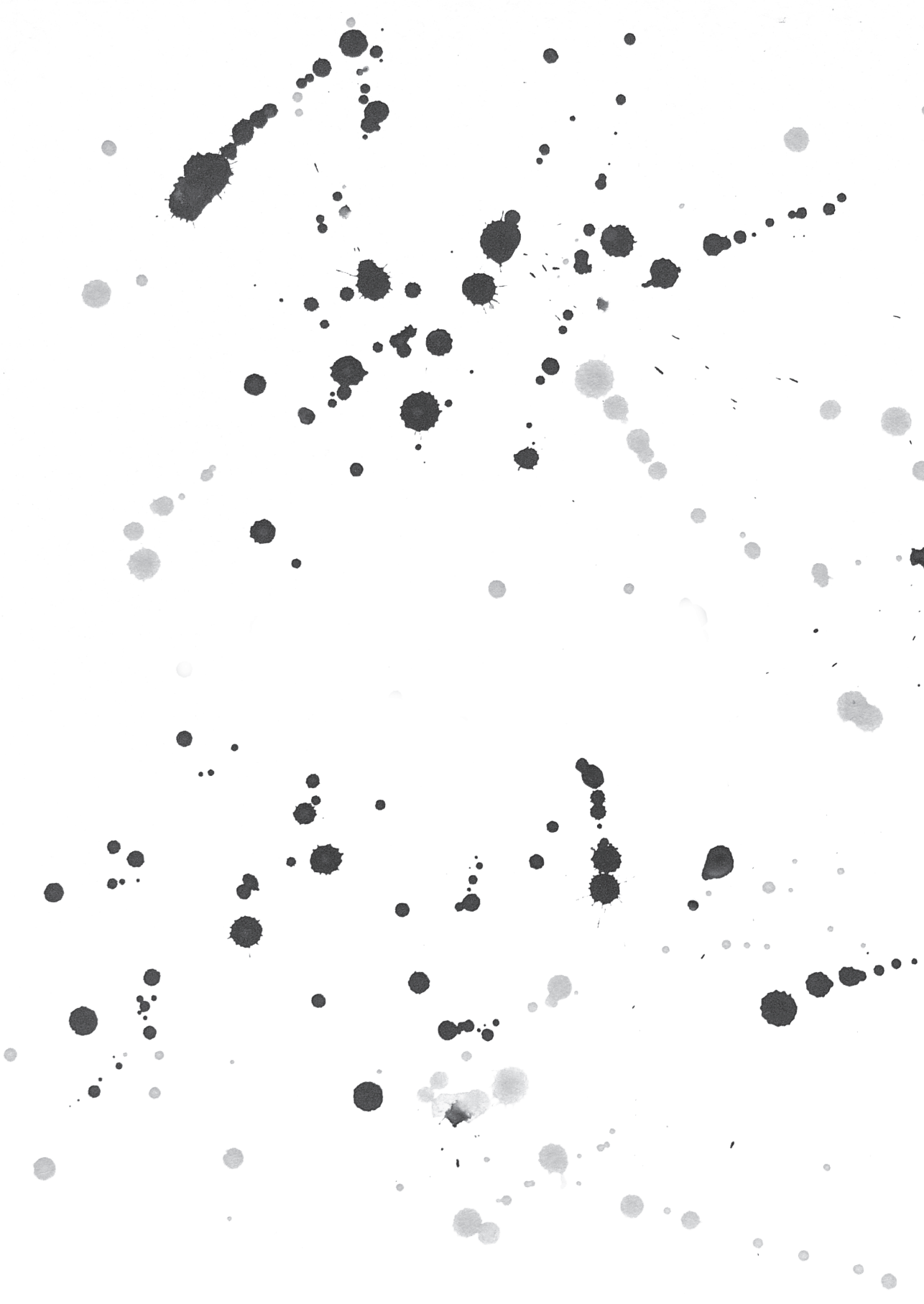


Part I – Nodal Staging



Chapter 2

Implementation of the 7th Edition AJCC Staging System: Effects on Staging and Survival for pT1 Melanoma. *A Dutch Population Based Study*

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Abstract

Background In the 7th edition of the AJCC staging system the mitotic rate criterion replaced Clark level to increase correct classification of high risk thin melanoma patients (pT1B). Additionally, sentinel node biopsy (SNB) was recommended for nodal staging for pT1B melanomas. Aim: to evaluate the effects on pT1 substaging and clinical implications in the national pT1 melanoma population.

Methods All pT1 melanomas diagnosed in the Netherlands from 2003 – 2014 were selected from the Netherlands Cancer Registry (IKNL). Patients were stratified by cohort: according to AJCC edition: 1) 2003–2009 (6th) and 2), 2010–2014 (7th). Relative survival was calculated to estimate melanoma specific survival.

Results A total of 29.546 pT1 melanoma patients were included. The pT1b proportion increased from 10.1% in cohort 1, to 21.5% in cohort 2. The proportion of performed SNBs per cohort increased: for pT1b melanomas alone from 4.5% to 13.0%. SNB positivity rate decreased from 10.5% to 8.8% for the entire pT1 population, and for pT1b melanomas from 11.3% to 8.6%. At 5 year, the relative survival rate was similar for pT1a and pT1b in both cohorts, namely pT1a 100% vs pT1b 97% (cohort 1), and pT1a 100% vs. pT1b 98% (cohort 2).

Conclusion The 7th edition of the AJCC staging system has caused an increased number of patients to undergo SNB, without an increase in SNB positivity rate. Survival between pT1 subgroups remains similar. The mitotic rate criterion for pT1b classification and the recommendation to perform SNB for pT1b melanomas should be reconsidered.

Introduction

The incidence of newly diagnosed cutaneous melanomas continues to increase globally and in the Netherlands[1, 2]. The majority of all new melanoma patients have a thin melanoma (pT1, Breslow thickness ≤1.00mm), which has a good prognosis (10-year survival is 92%-95%)[3-5]. A minority of these patients however will develop regional and distant metastases, and ultimately die due to melanoma.

One of the most important prognostic factors for primary melanomas is the Breslow thickness, which has become the main allocator for the different primary tumor (pT) categories[3, 6, 7]. In the 6th edition of the American Joint Committee on Cancer (AJCC) Staging System the presence of ulceration was major criterion for pT1b classification besides a high Clark level (4/5), and recommendations for Sentinel Node Biopsy (SNB) were based on classification as pT1b melanoma[8, 9].

In the 7th edition of the AJCC Staging System, high Clark level was replaced by mitotic rate; i.e. the microscopic presence of 1 or more mitotic cells per 1mm2. This was found to be a stronger prognostic factor than high Clark level[3, 10]. The extent of its power in thin melanomas is however debated; the cut-off point of 1 or more mitosis per mm2 may be too low to be used as discriminating prognostic feature, and the large variety in histopathological examination protocols (number of examined slides, number of searched high power fields, size of high power fields) make it highly operator dependent[11]. Importantly, the decision to perform further nodal staging with a SNB in pT1 melanomas is based on this criterion in the Dutch melanoma guidelines[4]. As SNB is a minimally invasive surgical staging procedure, but an invasive procedure nonetheless[12, 13]. Changes in melanoma classification due to the update from 6th to 7th edition may have a large effect on the absolute number of patients that will be offered a SNB. This will also affect the absolute number of patients at risk for morbidity, due to the fact that the incidence of pT1 melanomas is high compared to pT2-4 melanomas.

Besides the question if a SNB is warranted in thin melanomas, it is not known if the mitotic rate criterion stratifies thin melanomas more accurately as high risk patients in the Dutch population. A corresponding higher risk of a positive SNB would be expected.

Aim of this study is to evaluate the effects of implementation of the 7th edition of the AJCC staging system and subsequent change to the national guidelines on pT1 substaging and clinical implications in the national pT1 melanoma population.

Patients and Methods

Patients

Population-based data was retrieved for all pT1 melanoma patients diagnosed from 2003 – 2014 from the Netherlands Cancer Registry (NCR), which is embedded within the Netherlands Comprehensive Cancer Organisation[2]. The NCR is annually linked to the Municipal Personal Records database to retrieve information on vital status and date of death. The follow-up data were completed until January 1st 2015.

The following patient and melanoma features were collected: gender, age at diagnosis, year of diagnosis, Breslow thickness, pT classification, stage, SNB performed yes/no, SNB result, whether completion lymph node dissection was performed (CLND), CLND result, number of removed lymph nodes, number of removed positive lymph nodes, follow-up from date of diagnosis in months, and status at last follow-up (dead or alive).

Patients were divided into two cohorts based on year of diagnosis and use of the AJCC Staging System 6th edition, cohort 1 (2003-2009) or 7th edition, cohort 2 (2010-2014). Due to the registration methods used during cohort 1 data on SNB where not accurate for this cohort. In case of a positive SN, CLND data sometimes overruled the SN data. This way, it was not possible to distinguish between patients with a lymph node dissection (LND) following a positive SN or with clinically involved lymph nodes warranting therapeutic LND. The registration methods for SNB were adapted prior to 2010 with the option to register SN data separately from LND data: SNB data for cohort 2 thus were accurate.

A quality control on accuracy of tumor staging has been performed for the entire database in 2016 prior to selection of data.

Statistics

Primary patient and tumor features were analyzed using X^2-tests or Mann- Whitney U test, as appropriate. The proportion of pT1a and pT1b was determined per cohort and per year of diagnosis. Incidence-rates were standardized for age according to the world standard population (WSR, World Standardized Rate) and are presented per 100,000 person-years. The proportion of performed SNBs was determined per cohort and per pT1 category, as well as the proportion of positive SNs. Follow-up was calculated from date of diagnosis until last follow-up or death. Survival times were calculated from the melanoma diagnosis to death (any cause) and considered censored for patients alive at last follow-up. Cause of death was not known.

Survival time was defined as time from diagnosis to death, or January 1, 2015 for those patients who were still alive. Relative survival was used as proxy of disease specific survival and was calculated correcting for age- and gender-specific background mortality[14]. A two-sided p-value of <0.05 was considered statistically significant. Statistical analyses

were performed using SPSS Version 21.0 (IBM Corp. Released 2010. IBM SPSS Statistics for Windows Version 21.0 (IBM Corp., Armonk, NY, USA), and SAS version 9.4 (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA).

Results

Patients

A total of 29.546 pT1 melanoma patients was registered for the time period 2003 – 2014. Of these patients, 23,879 (80.8%) were classified as pT1a, 4,779 (16.2%) as pT1b, and 888 (3.0%) were not further classified due to missing data on either Clark level or mitotic rate of the presence of ulceration at the time of registration. See **Table 1** for patient and melanoma features. Breslow thickness was comparable between cohort 1 and 2 (median 0.60 and 0.58), although statistically significant ($p<0.001$), probably due to the large number of patients in the study population. In Cohort 2 more patients were of the male sex and median age was higher (**Table 1**). Median follow-up for cohort 1 was 92 months [IQR 72 – 115 months]; median follow-up for cohort 2 was 28 months [IQR 14 – 43 months]. The incidence of thin melanomas increased over the investigated time period, starting at 1503 new pT1 melanomas in 2003 (pT1a 2.6 /100,000 person-years and pT1b 0.3 100,000 person-years (WSR)), up to 3430 new pT1 melanomas in 2014 (pT1a 4.9 /100,000 person-years and pT1b 1.5 /100,000 person-years (WSR)) (**Figure S1**).

pT1b Classification

The pT1b proportion differed significantly between both entire cohorts: 10.1% in cohort 1 vs. 21.5% in cohort 2 ($p<0.0001$). There was a clear increase in the pT1b proportion from 2011 onwards (**Figure 1**).

Sentinel Node Biopsies

The proportion of pT1 patients undergoing SNB doubled over time; 2.0% in cohort 1 vs. 4.8% in cohort 2 ($p<0.0001$) (**Figure 2).** The proportion of pT1b patients undergoing SNB increased almost threefold in cohort 2 (**Figure 2**). The proportion of patients with a positive SN was comparable in both cohorts (10.6% vs 9.0% without patients with unknown results, $p=0.445$) (**Table 2**). Of all positive SN patients, 46% (n=44) underwent a CLND (20 (69%) in cohort 1, 24 (36%) in cohort 2). The proportion and number of positive non-SNs was unknown, as this was registered combined with the number of positive SNs in the NCR.

Previous AJCC staging systems and others report a cut-off at 0.75 mm Breslow. To analyze this point, we have used this distribution in both cohorts with the current pT1a and pT1b: ≤ 0.75 mm and 0.76 – 1.00 mm without/with mitoses (**Table 2**).

Table 1. Patient and tumor characteristics of all patients diagnosed with tumor stage I melanoma in the Netherlands, 2003-2014.

	Cohort I 2003 - 2009	Cohort II 2010 - 2014	
	N (%) / median [IQR]	N (%) / median [IQR]	p
Gender			
male	5,324 (38.8)	6,964 (44.0)	<0.0001
female	8,401 (61.2)	8,857 (56.0)	
Age	53 [41 - 64]	58 [46 - 68]	<0.0001
Breslow	0.60 [0.40-0.78]	0.60 [0.40 - 0.80]	<0.0001
pT category			
pT1 nos	744 (5.4)	144 (0.9)	
pT1a	11,600 (84.5)	12,279 (77.6)	<0.0001
pT1b	1,381 (10.1)	3.398 (21.5)	
All pT1	13,725 (100)	15,821 (100)	
SNB			
Yes	276 (2.0)	759 (4.8)	<0.0001
No	13,449 (98.0)	15,062 (95.2)	
SN result			
Negative	245 (88.8)	677 (89.2)	
Positive	29 (10.5)	67 (8.8)	0.279
Not found	2 (0.7)	15 (2.0)	
LND			
Yes	48 (0.3)	55 (0.3)	0.976
No	13.677 (99.7)	15.766 (99.7)	
LND result			
Negative	41 (85.4)	47 (85.5)	
Positive	7 (14.6)	7 (12.7)	0.626
Unknown	0 (-)	1 (1.8)	

Patient and tumor characteristics of all patients diagnosed with tumor stage I melanoma in the Netherlands, 2003-2014. Features were analyzed using X^2-tests or Mann- Whitney U test, as appropriate. Abbreviations: SD, standard deviation; IQR, interquartile range; nos, not otherwise specified; SNB, sentinel node biopsy; SN, sentinel node; LND, lymph node dissection.

Survival

At 5 year, the relative survival rate was similar for pT1a and pT1b in both cohorts, namely pT1a 100% (standard error (SE) 0.2%) vs 100% (SE 0.5%), and pT1b 97% (SE 0.8%) vs. 98% (SE 1.2%) (**Figure 3**). The small group of unspecified pT1 patients had survival rates comparable with pT1a (data not shown). Five-year relative survival rate for pT1a patients with/without SNB was both 100% (cohort 1) and 96% vs. 100% (cohort 2), 5-year relative survival for pT1b patients with/without SNB was both 97% (cohort 1) and 97% vs. 98% (cohort 2).

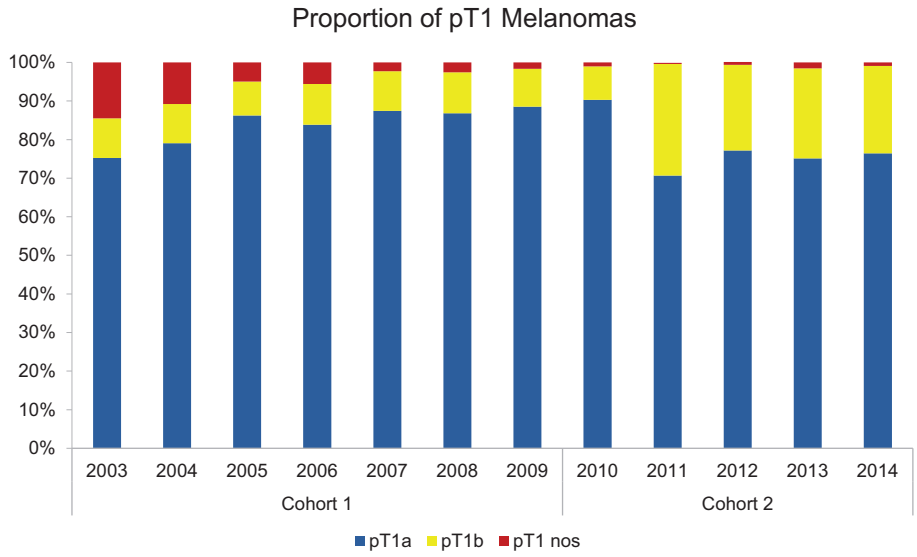


Figure 1. Proportion of pT1a (blue), pT1b (yellow) and pT1 nos (red) patients in percentages of the total annual number of patients. NOS; not otherwise specified.

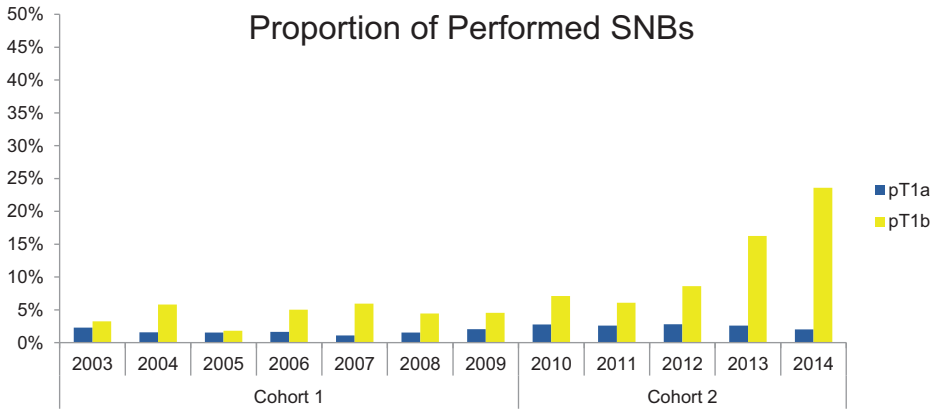
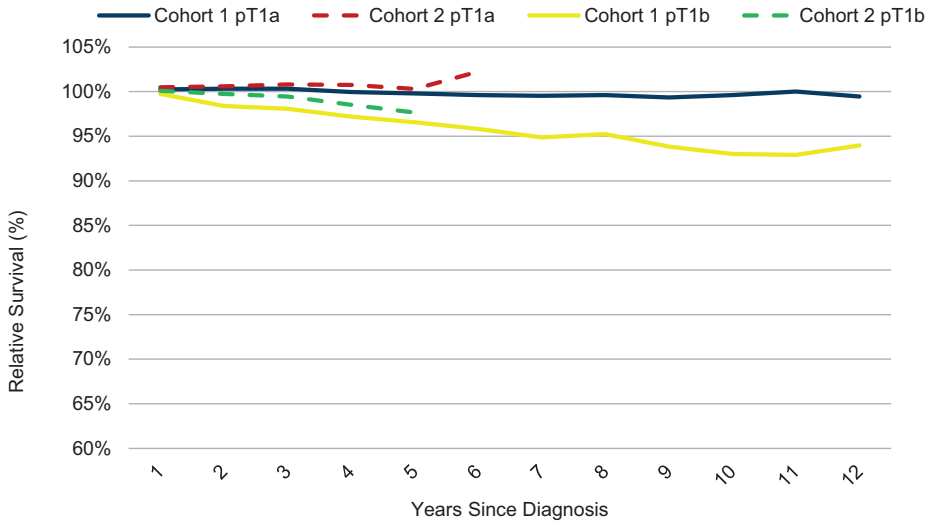


Figure 2. Proportion of performed sentinel node biopsies (SNBs) for pT1a (blue), pT1b (yellow), in percentages per year.

Five-year relative survival rate per SN status was 100% (SE 1.3%) vs. 88% (SE 6.5%) for SN negative vs. SN positive patients in cohort 1. In cohort 2 only 4-year relative survival rate could be calculated due to a low number of cases: this was 99% (SE 1.4%) for SN negative patients vs 85% (SE 8.2%) for SN positive patients. Relative survival for Breslow ≤0.75mm and >0.75mm was calculated for pT1a and pT1b, stratified per cohort (**Figure 4**).



No. at Risk*

Cohort 1												
pT1a	11576	11437	11289	11125	10911	9742	7679	5882	4343	2881	1581	511
pT1b	1378	1354	1317	1295	1263	1127	892	677	499	342	203	67
Cohort 2												
pT1a	11127	8463	5869	3506	1228	23						
pT1b	2008	1209	642	354	116							

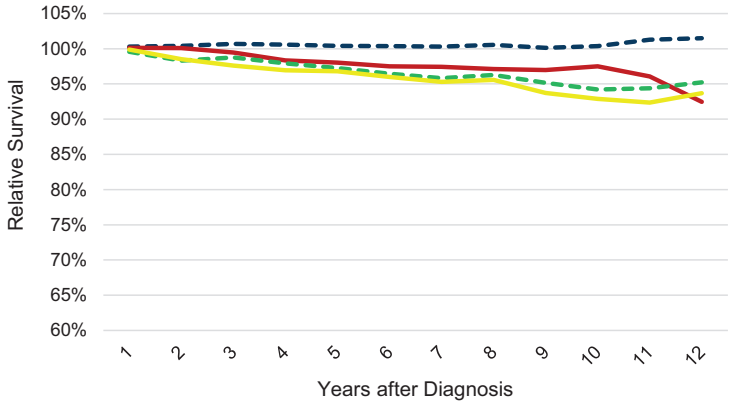
Figure 3. Relative Survival per pT1 category and Cohort. Relative survival for cohort 1 pT1a (blue), pT1b (yellow) and cohort 2 pT1a (red), pT1b (green). *: Effective number at risk.

Discussion

An increasing incidence of melanoma has been seen in the Netherlands already during many recent years[1, 15]. In the current report, we continue to see this increasing incidence of melanoma and see an increasing incidence of thin melanomas over the investigated time period (**Figure S1**) too**.** The proportion of pT1b patients increased considerably from 2011. One year later, (in 2012) the new version of the Dutch Melanoma Guideline was finalized, including the recommendation to consider SNB for pT1b melanoma and higher (previously for T2 melanoma and higher)[4]. The Dutch Cancer Registry started using the 7th edition of the AJCC staging system since 2010, but not all hospitals updated their staging system or clinical practice immediately, causing a temporary delay in the new classification of pT1b primaries according to the 7th AJCC edition.

The increased proportion of performed SNBs for pT1 patients (from 2% to 4.8%) was mainly caused by a steep increase in SNBs performed for pT1b patients in cohort 2. This could be expected considering the recommendation to offer SNB (but not mandatory to perform) for pT1b patients in the updated Dutch Melanoma Guideline in 2012.

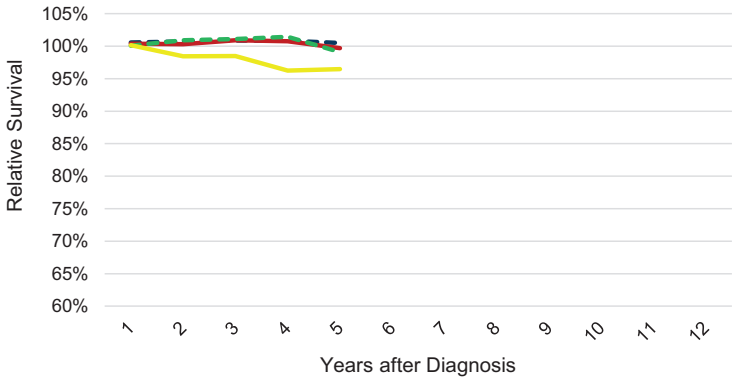
A
Cohort 1



No. at risk*

pT1a												
≤0.75mm	8598	8500	8393	8292	8154	7266	5708	4333	3186	2102	1137	365
>0.75mm	2739	2703	2666	2610	2542	2278	1803	1400	1025	661	359	114
pT1b												
≤0.75mm	609	598	583	579	565	511	418	327	245	170	99	32
>0.75mm	736	724	703	686	671	595	458	340	247	165	99	33

B
Cohort 2



No. at Risk*

pT1a					
≤0.75mm	8664	6565	4528	2703	949
>0.75mm	2192	1712	1217	735	257
pT1b					
≤0.75mm	981	589	306	162	52
>0.75mm	938	567	304	175	60

Figure 4. Relative Survival per Breslow Category and Cohort. Relative survival for Breslow ≤0.75mm and >0.75mm per pT1 category, for cohort 1 (A) and cohort 2 (B). *: Effective number at risk.

When taking into account that after 2012 all pT1b patients should be offered a SNB according to the updated guidelines, the proportion of performed SNBs in the second cohort (13.0%) is still relatively low compared to the proportion of performed SNBs for intermediate thickness melanomas (pT1b – T3b): this was approximately 40% between 2003-2011[5]. Again for intermediate thickness melanomas the guidelines recommend to offer a SNB, but it is not mandatory to perform a SNB. A significant increase in the actual number of patients being exposed to SNB surgery can be observed (from 62 to 443 pT1b patients). As pT1 melanomas form the majority of all newly diagnosed melanomas, this has clear clinical implications: a more stressed referral system, increased wait lists, and increased costs. Let alone the increased number of patients being exposed to potential, albeit low, chance of morbidity related to the SNB, and CLND in case of a positive SN.

The actual number of patients with a negative SNB increased from 245 to 677. If all the potential pT1b patients would undergo a SNB, the number of additional SNB procedures would increase with 2,955 nationwide (fourfold increase for all pT1 melanomas). This would have massive budget implications to the national health care system, but more importantly the potential absolute number of patients at risk for unnecessary morbidity (approximately 300 individuals).

An interesting finding was that CLND was performed less often in cohort 2, which did not result in a decreased survival. This is in line with the results of the MSLT-1 and DeCOG trials, showing no survival difference for patients with immediate or delayed CLND[16, 17].

Previous AJCC staging systems and others report a cut-off at 0.75 mm Breslow in order to offer patients a SNB[18, 19]. In cohort 2, SNB positivity rate for pT1a ≤0.75mm and 0.76 – 1.00mm was 5.7% vs. 10.0% (p=0.357), and for pT1b 7.4% vs. 9.4% (p=0.572) (**Table 2**). Although not significant in this series, SN positivity rate was higher for melanomas with a Breslow thickness of >0.75mm in both pT1a and pT1b patients. SN positivity rate appears to be mainly driven by Breslow thickness and not by mitotic rate as it is currently defined.

Relative survival was excellent for the Dutch population. An increase in survival difference between pT1 substages was not observed, indicating that the stratification of high risk patients did not improve.

Summarizing, our data show an increase in the proportion of pT1b melanomas and a parallel increase in the proportion of performed SNBs in pT1b melanomas, but no increase in the proportion of positive SNs or a clear worse prognosis for all pT1b melanomas in the second cohort (7th edition AJCC). This implies that there has been stage migration between pT1a and pT1b, more specifically, patients formerly classified as pT1a now being classified as pT1b solely on presence of at least 1 singly mitosis/mm2. In a Surveillance, Epidemiology, and End Results (SEER) cancer registry study, the proportion of pT1b melanomas increased from 16.1% to 22.4%, but the proportion of performed SNBs for pT1b melanomas decreased (from 40.9% to 33.3%), and no significant increase

Table 2. Proportion of Performed and Positive SNBs per Breslow Category and per Cohort.

Cohort, group	Breslow (mm)	SNB performed			SN status		
		No	Yes	p	Negative	Positive	p
		N (%)	N (%)		N (%)	N (%)	
I pT1a	<0.75	8375 (99.4)	47 (0.6)		43 (95.6)	2 (4.4)	
	≥0.75	2803 (95.4)	135 (4.6)	<0.0001	119 (88.1)	16 (11.9)	0.249
	Total	11,178 (98.4)	182 (1.6)		162 (90.0)	18 (10.0)	
I pT1b	<0.75	561 (99.1)	5 (0.9)		5 (100)	0	
	≥0.75	731 (93.6)	50 (6.4)	<0.0001	43 (86.0)	7 (14.0)	1.00
	Total	1,292 (95.9)	55 (4.1)		48 (87.3)	7 (12.7)	
I All	<0.75	9,464 (99.4)	55 (0.6)		51 (96.2)	2 (3.8)	
	≥0.75	3,702 (94.9)	201 (5.1)	<0.0001	175 (87.1)	26 (12.9)	0.081
	Total	13,166 (98.1)	256 (1.9)		226 (89.0)	28 (11.0)	
II pT1a	<0.75	9,346 (99.0)	91 (1.0)		82 (94.3)	5 (5.7)	
	≥0.75	2,335 (92.4)	193 (7.6)	<0.0001	171 (90.0)	19 (10.0)	0.357
	Total	11,681 (97.6)	284 (2.4)		253 (91.3)	24 (8.7)	
II pT1b	<0.75	1,704 (93.2)	124 (6.8)		113 (92.6)	9 (7.4)	
	≥0.75	1,153 (79.8)	291 (20.2)	<0.0001	259 (90.6)	27 (9.4)	0.572
	Total	2,857 (87.3)	415 (12.7)		372 (91.2)	36 (8.8)	
II All	<0.75	11,162 (98.1)	215 (1.9)		195 (93.3)	14 (6.7)	
	≥0.75	3,516 (87.9)	486 (12.1)	<0.0001	431 (90.2)	47 (9.8)	0.184
	Total	14,678 (95.4)	701 (4.6)		626 (91.1)	61 (8.9)	

Proportion of performed SNBs and SN results per cohort and per pT1 category, subdivided by Breslow thickness <0.75mm or ≥0.75mm. Patients with unknown pT1 category and/or unknown Breslow thickness were censored for analysis. P-values were calculated with X^2-test or Fisher's exact test as appropriate. Abbreviations: SNB, sentinel node biopsy; SN, sentinel node.

in the proportion SN positive pT1b patients (7.1 vs 8.5%) was found[20]. Since they only compared the year 2010 (7th edition) with 2004-2009 (6th edition) any changes due to the implementation of the 7th edition after 2010 are not included in their analyses, which is a drawback of that study.

On top of the increase of diagnosed pT1b melanomas due to the mitotic rate criterion, the recommendation to perform nodal staging with a SNB in these patients may have driven some pathologists to perform an active search for mitoses (possibly even make and examine additional slides) in order to be able to diagnose a melanoma as pT1b, leading to overdiagnosis. This will further trouble the identification of true high risk patients. Also this will diminish the potential benefits of nodal staging for pT1b melanoma patients, which may not weigh up against the costs of SNB surgery.

Several studies have reported on the prognostic value of mitotic rate in thin melanomas[21-24], which formed the basis of including this in the 7th edition of the AJCC staging system[3, 10]. Kirkland et al. question the mitotic rate criterion of the 7th AJCC staging edi-

tion considering the fact that it is associated with the decision to offer a SNB[11]. They conclude in their review that despite its statistically significant value as prognostic variable, the chosen cut-off of 1 mitosis/mm2 does not have a clear clinically relevant impact on survival rates (nor on SN positivity rates), and thus does not warrant the decision to perform SNB in these patients in a routine fashion. This is supported by Wat et al. who found no association between mitotic rate and SN status in pT1 melanomas[25], and by Speijers et al. who also could not confirm a relationship between mitotic rate and SN status, nor for mitotic rate and survival[26].

Due to the fact that the data used are derived from a national registry system, available data is restricted to the registration performed at the time of diagnosis. Unfortunately not all data on SNBs performed in cohort 1 are available, as explained in the methods section. Potentially there may have been more patients who underwent a SNB which is registered as an LND only.

The actual details for pT1 substaging were not available for the current study (i.e. mitotic rate, Clark level and ulceration), but all T staging was based on these criterions according to the AJCC staging system valid at the time of diagnosis.

In cohort 2 the male gender was overrepresented, and median age was higher. This may have biased results, but these features were adjusted for in survival analyses to minimize potential bias.

The cause of death is unknown for all patients, since the registry data are anonymized after initial registry. However, we used a method to calculate relative survival adjusting for tumor and patient specific features, which forms an adequate alternative for disease specific survival.

Despite these drawbacks, data are present from all melanomas diagnosed between 2003 and 2014 on a national level, including not only primary tumor data but also SN and LND data, and mature follow-up data. This provides valuable insight in incidence and prognosis of pT1 melanomas from the entire Dutch population.

Conclusions

pT1 incidence has increased in the Netherlands over the past decade. Introduction of the mitotic rate criterion for pT1b substaging and the recommendation to perform SNB for pT1b melanoma has led to a proportional increase in pT1b melanomas, and an increase in performed SNBs. SN positivity rate has not increased and survival remained stable for pT1b melanomas, indicating that mitotic rate alone as criterion for pT1b has not improved selection of high risk pT1 patients for optional further (nodal) staging.

Based on the results of this study, and considering the fact that survival of Cohort 2 was not different at 5 year follow-up, recommendation on SNB for pT1b melanomas may be reconsidered as well.

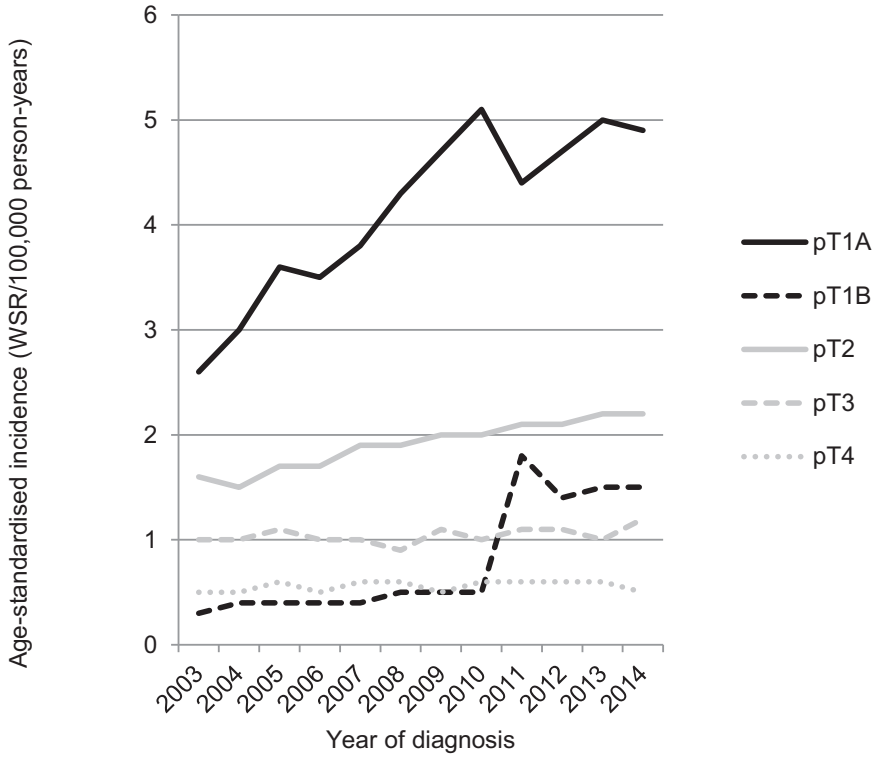


Figure S1. Melanoma Incidence per T Stage

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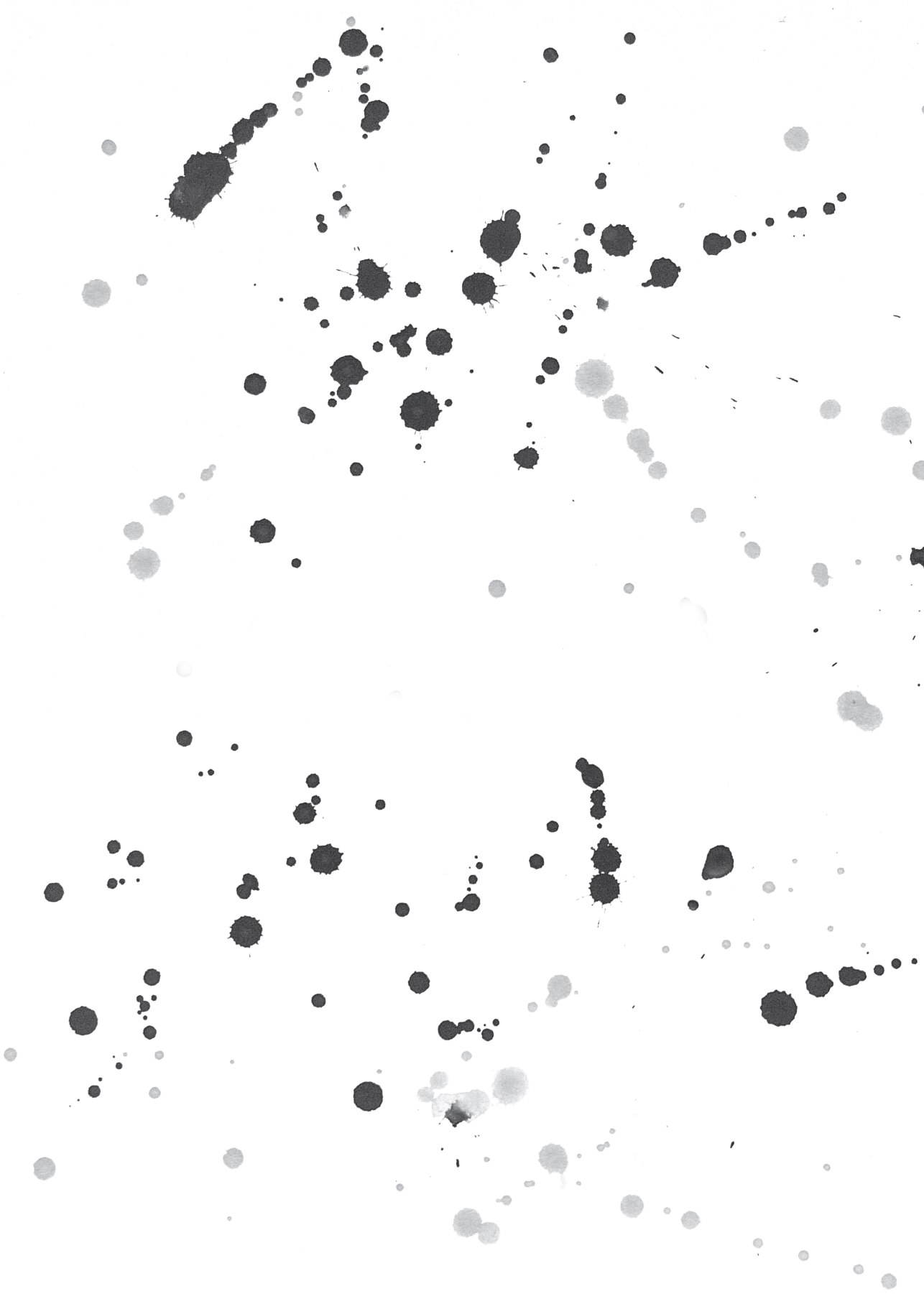

Chapter 3

Ultrasound of the Sentinel Node in Melanoma Patients: Echo Free Island (EFI) is a Discriminatory Morphologic Feature for Node Positivity

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Abstract

Objective Unlike breast and thyroid cancer, the use of Ultrasound (US) guided Fine Needle Aspiration Cytology (FNAC) for preoperative staging is limited in melanoma. New US morphology criteria have shown that US-FNAC can correctly identify 50% of all involved sentinel nodes (SN) in melanoma patients prior to surgical excision. Aim of this study was to examine a new criterion, the Echo-Free Island (EFI).

Methods 1,000 consecutively staged melanoma patients (Breslow thickness > 1mm or < 1mm, but ulcerated, Clark IV/V or regressed) scheduled for SN staging underwent preoperative US. US morphology items were assessed: Peripheral Perfusion (PP), Loss of Central Echoes (LCE), Balloon Shape (BS) and EFI. FNAC was performed in case of suspicious and malignant US patterns. All patients proceeded to undergo an SN biopsy or direct CLND (in case of positive FNAC).

Results 7% was male. Mean / median Breslow thickness was 2.58 / 1.57 mm. Mean / median follow-up was 56 / 53 months. SN was positive in 21%. EFI information was available in 95.3%. It was seen in 40 patients (4%). EFI sensitivity was 10.8%, specificity 97.6%, PPV 50% and NPV 80.2%. EFI was significantly correlated to PP (67.5%). There was no correlation to BS or LCE. 5-year MSS of patients with EFI was significantly worse: 80% versus 92% when absent.

Conclusions The Echo-Free Island (EFI) can be useful in the early detection of SN melanoma metastasis. It is an early sign of involvement and thus associated with a decreased survival.

Introduction

Unlike staging for breast or thyroid cancer, preoperative ultrasound (US) of the regional sentinel nodes (SNs) is not routinely performed for melanoma[1-4]. Previously, our experience with preoperative US guided fine needle aspiration cytology (FNAC) for the early detection of SN involvement in melanoma was described[5-7]. Amongst others, the pattern of an echo free island (EFI) was presented. Other morphologic factors, such as peripheral perfusion (PP), balloon shaped (BS) lymph node and loss of central echoes (LCE) were key characteristics in these first analyses. EFI was a relatively infrequent finding. With increased experience, the discriminative role of this US morphologic characteristic has now been investigated.

In a 2011 meta-analysis Catalano et al.[8] found that 31 out of 201 articles matched their inclusion criteria to provide a description of the use of US scanning for the detection of melanoma lymph node metastases. This compilation of articles showed that, in contrast to older systems, modern high-resolution scanners now allow for recognition of very subtle abnormalities within the lymph node. Predictive criteria should therefore be modified according to the improved devices and resolution.

Especially the color Doppler imaging should be seen differently nowadays. Even if set adequately for detecting slow flow, the scanners used in the past would frequently not have been able to allow for the recognition of relevant signals in malignant lymph nodes, simply because of the low intrinsic sensitivity of those machines[8]. Instead, modern equipment would be very sensitive to slow flow and optimal depiction of the abnormal angiographic architecture of superficial lymph node metastases[8]. The previous limitations of Doppler systems would help explain why many authors did not consider color Doppler findings in their assessment of melanoma lymph node metastases in the past[8].

Unfortunately though, all meta-analyses comprise a certain time-period, and Catalano et al. used the years 1989 – 2009[8]. Our pivotal papers from 2009-2010 and obviously the most recent from 2014, reporting the largest prospective collected database on melanoma lymph node US patterns, were not used in the meta-analysis[5-7]. In this large database up to 65% of all metastases could be potentially identified preoperatively[5]. The most sensitive morphologic criterion was PP, which could potentially identify 77% of metastases preoperatively, but had a low specificity of 52%[6]. BS and LCE had a lower sensitivity, but a high positive predictive value (PPV) of 96% and 65%, respectively[6]. Moreover, a clear correlation between these US patterns and SN tumor burden could be established. Smaller lesions showed PP, whereas only in advanced lesions BS and LCE was seen[5].

Aim of the current study was to report on the infrequently observed morphologic criterion, the echo free island (EFI), which often indicates presence of micrometastatic involvement of the lymph node in melanoma patients.

Patients and Methods

Patients

This prospective database included all patients presenting with a newly diagnosed, histopathologically proven, primary malignant melanoma (at least 1.00 mm Breslow thickness, or if less, at least Clark level IV/V, ulcerated and/or with regression) and who were planned for an SN procedure at the department of dermatology, Charité, University Medicine Berlin, Germany. The institutional ethical review board (ERB) approved the study and informed consent was obtained from all patients enrolled. For the current analyses of the US patterns, inclusion was halted after the first 1,000 consecutive patients with sufficient follow-up (July 2001 – November 2010). Results in the first 400 patients and the follow-up in the first 1,000 patients have been previously reported[5-7].

Methods

The US patterns were examined, determined and listed in a prospective database. All patients were scheduled for an SN procedure in either a 1 or 2 day protocol. In all cases, patients first underwent a lymphoscintigraphy, which allowed for a targeted US examination of the SNs and adjacent lymph nodes. In the timeslot between lymphoscintigraphy and surgery, patients were examined by US in B-Mode and Power Doppler. US was aimed at clearly depicting the location of the suspected SN and at clearly stating whether it seemed involved or not. If US depicted a suspicious or malignant SN, FNAC was performed for verification of the lesion (3-4 repeat FNACs were performed within one single procedure). If a clearly malignant US pattern could not be verified by FNAC, patients proceeded to undergo an SN nevertheless, since the decision to alter a planned SN directly into a CLND was always based on positive cytology.

Micro-anatomic location of the SN metastases was evaluated according to the criteria by Dewar et al.[9]. SN tumor burden was assessed according to the Rotterdam Criteria for SN tumor burden[10-12].

Ultrasound Technique and Image Analysis

All US examinations were performed using the high-end device MyLab 70 (ESAOTE, Genova, Italy) equipped with 3 transducers (1-18 MHz) (B-mode, 30 pictures per second, color Doppler, Power Mode). The lymph node was measured, the pattern was described and it was classified as benign [b], suspicious [s] or malignant [m].

In general, an US was considered suspicious, when PP was present or if the central echo was wandering towards the rim. US was considered malignant if there was a total LCE or if the lymph node was enlarged and BS. If an echo-poor disruption of the lymph node architecture was seen, the lymph node was described as EFI (**Figure 1**). Details of these Berlin US morphology criteria have been reported previously elsewhere[6].

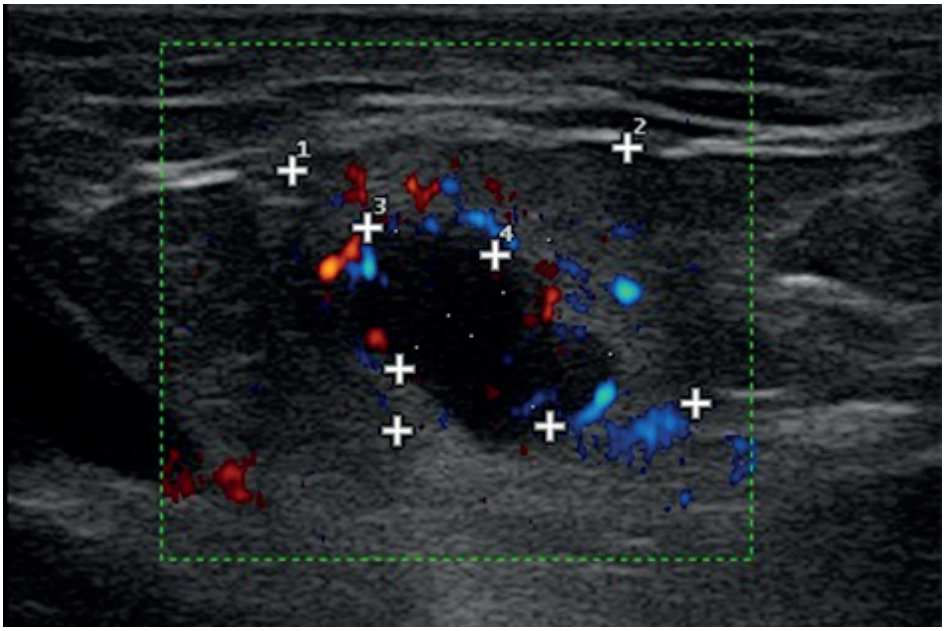


Figure 1. Illustration of the Echo-Free Island (EFI) Phenomenon on Ultrasound Examination. Line 1 and 2 indicate the maximum diameters of the lymph node, line 3 and 4 indicate the maximum diameters of the EFI within the lymph node.

Fine Needle Aspiration Cytology (FNAC) in detail

FNAC was performed with a hand-held Binder-valve as described in detail elsewhere[5]. The fine needle used for superficial lymph nodes has a diameter of approximately 0.4 mm (26G), especially for small targets. A smear was considered technically sufficient, if it contained approximately 100 cells.

Cytology results were reported to the surgeons and it was left to their discretion how to proceed with surgery, either SN or LND. If the US did not show any suspicious (EFI, PP) or malignant (BS, LCE) patterns or if the cytology was negative, the patient proceeded to undergo the scheduled SN.

Histopathological evaluation of excised SN

In brief, the EORTC Melanoma Group protocol according to Cook et al. was followed[13]. Lymph nodes were fixed for 24 hours in buffered formalin. After fixation they were cut in half through the hilum and its longest dimension, and embedded in paraffin. In rare cases, exceptionally larges lymph nodes were sectioned parallel to the first cut in order to fit into the blocks. Fiver serial step sections of 4 µm each were cut from each face of the lymph node and staining with H&E, S100 and HMB-45 was performed. Micro-anatomic location of the metastases and SN tumor burden were assessed according to the Dewar and Rotterdam Criteria, respectively[9-12].

Statistics

To assess the predictive value of individual and combination of US patterns for involved SNs; sensitivity, specificity, PPV (Positive Predictive Value) and NPV (Negative Predictive Value) were calculated. Associations were tested with Pearson's chi square test. Disease-Free Survival (DFS) and Melanoma Specific Survival (MSS) were calculated for SN date until recurrence (DFS) or death due to melanoma (MSS). Patients were censored at the date of last known follow-up if no events had taken place. Univariate analyses of survival were performed using the Kaplan-Meier method and log-rank test. Multivariate analyses to determine the prognostic value of covariates regarding survival were performed using the Cox's proportional hazard model. Statistical analyses were all performed with Stata®, version 10.0 (Stata Corporation, College Station, TX, USA). P values of less than 0.05 were considered as significant.

Results

Table 1 summarizes the most important patient, primary tumor, SN and US characteristics. Mean and median follow-up of all 1,000 patients was 56 and 53 months, respectively. The EFI pattern could be assessed in 953 patients (95.3%), the pattern was present in 40 patients (4%).

EFI showed a sensitivity of 10.8% for metastasis in the final histology of the SN. Specificity was high (97.6%), PPV was 50%, NPV was 80.2% (**Table 2**). Moreover, a clear correlation was seen with PP. In 27/40 cases with EFI, PP was also seen (67.5%) (**Table 2**).

However, in case of PP, the EFI was only seen in a minority of cases 27/273 (9.9%). There was no correlation between EFI and BS (P=0.852) (Table 2). In most cases, when an EFI was seen, the central echo would still be normally present 24/40 (60%) (**Table 2**).

Univariate analysis showed that patients with an EFI had a significantly worse 5-year MSS compared to patients without the EFI US pattern (80% vs. 92%) (P<0.001)(**Figure 2**). The univariate hazard ratio (HR) for MSS was 3.32 (95% CI 1.66 – 6.68) (P=0.001).

Multivariate analysis for MSS demonstrated that EFI was non-significant when analyzed together with other known independent prognostic factors (ulceration, Breslow thickness and PP, data not presented).

When the multivariate analysis for MSS was performed separately for US patterns only, the presence of any of these patterns was associated with a detrimental survival. EFI had a HR of 2.06 (95% CI 1.01 – 4.20) (P=0.048). BS had a HR of 2.62 (95% CI 1.01 – 6.79) (P=0.048). LCE has a HR of 3.05 (95% CI 1.26 – 7.40) (P=0.013). PP was associated with a HR of 2.57 (95% CI 1.49 – 4.41) (P<0.001).

Table 1. Baseline Patient, Primary Tumor, Sentinel Node and Ultrasound Characteristics of All 1,000 Patients

Characteristic	N	%	Characteristic	N	%
Gender			*SN Tumor Burden (n = 208)*		
Male	567	57%	≤ 0.1 mm	30	14%
Female	433	43%	0.1 – 1.0 mm	62	30%
Histological subtype			> 1.0 mm	62	30%
SSM	595	60%	LND / Unknown	54	26%
NM	242	24%	*PP*		
LMM	37	4%	Absent	663	66%
ALM	44	4%	Present	273	27%
Unknown / other	82	8%	Unknown	64	7%
Breslow Thickness			*LCE*		
Mean / Median (Range)	2.58 / 1.57 (0.2 – 44) mm		Central Echo Present (Normal)	791	79%
T1 (≤ 1.00 mm)	288	29%	Wandering to Rim	97	10%
T2 (1.01 – 2.00 mm)	308	31%	Lost	66	6%
T3 (2.01 – 4.00 mm)	231	23%	Unknown	46	5%
T4 (> 4.00 mm)	173	17%	*BS*		
Ulceration			Absent	881	88%
Absent	758	76%	Present	53	5%
Present	242	24%	Unknown	66	7%
SNs Removed			*EFI*		
Mean / Median	1.72 / 1 (1 – 13)		Absent	913	91%
SN result			Present	40	4%
Negative	792	79%	Unknown	47	5%
Positive	208	21%	*US-FNAC Result*		
Direct LND (after + FNAC)	43	4%	Not Performed & Negative	892 / 342	89% / 34%
	43 / 208	21%	Positive	98 / 342	10% / 26%

SN, sentinel node; SSM, superficial spreading melanoma; LND, lymph node dissection; NM, nodular melanoma; LMM, lentigo maligna melanoma; ALM, acrolentiginous melanoma; PP, peripheral perfusion; LCE, loss of central echo; BS, balloon shape; EFI, echo-free island; US-FNAC, ultrasound – fine needle aspiration cytology

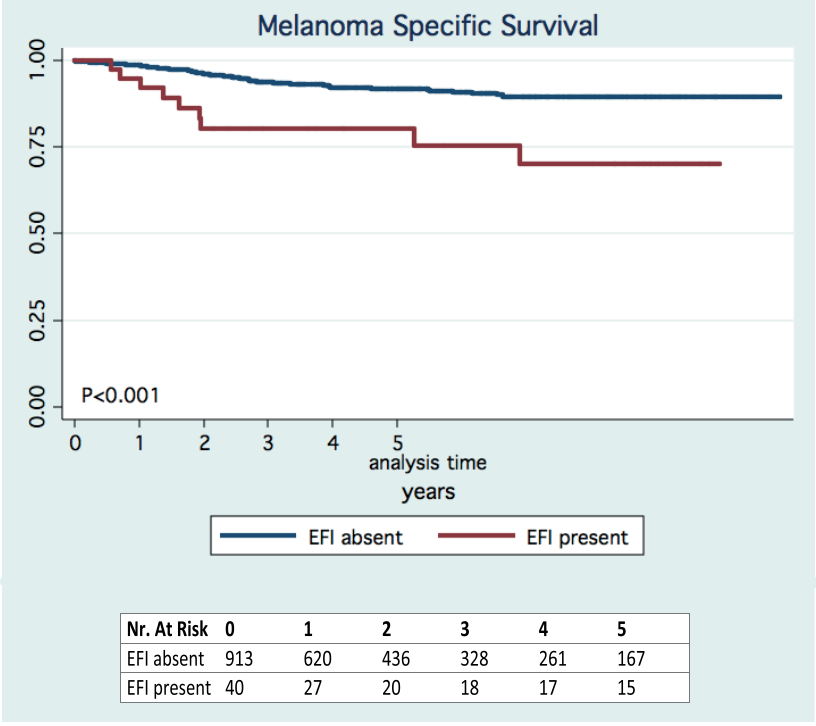
Discussion

The final results of the Multicenter Selective Lymphadenectomy Trial-1 (MSLT-1) have been published[14]. Although the MSLT-1 trial did not demonstrate a significant benefit for wide local excision (WLE) + SN staging (in case of a positive result followed by CLND) versus WLE + sequential nodal observation, the authors concluded that biopsy-management prolongs DFS for all patients and prolongs DMFS and MSS for node positive

Table 2. Sensitivity, Specificity, PPV and NPV of the Echo-Free Island (EFI) and Correlation to Peripheral Perfusion (PP), Loss of Central Echoes (LCE) and Balloon Shape (BS)

	SN negative	SN positive	P-value	
EFI absent	732	181		
EFI present	18	22	P<0.001	
Correlations	**PP absent**	**PP present**		
EFI absent	650	246		
EFI present	13	27	<0.001	
	BS absent	**BS present**		
EFI absent	843	51		
EFI present	38	2	0.851	
	Central Echo	**Wandering to Rim**	**LCE**	**P-Value**
EFI absent	766	86	61	
EFI present	24	11	5	<0.001
	Central Echo / Wandering to Rim		**LCE**	
EFI absent	852		61	
EFI present	35		5	0.156

SN, sentinel node; EFI, echo-free island; PP, peripheral perfusion; BS, balloon shape; LCE, loss of central echo



Nr. At Risk	0	1	2	3	4	5
EFI absent	913	620	436	328	261	167
EFI present	40	27	20	18	17	15

Figure 2. Kaplan-Meier 5-year Melanoma Specific Survival (MSS) for Patients With or Without Echo-Free Island (EFI)

patients with intermediate thickness melanoma[14]. These last conclusions are very much topic of an ongoing debate[15-17].

Perhaps more important is the fact that the SN biopsy provides accurate and important staging information[14, 15]. This SN result provides information on the chance of additional non-SN involvement of the CLND and with respect to survival. SN tumor burden further stratifies these risk assessments[10, 12]. Nowadays, patients with significant SN tumor burden (> 1 mm in maximum diameter), who have a high risk for relapse, might be eligible for adjuvant therapy (studies). Recently, the first positive results with respect to an improved RFS have been reported for patients undergoing adjuvant Ipilimumab[18, 19].

Even if SN staging itself does not provide a survival benefit to the individual patient, effective adjuvant therapy might. Moreover, patients should be adequately staged to be eligible for participation into new adjuvant therapy trials. It would be unethical to withhold them this possibility.

When comparing the situation in melanoma to that of breast or thyroid cancer, the role of US staging has been minimal. US-guided-FNAC is even less invasive than surgical SN staging. Previous results have shown that US-guided-FNAC can correctly identify 50% up to 80% in selected subgroups, of all SN metastases, prior to the surgical excision by SN biopsy[5, 6]. Important aspects to achieve these improved results are the use of newly defined US patterns and a low threshold for FNAC[7].

The current paper is an addendum to the previously reported Berlin morphology patterns. Another potential useful item is presented, the EFI. Although the EFI is a rare phenomenon, the EFI is significantly associated with PP in 67.5% of cases, and at the same time the Central Echo is still present in the majority of cases (60%), indicating that it is an early sign of disruption by a developing metastasis. This is illustrated by the survival curves, which show that EFI is associated with a significantly decreased MSS.

As these US morphologic signs can be used to detect early (sub) micrometastases in SNs, standard US of the lymph node basins prior to surgery may once more be considered. Ultimately, further improvement of the technique of US scanning and targeted FNAC of the SN may even replace the surgical SN procedure as the routine staging of new stage I/II melanoma patients. Currently ongoing research is being performed to investigate minimally invasive alternatives to the surgical SN procedure (Dutch trial: Gamma Probe and Ultrasound Guided Fine Needle Aspiration Cytology of the Sentinel Node - GULF trial, NL52091.078.15).

In conclusion, the Echo-Free Island (EFI) as an US morphologic sign can be useful in the early detection of SN metastases in melanoma patients. It is an early sign of involvement and thus associated with a decreased survival.

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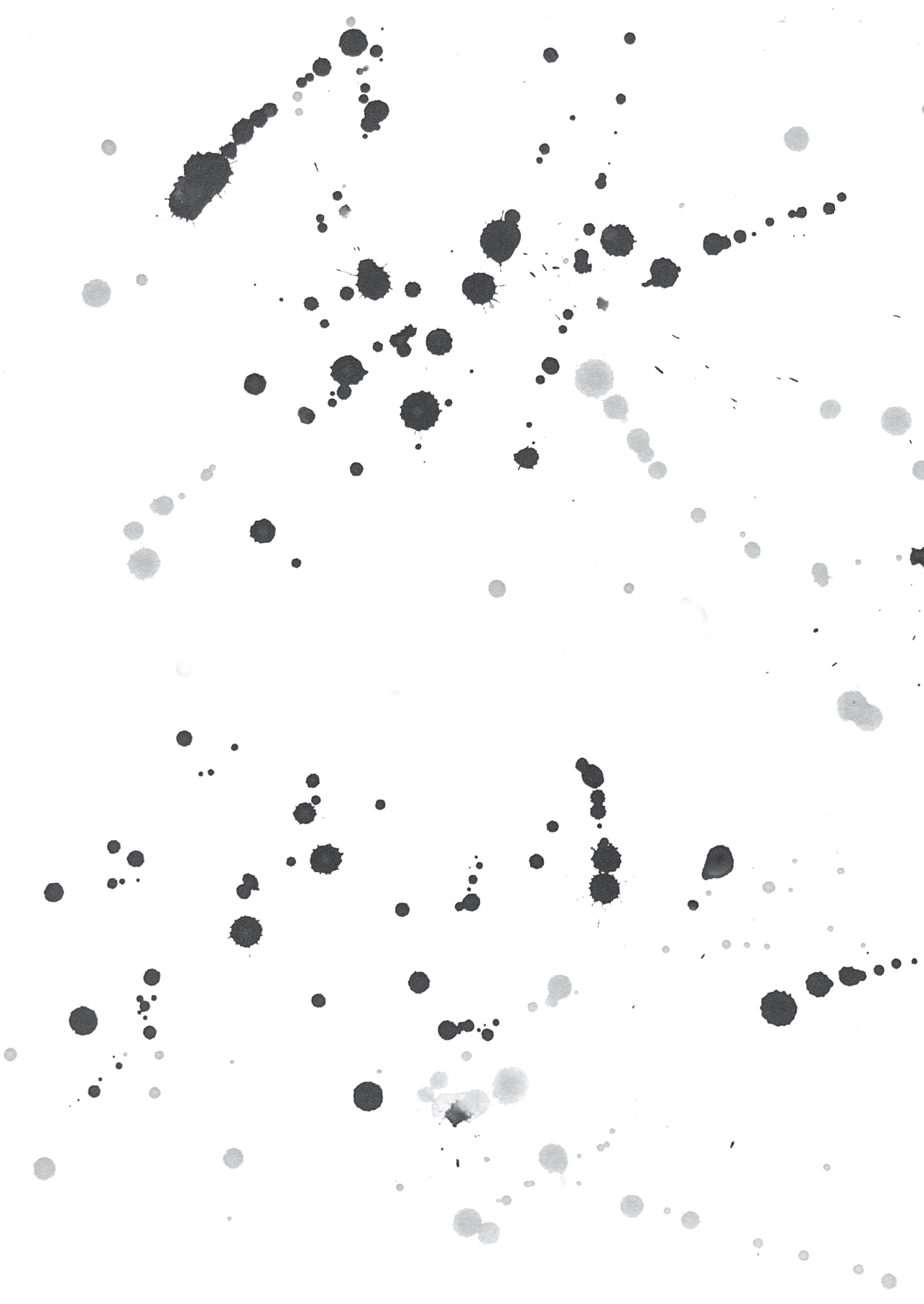

Chapter 4

Long-term Results of Ultrasound Guided Fine Needle Aspiration Cytology in Conjunction with Sentinel Node Biopsy Support Step-wise Approach in Melanoma

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Abstract

Background US-FNAC is a common diagnostic tool in the work-up of many cancers. Results in melanoma were initially poor (sensitivity 20-40%). Introduction of the Berlin Morphology criteria has shown potential improvement up to 65–80% in selected patients.

Aim This cohort study evaluates the long-term survival outcome of melanoma patients undergoing Ultrasound (US) guided Fine Needle Aspiration Cytology (FNAC) prior to sentinel node biopsy (SNB) or direct lymphadenectomy.

Methods from 2001-2010 over 1,000 consecutive melanoma patients prospectively underwent targeted US-FNAC prior to SNB. The Berlin US morphology criteria: peripheral perfusion (PP), loss of central echoes (LCE) and balloon shape (BS) were registered. FNAC was performed if any factor was present. All patients underwent SNB or lymphadenectomy in case of positive FNAC.

Results Median follow-up was 61 months (IQR 40-95). SN positivity rate was 21%. Survival analyses demonstrated that patients with positive US-FNAC had poor survival. After adjustment for SN status and other known prognostic features, patients with positive US-FNAC (hazard ratio (HR) 1.80, 95% CI 1.10-2.96) had worse survival than patients with normal US (reference). Patients with suspicious US and negative FNAC (HR 1.13, 95% CI 0.71-1.78) had survival comparable to patients with normal US.

Conclusions The long-term US-FNAC results support this step-wise approach to melanoma patients. Patients with positive US-FNAC have a poor survival and can be spared a SNB. Patients with suspicious US and negative FNAC should undergo SNB to detect microscopic occult disease. Completely US-FNAC negative patients might only require follow-up and no SN staging at all.

Introduction

Primary cutaneous melanoma used to be treated with aggressive surgery in absence of other successful treatment modalities. Elective lymphadenectomy was performed based on the hypothesis of sequential metastatic spread[1]. It aimed to potentially prevent metastatic spread of the disease, and to minimize the number of patients who would develop aggressive regional disease burden[1]. This prophylactic procedure came with a cost: only a minority of patients had involved lymph nodes at the time of surgery, a significant amount of patients suffered from long-term morbidity, and survival was not altered[2]. Morton et al. introduced a more sophisticated manner to identify those patients with regional nodal involvement; the sentinel node biopsy (SNB) [3].

To date, SNB remains the gold standard for adequate staging of the N-status in clinically node negative melanoma patients [4-6]. Meanwhile, its therapeutic power continues to be topic of debate. As the final trial report of the MSLT 1 did not find an overall survival benefit for melanoma patients undergoing wide local excision (WLE) + SNB versus WLE only and nodal observation[7], the search for less invasive diagnostic staging methods continues to be worthwhile.

Early diagnosis of regional nodal involvement is important not only for adequate staging, but also for potential participation in adjuvant therapy trials. Adjuvant therapy may be of a potential greater benefit in early stage III (SN-positive patients) compared to patients with palpable stage III disease. Stratification by stage III (N1: SN positive) vs Stage III (N2: palpable nodal disease) was performed in the two largest adjuvant IFN trials EORTC 18952 and 18991 demonstrated a significantly greater benefit in SN-positive patients [8-12]. Recently a recurrence free survival benefit for SN-positive patients was also demonstrated in the EORTC 18071 trial regarding adjuvant ipilimumab in stage III patients [13]. The ongoing EORTC 1325 trial regarding adjuvant pembrolizumab is stratified similarly [14]. Final results regarding recurrence free survival and overall survival will have to be awaited for these trials, but results of the EORTC 18071 are promising.

Ultrasound (US) guided Fine Needle Aspiration Cytology (FNAC) is a common diagnostic tool proven to be helpful in the work-up of breast cancer and thyroid cancer [15, 16]. The results of diagnostic preoperative US of the regional lymph node basins in melanoma patients were poor in the past decades, with a reported sensitivity of only 5 - 40% [17-21]. Hence preoperative US in combination with FNAC has not yet been adopted as a standard preoperative diagnostic tool.

Previously, our group has demonstrated that is it possible to identify the sentinel node (SN) using targeted US with a good accuracy [22]. Several others have performed targeted US of the SN area directly after lymphoscintigraphy as well [21, 23, 24]. Correct identification of tumor positive SNs prior to surgery is the ultimate goal of targeted US; results vary but can be promising when this technique is further improved. Marone et al correctly

identified 18 out of 122 positive SNs (15%) (from 831 excised SNs in total), and Testori et al. correctly identified 16 out of 16 positive SNs (100%) with targeted US, but also had 9 false positive patients at histological analysis (false positive rate = 36% (FP/ (TP + FP)) [21, 24].

In a second study by our group US-FNAC of the SN could identify up to 65% of all tumor positive SNs preoperatively, and additionally the Berlin morphology criteria have been presented, describing specific US patterns related to early involvement with which a high sensitivity for US-FNAC could be achieved [25, 26].

More recently our group reported on the first 1,000 prospective melanoma patients who underwent US-FNAC prior to a scheduled SNB: application of the Berlin Morphology criteria yielded a sensitivity of up to 76% in selected patients [27]. 5-year estimated Kaplan-Meier melanoma specific survival (MSS) and disease free survival (DFS) showed a significant difference in survival outcomes for each US-FNAC status [27], indicating its potential as a prognostic indicator.

The current study aims to evaluate the long-term survival outcomes of this now fully matured cohort of melanoma patients undergoing US-FNAC prior to SNB or direct lymphadenectomy.

Patients and Methods

Patients

The current study concerns the long-term follow-up of a previously collected cohort by Voit et al. published previously [27] of over 1,000 melanoma patients who underwent US-FNAC and SNB or immediate lymph node dissection (LND) in case of positive US-FNAC.

Briefly, the cohort consisted of over 1,000 stage I/II consecutive melanoma patients who prospectively underwent US-FNAC prior to Sentinel Node Biopsy (SNB) between 2001 and 2010. All patients had a histopathologically proven malignant melanoma (Breslow thickness ≥1.00mm, or at least one risk factor such as Clark level IV/V, ulceration or regression) and were scheduled for a SNB at the Department of Dermatology, Charité, University Medicine Berlin, Germany. The institutional ethics review board (ERB) approved the study and informed consent was obtained from all patients enrolled. For the current analysis the first 1,000 consecutive patients with sufficient follow-up (July 2001 – November 2010) were selected. A quality control of the database was carried out to assure maximum retrieval of any missing data from patient records at 5-year follow-up. Two duplicate cases were excluded and 6 patients with a second primary melanoma requiring a second US-FNAC and SNB were censored for survival analysis. Eight consecutive study patients were added to the cohort to restore a sample size of 1,000 patients (1,006 US-FNAC cases) for the current analyses.

Design

All patients underwent lymphoscintigraphy prior to US-FNAC. The Berlin US morphology criteria: Peripheral perfusion (PP), loss of central echoes (LCE) and balloon shaped (BS) were registered and FNAC was performed if any factor was present. If FNAC could not verify a clearly malignant US pattern, patients always proceeded to undergo a SNB. In the early phase of the study all patients proceeded to undergo a SNB even if FNAC was positive (n=47). During the course of the study, a change in hospital policy allowed the surgeon to proceed to an immediate LND after a positive FNAC. The decision to change a scheduled SNB to a LND was always based on a positive FNAC.

Definitions

US was considered malignant in case of LCE or BS. US was considered suspicious in case of PP or the wandering to the rim of the central echo. US-FNAC was considered positive if LCE or BS (with or without a FNAC verification) was seen or in case of a positive FNAC.

When an echo-poor disruption of the lymph node architecture was observed this was described as Echo free island (EFI). Results of EFI have been described in detail previously [28].

US-FNAC technique and analysis

The high-end US device MyLab 70 (ESAOTE, Genova, Italy) was used for all US examinations. An expert ultrasonographist identified the lymph node, measured it and described the morphologic pattern. The lymph node was classified as benign, suspicious or malignant according to the visualized pattern. Details of the ultrasound technique, image analysis using ultrasound morphology criteria and classification have been described previously [25, 27]. For FNAC a hand-held Binder-valve was used as described in detail previously [27]. A smear had to contain at least approximately 100 cells to be considered technically sufficient.

Details of pathologic examination of the SN have been described previously [27]. SN tumor burden was measured according to the Rotterdam criteria [29, 30]. Microanatomic location of SN metastases was evaluated according to the criteria by Dewar et al [31]. Final histology of the SN or LND was considered as the gold standard. The first 120 patients underwent both targeted US and FNAC of the SN regardless of the US classification, as a feasibility study.

Statistics

DFS and MSS were calculated from SN date until first recurrence or death or censored at the date of last known follow-up, if no events had taken place. 5 year DFS and MSS were estimated using the Kaplan Meier method and compared using the log-rank test. Cox's proportional hazard model was applied for univariable and multivariable analyses

to determine the prognostic value of covariates regarding MSS. Hazard ratios (HR) were estimated for: SN status, SN tumor burden, US-FNAC result, age, gender, primary tumor location, histologic subtype, Breslow thickness, and ulceration status. SN tumor burden was left out as covariate for the multivariate Cox regression model 1 as a significant correlation with SN status could be expected, and was tested in a separate model 2 without SN status. All statistical analyses were performed with SPSS version 21 (IBM Corporation, Armonk, NY, USA). P values of less than 0.05 were considered as significant.

Results

Baseline features of the first 1,000 US-FNAC cases have been described previously elsewhere [27]. After quality control (where 2 duplicate cases were excluded and 6 cases were identified concerning patients with a second primary melanoma and a second US-FNAC) eight consecutive study patients were added to the cohort to restore a sample size of 1,000 patients (1,006 cases) for the current analyses. Patient and tumor features are displayed in **Table 1 and Table 2.** Mean/median follow-up was 66/61 months (IQR 40 - 95).

Survival

5-year and 10-year Kaplan-Meier estimated MSS was significantly better for patients with negative US and FNAC: 90% (SE 1%) vs. 51% (SE 5%) for US-FNAC positive patients and 85% (SE 2%) vs. 34% (SE 6%) respectively (both p<0.0001) **(Figure 1a)**. This difference in MSS remained significant in the group of SN positive patients: 5-year MSS 71% (SE 5 %) for US-FNAC negative patients vs. 51% (SE 5 %) for US-FNAC positive patients and 10-year MSS 65% (SE 7%) vs. 33% (SE 6%) (both p<0.0001) respectively **(Figure 1b)**. Since there was only 1 SN negative patient with positive US-FNAC (whom turned out to have a false negative SNB), no log rank test comparison could be performed for SN negative patients. The corresponding 5-year and 10-year Kaplan-Meier estimated DFS rates for all patients were 84% (SE 3%) for US-FNAC negative patients vs. 33% (SE 5%) for US-FNAC positive patients, and 79% (SE 2%) vs. 24% (SE 6%) (both P<0.0001) respectively **(Figure 2).**

There were 778 both US (and/or FNAC) negative and SN negative patients. Of these patients, 49 (6%) developed regional lymph node metastases. False negative rate (FN/(FN+TP)) was 49 / (49 + 119) = 29%. The median time interval to nodal basin failure was 28 months (interquartile range 19 – 44 months). The majority of these patients had either a NM (n=23) or a SSM (n=21, 2); 2 patients had an ALM; 1 patient a LMM; and in 2 patients exact histology data were not available.

Table 1. Baseline characteristics of all melanomas (1,006 in 1,000 patients).

Characteristic	n (%) or mean/median (range)
Gender	
Female	435 (43)
Male	571 (57)
Histological subtype	
SSM	601 (60)
NM	242 (24)
LMM	37 (4)
ALM	44 (4)
Unknown	82 (8)
T stage	
T1 (≤1.00mm)	294 (29)
T2 (1.01-2.00mm)	309 (31)
T3 (2.01-4.00mm)	233 (23)
T4 (>4.00mm)	170 (17)
Ulceration	
Absent	763 (76)
Present	243 (24)
SNs removed	1.72/1 (1-13)
SN result	
Negative	797 (79)
Positive (incl. 43 direct LND for pos. FNAC)	209 (21)
immediate LND (after pos. FNAC)	43 (4) / 43/209 (21)
SN tumor burden Rotterdam criteria	(n=209)
<0.1mm	32 (15)
0.1 - 1.0mm	63 (30)
>1.0mm	64 (31)
immediate LND or unknown	50 (24)

Baseline characteristics of all melanomas with n and percentage or mean/median and range. Abbreviations: US, ultrasound; SSM, superficial spreading melanoma; NM, nodular melanoma, LMM, lentigo maligna melanoma; ALM, acrolentiginous melanoma; SN, sentinel node; LND, lymph node dissection, pos., positive; FNAC, fine needle aspiration cytology.

Classification of patients according to Berlin ultrasound morphology criteria showed that patients with a suspicious US (i.e. presence of PP or beginning LCE = 'wandering to the rim') had a slightly worse 5-year MSS than patients with a normal US; and patients with a clearly malignant US (i.e. presence of BS or total LCE) had the poorest survival: 5-year MSS 91% (normal US, SE 1%) vs. 81% (suspicious US, SE3%), and vs. 55% (malignant US, SE 6%), and 10-year MSS 86% (SE 2%) vs. 74% (SE 4%) and 38% (SE 8%) (all p<0.0001) **(Figure 3).**

Table 2. Ultrasound and Fine Needle Aspiration Cytology Results

Characteristic	N(%)
PP	
Absent	670 (67)
Present	273 (27)
Unknown	63 (6)
LCE	
central echo present (normal)	798 (79)
central echo wandering to rim	97 (10)
central echo lost	66 (7)
Unknown	45 (5)
BS	
Absent	887 (88)
Present	53 (5)
Unknown	66 (7)
US results	
US benign	683 (68)
US suspect	247 (25)
US malignant	76 (7)
FNAC results	n=341
benign	252 (74)
malignant	89 (26)
US/FNAC results	
Normal US/ FNAC negative	681 (68)
PP at US	206 (20)
BS/LCE and/or FNAC positive	119 (12)

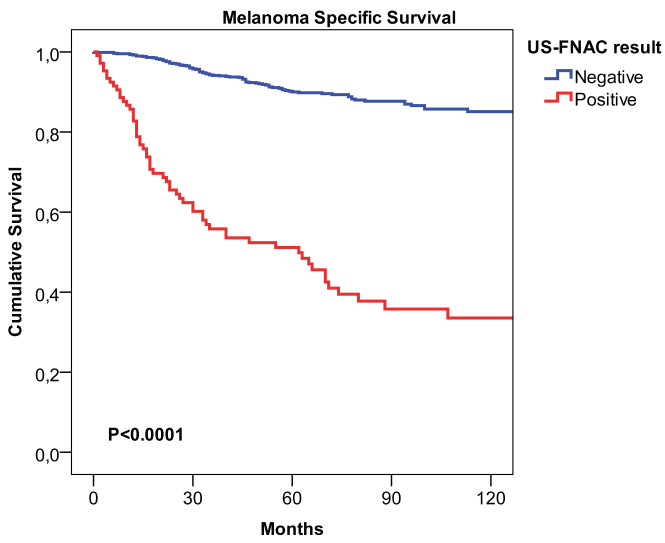
Ultrasound results of all 1,006 melanomas. Abbreviations: PP, peripheral perfusion; LCE, loss of central echo; BS, balloon shape; US, ultrasound; FNAC, fine needle aspiration cytology.

The unadjusted and adjusted HRs for 5 year MSS are shown in **table 3**.

US-FNAC results were categorized as follows: US normal and FNAC negative; US suspicious and FNAC negative; US positive and/or FNAC positive. After adjustment for SN status, gender, age, Breslow thickness, primary tumor location, histology type, and ulceration in model 1, suspicious US was no prognostic indicator, but positive US-FNAC did remain as a prognostic indicator for worse MSS.

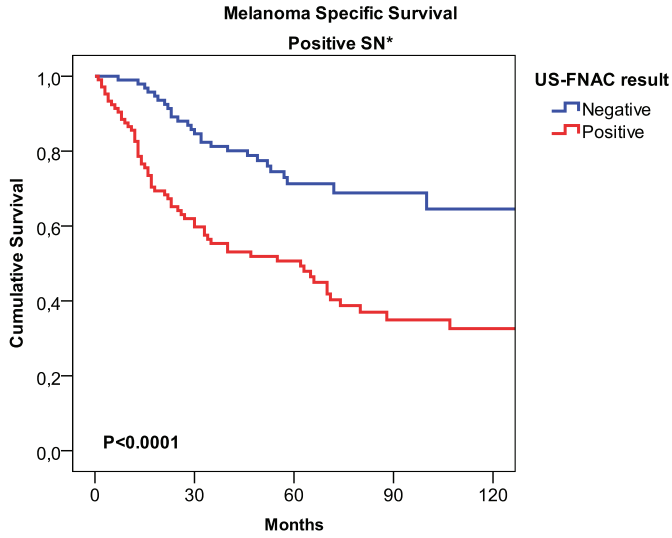
A second model was formed with SN tumor burden. An interaction term was calculated for SN tumor burden and US-FNAC result since they were found to be significantly correlated [27]. In a simple model with US-FNAC result and SN tumor burden, the interaction term was not significant (data not shown). The unadjusted and adjusted hazard ratios for 5 year MSS for model 2 are shown in **table 3**. In this model, US-FNAC was no prognostic indicator.

A



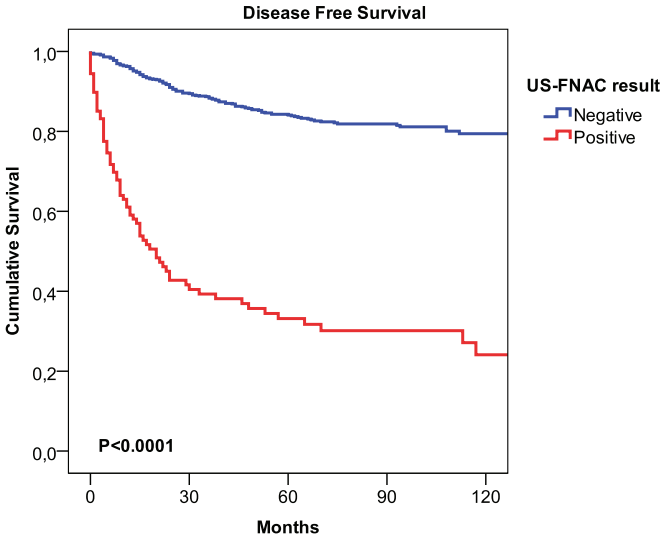
No. at risk	0	12	24	36	48	60	72	84	96	108	120
US-FNAC neg	892	865	818	738	637	483	368	288	232	158	89
US-FNAC pos	108	87	63	51	44	40	27	19	17	14	9

B



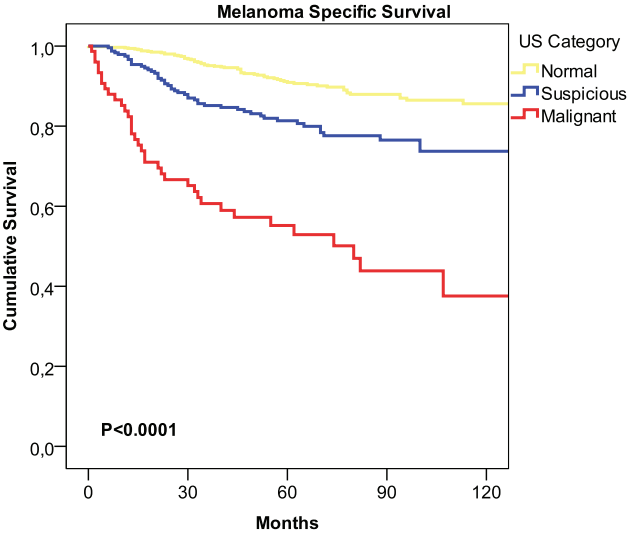
No. at risk	0	12	24	36	48	60	72	84	96	108	120
US-FNAC neg	101	93	79	71	58	43	29	23	18	11	6
US-FNAC pos	107	86	62	50	43	39	26	18	16	13	9

Figure 1. Estimated Kaplan-Meier melanoma specific survival of all patients (A) and of SN positive patients only (B) for ultrasound (US) – fine needle aspiration cytology (FNAC) negative result (blue line) and for US-FNAC positive result (red line) compared with the log-rank test.



No. at risk	0	12	24	36	48	60	72	84	96	108	120
US-FNAC neg	892	839	771	689	585	449	332	266	213	148	80
US-FNAC pos	108	61	40	34	30	26	19	13	13	10	7

Figure 2. Estimated Kaplan-Meier disease free survival of all patients for ultrasound (US) – fine needle aspiration cytology (FNAC) negative result (blue line) and US-FNAC positive result (red line) compared with the log-rank test.



no. at risk	0	12	24	36	48	60	72	84	96	108	120
US normal	679	663	631	570	493	369	281	217	176	122	66
US suspicious	245	230	204	179	158	128	94	77	61	45	29
US malignant	76	59	46	40	30	26	20	13	12	5	3

Figure 3. Estimated Kaplan-Meier melanoma specific survival of all patients for ultrasound (US) category normal (yellow line), suspicious (blue line) and malignant (red line) compared with the log-rank test.

Table 3. Cox Proportional Hazards Regression Analysis for Melanoma Specific Survival (n=1,000)

Variable	Univariable			Multivariable model 1			Multivariable model 2		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
SN status									
Negative	Ref			Ref			-		
Positive**	6.52	4.73-8.98	<0.0001*	3.65	2.37-5.63	<0.0001*	-		
SN tumor burden									
Negative	Ref			-			Ref		
<0.1mm	1.04	0.33-3.29	0.954	-			0.96	0.30-3.11	0.942
0.1-1.0mm	5.94	3.72-9.49	<0.0001*	-			4.67	2.81-7.79	<0.0001*
>1.0mm	8.09	5.28-12.4	<0.0001*	-			3.89	2.78-8.46	<0.0001*
Dir. LND/ missing	12.1	7.74-19.0	<0.0001*	-			4.25	2.08-8.65	<0.0001*
US-FNAC									
Both neg.	Ref			Ref			Ref		
US susp & FNAC neg	1.45	0.93-2.26	0.100	1.13	0.71-1.78	0.617	1.20	0.76-1.90	0.426
US malig/ FNAC pos	7.56	5.31-10.8	<0.0001*	1.80	1.10-2.96	0.019*	1.52	0.87-2.65	0.144
Gender									
Female	Ref			Ref			Ref		
Male	1.39	0.99-1.93	0.053	1.48	1.04-2.11	0.029*	1.45	1.02-2.08	0.041*
Age									
Cont.	1.01	0.99-1.02	0.136	1.01	1.00-1.02	0.045*	1.01	0.99-1.02	0.064
Location									
Extremity	Ref			Ref			Ref		
Trunk	0.95	0.67-1.35	0.782	1.12	0.76-1.64	0.567	1.10	0.74-1.62	0.641
Head & neck	1.72	1.08-2.75	0.022*	2.33	1.43-3.79	0.001*	2.27	1.38-3.74	0.001*
Breslow									
Cont.	1.11	1.10-1.13	<0.0001*	1.06	1.00-1.08	<0.0001*	1.06	1.03-1.09	<0.0001*
Ulceration									
Absent	Ref			Ref			Ref		
Present	3.19	2.32-4.39	<0.0001*	1.53	1.07-2.19	0.019*	1.51	1.05-2.17	0.026*
Histology									
SSM-LMM	Ref						Ref		
NM/ALM	2.88	2.08-4.00	<0.0001*	1.47	1.01-2.12	0.042*	1.53	1.06-2.22	0.024*
Unknown	1.14	0.54-2.37	0.735	1.07	0.51-2.24	0.859	1.04	0.49-2.17	0.928

Multivariable model 1 was adjusted for: gender, age, primary tumor location, Breslow thickness, ulceration status, histologic subtype, SN status (including 43 patients with direct lymph node dissection), and US-FNAC result. Multivariable model 2 was adjusted for: gender, age, primary tumor location, Breslow thickness, ulceration status, histologic subtype, SN tumor burden and US-FNAC result. A p-value of <0.05 was considered statistically significant (marked with an *).Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; SN, sentinel node; Ref, reference; Cont., continuous; SSM, superficial spreading melanoma; LMM, lentigo maligna melanoma; NM, nodular melanoma; ALM, acrolentiginous melanoma; US-FNAC, ultrasound-fine needle aspiration cytology result; neg., negative; susp, suspicious; malig, malignant; pos, positive. ** Positive SN patients include 43 patients undergoing direct lymph node dissection after positive US-FNAC.

Discussion

This study is the largest to date reporting on the value of preoperative assessment of the SN with US morphologic criteria in combination with FNAC in melanoma patients. We present the long term follow up results of this matured cohort of 1,006 US-FNAC examinations in 1,000 patients described previously by Voit et al. [27].

Crude 5-year estimated MSS was significantly worse for patients with suspicious US (PP or wandering of the central echo to the rim) and for patients with malignant US (BS or LCE) as well as for the combined result of a positive US and/or FNAC **(figure 1 and 3).**

The unadjusted HR for a suspicious US in absence of a positive FNAC was slightly higher than the reference value of a normal US (and negative FNAC), although not significant. The unadjusted HR for a malignant US and or a positive FNAC was significantly higher compared to patients with a normal US **(table 3).** In model 1, adjusted HR for positive US-FNAC remained as prognostic indicator with a HR of 1.80 (p=0.019), while in model 2, where a more detailed classification of SN tumor burden according to the Rotterdam criteria was applied, positive US-FNAC was not a significant prognostic indicator, despite the still slightly elevated HR of 1.52 (p=0.144).

Voit et al. found that US-FNAC outcome was clearly correlated with SN tumor burden; preoperative US-FNAC correctly identified 61% of SNs with a tumor burden of >1.0mm as malignant, and up to 91% of the patients who proceeded directly to LND was correctly identified as SN-positive [27]. This can explain why a positive US-FNAC result is a relevant prognostic indicator for MSS after adjustment for SN status and other prognostic indicators in model 1, and not in model 2 where SN tumor burden already is a covariate.

Routine US-FNAC in breast cancer patients has shown to upstage a significant amount of patients preoperatively, sparing them an unnecessary SNB in up to 18% [15]. The fact that US-FNAC results remain as prognostic indicator after a median follow-up of 5 years in this large cohort emphasizes the potential to incorporate the Berlin US morphology criteria combined with FNAC as was done with the Rotterdam criteria in staging of melanoma patients in this paradigm shifting era with upcoming systemic therapies for melanoma Especially in light of current adjuvant therapy (trials), in a field which lacked effective systemic therapy until 2010, the need for early and easy staging is desired by patients and physicians. As described previously by Voit et al[26, 32], this might be a cost-effective baseline staging for pT3-4 melanomas and/or primary ulcerated melanomas [33].

Limitations

PP and beginning LCE were no prognostic indicators, nonetheless these are helpful signs in selecting which patients should undergo FNAC as well in order to further differentiate between a negative or positive US-FNAC result. As was described previously, sensitivity of combined US-FNAC was significantly higher than in other studies, possibly due to the

fact that the threshold to perform FNAC because of a suspicious US Berlin criterion was lower than in other performed studies [27].

The correlation between US-FNAC and SN tumor burden may color survival outcome of US-FNAC status, causing significant differences in survival which may be more based on SN tumor burden than on US-FNAC status. Potentially US-FNAC can best be seen as an indicator of high SN tumor burden. Ultimately all patients undergoing US-FNAC will undergo a SNB or direct LND in case of positive US-FNAC, thus no potential nodal involvement will be missed. Histology of SN or dissected lymph nodes will still be used for pathological staging; but patients can skip and be spared a potentially unnecessary SNB in case of positive US-FNAC.

As all US-FNAC negative patients underwent a SNB, no answer can be given on whether these patients would have developed nodal basin failure over time if no SNB was performed, not taking into account the patients that turned out to be false negative after SNB. The 29% false negative rate and regional nodal recurrence rate in US-FNAC and SN negative patients is comparable to other reports [7, 34], which is reassuring.

One of the limitations of this study is that all US-FNACs were performed by a select group of 3 dedicated ultrasonographists, of whom one performed the first 400 alone. The reproducibility of US-FNAC results by another study team has yet to be investigated. Efforts have been made to educate others in recognizing and utilizing the Berlin morphology criteria for targeted US-FNAC of the SN by organizing EORTC Melanoma Group sentinel node ultrasound courses since 2012. More recently the GULF trial, a prospective multicenter study has started (Dutch trial registry number NTR5193, www.trialregister.nl). In this feasibility study 120 patients eligible for SNB (melanoma and breast cancer patients) will undergo gamma-probe and US guided FNAC of the SN prior to surgical removal of the SN, with sensitivity of the gamma-probe guided US-FNAC as main objective. Additionally, US images of all SNs will be classified according to the Berlin criteria.

Despite this drawback, US-FNAC of the sentinel node has proven to be accurate and sensitive in detecting patients with possible lymph node involvement prior to surgery, and has the potential to become a part of standard preoperative diagnostic work up like in breast cancer.

Conclusions

The long-term results of this study support the step-wise approach to melanoma patients. In case of positive FNAC and/or clearly malignant US (BS and/or LCE) they can be spared a SNB. In case of PP and negative FNAC, patients could be offered continue US surveillance or SNB for higher risk primary tumors. Completely US-FNAC negative patients might only require follow-up and no SN staging, with continue US surveillance as addendum for high risk T3/4 and/or ulcerated primaries.

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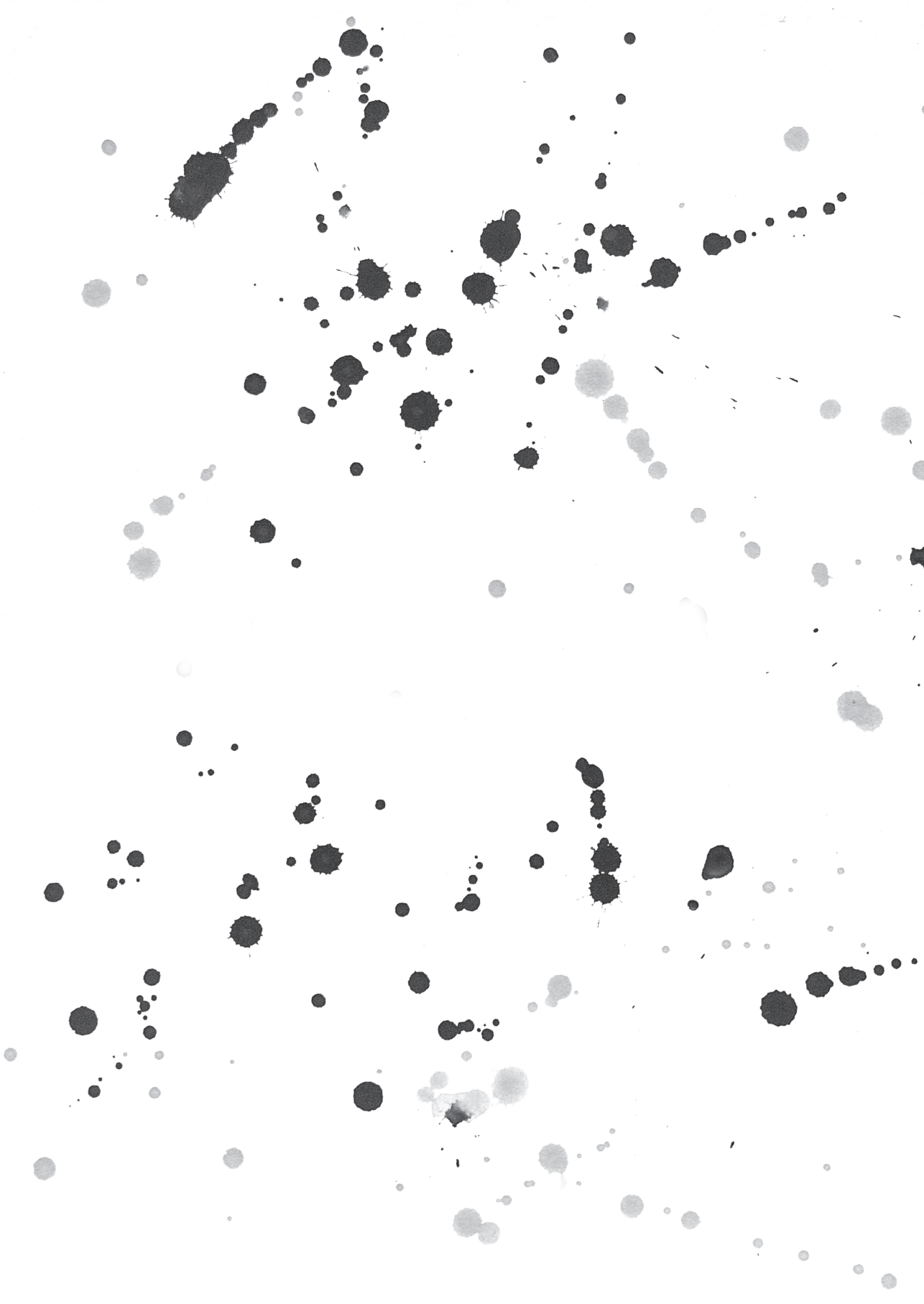

Chapter 5

Gamma Probe and Ultrasound Guided Fine Needle Aspiration Cytology of the Sentinel Node (GULF) Trial - Overview of the Literature, Pilot and Study Protocol

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Abstract

Background Sentinel node (SN) biopsy (SNB) detects clinically occult metastases of breast cancer and melanoma in 20-30%. Wound infections, seroma and lymph edema occur in up to 10%. Targeted ultrasound (US) of the SN, (with fine needle aspiration cytology (FNAC) if appropriate) has been investigated as a minimally invasive alternative, but reported sensitivity rates are too low to replace SNB. Our hypothesis is that the use of a handheld gamma probe concomitant with US may improve sensitivity.

Aim To provide an overview of the current literature on preoperative nodal staging of clinical N0 melanoma patients, report on a pilot, and present a study protocol for a minimally invasive alternative to the SNB: **G**amma probe and **UI**trasound guided **F**ine needle aspiration cytology of the sentinel node (GULF trial).

Methods The GULF trial is a multicenter open single arm observational trial. Newly diagnosed cT1b-4N0M0 cutaneous melanoma or cT1-3N0M0 breast cancer patients, aged >18 years, presenting for SNB are eligible. 120 patients will be included for preoperative targeted gamma probe guided US and FNAC of the SN. Afterwards all patients proceed to surgical SNB. Primary endpoint is the sensitivity of FNAC. Secondary endpoints include SN identification rate and the histopathological compatibility of Core Needle Biopsy and FNAC vs. SNB. Secondary endpoints were investigated in a pilot with 10 FNACs and marker placements, and 10 FNACs combined with Core Needle Biopsy.

Results A pilot in 20 patients showed that SN identification rate was 90%, supporting the feasibility of this technique.

Discussion There is broad experience with US (in combination with FNAC) prior to SNB, but sensitivity and specificity are too low to completely abandon SNB. Promising alternative techniques potentially will replace SNB in the future but more evidence is needed in the form of prospective studies. Accurate identification of the SN for US-FNAC has been proven feasible in our pilot. When adequate sensitivity can be reached, US-FNAC provides a minimally invasive alternative for the surgical SNB procedure.

Trial registration The GULF trial is registered in the Netherlands Trial Registry (NTR), ID: NTR5193. May 1st 2015.

Background

Sentinel node biopsy

With the introduction of sentinel node (SN) biopsy (SNB) as a less invasive alternative to elective lymph node dissection for melanoma and breast cancer with clinically negative lymph nodes, this has become the gold standard for adequate staging. Although less invasive than an elective lymph node dissection, SNB is still associated with some potential morbidity. Morbidity occurs in up to 10% of patients; wound infections and seroma are the most frequently seen complications[1, 2]. Rarely lymph edema is seen after SNB. Around 70-80% of SNB's are tumor negative after histological assessment, these patients cannot benefit from the SNB procedure. In that light the morbidity of a surgical SNB procedure is deemed considerable, and any less invasive procedure, if accurate enough, would be preferred.

The detection rate of submicrometastases has increased considerably in the past decades; adaptation of the melanoma and breast cancer SN sectioning protocols and use of standard immunohistochemistry staining enabled pathologists to detect even the smallest tumor deposits accurately[3-8]. This has clear clinical implications; more patients are diagnosed as SN positive and will be offered a completion lymphadenectomy (CLND)[6, 8]. It is questionable whether this morbid surgical procedure is justified in cases with minimal SN tumor burden[9], as several retrospective melanoma studies and recently the prospective DeCOG study have shown that survival for this group of melanoma patients is similar to SN negative patients[10-13]. In breast cancer, presence of isolated tumor cells (≤0.2mm) or micrometastases (>0.2≤2.0mm) is associated with a slightly worse prognosis[7, 14], but its clinical relevance is debated as well[15-17], and CLND is omitted in certain groups of patients with a positive SN[18].

Prospective studies currently investigating the therapeutic value of CLND in melanoma are the EORTC-1208MG (Minitub)[9], including patients with minimal SN tumor burden only, and the MSLT2, which included all SN positive patients[19]. Parallel to this, certain adjuvant therapy trials (EORTC 18071, EORTC 1325, Combi-AD) recruit stage IIIA patients only in case of ≥1mm SN tumor burden[20-22].

Primary results from the EORTC 18071 show that SN positive patients benefit the most from adjuvant treatment measured as recurrence free survival at 3 years[21, 22]. In this light, it remains worthwhile to keep selecting patients for adjuvant therapy in trial setting and/or CLND based on nodal staging, and a cut-off for detection of (sub)micrometastases (<1.0mm) may aid in prevention of overtreatment in low risk patients.

Ultrasound guided Fine Needle Aspiration Cytology

Ultrasound (US) guided fine needle aspiration cytology (FNAC) or core needle biopsy (CNB) may provide a good minimally invasive alternative to SNB. In breast cancer pa-

tients screening US of the regional lymph node basin is part of the preoperative staging process; this way up to 17% of patients undergo axillary lymph node dissection immediately and are spared a SNB[23, 24]. Melanoma patients do not routinely undergo a preoperative US of the regional lymph node basin, due to previously reported poor identification rates of occult lymph node metastases with US and FNAC[25, 26]. Several studies have been conducted in order to analyze if US (with FNAC or CNB) can replace SNB, but thus far reported sensitivity rates for US vary considerably, ranging between 9-94%[27, 28].

For the current study we will focus on melanoma, as the therapeutic value of both SNB and CLND are debated, and alternatives for SNB are more limited for this type of cancer.

In our search for a reliable and accurate minimally invasive alternative to SNB for staging of clinical N0 melanoma patients, we examined the current available literature and performed a systematic search of all major databases to explore whether other methods than US guided FNAC may have proven adequate alternatives to SNB.

Literature Overview

All relevant studies on US imaging of regional lymph nodes in melanoma patients scheduled for SNB are displayed in **Table 1** (search details are given in **supplementary file 1**). Some of the studies mentioned in **Table 1** are overlapping; the studies from Voit et al[29-33] concern the same database with more inclusions over time. In the studies that performed US prior to lymphoscintigraphy, sensitivity rates were low, ranging from 4.7% to 39%, and specificity rates were high, ranging from 86% to 100%. Two studies did not mention the exact timing of US in relation to lymphoscintigraphy; Hocevar et al. reached a sensitivity and specificity of 71% and 84%, and Testori et al. reached a sensitivity of 94% and 90%.

In the studies that performed a targeted US (i.e. US of the marked "SN" area on the skin after lymphoscintigraphy), sensitivity ranged from 22% to 100%, and specificity ranged from 62% to 100% **(table 1).**

Besides US and targeted US with FNAC prior to SNB, several groups have focused on development of new imaging techniques for examination of the SN/lymph nodes and detection of SN tumor deposits, such as sonoelastography[34, 35], contrast enhanced US[36], and multispectral optoacoustic tomography (MSOT)[37] (**Table 2)**. Sonoelastography measures tissue consistency; which can be visualized on top of US images using different color shades; red indicating soft tissue, and blue indicating rigid tissue[34, 35]. As metastases tend to be more solid than normal lymph node tissue regions of interest for FNAC can be identified. For contrast enhanced US an intravenous contrast agent is applied to detect possible areas of hyperperfusion or hypoperfusion; indicating potential metastatic lesions[36]. These techniques reached a high sensitivity for identification of SN metastases (**Table 2**).

Two recently developed techniques for improved SN identification peri-operatively are SPECT-US[38, 39], and near infrared light fluorescence imaging[40-44]. SPECT-US displays the location of a radio-active SN in the US images; making it easier for the surgeon to locate SN's in anatomically challenging area's such as the cervical and occipital area; or to guide radiologists for FNAC[38, 39]. Near infrared light fluorescence imaging is conducted with Indocyanine green as tracer, which can be combined with 99Tc nano-colloids to form a hybrid tracer[41]. Intraoperative identification is similarly accurate to 99Tc-colloid; and particularly helpful for SN localization in the cervical and occipital area, where overprojection from the 99TC-colloid injection site is a common obstacle. Preoperative (transcutaneous) SN identification has reached lower identification rates, due to the limited penetration depth of the fluorescent tracer[45].

Summarizing, few US imaging studies have used a method to accurately identify the SN prior to US examination and FNAC. This may have contributed to lower than expected sensitivity rates for detection of SN metastases in studies where this was not applied. It explains why to date no alternative method for SN staging has been adopted in daily clinical practice, and the need for such a method remains.

Rationale for a new trial

SN identification

To overcome the problem of suboptimal identification of the SN, we hypothesize that use of a handheld gamma probe (Geiger teller) to detect the SN post lymphoscintigraphy may further aid the radiologist in accurately identifying the SN for ultrasound guided FNAC. Several pilot studies have been performed using this technique in breast cancer patients; correct localization of the SN occurred in 75% - 100%[46-49]. This formed the rationale for the GULF Trial (**G**amma probe and **Ul**trasound guided **F**ine needle aspiration cytology of the sentinel node).

Cytology or Histology?

In order to reach the sample size needed for proof of concept with accurate power and within an acceptable term, both melanoma patients and breast cancer patients will be included in the GULF trial. The SN procedure is uniformly applied for both melanoma and breast cancer, and breast cancer patients may equally benefit from a minimally invasive alternative for the SN. All patients will undergo FNAC. Since metastatic size may have clinical implications for breast cancer patients[18], a subset of 10 breast cancer patients will undergo CNB additionally after FNAC. This allows for a comparison of results between CNB, FNAC and SNB.

Table 1. Studies of Ultrasound Imaging of Regional Lymph Nodes in Melanoma Patients Scheduled for Sentinel Node Biopsy

Author, year	Study Design	N	US Setting	FNAC/other technique	Sens (%)	Spec (%)	PPV (%)	NPV (%)
Rossi[61], 2000	Not mentioned	69	Pre-lympho	No	33	100	100	86
Rossi[25], 2003	Prospective, monocentric	125	Pre-lympho	FNAC if US suspicious	US alone:Not mentioned	-	-	-
					US-FNAC: 39	100	100	85
Hocevar[62], 2004	Prospective, monocentric	57	unknown	FNAC if US suspicious	US alone:71	84	59	90
					US-FNAC:Not mentioned	-	-	-
Testori[63], 2005	Retrospective, monocentric	88	unknown	No	94	90	64	99
Starritt[64], 2005	Prospective, monocentric	31 all SN +	Post-lympho	No	NA	NA	NA	NA
Voit[65], 2006	Prospective, monocentric	127	Post-lympho	FNAC if US suspicious	US alone: 79	72	53	90
					FNAC alone:59	100	100	85
					US-FNAC:82	72	54	91
Van Rijk[26], 2006	Retrospective, monocentric	107	Pre-lympho	FNAC if US suspicious	US alone: 34	87	-	-
					US-FNAC: 4.7	100	-	-
Sibon[66], 2007	Prospective, monocentric	131	Pre-lympho	No	9	96	43	-
Kunte[67], 2009	Prospective, monocentric	25	Pre- and post-lympho	No	33	100	100	88
Voit[30], 2009	Prospective, monocentric	400	Post-lympho	FNAC if US suspicious	65	99	93	92
Sanki[60], 2009	Prospective, monocentric	716	Post-lympho	No	33	97	60	88
De Giorgi[36], 2010	Prospective monocentric	15	Post-lympho	Standard CEUS	CEUS: 100	62	55	100
Voit[31], 2010	Prospective monocentric	400	Post lympho	FNAC if US suspicious	All Berlin criteria combined: 82	80	52	94
Hinz[58], 2011	Prospective monocentric	81	Pre and post lympho	No	22	100	100	96
Chai[68], 2012	Retrospective monocentric	325	Pre-lympho	FNAC if US suspicious	34	86	37	84
Marone[69], 2012	Prospective monocentric	623	Pre-lympho	No	15	100	100	87
Pilko[70], 2012	Retrospective Monocentric	405	Pre-lympho	FNAC if US suspicious	Not mentioned	-	-	-
Stoffels[71], 2012	Retrospective Monocentric	221	Pre-lympho	FNAC if US suspicious	14	97	100	97
Hinz[72], 2013	Retrospective Monocentric	20	Pre-lympho &, pre PET-CT	No If US malig. Direct LND	12	100	100	74

Table 1. Studies of Ultrasound Imaging of Regional Lymph Nodes in Melanoma Patients Scheduled for Sentinel Node Biopsy (continued)

Author, year	Study Design	N	US Setting	FNAC/other technique	Sens (%)	Spec (%)	PPV (%)	NPV (%)
Ulrich[73], 2014 *In German*	Prospective monocentric	800	Post lympho	FNAC if US suspicious	US-FNAC: 56	99	92	89
Voit[32], 2014	Prospective monocentric	1000	Post lympho	FNAC if US suspicious	US alone: 71	-	-	-
					US-FNAC: 51	99	99	89
Voit[33], 2016	Prospective monocentric	1000	Post lympho	FNAC if US suspicious	US alone: 71	-	-	-
					US-FNAC: 51	99	99	89

Overview of studies reporting on ultrasound imaging of regional lymph nodes in melanoma patients prior to sentinel node biopsy. Abbreviations: US, ultrasound; FNAC, fine needle aspiration cytology; Sens, sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value; lympho, lymphoscintigraphy; NA, not applicable.

Table 2. Pilot studies on novel techniques for pre-operative non-invasive detection of melanoma metastases in lymph nodes.

Author, yr	Topic	No. of patients	Technique	Sens (%)	Spec (%)
Hinz[72], 2013	Elastography	36	US + power Doppler:	81	76
			Elastography:	91	76
			Combined:	95	76
Ogata[35], 2014	Elastography	12	US:	77	57
			Elastography:	100	71
Stoffels[37], 2015	MSOT and indocyanin green	20	MSOT	100	48.6

Overview of pilot studies investigating non-invasive detection of melanoma lymph node metastases. Abbreviations: yr, year; Sens, sensitivity; Spec, specificity; US, ultrasound; MSOT, multispectral optoacoustic tomography.

Hypotheses GULF trial

We hypothesize that a sensitivity of 90% with a 95% confidence interval of 80% - 100% is achievable. Secondly, we expect that a SN identification rate of more than 75% is feasible.

Study aims

- To present a study protocol for a minimally invasive alternative to the sentinel node biopsy (GULF trial), with as primary objective to determine whether an acceptable sensitivity for US and gamma probe guided FNAC can be achieved.
- Secondary objective is 1) the identification rate of the SN and 2) the histological results of CNB versus FNAC and versus SNB.

Prior to starting the GULF-trial, we had to prove the concept of adequate identification of the SN. A pilot study focusing on the adequate detection rate of the SN was conducted.

Pilot

After approval of the Ethical Review Board a pilot was performed in 20 patients presenting at the Erasmus MC Cancer Institute. All patients underwent gamma probe guided US-FNAC after written informed consent. In the 10 first melanoma patients additional metallic marker placement (O-Twist-Marker, BIP) was performed after local infiltration of the skin and surrounding tissue with 1-10mL lidocaine 2%. Correct identification of the SN was assessed by examining the excised SN(s) on presence of the marker. Separately, in the first 10 breast cancer patients CNB was performed after FNAC with a 14G needle, after local infiltration similar to marker placement. CNB was done for assessment of concordance with FNAC results and to detect potential superiority of either technique.

All patients proceeded to OR for SNB, which was performed according to the triple technique: preoperative 99Tc lymphoscintigraphy <24h prior to surgery, intradermal

injection of patent blue near the primary tumor site prior to first incision, and peroperative use of a handheld gamma-probe to locate SN(s)[50, 51], Lymph nodes were considered SN when radioactive and/or blue. A marker was retrieved from the SN in 9 out of 10 patients; which meant the SN identification rate was 90%. CNB samples were investigated on presence of lymphoid tissue. This was present in 6 out of 10 patients. 40% of CNBs was not representative. In comparison: FNAC color staining was representative in 19 out of 20 patients (95%), and FNAC immunohistochemistry staining was representative in 14 out of 20 patients (70%).

During the pilot study no safety issues occurred. In the second enrolled study patient none of the 2 placed markers were found at histopathological examination of the SN and in another patient only 1 of 2 placed markers was found. A detailed shoulder X-ray confirmed the markers were still in situ in both patients. In the latter patient the X-ray images were suggestive of marker displacement towards mamma tissue; this was probably due to intraoperative displacement of the marker during SN removal.

Considering the positive results from this pilot, the study will be continued with an expansion of the pilot population in order to reach a sufficient sample size according to the presented study protocol.

GULF Design

Patients with a newly diagnosed cT1b-4N0M0 cutaneous melanoma or cT1-3N0M0 breast cancer presenting at the outpatient clinic of the Erasmus MC Cancer Institute, and the Netherlands Cancer Institute – Antoni van Leeuwenhoek (only melanomas) will be assessed for inclusion. All patients will undergo US and gamma probe guided FNAC of the SN. The pilot patients received additional marker placement (n=10) for identification purposes, or additional CNB (n=10) for assessment of potential benefit of CNB (i.e. histology and size measurement possible) (**Figure 1**).

Study population

Inclusion criteria

Age ≥ 18 years, new diagnosis of cT1b-4N0M0 cutaneous melanoma or cT1-3N0M0 breast cancer.

Prior to start of any study related procedure, written informed consent must be given according to ICH/GCP and national legislation.

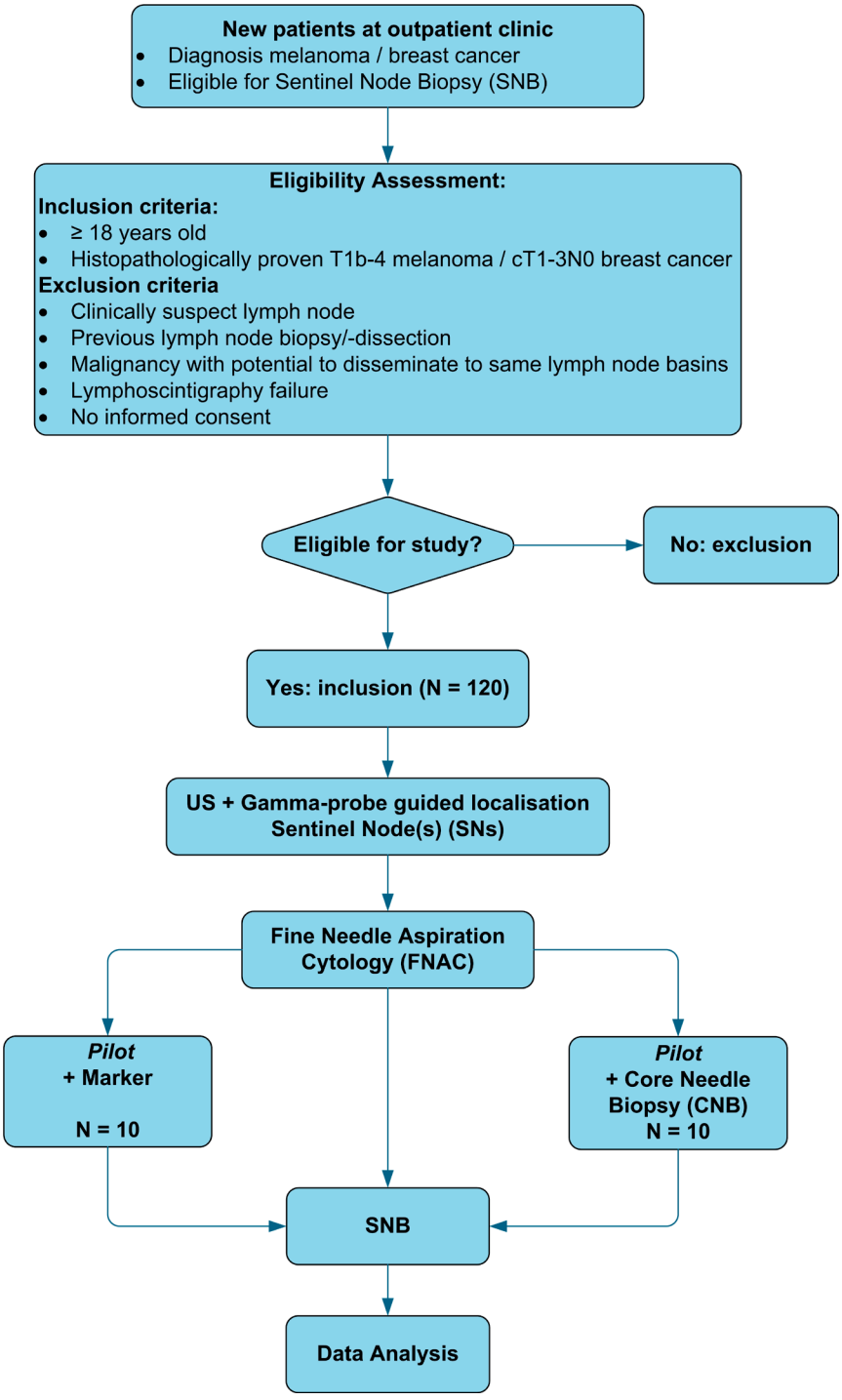

Figure 1. Study Flowchart GULF Trial

Exclusion criteria

Clinically suspect lymph node, other known malignancy with potential to disseminate to axillary or groin lymph node basins, prior lymph node biopsy, no SN visible at lymphoscintigraphy / not identifiable with gamma probe.

Study procedures

US-FNAC

All patients will be admitted to the surgical ward on the day of surgery. First, a lymphoscintigram <24 hours prior to SNB will be performed, as is standard procedure. Following successful lymphoscintigraphy (i.e. SN(s) is/are visible) the study procedures can start. A dedicated radiologist will perform US imaging of the lymph node basin where a SN or multiple SNs was/were identified by lymphoscintigraphy. The exact location of the SN(s) will be determined using a handheld gamma probe, and by combination with US; the assumed SN(s) will be visualized (being a visible lymph node at the center of the hotspot found with the gamma probe). FNAC will be performed of all visualized assumed SN(s). In case of multiple SNs in one lymph node basin or multiple draining lymph node basins with an SN in every basin (for instance a melanoma on the back draining to both axilla and groin), FNAC will be performed of all lymph nodes pointed out as primary tier SN by the nuclear medicine specialist. For FNAC 1-4 cortical samples will be taken. Whenever additional clearly suspect lymph nodes are visible, the radiologist will perform FNAC from these nodes as well, as is standard of care. All samples will be transported to the pathology lab for analysis. US findings will be recorded according to the Berlin morphologic Criteria to create uniformity in recording per center[30]. After FNAC, all patients will proceed to the operating room for SNB according to standard procedure (as described in the Pilot section). Lymph nodes were considered SN when radioactive and/or blue. No diagnostic procedure or treatment is postponed or elongated. No additional visits to the outpatient clinic are required.

Endpoints:

Primary endpoint: Primary outcome is the sensitivity of gamma probe and US guided CNB or FNAC.

Secondary endpoints: Secondary outcome is 1) the identification rate of the SN 2) the histological results of CNB versus FNAC and versus SNB.

Ad 1) an identification rate of at least 75% is deemed acceptable (concordant with literature). This has been proven feasible in the pilot study.

Statistical considerations

Sample size and accrual

Based on retrospective data, the prevalence of metastatic SNs is expected to be 30%. Our gold standard is the histological outcome of SNB (absence or presence and size of metastases in the SN). Submicrometastases (i.e. <0.1 mm at any site or 0.4mm subcapsular) in melanoma patients, and isolated tumor cells (i.e. ≤0.2mm) in breast cancer patients will be considered negative: a negative FNAC is accepted in these cases. Based on previous reports, we expect to find around 10% of these submicrometastases and isolated tumor cells in both melanoma and breast cancer patients [52, 53]. Considering this, the maximum achievable sensitivity of FNAC will be 90%. For this sensitivity, and a 95% confidence interval of 80-100% (With a two-sided significance level $\alpha = 0.05$ and power $1 - \beta = 0.8$), the required sample size is 116 considering a 30% prevalence of metastatic SNs. Around 3% of patients are expected to have a negative lymphoscintigram: the sample size will be increased to 120 patients. With an average accrual rate of 60 patients per year, maximum accrual will be met at 2 years post start of study.

Statistical analysis plan

The main analysis addressing the primary endpoint will be performed after inclusion of all 120 patients. No interim analysis is planned for this endpoint.

Ethical considerations

This study has been approved by the Erasmus MC medical-ethical committee. The study will be conducted according to the principles of the Declaration of Helsinki and in accordance with national and regional legislation, guidelines, regulations and acts.

Discussion

Currently SNB is the most important staging procedure for clinically N0 melanoma patients, especially in the light of trial participation for adjuvant therapies based on N-status [20, 21, 54]. The therapeutic role of SNB for melanoma is still under debate [55-57]. Considering the fact that this is a surgical staging procedure associated with complications in up to 10% of patients, our group sought to investigate a more minimally invasive alternative.

The ongoing improvement of imaging techniques (i.e. more accurate and detailed US imaging) and increased experience with FNAC renders combined US-FNAC as a high potential minimally invasive alternative for surgical SNB [31, 32]. Correct transcutaneous identification of the SN forms the main obstacle for broad application of this technique as this is key in obtaining reliable FNAC.

The current study aims to give an overview of the current melanoma literature, report a pilot and present a study protocol for a minimally invasive technique to investigate the SN using gamma probe guided US-FNAC.

Overview of the Literature

The studies presented provide evidence that it is difficult to detect clinically occult lymph node metastases in melanoma patients, and although some studies have achieved high sensitivity and specificity rates, these results have not been reproduced by other groups. There are many differences between the reported studies; namely retrospective vs. prospective study setting; US prior to lymphoscintigraphy vs. targeted US after lymphoscintigraphy; the number of persons performing US and their expertise; variation in US morphology criteria used to discriminate between benign and suspicious or malignant lymph nodes; and use of FNAC or not. Al these factors will have contributed to the outcome of these studies. It is interesting to see that sensitivity rates are low in the studies that performed an US of the entire lymph node basin without knowing the location of the SN(s), but that even in the studies were targeted US of the SN area was applied, sensitivity rates could be as low as 22%[58] and as high as 82%[31] or even a perfect 100%[36] as well. Thompson et al. proposed a possible explanation for these disparate results; many of the micrometastases present in SNs are too small to be detected by the US-equipment used[59, 60]. However, Voit et al. demonstrated that it was possible to successfully perform a FNAC in a lesion as small as 0.4 mm. Nevertheless, most smaller SN metastases will be overlooked by US and/or missed by FNAC. The question is if this has any clinical implications.

As long as US-imaging is limited by a detection limit, and alternative imaging techniques are tested in pilot settings, the need for a reliable, minimal invasive easy to perform and replicate method to assess SN status remains. Hence the presentation of the GULF trial study protocol here.

Pilot

Our pilot results show that correct identification of the SN for FNAC was possible in 90%, and that the sampled material was representative in 95% of FNAC samples. CNB was representative in only 60%. This confirms that the described technique for targeted US-FNAC of the SN is feasible. CNB will not be added to the study procedure considering the low rate of representative tissue in the pilot phase.

If an acceptable sensitivity can be achieved for FNAC, patients can proceed to undergo radical lymph node dissection immediately in case of positive FNAC, bypassing the SNB procedure. When the FNAC sample is negative, surgeons can choose to perform a SNB or continue with only surgical excision of the primary tumor and monitoring of potential lymph node involvement at follow up visits. This way up to 80% of patients

eligible for SNB can be spared this invasive procedure and the risk of morbidity related to this procedure. Furthermore, for melanoma patients this would mean that general anesthesia is no longer needed, as WLE can be performed under local anesthesia. Ultimately operative nodal staging may become completely obsolete.

Conclusions

The literature on pre-operative assessment of regional lymph nodes with US in clinically N0 melanoma patients is disparate. Targeted US of the SN area in combination with FNAC or other new techniques has potential to become a minimally invasive alternative for the SNB, however, findings need to be replicated in prospective clinical trials first. A pilot with gamma probe guided US-FNAC show that accurate SN identification in up to 90% of patients is feasible. Our group presents a study protocol of the *Gamma probe and ULtrasound guided Fine needle aspiration cytology of the sentinel node Trial* (GULF trial) as a potential improvement to the reported US-FNAC techniques and ultimately even a possible replacement of the SNB.

Supplementary file 1. Literature Search 22 April 2016
Subject: Melanoma echography lymph nodes

Database	Number of studies	Number of unique studies
Embase.com	347	340
Medline Ovid	213	28
Web of science	286	122
Cochrane	5	0
PubMed publisher	5	4
Google scholar	100	69
Total	**956**	**563**

Embase.com n=347

('melanoma'/exp OR (melanom*):ab,ti) AND ('lymph node'/exp OR 'lymph node biopsy'/exp OR 'lymph node metastasis'/exp OR (lymph-node* OR sentinel-node* OR SLNB OR SLN OR SLNs OR SN):ab,ti) AND ('echography'/exp OR ultrasound/de OR (echogra* OR ultraso* OR (us NEXT/1 (guid* OR examin* OR imag*)) OR preoperative-US OR sonogra* OR optoacoust*):ab,ti) AND ('diagnostic accuracy'/exp OR 'diagnostic test accuracy study'/de OR 'diagnostic error'/exp OR 'predictive value'/de OR 'diagnostic value'/de OR 'sensitivity and specificity'/exp OR 'early diagnosis'/de OR 'reproducibility'/de OR 'observer variation'/de OR (((diagnos* OR ultrasound OR us) NEAR/6 (accurac* OR error* OR metast* OR abilit* OR value OR improv* OR mis OR missed)) OR ((Micrometasta* OR metasta* OR sentin* OR sn OR sln) NEAR/6 (identif* OR detect*)) OR (false NEXT/1 (positive* OR negative*)) OR predictive-value* OR npv OR ppv OR sensitiv* OR specific* OR (early NEAR/3 (diagnos* OR detect*)) OR reproducib* OR Misdiagnos* OR ((observer* OR interobserver* OR intraobserver*) NEAR/3 (varia* OR bias))):ab,ti) NOT ([Conference Abstract]/lim OR [Letter]/lim OR [Note]/lim OR [Editorial]/lim)

Medline Ovid n=213

("melanoma"/ OR (melanom*).ab,ti.) AND ("Lymph Nodes"/ OR "Sentinel Lymph Node Biopsy"/ OR (lymph-node* OR sentinel-node* OR SLNB OR SLN OR SLNs OR SN).ab,ti.) AND (exp "Ultrasonography"/ OR "Ultrasonography".xs. OR Ultrasonics/ OR (echogra* OR ultraso* OR (us ADJ (guid* OR examin* OR imag*)) OR preoperative-US OR sonogra* OR optoacoust*).ab,ti.) AND (exp "Diagnostic Errors"/ OR "diagnostic value"/ OR exp "sensitivity and specificity"/ OR exp "early diagnosis"/ OR "Reproducibility of Results"/ OR (((diagnos* OR ultrasound OR us) ADJ6 (accurac* OR error* OR metast* OR abilit* OR value OR improv*)) OR ((Micrometasta* OR metasta* OR sentin* OR sn OR sln) ADJ6 (identif* OR detect*)) OR (false ADJ (positive* OR negative*)) OR predictive-value* OR npv OR ppv OR sensitiv* OR specific* OR (early ADJ3 (diagnos* OR detect*)) OR reproducib* OR Mis-

diagnos* OR ((observer* OR interobserver* OR intraobserver*) ADJ3 (varia* OR bias))).ab,ti.) NOT (letter OR news OR comment OR editorial OR congresses OR abstracts).pt.

Cochrane n=5

((melanom*):ab,ti) AND ((lymph-node* OR sentinel-node* OR SLNB OR SLN OR SLNs OR SN):ab,ti) AND ((echogra* OR ultraso* OR (us NEXT/1 (guid* OR examin* OR imag*)) OR preoperative-US OR sonogra* OR optoacoust*):ab,ti) AND ((((diagnos* OR ultrasound OR us) NEAR/6 (accurac* OR error* OR metast* OR abilit* OR value OR improv* OR mis OR missed)) OR ((Micrometasta* OR metasta* OR sentin* OR sn OR sln) NEAR/6 (identif* OR detect*)) OR (false NEXT/1 (positive* OR negative*)) OR predictive-value* OR npv OR ppv OR sensitiv* OR specific* OR (early NEAR/3 (diagnos* OR detect*)) OR reproducib* OR Misdiagnos* OR ((observer* OR interobserver* OR intraobserver*) NEAR/3 (varia* OR bias))):ab,ti)

Web of science n=286

TS=(((melanom*)) AND ((lymph-node* OR sentinel-node* OR SLNB OR SLN OR SLNs OR SN)) AND ((echogra* OR ultraso* OR (us NEAR/1 (guid* OR examin* OR imag*)) OR preoperative-US OR sonogra* OR optoacoust*)) AND ((((diagnos* OR ultrasound OR us) NEAR/5 (accurac* OR error* OR metast* OR abilit* OR value OR improv* OR mis OR missed)) OR ((Micrometasta* OR metasta* OR sentin* OR sn OR sln) NEAR/5 (identif* OR detect*)) OR (false NEAR/1 (positive* OR negative*)) OR predictive-value* OR npv OR ppv OR sensitiv* OR specific* OR (early NEAR/2 (diagnos* OR detect*)) OR reproducib* OR Misdiagnos* OR ((observer* OR interobserver* OR intraobserver*) NEAR/2 (varia* OR bias)))))

PubMed publisher n=5

("melanoma"[mh] OR (melanom*[tiab])) AND ("Lymph Nodes"[mh] OR "Sentinel Lymph Node Biopsy"[mh] OR (lymph-node*[tiab] OR sentinel-node*[tiab] OR SLNB OR SLN OR SLNs OR SN)) AND ("Ultrasonography"[mh] OR "Ultrasonography"[sh] OR Ultrasonics[mh] OR (echogra*[tiab] OR ultraso*[tiab] OR us guid*[tiab] OR us examin*[tiab] OR us imag*[tiab] OR preoperative-US[tiab] OR sonogra*[tiab] OR optoacoust*[tiab])) AND ("Diagnostic Errors"[mh] OR "diagnostic value"[mh] OR "sensitivity and specificity"[mh] OR "early diagnosis"[mh] OR "Reproducibility of Results"[mh] OR (diagnostic accurac*[tiab] OR diagnostic error*[tiab] OR diagnostic abilit*[tiab] OR diagnostic value*[tiab] OR false positive*[tiab] OR false negative*[tiab] OR predictive-value*[tiab] OR npv OR ppv OR sensitiv*[tiab] OR specific*[tiab] OR early diagnos*[tiab] OR early detect*[tiab] OR reproducib*[tiab] OR Misdiagnos*[tiab] OR observer varia*[tiab] OR interobserver varia*[tiab] OR intraobserver varia*[tiab] OR observer bias*[tiab] OR interobserver

bias*[tiab] OR intraobserver bias*[tiab])) NOT (letter[pt] OR news[pt] OR comment[pt] OR editorial[pt] OR congresses[pt] OR abstracts[pt]) AND publisher[sb]

Google scholar n=100

melanoma "lymph|sentinel node|nodes" echography|ultrasound|ultrasonographic|ultrasonography "diagnostic|predictive accuracy|errors|ability|value"|misdiagnosis|"false positives|negatives"

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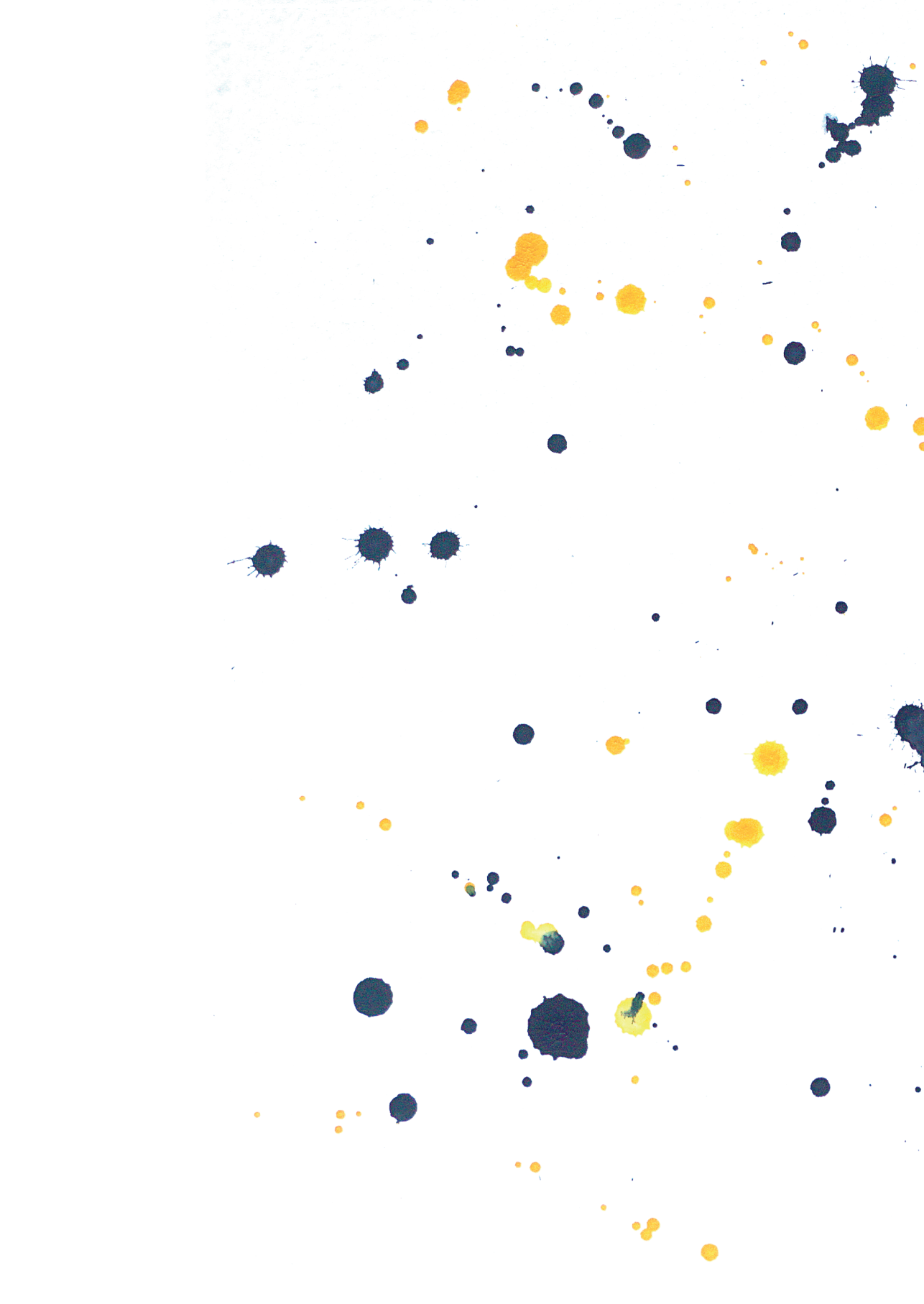
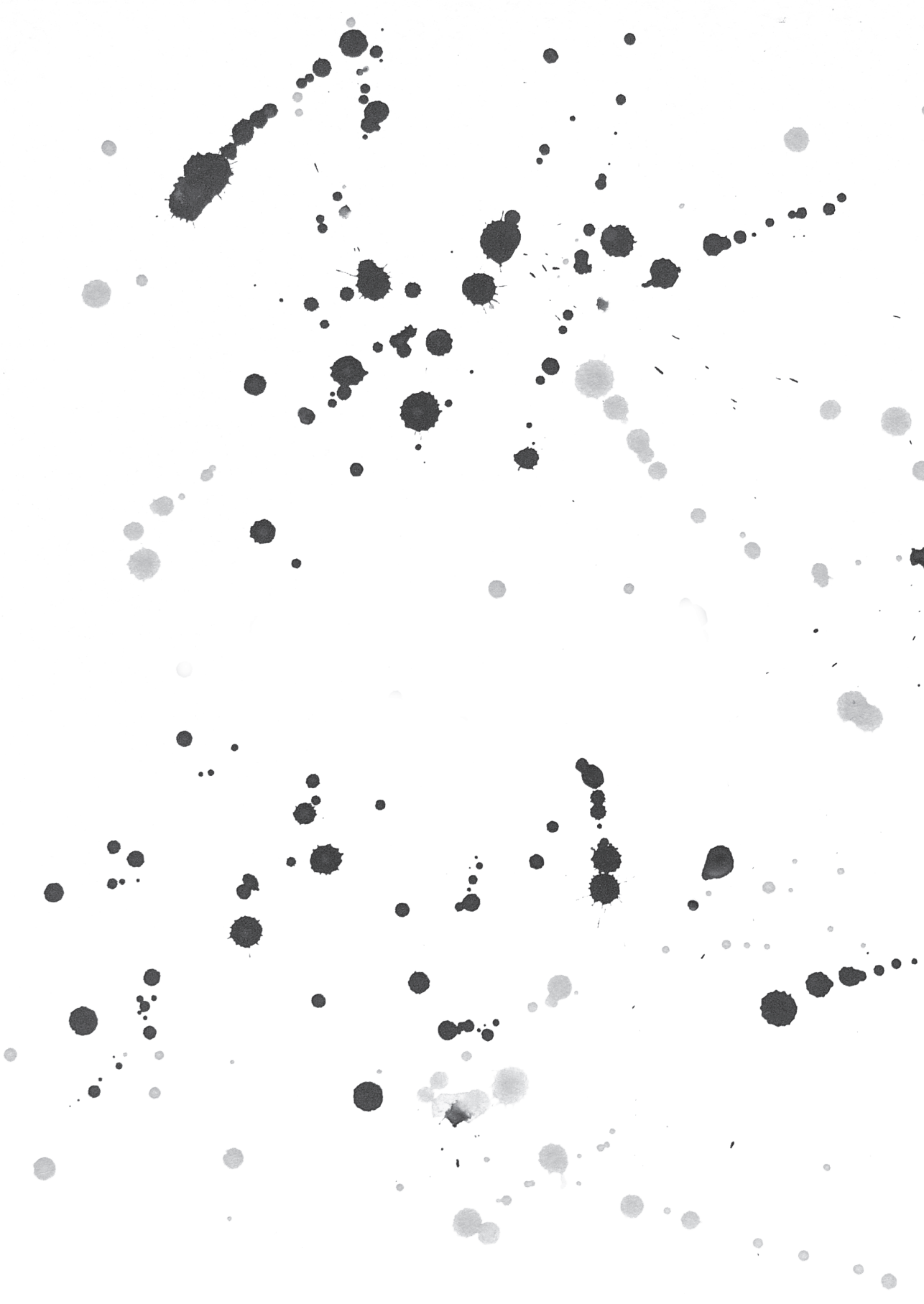


Part II – Timing of Surgery



Chapter 6

The Interval between Primary Melanoma Excision and Sentinel Node Biopsy Is Not Associated with Survival in Sentinel Node Positive Patients - *An EORTC Melanoma Group study*

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Abstract

Background Worldwide, sentinel node biopsy (SNB) is the recommended staging procedure for stage I/II melanoma. Most melanoma guidelines recommend re-excision plus SNB as soon as possible after primary excision. To date, there is no evidence to support this timeframe. Aim: To determine melanoma specific survival (MSS) for time intervals between excisional biopsy and SNB in SNB positive patients.

Methods Between 1993-2008, 1 080 patients were diagnosed with a positive SNB in nine Melanoma Group centers. We selected 1 015 patients (94%) with known excisional biopsy date. Time interval was calculated from primary excision until SNB. Kaplan-Meier estimated MSS was calculated for different cutoff values. Multivariable analysis was performed to correct for known prognostic factors.

Results Median age was 51 years (Inter Quartile Range (IQR) 40-62 years), 535 (53%) were men, 603 (59%) primary tumors were located on extremities. Median Breslow thickness was 3.00mm (IQR 1.90-4.80mm), 442 (44%) were ulcerated. Median follow-up was 36 months (IQR 20-62 months). Median time interval was 47 days (IQR 32-63 days). Median Breslow thickness was equal for both <47 days and ≥47 days interval: 3.00mm (1.90-5.00mm) vs 3.00mm (1.90-4.43mm) (p=0.402). Sentinel node tumor burden was significantly higher in patients operated ≥47 days (p=0.005). Univariate survival was not significantly different for median time interval. Multivariable analysis confirmed that time interval was no independent prognostic factor for MSS.

Conclusions Time interval from primary melanoma excision until SNB was no prognostic factor for MSS in this SNB positive cohort. This information can be used to counsel patients.

Introduction

Parallel to the increasing incidence of primary cutaneous melanomas, sentinel node biopsies (SNB) are being performed more often. This is the current standard to detect early lymph node micrometastases[1-3].

As recommended by the American Joint Committee on Cancer (AJCC), as well as the American Society of Clinical Oncology (ASCO) and the Society of Surgical Oncology (SSO), by performing a SNB, it is possible to provide accurate staging of intermediate thickness (Breslow 1.0 – 4.0mm) primary cutaneous melanoma[4, 5]. This way, patients can be provided more information about their prognosis [4, 6, 7]. Sentinel node (SN) status can help to select patients who might benefit from completion lymph node dissection (CLND) and / or adjuvant systemic therapies in trial setting, for instance the EORTC 18991 study on pegylated interferon alfa and the EORTC 18071 study on ipilimumab[8, 9]. Currently no uniform recommendation exists on the maximum allowed time interval between primary melanoma excision and wide local excision (WLE) combined with SNB. Most national melanoma guidelines advise to perform WLE and SNB as soon as possible within an acceptable time frame. The Dutch national melanoma guideline advocates a strict maximum time interval of six weeks[10]. This suggests a detrimental effect on survival if not adhered to. To date, only two studies have reported on this topic. Parrett et al. found no adverse effects on survival for a time interval of <40 days vs. > 40 days, while Tejera-Vaquerizo et al. reported a detrimental effect of a time interval of <40 days at the expense of SN negative patients[11, 12]. These contradicting findings are not sufficient to answer the question which effects, if any, time interval may have on survival.

One of the negative aspects of advising a short time frame for SNB is the incentive for general practitioners (GP's) and dermatologists to perform high urgency referrals. The potentially increased patient anxiety due to longer wait times (depending on the country's healthcare system) may also play a considerable role in this. Altogether this poses the need to objectively describe the possible influences of the time interval between primary diagnosis and WLE plus SNB on survival. We hypothesize that this time interval may be associated with a difference in survival. Aim of the study is to investigate if time interval between primary diagnosis and WLE plus SNB is associated with survival differences in a SN positive melanoma population.

Methods

Patients

For purposes of this current study, a retrospective cohort of SN positive patients, previously collected and described, was used[13]. In brief, this cohort contained 1 080 SN positive patients from nine European Organization for Research and Treatment of Cancer (EORTC) Melanoma Group centers, undergoing SNB between 1993 and 2008. The study was performed in accordance with local ethics committee guidelines. In total, 1 015 patients (94%) were selected with known date of primary melanoma excision. Collected data included: gender, age, date of primary excision, date of SNB, primary tumor characteristics i.e. location, Breslow thickness, ulceration, CLND data i.e. performed yes/no, positive non-SNs yes/no, and follow-up (FU).

Melanoma Diagnosis

Diagnosis of the primary melanoma was based on histopathologic examination of an excisional biopsy in all cases. Excisional biopsy was performed with total thickness excision and a narrow margin, as described in the American Association of Dermatology Guidelines and the National Cancer Comprehensive Network Clinical Practice Guidelines[14, 15]. Date of diagnosis was defined as the date of excisional biopsy. All patients treated at the participating centers were worked up for SNB in line with the recommendations stated by the European Society of Medical Oncology [16].

Surgical Procedure and Pathology:

SNB was performed if Breslow thickness was > 1.0 mm or if risk factors were present such as ulceration or high Clark level (IV or V), regression, or high mitotic rate (>1 count/field). Generally, WLE (with a margin of 1-2 cm depending on the Breslow thickness) and SNB were performed in the same setting. In all centers the triple technique was used for SNB; consisting of pre-operative lymphoscintigraphy within 24 hours prior to the procedure; perioperative injection of patent blue near the primary tumor site and use of an intraoperative handheld gamma detection probe to locate the SN(s) [17, 18]. A lymph node was defined as SN, if it was blue and / or hot (in situ: intraoperative gamma detector count of at least 3x background count, ex situ: intraoperative gamma detector count of at least 10x background count)[13]. Pathology review and reports were conducted according to the EORTC Melanoma Group Pathology Protocol, including scoring of SN tumor burden according to the Rotterdam criteria[19-21].

Outcome measures:

Time interval until SNB was the variable of interest (dependent variable) in this study. The primary endpoint was melanoma specific survival (MSS). Secondary endpoints were disease free survival (DFS); overall survival (OS); and SN tumor burden.

Statistics

Time until SNB was calculated from date of diagnosis until SNB date. FU was calculated from SNB date to last FU date or death. DFS was calculated from SNB date until date of first recurrence (any site). OS was calculated from SNB date until death (any cause) or last FU. MSS was calculated from SNB date until death by melanoma or last FU, deaths by other causes were censored (considered as withdrawal from population).

Patients were divided into two categories based on time interval: early SNB (< median) vs, late SNB (≥ median). Additionally, the first (Q1) and third quartile (Q3) of time interval were tested as binominal categories, and first (Q1) and last quartile (Q4) were tested against each other to detect differences between both outer quartiles. Breslow thickness, ulceration, SN tumor burden, gender and location of the primary tumor were analyzed per time interval category in order to investigate the possibility of differences in distribution indicating a selection bias in favor of early or late SNB. Kaplan-Meier estimated MSS was calculated per time interval category. Cox proportional hazard multivariable analysis was performed adjusting for age, gender, Breslow thickness, histology type, ulceration, Clark level, SN tumor burden, CLND category (performed/not performed), additional positive non-SNs and time interval as continuous variable. The maximum allowed degrees of freedom in the model were based on the number of events, not exceeding one tenth of the number of events.

A p-value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS Version 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.).

Results

In total 1 015 patients (93.9%) were selected of whom diagnosis date was known and time interval was less than 154 days (22 weeks). Median age at diagnosis was 51 years (IQR 40-62 years). Median FU was 36 months (IQR 20 -62 months), median DFS was 27 months (11 – 57 months). **Table 1** summarizes the baseline characteristics of the study population per time interval category.

Regression and mitotic rate were only recorded in a minority of patients, hence these variables were not included for further analysis. Median Breslow thickness was 3.00 mm (IQR 1.90 – 4.80mm). Median time interval per center is shown in **table 2.**

Table 1. Baseline Characteristics of Sentinel Node Positive Patients (N = 1 015)

Characteristic	< 47 days	≥ 47 days	All	p
N (%)	507 (50)	508 (50)	1 015 (100)	
Center				
1	53 (10.5)	62 (12.2)	115 (11.3)	
2	82 (16.2)	4 (0.8)	86 (8.5)	
3	101 (19.9)	120 (23.6)	221 (21.8)	
4	102 (20.1)	102 (20.1)	204 (20.1)	
5	22 (4.3)	70 (13.8)	92 (9.1)	
6	25 (4.9)	41 (8.1)	66 (6.5)	
7	60 (11.8)	46 (9.1)	106 (10.4)	
8	20 (3.9)	36 (7.1)	56 (5.5)	
9	42 (8.3)	27 (5.3)	69 (6.8)	0.005*
Gender				
Female	228 (45.0)	252 (49.6)	480 (47.3)	
Male	279 (55.0)	256 (50.4)	535 (52.7)	0.139
Age, years				
≤ 51	255 (50.3)	264 (52.0)	520 (51.1)	
> 51	252 (49.7)	244 (48.0)	496 (48.9)	0.594
Location				
Extremity	314 (61.9)	289 (56.9)	603 (59.4)	
Trunk	177 (34.9)	204 (40.2)	381(37.5)	
Head/neck	16 (3.2)	15 (3.0)	31 (3.1)	0.122
Histology				
SSM	179 (35.3)	197 (38.8)	376 (37.0)	
NM	172 (33.9)	157 (30.9)	329 (32.4)	
Other	25 (4.9)	15 (3.0)	40 (4.0)	
Unknown	131 (25.8)	139 (27.4)	270 (26.6)	0.538
Breslow Thickness, mm				
T1 (</= 1.00)	29 (5.7)	20 (3.9)	49 (4.8)	
T2 (1.01-2.00)	118 (23.3)	139 (27.4)	257 (25.3)	
T3 (2.01-4.00)	201 (39.6)	210 (41.3)	411 (40.5)	
T4 (>4.00)	159 (31.4)	137 (27.0)	296 (29.2)	
Missing	-	2 (0.4)	2 (0.2)	0.236
Clark level				
II	12 (2.4)	20 (3.9)	32 (3.2)	
III	120 (23.7)	133 (26.2)	253 (24.9)	
IV	309 (60.9)	276 (54.3)	585 (57.6)	
V	48 (9.5)	52 (10.2)	100 (9.9)	
Unknown	18 (3.6)	27 (5.3)	45 (4.4)	0.567
Ulceration				

Table 1. Baseline Characteristics of Sentinel Node Positive Patients (N = 1 015) (continued)

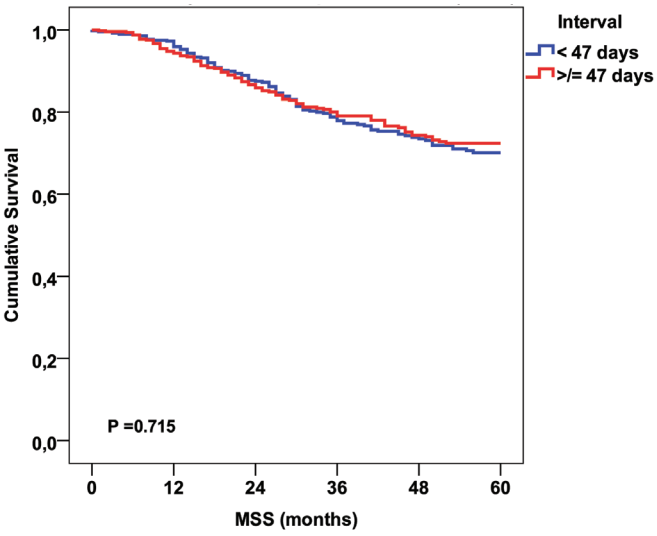
Characteristic	< 47 days	≥ 47 days	All	p
Absent	249 (49.1)	262 (51.6)	511 (50.3)	
Present	229 (45.2)	213 (41.9)	442 (43.5)	
Unknown	29 (5.7)	33 (6.5)	62 (6.1)	0.550
SN tumor burden				
<0.1mm	60 (11.8)	52 (10.2)	112 (11.0)	
0.1 – 1.0mm	238 (46.9)	199 (39.2)	437 (43.1)	
>1.0mm	209 (41.2)	257 (50.6)	466 (45.9)	0.005*
CLND performed				
No	24 (4.7)	22 (4.3)	46 (4.5)	
Yes	468 (92.3)	482 (94.9)	950 (93.6)	
Unknown	15 (3.0)	4 (0.8)	19 (1.9)	0.276
Positive non SNs				
No	380 (75.0)	415 (81.7)	795 (78.3)	
Yes	110 (21.7)	87 (17.1)	197 (19.4)	
Unknown	17 (3.4)	6 (1.2)	23 (2.3)	0.009*
Time interval, median (IQR)	32 (26 – 40)	63 (54 – 75)	47 (32 – 63)	0.331

N, number of patients; IQR, inter quartile range; SSM, superficial spreading melanoma; NM, nodular melanoma; LMM, lentigo maligna melanoma; ALM, acrolentiginous melanoma; CLND, completion lymph node dissection; SN, sentinel node. * significance reached at p<0.05.

The proportion of patients undergoing SNB early (<47 days) differed significantly per center, due to more early surgical procedures in Center 2 and Center 9 (operated within 47 days: 95.3% and 60.9% vs. 23.9% - 56.6% in the remaining centers) (**table 1, table 2)**. Median FU did not differ between patients operated at <47 days (37 months, IQR 19 – 62 months) vs. patients operated at ≥47 days (35 months, IQR 21 – 62 months) (p=0.632).

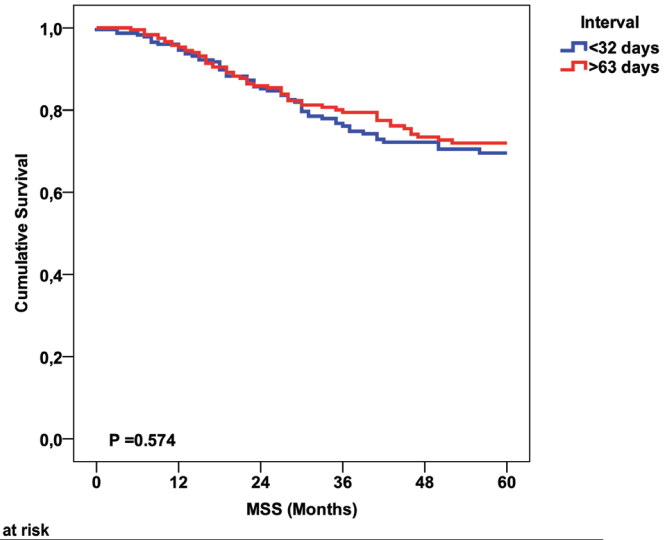
Table 2. Time Interval Between Melanoma Diagnosis and Sentinel Node Biopsy per Center

Center	Time interval in days: median, (inter quartile range)
1	48 (36 – 61)
2	9 (0 - 30)
3	49 (36 – 63)
4	47 (33 – 61)
5	63 (48 – 73)
6	53 (37 – 69)
7	41 (29 – 62)
8	50 (41 – 64)
9	37 (21 – 59)



Nr. at risk						
< 47 days	507	435	353	261	194	136
≥ 47 days	508	445	354	253	198	142

Figure 1. 5 Year Estimated Melanoma Specific Survival (MSS) for Median Sentinel Node Biopsy (SNB) Time Interval. <47 days (blue line) and ≥47 days (red line).



Nr. at risk						
≤ 32 days	246	204	166	124	89	62
> 63 days	254	224	177	130	107	81

Figure 2. 5 Year Estimated Melanoma Specific Survival (MSS) for Sentinel Node Biopsy (SNB) Time Interval Outer Quartiles. First quartile Q1, <32 days (blue line) and fourth quartile Q4, >63 days (red line).

Table 3. Univariable and Multivariable Analysis on 5 Year Melanoma Specific Survival (MSS) (N = 1 015)

Covariate	Univariable			Multivariable		
	HR	95% CI	*P*	HR	95% CI	*P*
Age, continuous	1.01	1.00 – 1.02	0.043*	1.01	0.99 – 1.01	0.322
Gender						
Female	1			1		
Male	1.31	1.00 - 1.72	0.046*	1.37	1.04 – 1.81	0.024*
Histology						
SSM	1			1		
NM	1.40	1.01 - 1.93	0.042*	0.96	0.68 – 1.35	0.959
Other	2.04	1.10 - 3.76	0.023*	1.75	0.92 – 3.34	0.088
Unknown	1.39	0.98 - 1.99	0.065	1.32	0.89 – 1.95	0.170
Clark level						
II	1			1		
III	1.34	0.48 - 3.75	0.576	1.61	0.57 - 4.57	0.372
IV	1.98	0.73 - 5.36	0.178	2.09	0.77 - 5.73	0.150
V	3.84	1.37 - 10.8	0.011*	2.42	0.84 - 6.96	0.101
Unknown	2.70	0.86 - 8.47	0.090	2.21	0.67 - 7.28	0.194
Ulceration						
Absent	1			1		
Present	2.19	1.65 - 2.91	<0.0001*	1.67	1.24 - 2.26	0.001*
Unknown	1.73	0.98 - 3.05	0.059	1.44	0.77 – 2.70	0.254
Breslow, continuous	1.07	1.06 – 1.09	<0.0001*	1.05	1.02 – 1.07	0.0002*
Tumor burden						
< 0.1 mm	1			1		
0.1 – 1.0 mm	3.20	1.48 - 6.93	0.003*	2.85	1.31 - 6.21	0.008*
> 1.0 mm	5.96	2.79 - 12.7	<0.0001*	4.14	1.91 – 9.00	0.0003*
CLND done						
No	1			1		
Yes	1.12	0.53 – 2.37	0.775	0.63	0.29 – 1.37	0.244
Unknown	1.99	0.58 – 6.81	0.271	0.61	0.06 – 6.29	0.674
Positive non-SNs						
No	1			1		
Yes	2.47	1.86 – 3.28	<0.0001*	2.27	1.68 – 3.05	<0.0001*
Unknown	2.37	0.97 – 5.79	0.058	2.51	0.34 – 18.4	0.366
Time interval, continuous	1.00	0.99 – 1.01	0.721	1.00	0.99 – 1.01	0.567
Unknown	1.73	0.98 - 3.05	0.059	1.44	0.77 – 2.70	0.254

Abbreviations: MSS, melanoma specific survival; N, number of patients; HR, Hazard Ratio; 95% CI, 95% Confidence Interval; *, significant at p<0.05; SSM, superficial spreading melanoma; NM, nodular melanoma; n.s., not significant; CLND, completion lymph node dissection; SN, sentinel node.

5-year Estimated Kaplan-Meier MSS showed no significant difference in survival for early SNB (<47 days) vs. late SNB (≥ 47 days) **(Figure 1)**. For time interval categories Q1 and Q3 respectively, also no significant difference in MSS or DFS was seen (data not shown). Survival was not different between both outer quartiles; 5-yr MSS for Q1 (<32 days) was 70% vs. 72% for Q4 (>63 days), p=0.574 **(Figure 2).**

Univariable logistic Cox regression analyses showed a significant difference in 5-year estimated MSS for the following variables: older age (as continuous variable), gender, histological subtype, Clark level, ulceration, Breslow thickness, SN tumor burden, and positive non-SNs at CLND **(table 3)**. Non-significant on univariable analyses were: primary tumor location, center, CLND category and time interval (as continuous variable).

A Cox proportional hazard multivariable analysis was performed with inclusion of the significant factors on univariable analyses as mentioned above, CLND category and time interval to adjust for any possible occult selection bias on univariable analysis. Only male gender, presence of ulceration, higher Breslow thickness, SN tumor burden >0.1mm and positive non-SNs at CLND remained as independent prognostic factors for 5 year MSS **(table 3)**.

Time interval from primary excision to SNB was no independent prognostic factor for 5 year MSS after adjustment for potential confounding factors on multivariable analysis. DFS and OS were calculated for the entire cohort and each co-variable per time interval category (results not shown). Results were similar to the MSS data, namely that time interval was not a prognostic factor.

For DFS, the following additional prognostic indicators were found: increasing age (HR 1.01, 95% CI 1.00 – 1.01, p=0.050), center 2 (HR 0.59, 95% CI 0.35 – 0.98, p=0.040), center 4 (HR 0.51, 95% CI 0.31 – 0.83, p=0.006), center 5 (HR 0.47, 95% CI 0.29– 0.77, p=0.002), 8 (HR 0.33, 95% CI 0.17 – 0.62, p=0.001) and 9 (HR 0.24, 95% CI 0.12 – 0.48, p=0.001), and Clark level IV (HR 2.07, 95% CI 1.01 – 4.24, p=0.048) and V (HR 2.21, 95% CI 1.03 – 4.76, p=0.042).

Discussion

The MSLT 1 final report showed no difference in 10-year MSS for WLE and SNB followed by immediate CLND versus WLE alone and nodal observation followed by delayed therapeutic lymph node dissection if necessary[22]. Sub analyses in node positive patients with intermediate-thickness melanoma (1.2-3.5mm) showed a significantly improved 10-year distant DFS and MSS in favor of SNB. Considering this, any potential impact of the time interval until SNB on survival might more likely become detectable in patients with nodal disease, i.e. a positive SN. This formed the rationale to perform the current study with *SN positive patients.*

In this study, 5-year estimated MSS is not significantly different for short versus longer time intervals **(Figure 1, figure 2).** SN tumor burden according to the Rotterdam criteria[13, 20] is significantly more often high in those patients undergoing SNB after a time interval of 47 days or more. Thus it may seem that late performance of SNB might have an adverse effect on tumor burden. Oppositely of the increase of SN tumor burden with a longer time interval, the risk of additional positive non-SNs at CLND was higher in patients with early SNB (≤47 days). In multivariable logistic regression (data not shown), time interval was not correlated to CLND outcome, but Center was. This has been addressed by van der Ploeg et al[23]. Since time intervals are different between centers **(table 2),** there is a strong correlation between center and time interval. This could explain why it would seem that time interval has influence on the proportion of patients with positive non-SNs at CLND while in truth proportion of positive non-SNs is associated with the center of treatment.

After correcting for tumor burden, CLND outcome, and other known prognostic factors in a multivariable model time interval cannot be identified as a detrimental prognostic factor for MSS. This is in line with the study of Parrett et al. which concerned 491 SN positive and negative patients from a single institution[12]. With a median time interval of 40 days, no differences in DFS, OS and MSS were found, nor any significant difference in SN positivity rates.

Importantly, the current study consists of SN positive patients only. Since SN positive patients have a worse prognosis, the outcome of this study strengthens the findings of Parrett et al. SN negative patients have not been investigated in the current study, but effect of time interval on survival is not expected in these low-risk patients. Interestingly, Tejera-Vaquerizo et al. did find a detrimental effect of a short time interval on survival for SN negative patients[11]. They hypothesized that a shorter time interval and worse prognosis were associated due to surgeons prioritizing patients for surgery when primary tumor features were worrisome. Validation of these data is needed, as the described findings are counterintuitive.

The phenomenon that high SN tumor burden was more frequently observed in those patients undergoing SNB at a later time interval might cause one to consider a correlation between SN tumor burden and time interval.

When stratifying for SN tumor burden in Kaplan-Meier estimated survival analyses, no significant differences in MSS are seen for time interval (data not shown). After stratification for time interval <47 days vs. >47 days, SN tumor burden did distinguish clearly between good, intermediate and poor prognosis (**Figure S1**). The fact that there was no unadjusted survival difference between the group with a time interval of <47 days versus the group with a time interval of ≥ 47 days while the proportion of patients with a high SN tumor burden was slightly larger in the latter may be explained by the fact that survival is influenced by many variables and that the net effect canceled out the slightly

more frequent high SN tumor burden in the latter group. The fact that survival for high SN tumor burden found with early SNB versus high SN tumor burden found with late SNB is not different confirms that SN tumor burden is a prognostic factor regardless of SNB timing.

It is remarkable that there are differences in DFS across centers and not in MSS. Due to the retrospective nature of this study, the exact timing of follow-up visits is not known for all centers. It could be that more frequent follow-up visits in these centers led to a lead-time bias effect. Another possibility could be that these centers treated more patients with low risk primaries. This was not the case, since centers with a low median Breslow thickness had more ulcerated tumors and vice versa. As detailed follow-up information including date and site of first recurrence was not known for all patients, the lower HR for DFS in five out of nine participating centers may also be explained by a selection bias due to missing data.

There is sparse literature with regard to the maximum allowable time interval for SNB. Two large prospective trials have included a maximum time interval as inclusion criterion. These are the MSLT I trial, with a maximum allowed time interval to SNB of 12 weeks [24], and the SUNBELT trial, where the maximum allowed time interval was 90 days (=13 weeks) [25]. This maximum time interval is at least two times as high as the median time interval found in the current cohort. These time intervals seem to be reasonable in the light of providing treatment within a timely manner, and at the same time allowing an adequately broad window for scheduling SNB. As for WLE, which is often combined with SNB: McKenna et al. reported long term survival data of a large retrospective cohort showing no differences in recurrence free survival and OS regardless of the time interval until WLE [26]. While time interval until SNB is not prognostic for survival in the current study, it can be used as a quality measure for hospitals performing SNB. This could form an addition to registration of SN positivity rate per hospital, another recently proposed quality measure[27].

There are limitations in the current study. It is a retrospectively collected cohort from nine tertiary referral melanoma institutes across Europe. Inevitably, this can cause a selection bias, due to differences in local patient population, patient selection and protocols per center. As all centers are EORTC Melanoma Group centers, there is much expertise in work up and treatment of melanoma patients. Uniform work up of patients eligible for SNB, surgery and histopathological analysis of the SN was already applied in all these centers prior to implementation into European consensus guidelines. Local differences will have mainly consisted of referral policies, wait lists and case-mix rather than technical approaches to melanoma patients.

In the current cohort adjuvant interferon therapy was not used as a covariate, as only a minority of patients received interferon in adjuvant trial setting (n=36), and for one third of all patients no information was available on trial participation. Primary melanomas in

the head and neck region seem to be underrepresented, and median Breslow thickness varies considerably per center**.** Also the number of thick melanomas is high **(Table 1).** One possible explanation for this may be the fact that all participating centers are EORTC Melanoma Group referral centers, with a corresponding high risk case-mix. Considering this, the current cohort may not be entirely representative for the general population that would normally be offered a SNB. To overcome this, multivariable analyses have been performed to correct for known prognostic and potential confounding factors. The FU is limited, and an update of follow up data would definitely improve the value of the current study. During the median FU of 36 months (3 years) DFS and MSS were not affected by time interval, which is considerable. As this cohort consists of SN positive patients only, it is by definition not representative for the entire population undergoing SNB. It does reflect a wide variety of SN positive patients, including patients with thin melanomas and patients with thick melanomas.

Since no differences in survival are found for different time intervals in this high risk SN group, survival differences for the more beneficial SN negative patient population are unlikely. One has to take into account that although no effects on survival were seen for SN positive patients, SN-positivity rate might be adversely influenced by a longer time interval. No conclusions can be drawn on this aspect with the current SN positive cohort alone.

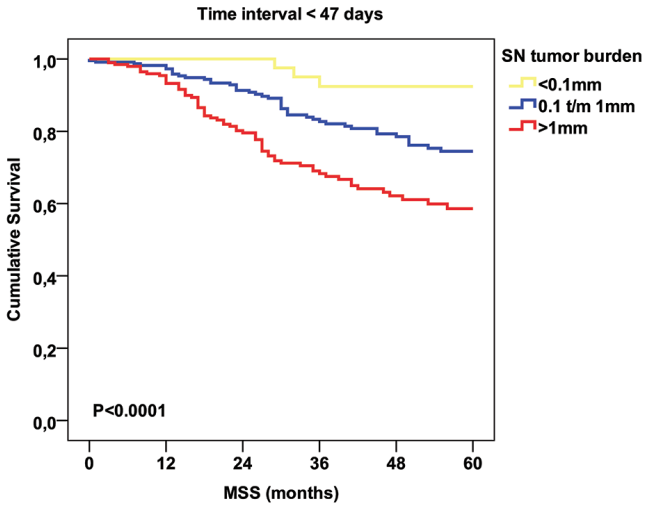
Finally, although a fixed maximum time interval based on survival does not seem to be necessary, minimizing the amount of wait time to surgery is still important to ease patient anxiety, as it affects the daily life of most patients. A survey by Eskander et al. in patients undergoing elective malignant thyroid surgery showed that anxiety levels significantly decreased after surgery, suggesting that stress and anxiety levels can be minimalized by performing surgery timely[28]. Another study by Oudhoff et al. concerning surgery for benign disorders, reported an increase in negative emotional reactions to waiting, significantly associated with wait time, which decreased significantly after surgery[29].

Taking all of the above into consideration, the need to perform early SNB as advised by specific melanoma guidelines should be reconsidered. As there is no solid base to adhere to a maximum time interval between WLE and SNB as stated in the above, maintaining a time interval falsely suggests that there still is a clinicopathological ground for performing SNB as soon as possible. This may facilitate unnecessary patient anxiety for patients on waiting lists. We therefore suggest that international melanoma guidelines should revise the need of a timeframe for SNB after primary melanoma excision in order to reduce patient anxiety and pressure on surgeon's schedule.

Conclusions

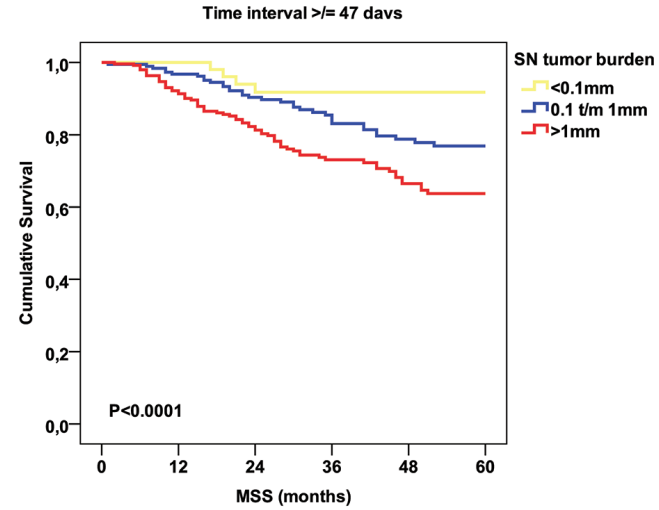
Time interval between primary melanoma excision and wide local excision (WLE) combined with Sentinel Node Biopsy (SNB) did not influence 5-year estimated DFS and MSS in a population of SN positive patients. Patients who got their SNB later had a slightly larger disease deposit and although this may have implications for prognosis this study did not detect any difference. These findings indicate that it is safe and equally informative to perform SNB after a prolonged interval of >9 weeks (4^{th} quartile). This information can be used to counsel patients.

A



Nr. at risk						
<0.1mm	60	53	47	36	28	18
0.1 – 1.0mm	238	205	174	134	105	78
> 1mm	209	177	132	91	61	40

B



Nr. at risk						
<0.1mm	52	51	42	38	34	24
0.1 – 1.0mm	199	175	146	110	86	68
> 1mm	257	219	166	105	78	50

Figure S1. 5-year estimated melanoma specific survival (MSS) in months stratified for a time interval until SN biopsy of <47 days (A) and a time interval of ≥ 47 days (B) for sentinel node (SN) tumor burden categories <0.1 mm (yellow line), 0.1-1.0mm (blue line), and >1.0mm (red line)

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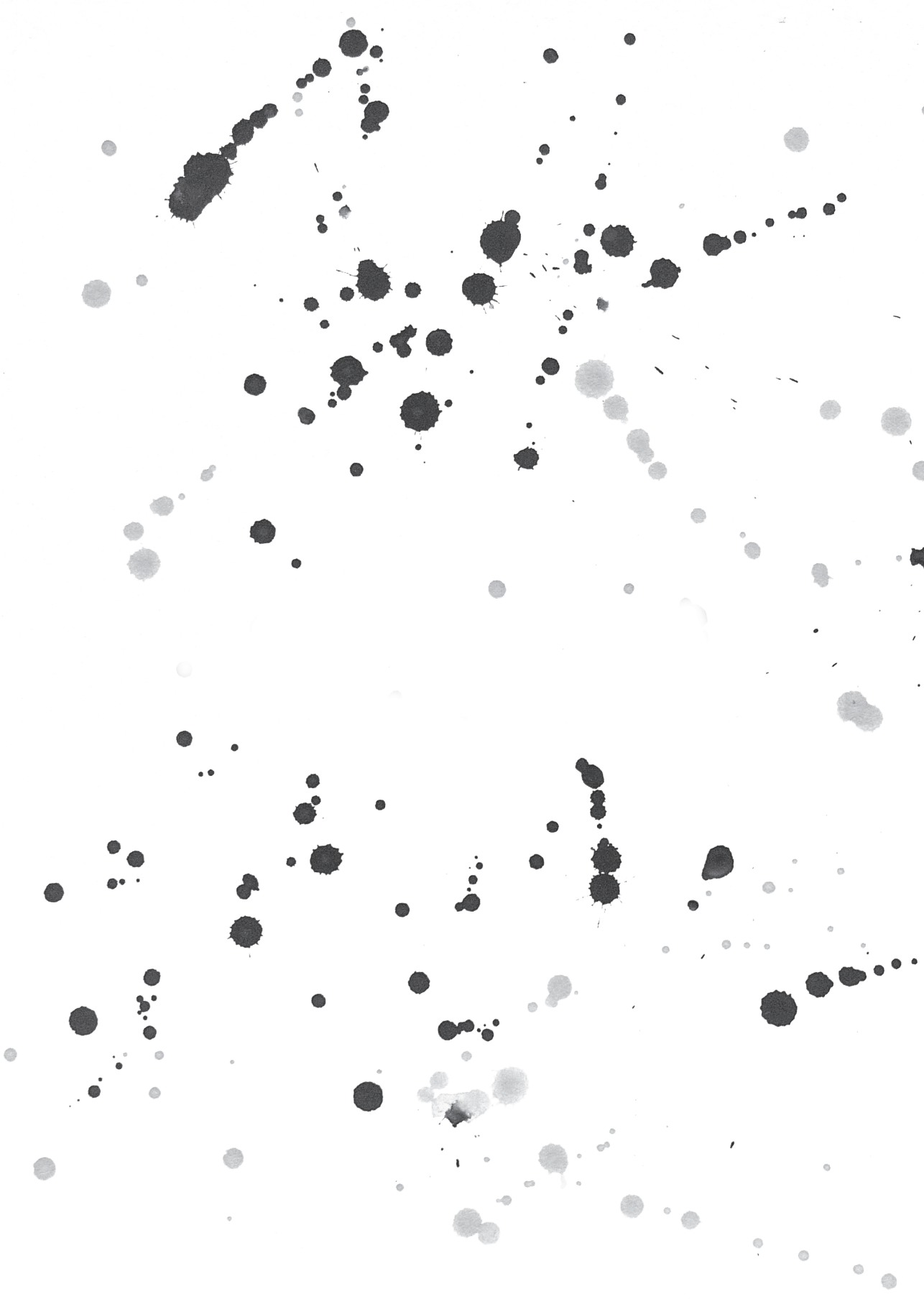

Chapter 7

Effects of Time Interval between Primary Melanoma Excision and Sentinel Node Biopsy on Positivity Rate and Survival

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Abstract

Background Sentinel node biopsy is essential for adequate melanoma staging. Most melanoma guidelines advocate to perform wide local excision and sentinel node biopsy as soon as possible, causing time pressure. Objective: To investigate the role of time interval between melanoma diagnosis and sentinel node biopsy on sentinel node positivity and survival.

Methods This is a retrospective observational study concerning a cohort of melanoma patients from four European Organization for Research and Treatment of Cancer Melanoma Group tertiary referral centers from 1997-2013. 4,124 melanoma patients underwent sentinel node biopsy. Patients were selected if date of diagnosis and follow-up information were available, and sentinel node biopsy was performed in <180 days. A total of 3,546 patients were included. Multivariable logistic regression and cox regression analyses were performed to investigate how baseline characteristics and time interval until sentinel node biopsy are related to positivity rate, disease free survival and melanoma specific survival.

Findings Median time interval was 43 days (interquartile range (IQR) 29–60 days), 705 (19·9%) of 3,546 patients had a positive sentinel node. Sentinel node positivity was equal for early surgery (≤43 days) vs. late surgery (>43 days): 19·7% vs. 20·1% (p=0·771). Median follow-up was 50 months (IQR 24–84 months). Sentinel node metastasis (hazard ratio (HR) 3·17, 95% CI 2·53–3·97), ulceration (HR 1·99, 95% CI 1·58–2·51), Breslow thickness (HR 1·06, 95% CI 1·04–1·08), and male gender (HR 1·58, 95% CI 1·26–1·98) (all p<0·00001) were independently associated with worse MSS and DFS, time interval was not.

Interpretation No effect of time interval between melanoma diagnosis and sentinel node biopsy on five year survival or sentinel node positivity rate was found for a time interval of up to three months. This information can be used to counsel patients and remove strict time limits from melanoma guidelines.

Introduction

Worldwide, sentinel node biopsy (SNB) has become essential for adequate staging of melanoma patients. It is the current gold standard to detect early lymph node involvement, as recommended by the American Joint Committee on Cancer (AJCC), the American Society of Clinical Oncology (ASCO), the Society of Surgical Oncology (SSO) as well as the European Society of Medical Oncology (ESMO)[1-3].

Currently no uniform recommendation exists on the maximum allowable time interval between melanoma diagnosis (i.e. date of excisional biopsy), and wide local excision (WLE) combined with SNB. Most melanoma guidelines advise to perform WLE and SNB as soon as possible, within an acceptable time frame. The Dutch national melanoma guideline for instance advocates a strict maximum time interval of six weeks after primary melanoma diagnosis[4]. Promoting a relatively short time frame for performing WLE and SNB suggests a detrimental effect if not adhered to.

Advising a short time frame for WLE and SNB negatively affects the referral system, as it forms an incentive for general practitioners (GP's) and dermatologists to perform high urgency referrals. High urgent referral implies influence of time interval (i.e. a longer interval may be detrimental) and therefore wait time to surgery can increase patient anxiety.

However, most melanoma specialists will not be expecting a link between SNB time interval and prognosis for two main reasons: first of all, to date SNB has been a strong predictor of prognosis, but whether the procedure has a prognostic effect itself remains subject to debate[5], let alone, the interval to the procedure. Second, the time interval between diagnosis and SNB is likely to be very short as compared to the duration of melanoma development pre-diagnosis. Variation in SNB timing of a few weeks (30 to 60 days) will probably represent only a fraction in the whole melanoma development story, and thus is unlikely to be of any effect on melanoma course.

The aim of this study is to investigate if time interval until WLE and SNB is associated with sentinel node (SN) positivity rate, disease free survival (DFS) and melanoma specific survival (MSS) in a large European melanoma population.

Methods

Patients

For purposes of the current study a retrospective cohort was collected of melanoma patients undergoing SNB in one of four European Organization for Research and Treatment of Cancer (EORTC) Melanoma Group centers. The study was approved and performed in accordance with local ethics committee guidelines and national legislation.

Between 1997 and 2013, 4,124 patients underwent SNB in one of four EORTC Melanoma Group centers. In total, 3,546 patients were selected with known date of primary melanoma diagnosis (i.e. diagnostic excisional biopsy) and SNB within 180 days, and available follow-up information. Collected data included: gender, age, diagnosis date, date of SNB, primary tumor characteristics; i.e. location, Breslow thickness, ulceration, histological subtype, outcome of SNB, details on completion lymph node dissection (CLND), and follow-up (FU).

Diagnosis

Diagnosis of the primary melanoma was based on histopathologic examination of an excisional biopsy in all cases. Excisional biopsy was performed with total thickness excision and a narrow circumferential margin, as described in the European Consensus-based Interdisciplinary Guideline, American Association of Dermatology Guidelines and the National Cancer Comprehensive Network Clinical Practice Guidelines[6-8]. Melanoma diagnosis was defined as the date of excisional biopsy.

Surgical Procedure and Pathology:

In all centers, eligibility for SNB was assessed according to international guideline criteria; i.e. Breslow thickness of >1·0mm or presence of risk factors ulceration, Clark level IV or V (AJCC staging 6th edition for patients operated up to 2009[9]), regression or mitosis >1/mm^2 (AJCC staging 7th edition for patients from 2009 up to 2013[1]). Generally, WLE (with a margin of 1-2 cm depending on the Breslow thickness) and SNB were performed in the same setting. SNB was performed according to the triple technique; i.e. pre-operative lymphoscintigraphy within 24 hours prior to the surgical procedure; perioperative intradermal injection of patent blue near the primary tumor site; and intraoperative use of a handheld gamma detection probe to locate the sentinel node(s) (SN)[10, 11]. A lymph node was defined as SN, if it was blue and / or hot (in situ: Geiger teller count of at least more than three times the background count, ex situ: Geiger teller count of at least ten times the background count) as described in detail elsewhere [12, 13]. Histopathological analysis of the SN was conducted according to the EORTC Melanoma Group Pathology Protocol[14].

Statistics

Time interval was defined as the time between the date of melanoma diagnosis and the date of WLE and SNB in days. FU was calculated from date of SNB to date of last FU or death. DFS was calculated from date of SNB to date of first recurrence (any site) or until death (unrelated cause) or end of FU. Overall survival (OS) was calculated from date of SNB to death (any cause) or last FU. MSS was calculated from date of SNB until death by melanoma or last FU, deaths by other causes were censored (considered

as withdrawal from the population). Primary tumor characteristics including Breslow thickness, ulceration, histological subtype, SN tumor burden, gender, and location of the primary tumor, as well as time interval were analyzed using X^2-tests or Mann-Whitney U tests, as appropriate. Survival was estimated with the Kaplan-Meier method and compared per time interval category (<median vs. >median, <first quartile vs. >first quartile, <fourth quartile vs. >fourth quartile, respectively) using the log-rank test.

To reduce bias due to missing values, we imputed missing covariates using multiple imputation[15]. Briefly, ten datasets were created that differed only in the imputed values and analyses were performed on each of the sets. The derived effect estimates were pooled using Rubin's rules[16] to obtain the final results that we report here. All univariable and multivariable binominal logistic regression and cox regression models were performed with the multiply imputed data sets (10 imputations).

Binominal logistic regression analysis was performed to identify predictors for SN-positivity with adjustment for: gender, age, primary tumor location, histologic subtype, Breslow thickness, ulceration status, Clark level, number of removed SNs, center, and time interval. Cox proportional hazard multivariable analysis was performed to identify prognostic factors for survival, with adjustment for all the variables used for multivariable binominal logistic regression as mentioned in the above, and SN status. All tests were performed two-sided. To correct for multiple testing we used the Bonferroni correction and considered p-values of less than 0·0005 to be statistically significant. All statistical analyses were performed using SPSS Version 21·0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21·0. Armonk, NY: IBM Corp.).

Role of the funding source No funding was obtained for the current study.

Results

Patients

For the current study 3,546 patients with a SNB within 180 days after diagnosis were selected. Data were available for patients with a SNB performed from 1997 up to 2013 in center one, from 1997 up to 2004 in center three and from 2000 up to 2012 in center two and four. Of all patients, 1,849 were women (52·1%), and 1697 (47·9%) were men. The median age was 54 years (interquartile range (IQR) 43 – 66 years) and median FU was 50 months (IQR 24 - 84 months). **Table 1** summarizes the baseline characteristics of the study population.

Table 1. Baseline characteristics (N=3,546)

Characteristic	N (%) or Median [IQR]
Center	
1	563 (15·9)
2	1,005 (28·3)
3	1,033 (29·1)
4	945 (26·6)
Gender	
Female	1,849 (52·1)
Male	1,697 (47·9)
Age in years	54 [43 – 66]
Location	
Extremity	1,666 (47·0)
Trunk	1,541 (43·5)
Head/neck	338 (9·5)
Missing	1 (0·0)
Histology	
SSM	1,880 (53·0)
NM	1,072 (30·2)
ALM	96 (2·7)
LMM	135 (3·8)
Other	47 (1·3)
Unspecified/missing	316 (8·9)
Breslow in mm	2·00 [1·20 – 3·30]
Missing	52 (1·5)
Tumor thickness	
T1 <1·0mm	632 (17·8)
T2 1·1 – 2·0mm	1,294 (36·5)
T3 2·1 – 4·0mm	991 (27·9)
T4 >4·0 mm	577 (16·3)
Missing	52 (1·5)
Ulceration	
Absent	2,420 (68·2)
Present	996 (28·1)
Missing	130 (3·7)
Time Interval	43 [29 – 60]
SN status	
Negative	2,841 (80·1)
Positive	705 (19·9)
No. SNs removed	1 [1 – 2]
Missing	119 (3·4)

Table 1. Baseline characteristics (N=3,546) (continued)

Characteristic	N (%) or Median [IQR]
No. SNs positive	1 [1 – 1]
Rotterdam Criteria	705 (100)
<0·1mm	65 (9·2)
0·1-1·0mm	212 (30·1)
>1·0mm	291 (41·3)
Missing	137 (19·4)
CLND	705 (100)
No	94 (13·3)
Yes	578 (82·0)
Missing	33 (4·7)
Additional positive LNs	578 (100)
No	458 (79·2)
Yes	118 (20·4)
Missing	2 (0·3)
Recurrence	
No	2,810 (79·2)
Yes	736 (20·8)
First recurrence	736 (100)
Local	54 (7·3)
In transit	140 (19·0)
Regional LN	156 (21·1)
Distant	237 (32·2)
Missing	149 (20·2)
FU	50 [24 – 84]

Distribution of patient and tumor characteristics per N and % or median and interquartile range. Abbreviations: IQR, inter quartile range; SSM, superficial spreading melanoma; NM, nodular melanoma; ALM, acrolentiginous melanoma; LMM lentigo maligna melanoma; SN, sentinel node; CLND, completion lymph node dissection; LNs, lymph nodes; FU, follow-up.

Time interval

The median time interval between melanoma diagnosis and SNB was 43 days (IQR 29 - 60 days) for all patients. There was significant variation between centers: 44 days (IQR 35 – 57 days) in center one; 25 days (IQR 17 – 34 days) in center two; 48 days (IQR 35 – 61) in center three; and 58 days (IQR 44 – 71 days) in center four (p<0·00001) (**Figure 1**).

In a multivariable logistic regression analysis treatment in center two (odds ratio (OR) 0·14, 95% confidence interval (95% CI) 0·11 – 0·18, p<0·00001) and in center four (OR 3·06, 95% CI 2·43 – 3·84 p<0·00001) were relevant predictors for early SNB (data not shown). Breslow thickness, ulceration status, location, histologic type, Clark level, and age were not.

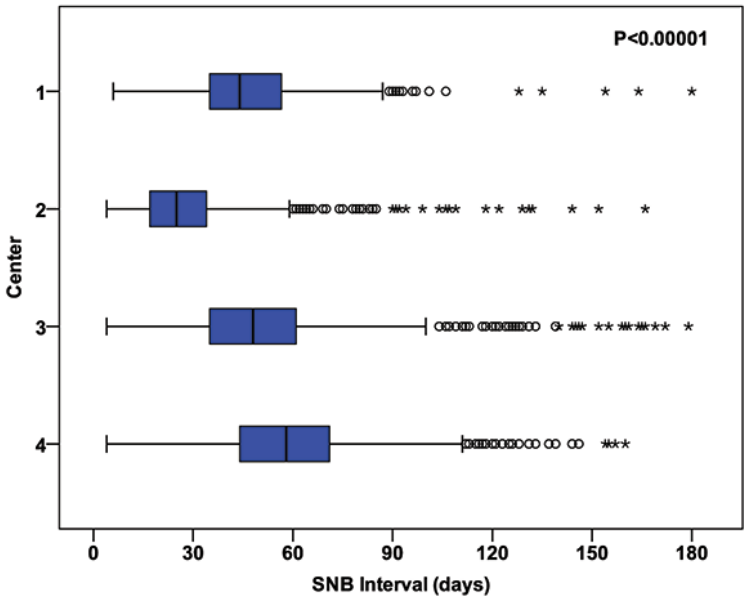

Figure 1. Distribution of Sentinel Node Biopsy Time Interval per Center. Boxplots indicating median (vertical line) and interquartile range (Box) of sentinel node biopsy (SNB) time interval in days per center. ○ (circles) indicate outliers >1.5 Box lengths (>1.5 SD), * (asterisks) indicate outliers >3 Box lengths (>3 SD).

SN Positivity

Distribution of SN positive patients per center was as follows: center one: 30·2% (170/563), center two: 17·3% (174/1005), center three: 15·3% (158/1033), and center four: 21·5% (203/945) (p<0·00001). The proportion of patients with a low, intermediate or high SN tumor burden (<0·1mm, 0·1-1mm, and >1·00mm respectively according to the Rotterdam criteria[17]) did not differ between patients undergoing SNB in ≤43 days vs. patients undergoing SNB after 43 days (p=0·122).

Table 2 displays the unadjusted and adjusted OR and 95% confidence interval for SN positivity per risk factor. Time interval was no relevant predictor for a positive SN (Table 2).

Overall, 6% (170/2806) of SN negative patients developed regional lymph node recurrence. The proportion of SN negative patients with regional lymph node recurrence did not differ significantly between early and late SNB: 6·3% (90/1438) vs. 5·8% (80/1368) (p=0·648).

Survival

The estimated 5-year DFS and MSS were not significantly different for early (≤43 days) vs. late (> 43 days) SNB: 76·8% (standard error (SE) 1·2%) vs. 76·8% (SE 1·1%) (p=0·729); and 86·4% (SE 1·0%) vs. 87·2% (SE 0·9%) (p=0·617) respectively **(Figure 2)**. Patients with a time interval of less than 29 days (first quartile) did not have a different survival from

Table 2. Univariable and Multivariable Binary Logistic Regression Analysis Positive SN Status (N=3,546).

Factor	Univariable			Multivariable		
	OR	95% CI	p-value	OR	95% CI	p-value
Center						
1	Ref			Ref		
2	0·48	0·38 – 0·62	<0·001*	0·54	0·41 – 0·71	<0·001*
3	0·42	0·33 – 0·53	<0·001*	0·41	0·31 – 0·54	<0·001*
4	0·63	0·50 – 0·80	<0·001*	0·58	0·45 – 0·75	<0·001*
Gender						
Female	Ref			Ref		
Male	1·27	1·08 – 1·50	0·005	1·07	0·89 – 1·29	0·46
Age, cont.	0·99	0·98 – 0·99	0·012	0·99	0·98 – 0·99	0·001*
Location						
Extremity	Ref			Ref		
Trunk	1·45	1·22 – 1·72	<0·001*	1·42	1·16 – 1·74	<0·001
Head/neck	2·25	1·73 – 2·94	<0·001*	2·12	1·57 – 2·88	<0·001*
Histology						
SSM	Ref			Ref		
NM	1·49	1·25 – 1·79	<0·001*	0·95	0·77 – 1·18	0·63
ALM	1·65	1·04 – 2·61	0·03	1·62	0·98 – 2·66	0·06
Other	0·56	0·27 – 1·18	0·12	0·54	0·25 – 1·19	0·12
Breslow, cont.	1·20	1·16 – 1·24	<0·001*	1·16	1·11 – 1·20	<0·001*
Ulceration						
No	Ref			Ref		
Yes	2·12	1·79 – 2·52	<0·001*	1·65	1·36 – 2·01	<0·001*
Clark level						
I-III	Ref			Ref		
IV-V	2·24	1·87 – 2·68	<0·001*	1·60	1·31 – 1·96	<0·001*
# SNs, cont.	1·20	1·14 – 1·27	<0·001*	1·12	1·06 – 1·19	<0·001*
Interval, cont.	1·00	0·99 – 1·00	0·58	1·00	0·99 – 1·00	0·92

Pooled coefficients from multiply imputed data (10 imputations). The multivariable models were adjusted for gender, age, primary tumor location, histologic subtype, Breslow thickness, ulceration status, Clark level, number of removed SNs, center, and time interval. A p-value of <0·0005 was considered statistically significant (marked with an *). Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; cont., continuous; SSM, superficial spreading melanoma; NM, nodular melanoma; ALM, acrolentiginous melanoma; # SNs, number of removed sentinel nodes.

patients with a time interval of more than 29 days, and patients with a time interval of less than 60 days did not have a different survival from patients with a time interval of more than 60 days (fourth quartile) (data not shown). Patients operated within 29 days also did not have a different survival from patients with a time interval of more than 60 days (first quartile vs. fourth quartile, data not shown). After stratification for SN status

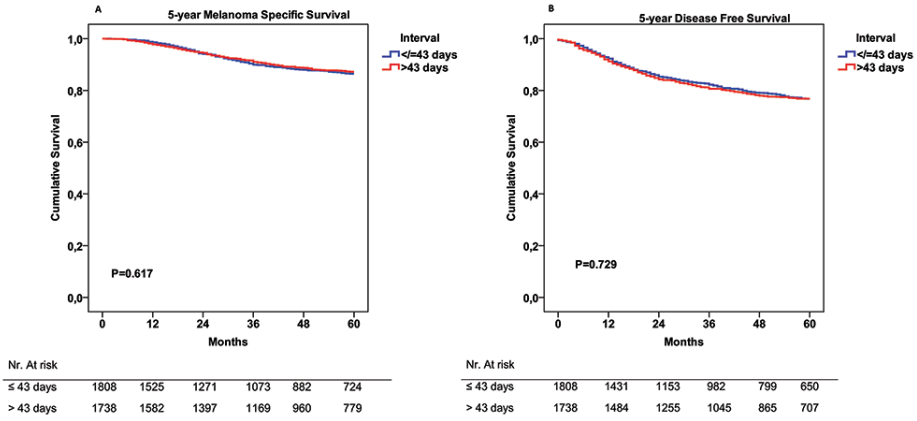

Figure 2. 5-year estimated survival curves per time interval. Kaplan-Meier curve of melanoma specific survival (A) and disease free survival (B) in months for a time interval ≤median (blue line) and >median (red line).

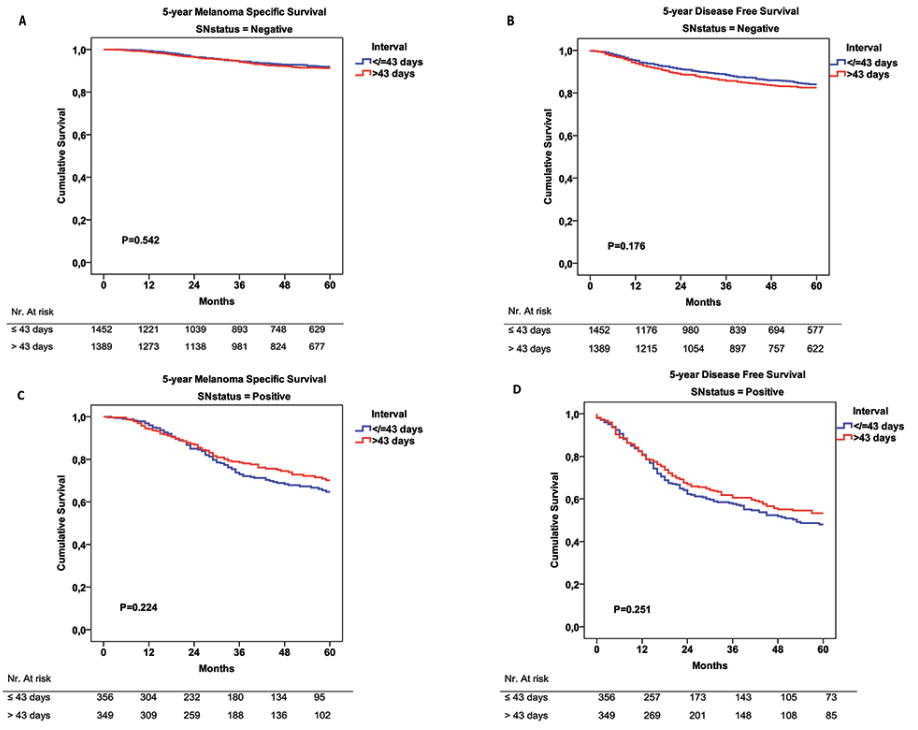

Figure 3. 5-year estimated survival curves per time interval and SN status. Kaplan-Meier curve of SN negatives (A,B) and SN positives (C,D) showing Melanoma Specific Survival MSS (A,C) and Disease Free Survival DFS (B,D) in months for a time interval < median (blue line) and > median (red line).

again no significant difference in survival was observed for median time interval as cut-off value **(Figure 3).** Univariable and multivariable Cox regression analyses were carried out to detect effects of time interval on survival **(Table 3).** Time interval as continuous variable predicting survival was neither significant in univariable analysis, nor after adjustment for known prognostic and confounding factors.

Discussion

In the current study we investigated if time interval from diagnosis until SNB had an effect on SN positivity rate and survival in a retrospective European melanoma cohort from four leading Melanoma centers. In the included 3,546 SNB patients no significant relationship between time interval and SN positivity rate **(Table 2)**, 5-year DFS nor 5-year MSS **(Figure 2 and Figure 3)** was found. There also was no significant difference between SN tumor burden categories and early vs. late time interval.

SN Positivity

Younger age, head & neck melanomas, higher Breslow thickness, ulceration, high Clark level, and a higher number of SNs removed were found to be predictors for a positive SN, which is in line with previous reports[18, 19]. Overall SN positivity rate was 19·9%, which is in line with previous studies as well, and the 15% to 30% SN positivity rate for each center also lies within previously reported SN positivity rates[18, 20-23]. It is known that there can be a wide variety in SNB positivity due to differences in expertise of surgeons, nuclear physicists and pathologists, as well as differences in patient characteristics according to volume and referral population[24]. Since all centers are EORTC Melanoma Group Centers with a high level of experience and expertise, a uniform approach to the work up and treatment of patients undergoing SNB can be expected, and any remaining differences between centers will probably be due to a different case mix as well as inevitable minor variations in execution of the same protocols.

As indicated in the above no significant difference in SN tumor burden categories was found between early and late SNB. Whether there may have been a minimal increase in tumor burden during the time interval between excisional biopsy and WLE plus SNB can neither be confirmed nor denied with the current study.

Time Interval and Survival

The only covariate influencing interval until SNB (<43 days) was center of treatment. This implies that primary tumor characteristics (e.g. high-risk melanoma features or an irradical excisional biopsy) were no significant factor for more urgent WLE and SNB in the process of scheduling patients for surgery.

Table 3. Univariable and Multivariable Cox Regression Analysis 5-Year Melanoma Specific Survival (MSS). (N=3,546)

Factor	Univariable			Multivariable		
	HR	95% CI	p-value	HR	95% CI	p-value
SN status						
Negative	reference			reference		
Positive	4·45	3·61 – 5·49	<0·001*	3·17	2·53 – 3·97	<0·001*
Center						
1	reference			reference		
2	0·73	0·53 – 1·01	0·06	0·82	0·59 – 1·16	0·26
3	0·92	0·70 – 1·22	0·56	0·99	0·73 – 1·34	0·94
4	0·58	0·42 – 0·79	<0·001	0·61	0·44 – 0·86	0·003
Gender						
Female	reference			reference		
Male	1·85	1·49 – 2·29	<0·001*	1·58	1·26 – 1·98	<0·001*
Age, cont.	1·01	1·00 – 1·02	0·03	1·01	0·99 -1·02	0·08
Location						
Extremity	reference			reference		
Trunk	1·75	1·56 – 1·97	<0·001*	1·53	1·19 – 1·96	<0·001
Head/neck	2·22	1·68 – 2·94	<0·001*	1·57	1·08 – 2·28	0·018
Histology						
SSM	reference			reference		
NM	2·54	2·02 – 3·20	<0·001*	1·53	1·19 – 1·96	<0·001
ALM	1·98	1·04 – 3·75	0·04	1·74	0·89 – 3·39	0·10
Other	0·85	0·42 – 1·71	0·65	0·93	0·46 – 1·90	0·85
Breslow, cont.	1·10	1·09 – 1·12	<0·001*	1·06	1·04 – 1·08	<0·001*
Ulceration						
No	reference			reference		
Yes	3·29	2·66 – 4·07	<0·001*	1·99	1·58 – 2·51	<0·001*
Clark level						
I-III	reference			reference		
IV-V	1·97	1·57 – 2·48	<0·001*	1·44	1·12 – 1·85	0·004
# SNs, cont.	1·05	0·97 – 1·12	0·23	0·97	0·90 – 1·05	0·50
Interval, cont.	0·99	0·99 – 1·00	0·35	1·00	0·99 – 1·01	0·74

Pooled coefficients from multiply imputed data (10 imputations). The multivariable models were adjusted for SN status, gender, age, primary tumor location, histologic subtype, Breslow thickness, ulceration status, Clark level, number of removed SNs, center, and time interval. A p-value of <0·0005 was considered statistically significant (marked with an *). Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; cont., continuous; SSM, superficial spreading melanoma; NM, nodular melanoma; ALM, acrolentiginous melanoma; # SNs, number of removed sentinel nodes.

To date, the effects of a longer time interval until SNB on survival have only been reported by three studies; Parrett et al.[21] and more recently Tejera-Vaquerizo et al[23] and Oude Ophuis et al[25]. The median time interval of 43 days (six weeks) found in the current study is in line with these studies[21, 23, 25]. Parrett et al. did not find any significant difference in DFS, OS and MSS, nor SN positivity rates[21]. Oude Ophuis et al. reported that interval had no effects on survival in a SN positive cohort of 1,015 patients[25]. The current study confirms these findings in both SN positive and SN negative patients, and demonstrates that SN positivity rate was not influenced by time interval either. Tejera-Vaquerizo et al. did find a *detrimental effect of a short time interval* on survival for 1,498 SN negative patients[23]. The current study contained 2,841 SN negative patients: no effects of time interval are found in this relatively low risk melanoma population. It could be that local differences in population or a selection bias favoring more aggressive melanomas caused the survival differences found by Tejera-Vaquerizo et al., but we can only speculate on the true cause of these contradictive findings. The fact that no survival differences were found in a larger group of SN negative patients in this study is reassuring.

It is the question whether performing WLE and SNB as soon as possible is necessary considering the above. Tejera-Vaquerizo et al. suggest to wait *longer* before performing SNB based on their theory that removal of the primary melanoma enables the start/continuation of an immunologic reaction in the SN, which would be disrupted by early removal of the SN[23]. Our findings do not confirm the findings of Tejera-Vaquerizo et al. that SN negative patients with a shorter time interval have a worse prognosis. The fact that a different interval does not change SN positivity rate or survival outcome in the current study is in fact compatible with the hypothesis that SNB could just be of no therapeutic impact.

Limitations

The SNB time intervals in the current study ranged between 4 and 180 days, with 50% of patients being operated between 29-60 days (IQR), and the variance of time interval is low between centers (**Figure 1**). This small range limits the possibility to study effects of a wider time interval; no conclusions can be drawn on a time interval beyond the maximum range. Potentially there could be an effect when comparing the outlier time intervals, but investigating this was not possible due to the design of the study where the majority of patients was operated around the IQR of 29-60 days.

This is a retrospectively collected cohort from four leading tertiary referral centers across North-East Europe. Inevitably, this can cause a selection bias, due to differences in case-mix, patient selection and protocol execution per center as reported in the above.

Median time interval varied significantly per center, depending on local referral systems and wait lists. SN positivity rate varied as well. This was not due to the time interval

to SNB, as was shown with logistic regression analysis. Considering that each center will have had a different population, and thus a different a priori risk of a positive SN, we cannot be sure that minor differences in surgical procedure and pathology review have occurred despite the adherence to the melanoma guidelines active at the time of surgery. To overcome these potential biases multivariable regression analyses have been performed with adjustment for center and primary tumor characteristics, as well as SN status.

There are generally two motives for high urgency referral; expedited performance of SNB, and expedited performance of WLE (for instance in case of a narrow excisional biopsy margin). The first has been addressed in the current study; early timing of SNB was found to have no effect on SN positivity rate or survival. On WLE only limited data was available; namely that it generally was performed in the same setting as SNB and a clinical margin of 1-2cm was applied depending on Breslow thickness. No data were available on the exact width of the margin of the excisional biopsy or the WLE, nor whether either one of these margins was tumor positive. Data were also lacking on whether any additional WLE had been performed in order to achieve a tumor negative margin. This could have affected survival outcomes, as Haydu et al.[26] demonstrated an association between excision margins <1cm and higher risk of recurrence for T2 melanomas. More recently Hayes et al.[27] reported the long term FU results of a RCT with T3 and T4 melanomas undergoing 1cm excision margin vs. 3cm excision margin[28] showing a worse MSS for patients with 1cm margin vs >1cm margin at a median FU of 8·8 years. Concerning the timing of WLE; McKenna et al. reported on 986 patients with an adequate diagnostic excisional biopsy followed by WLE (with a >1cm margin in 80%), no effects on recurrence free survival or OS were seen for any time interval[29].

This is in line with the current study, although lack of margin data is a definite drawback.

Conclusion

This is the largest SNB population to date to report on effects of time interval to WLE and SNB on SN positivity rate and survival. The current study sought to investigate possible effects of time interval to WLE and SNB on SN positivity rate and survival and found none in the 3,546 patients investigated. As expected a short change in time interval (up to +/- 1 months) has no obvious impact on SN positivity rate and prognosis. Whether intervals longer than 3 months may have an effect cannot be determined by this study. This reassuring information supports the removal of strict time intervals for WLE and SNB from melanoma guidelines, and can be used in daily clinical practice to counsel patients and reduce the number of high urgency referrals.

Research in context

Evidence before this study

An extensive search was performed in Embase, Medline, Cochrane Central, and Web-of-Science for studies describing timing of sentinel node biopsy in melanoma patients and the influence on survival. Search terms were: "melanoma AND sentinel node biopsy OR lymph node dissection OR lymphadenectomy AND timing OR wait list OR wait time OR delay OR delayed OR prognosis OR survival OR positivity rate". No date or language restrictions were applied to the search. Case reports were excluded. Two retrospective, non-randomized studies reported data regarding this subject. The findings of the first study are in line with our analyses. The second study reported a significant adverse effect on survival for early operated sentinel node negative patients. Our group has recently published a third relevant article focusing on sentinel node positive patients, results are in line with the current study.

Added value of this study

To our knowledge, this is the largest cohort study investigating the effects of sentinel node biopsy timing on SN positivity rate and survival. As opposed to the study describing adverse survival outcome for SN negative patients undergoing early surgery, which suggests that SNB timing can play a role in survival, no adverse effects on SN positivity rate, disease free survival, or melanoma specific survival were found.

Implications of all the available evidence

Overall, these findings did not show an influence of SNB timing on SN positivity rate or survival. The findings of the study reporting a worse survival for SN negative patients have not been validated in this largest cohort of SN melanoma patients, which confirms the assumption that the effect of SNB timing on survival, if at all existing, is very limited,. Based on all studies, it appears to be safe to delay SNB for more than six weeks. This information can be used to adapt current melanoma guidelines and counsel patients.

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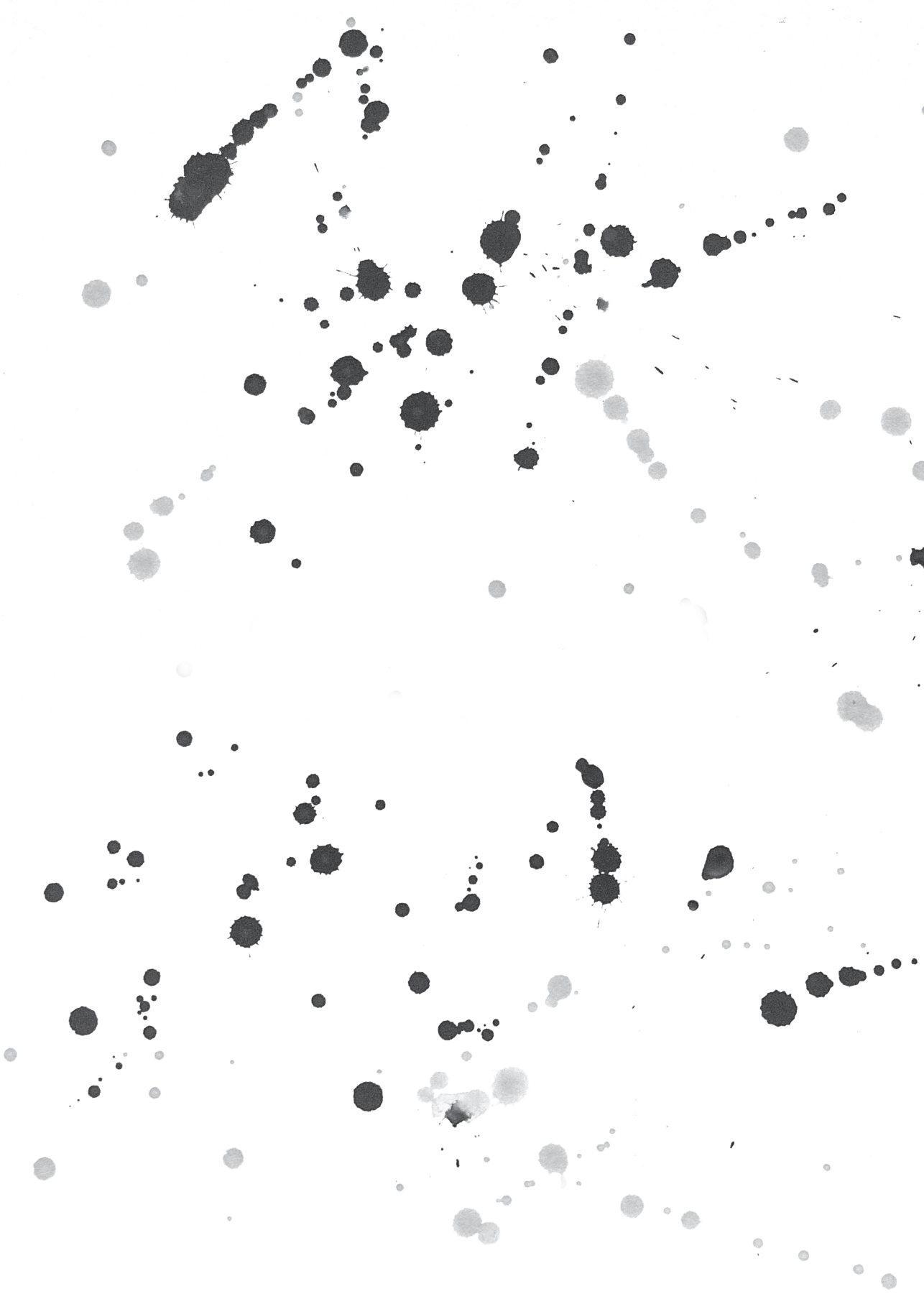

Chapter 8

Timing of Completion Lymphadenectomy after Positive Sentinel Node in Melanoma Patients

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Abstract

Background Nodal staging with sentinel node biopsy (SNB) and completion lymphadenectomy (CLND) informs melanoma patients and physicians on their prognosis. It is not known whether CLND timing is associated with survival outcome/CLND tumour load. The aim was to investigate if CLND timing is associated with CLND tumour load, disease free survival (DFS) and/or melanoma specific survival (MSS).

Methods A retrospective cohort of SNB positive melanoma patients from 9 EORTC Melanoma Group Centres undergoing surgery from 1993-2009 was examined. Patients were selected based on availability of CLND and follow-up data. CLND interval was defined as the number of days between diagnosis and CLND. Patient and tumour characteristics were collected. 5-year DFS and MSS were calculated. Cox and logistic regression analysis were performed adjusting for known prognostic/predictive indicators.

Results 784 patients were selected. Median age was 51 years (interquartile range (IQR) 40-62 years), 418 (53.3%) patients were male. Median Breslow thickness was 3.00mm (IQR 2.00-5.00mm), 148 patients (18.9%) had residual tumour load. Median CLND interval was 84 days (IQR 65-105 days). 5-year DFS and MSS were not significantly different for patients operated <84 days and ≥84 days: 54.2% vs. 53.3%, and 66.9% vs. 65.1%. In a multivariable Cox model, CLND interval was not a significant prognostic indicator. CLND interval was negatively correlated with positive non-SNs, after adjustment for known risk factors this effect was no longer found.

Conclusions The time interval between diagnosis and CLND did not influence CLND tumour load, DFS or MSS. This information can be used to counsel patients.

Introduction

A positive sentinel node biopsy (SNB) is generally followed by a completion lymph node dissection (CLND) as suggested by most current melanoma guidelines[1-3]. With a CLND any potential additional occult nodal metastases are removed, which can potentially improve survival. Moreover, the detailed pathological information on the extent of nodal disease can be used for adequate N-staging according to the AJCC Melanoma Staging System (7th edition) [1]. This can inform patients and their physicians more precisely on their prognosis, can select patients for adjuvant radiotherapy and allow inclusion of patients into adjuvant therapy trials.

Previously, several studies have reported on the timing of SNB and its potential association with survival, results are conflicting: Parrett et al. found no effect[4], Tejera-Vaquerizo et al. found a negative effect of early SNB for SN negative patients[5], while more recently Fortes et al. stated that early SNB had a positive effect on survival for SN positive patients[6]. Oude Ophuis et al. have investigated this topic as well, finding no difference in survival in a larger series of 1,015 SN positive patients[7]. Nor did SNB timing influence SN positivity in a group of 3,546 SN positive and SN negative patients[8].

The MSLT-2, Minitub and DeCOG studies[9-11] examine if a CLND has any therapeutic effect. Besides this question, is not known whether timing of the CLND after a positive SN can affect tumour burden and subsequent survival outcomes.

Previous retrospective studies report only on immediate CLND after a positive SN versus delayed therapeutic LND (in case of a false negative SNB or if no SNB was performed at all)[12-16]. Since the first group includes not only the patients with positive non-SNs but also up to 80% of patients with no additional positive nodes, while the latter group includes only the 20% of patients with occult positive lymph nodes at the time of diagnosis that have developed into clinically evident lymph nodes, these two heterogeneous groups cannot be compared one to one.

Aim of this study was to investigate if timing of CLND after a positive SNB is associated with tumour burden in CLND, disease free survival (DFS) and/or melanoma specific survival (MSS) differences in a large European cohort of melanoma patients.

Patients and Methods

Patients

A retrospective cohort described previously consisting of SN positive melanoma patients from 9 EORTC Melanoma Group Centres undergoing a SNB between 1993 and 2009 was used for this study[17]. For the current study additional information was gathered on date of CLND, number of excised lymph nodes and number of tumour positive non-SNs.

An update of follow-up data up to 2016 was performed in order to achieve long term follow-up results. Date of diagnosis and date of SNB were previously collected[7].

In brief, this cohort consisted of 1,080 patients with a melanoma of at least 1.0 mm Breslow thickness, or presence of at least one risk factor such as ulceration or Clark level IV/V (according to the 6th edition of the AJCC staging system used at the time of diagnosis)[18]. All patients had undergone a SNB and SN was considered positive after regular pathological work-up of the SN according to the EORTC protocol (see below). Patients were selected based on availability of detailed information on CLND: i.e. date of CLND, number of excised lymph nodes and number of additional positive non-SNs, and sufficient follow-up. Patients without CLND; with a CLND more than 180 days after diagnosis; or with a CLND more than 100 days after SNB were excluded, as this was considered to be aberrant from standard practice in our opinion.

Sentinel Node Biopsy

All patients underwent SNB according to the triple technique as described in detail previously [17, 19]. Histopathological examination of the removed SNs was performed according to the EORTC Melanoma Group pathology protocol[20]. Microscopic tumour burden and localization was scored according to the combined Rotterdam-Dewar Criteria[17, 21].

Completion lymphadenectomy

CLND consisted of either an axillary, inguinal, ilio-inguinal or a cervical lymphadenectomy, depending on the localization of the positive SN. The total number of surgically removed lymph nodes and the number of involved lymph nodes was registered for each patient.

Statistics

CLND interval was defined as the time between diagnostic excision of the primary melanoma and CLND in days. Separately the time interval between diagnosis and SNB, and the time interval between SNB and CLND were explored. As the time interval between diagnostic excision of the primary melanoma and SNB has been described previously[7] the current study will focus only on the SNB-CLND interval; defined as the time between SNB and CLND in days. Follow-up was calculated from date of diagnosis (i.e. date of diagnostic excision) until last follow-up or death of any cause. DFS was calculated from the date of diagnosis until first recurrence, deaths due to other cause and patients without recurrence at last follow-up were censored. MSS was calculated from date of diagnosis until the date of death due to melanoma, deaths due to other cause and patients alive at last follow-up were censored. Kaplan-Meier estimated 5 year DFS and MSS were calculated and the log rank test was applied for comparison between early and late (<median vs. >median / Q1 vs Q4) CLND interval and SNB-CLND interval. As maximum SN tumour

diameter (Rotterdam Criteria) was the most prognostic SN tumour burden factor[17] this was chosen for further evaluation in the current series. Logistic regression analysis and Cox regression analysis were performed univariable and multivariable, adjusting for: centre, gender, age, location of the primary, histology type, Breslow thickness, ulceration status, SN tumour burden, number of positive SNs/ SN ratio, number of removed lymph nodes at CLND, number of positive lymph nodes (non-SNs) in CLND resection specimen, and CLND interval. A two-sided p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS Version 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.).

Results

Of the 1,080 patients in the initial database, 296 patients were excluded for various reasons. Sixty-three patients had not undergone CLND, 200 patients had incomplete CLND data or FU data, 28 patients had a CLND > 180 days after diagnosis, and 5 patients had a CLND > 100 days after SNB. A total of 784 patients from 8 centres remained for the current study. Baseline patient, tumour and CLND features are displayed in **Table 1.**

Median follow-up was 65 months (IQR 28-113 months). All CLNDs were performed within a range of 0 - 178 days (median 84 days, IQR 65 – 105 days) after diagnosis, and median 37 days (IQR 27 – 48 days, range 0 - 97) after SNB. Again, none of them because of palpable nodes.

Non-SN Positivity

Positive non-SNs were found in 83/384 (221.6%) patients with a CLND <84 days after diagnosis (<median), and in 65/400 (16.3%) patients with a CLND ≥84 days (≥median) after diagnosis, which was not significantly different (p=0.055). The median number of positive non-SNs was similar between patients undergoing CLND <84 days and patients undergoing CLND ≥84 days after diagnosis (p=0.103). The proportion of patients with positive non-SNs was higher for patients operated within 65 days (Q1) than for patients operated more than 105 days (Q4) after diagnosis: 25.9% vs. 15.8% (p=0.014). Binominal logistic regression analysis was performed to investigate whether CLND interval was predictive for positive non-SNs; unadjusted OR was 0.99 (95% CI 0.985-0.997, p=0.003). After adjustment for: centre of treatment, gender, Breslow thickness, Ulceration, SN tumour burden and positive SN ratio (all p<0.05univariate), CLND interval was not a significant predictor (**Table 2**).

Table 1. Baseline Features (N = 784)

		N(%) or median [IQR]
Centre	1	102 (13.0)
	2	55 (7.0)
	3	228 (29.1)
	4	114 (14.5)
	5	110 (14.0)
	6	63 (8.0)
	7	55 (7.0)
	8	57 (7.3)
Gender	Female	366 (46.7)
	Male	418 (53.3)
Age	cont.	51 [40 – 62]
Location	Extremity	380 (48.5)
	Trunk	376 (48.0)
	Head/neck	28 (3.6)
Histology	SSM	330 (42.1)
	NM	300 (38.3)
	LMM	13 (1.7)
	ALM	29 (3.7)
	Other	4 (0.5)
	Unknown	108 (13.8)
Breslow	cont.	3.00 [2.00 – 5.00]
T Stage	T1	35 (4.5)
	T2	180 (23.0)
	T3	314 (40.1)
	T4	254 (32.4)
	Unknown	1 (0.1)
Ulceration	Absent	370 (47.2)
	present	379 (48.3)
	Unknown	35 (4.5)
# of SNs	cont.	2 [1 – 3]
# of positive SNs	cont.	1 [1 – 1]
Positive SN ratio	Cont.	1 [0.50 - 1]
SN tumour burden	<0.1mm	73 (9.3)
	0.1-1.0mm	334 (42.6)
	>1mm	377 (48.1)
SN tumour localisation	Subcapsular	138 (17.6)
	Non-subcapsular	534 (68.1)
	Unknown	112 (14.3)
CLND location	Axillary	375 (47.8)

Table 1. Baseline Features (N = 784) (continued)

		N(%) or median [IQR]
	Inguinal	291 (37.1)
	Ilio-inguinal	50 (6.4)
	Cervical	34 (4.3)
	multiple sites	33 (4.2)
	Unknown	1 (0.1)
# removed LN at CLND	cont.	14 [10 – 20]
# positive non-SNs	cont.	0 [0 – 0] (range 0 - 3)
CLND tumour load	Negative	636 (81.1)
	Positive	148 (18.9)
Interval diagnosis - CLND (days)	cont.	84 [65 – 105]
Interval diagnosis - SNB (days)	cont.	47 [33 – 62]
Interval SNB – CLND (days)	cont.	37 [27 - 48]

Baseline features of all 784 patients as number (%) or median [IQR]. Positive SN ratio was calculated as the number of positive SNs divided by the number of retrieved SNs. Abbreviations: IQR, inter quartile range; cont., continuous; SSM, superficial spreading melanoma; NM, nodular melanoma; LMM, lentigo maligna melanoma; ALM, acrolentiginous melanoma; SN, sentinel node; CLND, completion lymph node dissection; LN, lymph node(s); SNB, sentinel node biopsy.

Survival

Survival rates were not significantly different for patients undergoing CLND within 84 days (<median) after diagnosis and after 84 days or more (≥median); 5-year DFS was 53.3% (SE 2.6%) vs. 54.2% (SE 2.7%), 5-year MSS was 66.9% (SE 2.5%) vs. 65.1% (SE 2.5%) (**Figure 1 and Figure 2**). Different cut-off values for CLND time interval (Q1 vs Q2-4, <Q1-3 vs Q4, Q1 vs. Q4) did not show significant differences in survival either (data not shown). Five-year DFS and MSS were also calculated for the time interval between SNB and CLND with the median of 37 days as cut-off: DFS was 55.0% (SE 2.7%) vs. 52.6% (SE 2.6%) (p= 0.913), and MSS was 68.5% (SE 2.5%) vs. 63.8% (SE 2.5%) (p=0.479) respectively.

Prognostic Indicators

Results of univariable and multivariable Cox regression analysis for all patients are displayed in **Table 3.** CLND interval was not significant as prognostic indicator on univariable analysis. In order to adjust for a potential occult effect masked by other covariates, CLND interval was also included in the multivariable model, along with significant covariates at univariable analysis. Centre 4, Higher Breslow thickness, ulceration, SN tumour burden 0.1-1.0mm and >1.0mm, and number of positive non-SNs in the CLND resection specimen were significant prognostic indicators for 5-year MSS, time interval until CLND was not.

Table 2. Uni- and Multivariable Binominal Logistic Regression Analysis for Positive non-SN Status

			Univariable			Multivariable		
		n	OR	95% CI	p	OR	95% CI	p
Centre	1	102	ref			ref		
	2	55	2.56	1.06 - 6.19	0.037*	2.18	0.83-5.74	0.114
	3	228	2.69	1.34 - 5.39	0.005*	2.00	0.97 - 4.14	0.061
	4	114	1.16	0.50 - 2.68	0.732	1.32	0.55 - 3.17	0.530
	5	110	1.21	0.52 - 2.80	0.662	1.13	0.48 - 2.69	0.779
	6	63	1.38	0.54 - 3.54	0.504	1.61	0.61 - 4.26	0.333
	7	55	1.84	0.73 - 4.65	0.198	2.65	1.02 - 6.92	0.047*
	8	56	4.83	2.11 - 11.0	<0.0001*	5.20	2.21 - 12.3	<0.0001*
Gender	Female	366	Ref			ref		
	Male	418	0.70	0.49 - 0.99	0.047*	0.70	0.47 - 1.02	0.062
Breslow	cont.	783	1.13	1.08 - 1.19	<0.0001*	1.11	1.05 - 1.17	<0.0001*
Ulceration	Absent	370	ref.			ref.		
	Present	379	1.52	1.05 - 2.20	0.025*	1.05	0.70 -1.59	0.805
	Unknown	35	0.68	0.23 - 1.99	0.483	0.79	0.24 - 2.57	0.696
SN tumour burden	<0.1mm	73	ref.			ref.		
	0.1-1.0mm	334	2.06	0.85 - 4.99	0.110	2.07	0.82 - 5.23	0.124
	>1mm	377	3.50	1.47 - 8.34	0.005*	3.11	1.22 - 7.90	0.017*
Pos SN ratio	cont.	784	2.10	1.11 - 3.98	0.022*	2.53	1.27 - 5.01	0.008*
CLND interval	cont.	784	0.99	0.99 - 0.99	0.003*	0.99	0.98 - 1.00	0.133

Univariable and multivariable binominal logistic regression model for presence of positive non-sentinel nodes. Positive SN ratio was calculated as the number of positive SNs divided by the number of retrieved SNs. Abbreviations: SN, sentinel node; OR, odds ratio; 95% CI, 95% confidence interval; ref, reference value; cont., continuous; Pos, positive; CLND, completion lymph node dissection.

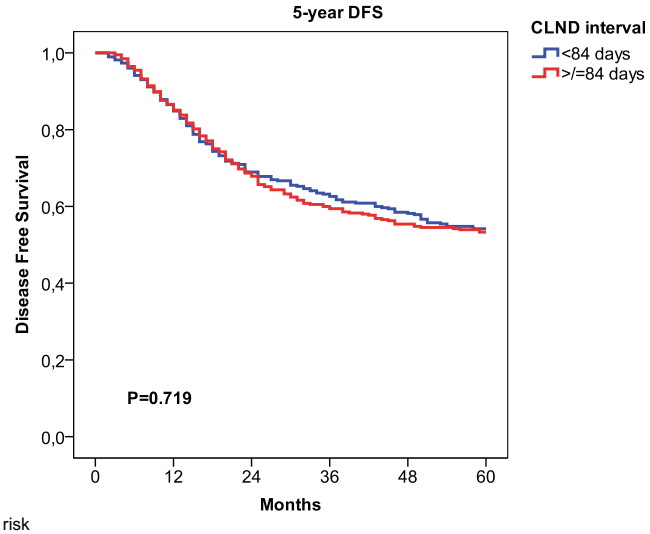
Discussion

The current study investigated whether timing of CLND after a positive SNB is associated with non-SN positivity, DFS and/or MSS differences in a large European cohort of melanoma patients.

No association between CLND interval and DFS or MSS was found in this study.

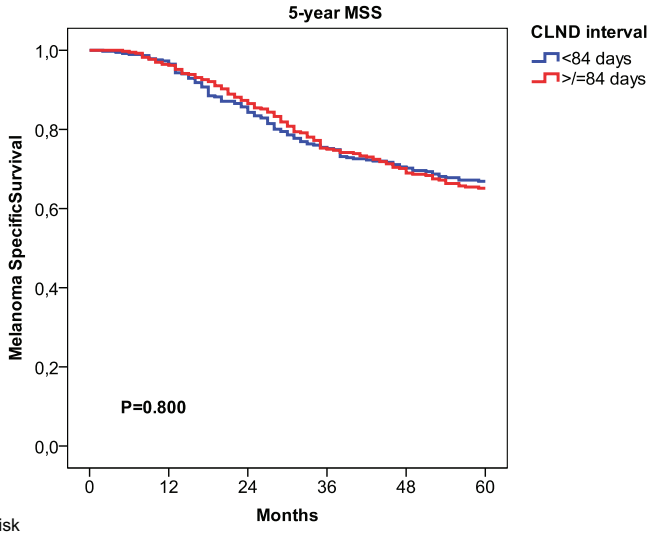
Nineteen percent of all patients had positive non-SNs, which is in line with other studies[22].

The prognostic indicators found in the multivariable Cox model (Breslow thickness, ulceration status, SN tumour burden, and number of positive non-SNs) are also in line with previous reports[13, 22], indicating the fact that the current cohort consists of a common, representative SN positive melanoma population.



no. at risk						
<84 days	384	315	241	216	195	169
>=84 days	400	339	255	217	187	172

Figure 1. Five-year Disease Free Survival per CLND Interval Category. Kaplan-Meier curve displaying 5-year disease free survival for completion lymphadenectomy (CLND) within 84 days (blue line) vs. CLND after more than 84 days (red line) post diagnosis. Difference in survival calculated with the log-rank test.



no. at risk						
<84 days	384	356	305	261	239	213
>=84 days	400	377	324	272	239	214

Figure 2. Five-year Melanoma Specific Survival per CLND Interval Category. Kaplan-Meier curve displaying 5-year melanoma specific survival for completion lymphadenectomy (CLND) within 84 days (blue line) vs. CLND after more than 84 days (red line) post diagnosis. Difference in survival calculated with the log-rank test.

The number of positive non-SNs is one of the most potent prognostic indicators (HR 1.24 per 1 node increase) (**table 3**). The fact that CLND interval was not associated with an increased number of positive non-SNs implies that at least for the time frame in which all CLNDs were performed in this study (0 – 178 days after diagnosis, IQR 65 – 105 days), the risk of metastatic spread to adjacent lymph nodes was not significantly increased for patients with a longer interval to undergo their CLND. This is reflected in the similar survival outcomes for patients with early CLND vs. late CLND in the current study.

Additionally, this study investigated whether the time interval between SNB and CLND was associated with survival; this was not the case. Previously Oude Ophuis et al. have reported on the timing of SNB after diagnosis of a new melanoma in SN positive patients; no significant effects on DFS or MSS were found in a cohort of over 1,000 SN positive patients[7]. Recently 2 series have been published reporting on SNB timing for both SN negative patients and a smaller number of SN positive patients, with strikingly conflicting results[5, 6]. Tejera-Vaquerizo et al. concluded that SN negative patients would profit from a longer time interval, while Fortes et al. stated that SN positive patients would benefit from a short time interval. Oude Ophuis et al. performed a larger study concerning SNB timing for over 3,500 SN positive and SN negative patients in which SNB timing was also not associated with survival or SN positivity. The fact that both studies from Tejera-Vaquerizo and Fortes show contradicting results, based on post-hoc analyses, and that Oude Ophuis et al. have investigated the largest SN series to date[8], affirm that can be assumed with a high degree of certainty that the effect of SNB timing is negligible.

As the therapeutic value of CLND itself continues to be questioned, it can be argued that CLND may no longer need to be performed at all, considering the invasive nature of the procedure, and association with considerable morbidity in a relevant proportion of patients[11]. Recently the results of the DeCOG have been reported, which showed no survival benefit of CLND vs. nodal observation for patients with a tumour positive SN at 3 years follow-up in 483 patients[10]. As they have mentioned, the study was underpowered due to a lower accrual rate than anticipated, moreover the majority of patients had a low SN tumour burden (≤1 mm) contributing to a low event rate, and reported results cover only the first three years of follow-up. The final results of DeCOG have to be awaited, as well as and more importantly the final results of the MSLT2 in order to be able to fully assess the value of CLND. Meantime, the presented data show that a limited delay in CLND (max 178 days after diagnosis) can be considered safe, as it did not affect survival or CLND tumour burden.

Due to the retrospective nature, this study is inevitably associated with limitations due to selection bias and missing data. Thus, the findings of this study should be interpreted with caution. From the initial study cohort consisting of 9 centres, only data from 8 centres could be included. Data from these centres were checked meticulously

Table 3. Uni- and Multivariable Cox Regression Analysis Melanoma Specific Survival

			Univariable			Multivariable		
Covariate		n	HR	95% CI	p	HR	95% CI	p
Centre	1	102	ref			ref		
	2	55	0.83	0.45 - 1.53	0.554	0.71	0.37 - 1.39	0.319
	3	228	1.62	1.10 - 2.39	0.016*	1.14	0.75 - 1.72	0.537
	4	114	0.61	0.36 - 1.03	0.066	0.50	0.28 - 0.89	0.019*
	5	110	0.84	0.51 - 1.36	0.469	0.71	0.43 - 1.19	0.193
	6	63	0.85	0.48 -1.50	0.576	0.74	0.41 -1.36	0.331
	7	55	0.50	0.25 - 1.01	0.055	0.68	0.33 - 1.40	0.294
	8	57	0.98	0.54 - 1.81	0.958	0.93	0.47 - 1.82	0.824
Gender	female	366	ref			ref		
	male	418	1.31	1.02 - 1.69	0.036*	1.29	0.98 - 1.69	0.070
Age	cont.	784	1.01	1.00 - 1.02	0.026*	1.01	0.99 - 1.02	0.188
Location	Extremity	380	ref			ref		
	Trunk	376	1.40	1.08 - 1.81	0.011*	1.29	0.97 - 1.72	0.079
	Head/neck	28	1.26	0.66 - 2.41	0.487	1.41	0.66 - 3.03	0.372
Histology	SSM	330	ref			ref		
	NM	300	1.54	1.16 - 2.06	0.003*	0.99	0.73 - 1.36	0.981
	Other	46	1.60	0.95 - 2.69	0.08	1.47	0.84 - 2.55	0.174
	Unknown	108	1.70	1.16 - 2.50	0.006*	1.32	0.87 - 2.03	0.196
Breslow	cont.	783	1.08	1.06 - 1.10	<0.0001*	1.06	1.04 - 1.08	<0.0001*
Ulceration	Absent	370	ref			ref		
	Present	379	2.22	1.69 - 2.90	<0.0001*	1.54	1.16 - 2.05	0.003*
	Unknown	35	2.01	1.12 - 3.60	0.020*	2.09	1.05 - 4.15	0.036*
SN tumour burden	<0.1mm	73	ref			ref		
	0.1-1.0mm	334	2.95	1.29 - 6.77	0.011*	2.59	1.11 - 6.07	0.028*
	>1mm	377	7.13	3.16 - 16.1	<0.0001*	5.55	2.05 - 11.1	<0.0001*
# nodes CLND	cont.	775	1.01	0.99 - 1.03	0.083	1.00	0.98 - 1.02	0.962
# positive non SNs	cont.	784	1.21	1.15 - 1.29	<0.0001*	1.24	1.15 - 1.33	<0.0001*
CLND interval	cont.	784	0.99	0.99 - 1.00	0.490	1.00	0.99 - 1.01	0.846

Univariable and multivariable Cox regression model for 5-year melanoma specific survival. Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; ref, reference value; cont., continuous; SSM, superficial spreading melanoma; NM, nodular melanoma; SN, sentinel node; CLND, completion lymph node dissection.

for any missing data on CLND (i.e. date of CLND, number of removed nodes and number of positive nodes) which was added when available; and additionally all follow-up was updated, creating a mature cohort of SN positive patients from 8 tertiary referral mela-

noma group centres. There was a significant difference between centres for the median time interval until CLND and for the proportion of patients with positive non-SNs, which is illustrated by the high odds ratios of some of the centres at logistic regression (**table 2**), and by the significantly lower HR for centre 4 at multivariable analysis. These findings can be explained partially by the variety of case-mix between centres; some centres had more patients with a high or low Breslow thickness or performed all surgeries within 60 days. To correct for these potentially confounding differences, adjustment for centre was performed in all multivariable analyses.

The time frame in which CLNDs were performed ranged from 0 – 178 days (IQR 65 – 105 days), making it impossible to draw any conclusions on the effects of CLNDs performed after this time frame. It could be that the turning point for potential therapeutic benefit lies outside the time interval reported in this study, but before the point of development of clinically evident lymph node involvement as used in the MSLTI and DeCOG[10, 22]. Unfortunately, extracapsular extension (ECE), known to be a prognostic indicator[23], was not available for the majority of the patients in this study. However, ECE is rarely seen in SN and CLND cases (2.2%)[12]. SN tumour burden was available for all patients, which also could clearly identify high risk patients at multivariable Cox regression survival analysis. As CLND timing was not significant at univariable nor at multivariable analysis after adjustment for confounding and prognostic indicators, the current study provides valuable information for daily clinical practice in which surgery is often still prioritized based on patient anxiety and potential reduction of doctor's delay due to long referral times. Ultimately MSLT 2 will provide a definitive answer on the question whether CLND is therapeutic (10 year follow up results anticipated in 2022)[9]. Until then, this study provides a valuable insight that there is no rush to perform a CLND, if chosen to be performed.

Conclusions

The current study shows that in this retrospective cohort of 784 SN positive melanoma patients, non-SN positivity and survival were not associated with CLND timing; indicating that it is safe to wait for at least 3 months (105 days, third quartile) after diagnosis, as there is no need to perform CLND as soon as possible. This information can be used to counsel patients and referring physicians and can potentially relieve pressure on the wait list.

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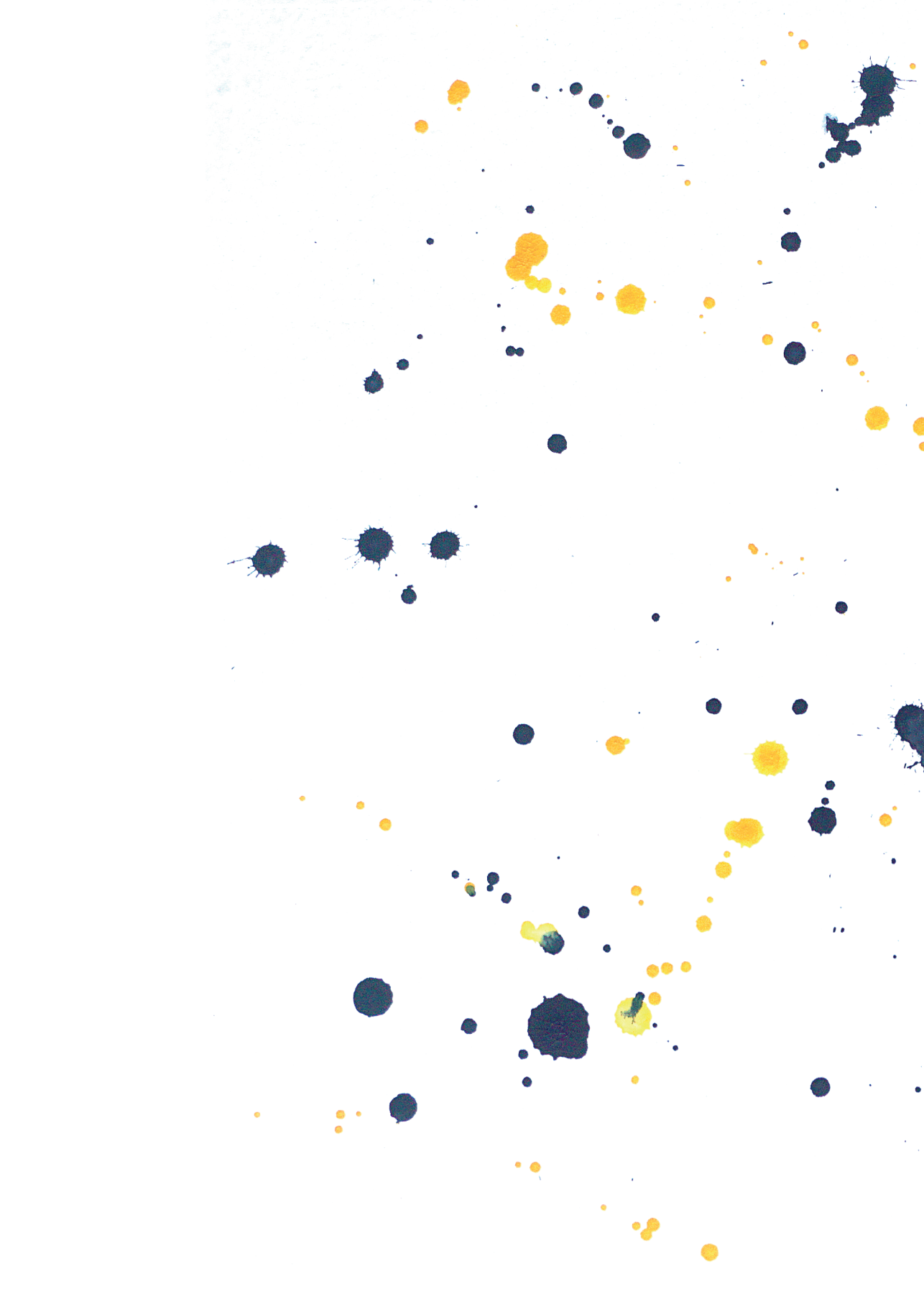
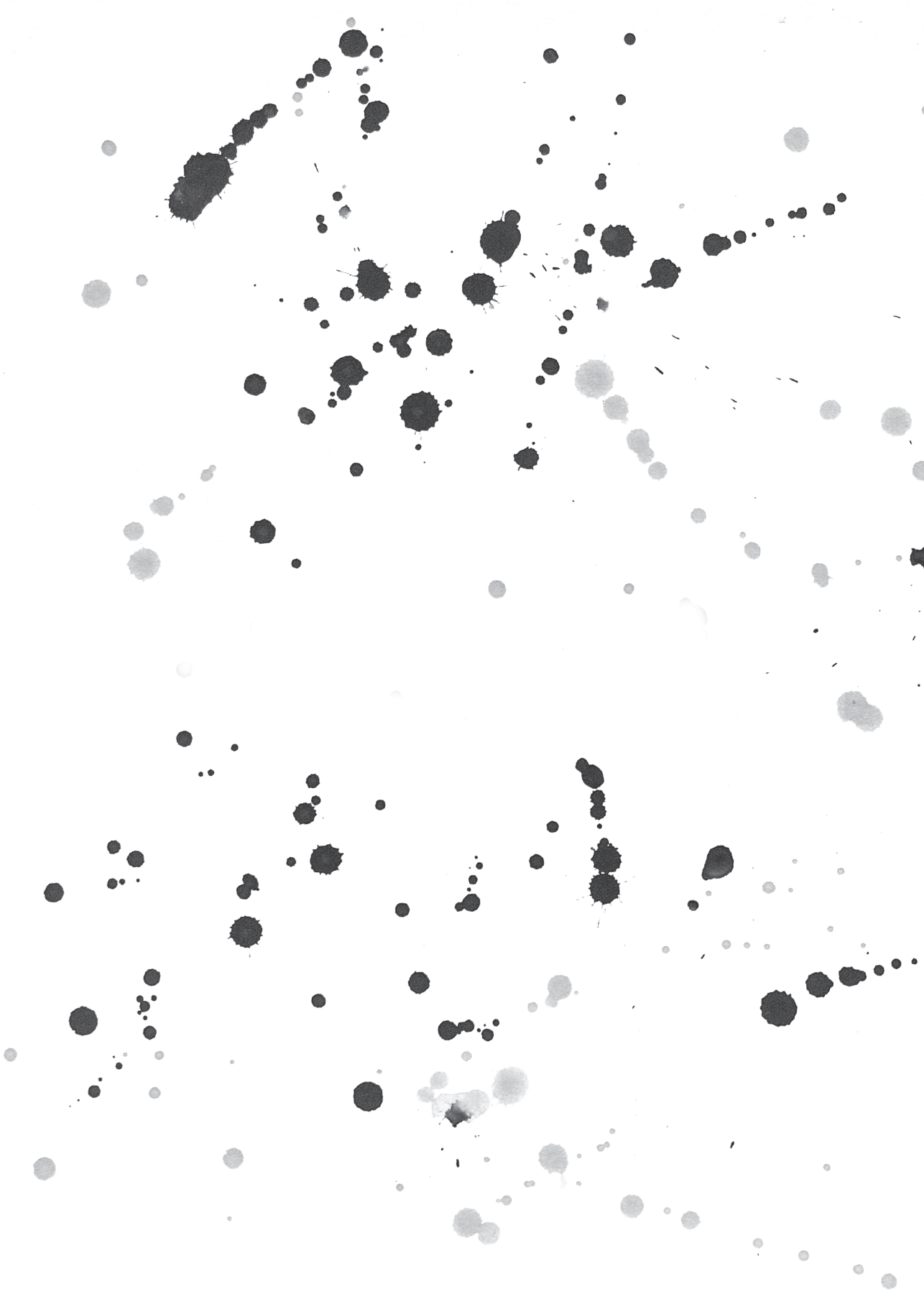


Part III – (Extent of) Groin Dissections



Chapter 9

Risk Factors for Positive Deep Pelvic Nodal Involvement in Patients with Palpable Groin Melanoma Metastases: Can the Extent of Surgery Safely Be Minimized? *A Retrospective Multicenter Cohort Study*

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Abstract

Background Patients with palpable melanoma groin metastases have a poor prognosis. There is debate whether a combined superficial (SGD) and deep groin dissection (CGD) is necessary or if SGD alone is sufficient. This study analyses risk factors for deep pelvic nodal involvement.

Methods This retrospective multicenter cohort study concerned 209 therapeutic CGDs from four tertiary centers in the Netherlands (1992–2013), selected based on complete preoperative imaging and pathology reports. Analyzed risk factors included baseline and primary tumor characteristics, total and positive number of inguinal nodes, inguinal lymph node ratio (LNR) and positive deep pelvic nodes on imaging (CT ± PET, or PET - low dose CT).

Results Median age was 57 years, 54% was female, median follow-up was 21 months (inter quartile range (IQR) 11-46 months). Median Breslow thickness was 2.10mm (IQR 1.40-3.40mm), 26% was ulcerated. Positive deep pelvic nodes occurred in 35% of CGDs. Significantly fewer inguinal nodes were positive in case of negative deep pelvic nodes; median 1 (IQR 1-2) versus 3 (IQR 1-4) for positive deep pelvic nodes ($p<0.001$). LNR was significantly lower for negative versus positive deep pelvic nodes; median 0.15 (IQR 0.10-0.25), versus 0.33 (IQR 0.14-0.54) ($p <0.001$). Combination of negative imaging, low LNR, low number of positive inguinal nodes and no extracapsular extension could accurately predict absence of pelvic nodal involvement in 84%.

Conclusion Patients with negative imaging, few positive inguinal nodes, no extracapsular extension and low LNR, have low risk of positive deep pelvic nodes and may safely undergo SGD alone.

Introduction

Patients with clinically palpable nodal metastases of cutaneous melanoma in the groin have a poor prognosis. Balch et al. reported a 5-year overall survival (OS) rate of 59% for stage IIIB melanoma in the 2009 AJCC melanoma staging system analysis[1]. Reported 5-year OS rates for the subgroup of patients with palpable groin metastases range from 52% for superficial involvement to 12% for deep involvement[2-7].

Standard of care for these patients consists of therapeutic lymph node dissection (TLND)[2, 8-10]. There is ongoing debate, whether this should consist of either a combined superficial and deep groin dissection (CGD) or that merely a superficial groin dissection (SGD) would suffice.

Several cohort studies seem to indicate no difference in survival between these two procedures, and patients may benefit from SGD alone if no positive deep pelvic nodes are present on preoperative imaging [2, 8, 10-12].

Since the estimated prevalence of positive deep pelvic nodes in patients with palpable inguinal lymph nodes is 30%, the majority of patients undergoing CGD may not benefit from deep groin dissection (DGD)[6, 12]. As CGD is a more extensive procedure than SGD, the risk of morbidity is potentially higher[6]. A clear need exists to select those patients who can be safely spared a DGD in absence of deep pelvic nodal involvement[10, 11, 13-15].

Preoperative imaging techniques such as CT and positron emission tomography (PET) form a valuable adjunct to staging. Up to 27% of patients presenting with palpable lymph node metastases have synchronous distant metastases at preoperative PET/CT, which changes the indication for surgery into palliative resection and/or systemic therapy[16]. Additionally, imaging provides assessment of suspicious deep pelvic nodes prior to surgery. High positive (PPV) and negative predictive value (NPV) have been achieved by Allan et al, (100% and 86% respectively)[3]. Other series reported PPV and NPV of 40-60%, which is too low to confirm or reject presence of positive deep pelvic nodes based on preoperative imaging alone[2, 17, 18]. Once suspicious deep pelvic nodes are detected on preoperative imaging, one cannot ignore their presence, and CGD is highly recommended. Absence of suspicious deep pelvic nodes on imaging does not rule out deep pelvic nodal involvement. Once imaging has been performed the focus should be on identification of further risk factors for positive deep pelvic nodes[2, 7, 11, 15, 17-21].

Aim: to analyze risk factors for deep pelvic nodal involvement in a retrospective multicenter cohort of palpable groin melanoma metastases. This could aid in the development of an algorithm for selective surgery in the future.

Patients and Methods

Patients

This retrospective multicenter cohort study describes 209 therapeutic CGDs performed at four tertiary melanoma centers in the Netherlands between 1992 and 2013. Patient selection was based on presence of a palpable nodal metastasis to the groin, complete pathology reports of the performed CGD, (i.e. clearly describing the dissected lymph nodes as inguinal or iliac, including obturator area), and preoperative imaging (CT, PET or PET/CT). Patients without imaging, with prior lymph node dissections in the groin area or with isolated limb perfusion or positive sentinel node(s) as indication for CGD were excluded. Analyzed preoperative imaging modalities were: CT scan, PET, and combined PET with low dose CT (PET-CT).

All patient characteristics were obtained from medical records and collected in a database for the current retrospective multicenter cohort study, according to local institutional review committee guidelines and national legislation.

Surgical Procedure

CGD was performed either via two separate transverse incisions, or via an inguinal ellipse shaped incision extending cranially according to local preferences per center, as described in detail elsewhere[6, 22].

Pathology

CGD pathology reports were considered adequate when clear description was given of the total number of inguinal nodes, as well as the number of tumor positive inguinal nodes, and similar description was given of the number of dissected deep pelvic nodes (iliac nodes and obturator nodes) and the number of tumor positive deep pelvic nodes.

Statistics / Data analysis

Patients were divided into two categories based on deep pelvic nodal status: positive or negative. Univariable Chi-squared tests were performed to test for significant differences in prevalence of gender, primary tumor located on the trunk, primary tumor stage (T1-T4), ulceration, and inguinal extracapsular extension (ECE). Nonparametric tests were performed to test for differences in age, median Breslow thickness, total number of inguinal nodes and number of positive inguinal nodes, total number of excised nodes and number of positive nodes, total number of deep pelvic nodes, number of positive deep pelvic nodes, and LNR. Sensitivity, specificity, PPV, NPV, and accuracy were calculated for all imaging modalities using number of true positives (TP), false positives (FP), true negatives (TN) and false negatives (FN).

Differences in baseline characteristics were tested with univariable logistic regression analysis. Multivariable models were calculated using variables significant at univariable

analysis. Binary logistic multivariable regression analyses were performed to test for independent predictors of deep pelvic nodal involvement.

Ridge regression analysis was performed to exclude the influence of multicollinearity in a prediction model based on independent predictive variables. An area under the receiver operating characteristic curve (AUC) was calculated for the model. The AUC indicates the probability that patients with observed positive deep pelvic nodes had a higher predicted probability than patients with observed negative deep pelvic nodes, providing information about the predictive value of the model.

All statistical analyses except Ridge regression were performed using SPSS Version 21.0 (IBM Corp. Released 2012. Armonk, NY, United States). Ridge regression was performed using RStudio (RStudio Inc. Boston, Massachusetts, United States). An $\alpha < 0.05$ was considered significant.

Results

Patients

Table 1 gives an overview of baseline characteristics. The majority of patients (n=201, 96%) had palpable stage IIIB disease, eight patients (4%) had stage IV disease. Median Breslow thickness was 2.10 mm (IQR 1.4 - 3.4 mm). Twelve patients had a history of negative sentinel node. Median follow-up was 21 months (IQR 11 - 46 months).

Imaging and Pathology

Four patients underwent both CT and PET/CT, they were scored as PET/CT since the additional information obtained from PET/CT was used for final determination of clinical nodal status. Predictive accuracy per imaging modality is shown in **table 2**. The different imaging modalities were used equally between the two groups (i.e. positive or negative deep pelvic nodes).

Logistic regression analysis

Variables significantly different on univariable analysis (**table 1**) were included in multivariable binary logistic regression analyses. LNR and number of positive inguinal lymph nodes were assessed in separate models due to evident multicollinearity. Remaining significant independent predictors were: suspicious deep pelvic nodes on imaging (odds ratio (OR) 9.64, 95% CI 4.35 – 21.3, $p < 0.001$), increasing LNR (OR 34.2, 95% CI 5.47 – 214, $p < 0.001$), presence of ECE (OR 2.13, 95% CI 1.01 – 4.48, $p = 0.046$), and in a separate multivariable model without LNR: increasing number of positive inguinal lymph nodes (OR 1.27, 95% CI 1.06 – 1.53, $p = 0.010$).

Table 1. Baseline Characteristics.

Characteristic	*Total n=209 (100%)*	*Pelvic Nodes - n=135 (65%)*	*Pelvic Nodes + n=74 (35%)*	*P*
	n,(%)	*n, (%)*	*n, (%)*	
Gender				
Female	114 (54)	76 (56)	38 (51)	
Male	95 (46)	59 (44)	36 (49)	0.49
Age (yrs) median (IQR)	57 (45 - 65)	55 (46 - 65)	59 (44 - 65)	0.63
Center				
1	60 (29)	42 (31)	18 (24)	
2	57 (27)	38 (28)	19 (26)	
3	24 (12)	11 (8)	13 (18)	
4	68 (32)	44 (33)	24 (32)	0.21
Tumor stage				
T1	22 (11)	9 (7)	13 (18)	
T2	57 (27)	36 (27)	21 (28)	
T3	60 (29)	39 (29)	21 (28)	
T4	30 (14)	22 (16)	8 (11)	
Unknown primary	10 (5)	8 (6)	2 (3)	
Missing	30 (14)	21 (15)	9 (12)	0.16
Ulceration				
Absent	125 (60)	74 (55)	51 (69)	
Present	54 (26)	38 (28)	16 (22)	
Missing	30 (14)	23 (17)	7 (9)	0.16
Clark level**				
II	1 (0.5)	0 (-)	1 (1)	
III	35 (17)	22 (16)	13 (17)	
IV	84 (40)	54 (40)	30 (41)	
V	13 (6)	11 (8)	2 (3)	
Missing	76 (36.5)	48 (36)	28 (38)	0.29
Location				
Leg	166 (80)	106 (79)	60 (81)	
Trunk	28 (13)	17 (13)	11 (15)	
Unknown primary	10 (5)	8 (6)	2 (3)	
Missing	5 (2)	4 (3)	1 (1)	0.62
Histology				
SSM	67 (32)	44 (32)	23 (31)	
NM	37 (18)	28 (21)	9 (12)	
Other	15 (7)	9 (7)	6 (8)	
Unknown primary	10 (5)	8 (6)	2 (3)	
Missing	80 (38)	46 (34)	34 (46)	0.24

Table 1. Baseline Characteristics. (continued)

Characteristic	*Total n=209 (100%)*	*Pelvic Nodes - n=135 (65%)*	*Pelvic Nodes + n=74 (35%)*	*P*
	n,(%)	*n, (%)*	*n, (%)*	
Nº nodes, median (IQR)				
Inguinal	10 (7 - 13)	10 (7 - 13)	9 (7 - 12)	0.54
Deep	6 (4 - 10)	6 (4 - 9)	8 (5 - 11)	0.039*
Total	17 (13 - 22)	17 (13 - 21)	17 (14 - 22)	0.39
Nº positive nodes, median (IQR)				
Inguinal	2 (1 - 3)	1 (1 - 2)	3 (1 - 4)	<0.001*
Deep	0 (0 - 1)	0 (0)	2 (1 - 3)	<0.001*
Total	2 (1 - 4)	1 (1 - 2)	5 (3 - 7)	<0.001*
LNR				
median (IQR)	0.20 (0.11 - 0.33)	0.15 (0.10 - 0.25)	0.33 (0.14 - 0.54)	<0.001*
Inguinal ECE				
No	134 (64)	94 (70)	40 (54)	
Yes	75 (36)	41 (30)	34 (46)	0.025*

n, number of patients; P, p-value; yrs, years; IQR, inter quartile range; T1, Breslow <1.00mm; T2, Breslow 1.01-2.00mm; T3, Breslow 2.01-4.00mm; T4, Breslow >4.00mm; Nº, number of; LNR, inguinal lymph node ratio; ECE, extracapsular extension.
**significant, p<0.05. Calculated with Chi-square and non-parametric tests ** Clark level II and III were combined for Chi-Square test.*

Table 2. Identification of Positive Deep Pelvic Lymph Nodes Using Preoperative Imaging Techniques (n=209).

	CT (n=67)	CT and/ or PET (n=57*)	PET/CT (n=85**)
Sensitivity	57%	36%	61%
Specificity	93%	94%	83%
PPV	80%	73%	68%
NPV	83%	70%	79%
Accuracy	82%	70%	75%

Abbreviations: CT, computed tomography; PET, position emission tomography; PET/CT, combined PET and low dose CT; PPV, positive predictive value; NPV, negative predictive value.*; 13 patients underwent PET alone. **, 4 patients underwent separate CT as well.

Subgroup analysis negative imaging

Suspicious deep pelvic nodes on imaging were highly predictive for positive deep pelvic nodes. A subgroup of 155 patients without suspicious deep pelvic nodes on imaging was selected for further analysis of additional risk factors for positive deep pelvic nodes. Thirty-five of these patients (23%) had positive deep pelvic nodes at histopathological examination with H&E staining, i.e. imaging was false negative. Univariable analysis results are displayed in **table 3**. Multivariable analysis was performed including all

Table 3. Baseline Characteristics for patients with negative preoperative imaging.

Characteristic	Total (n=155)	Pelvic nodes – (n= 120)	Pelvic nodes + (n= 35)	P
	n (%)	n (%)	n (%)	
Gender				
Female	83 (54)	67 (56)	16 (46)	
Male	72 (46)	53 (44)	19 (54)	0.29
Age (yrs) median (IQR)	56 (45 - 64)	55 (46 - 65)	57 (44 - 64)	0.99
Center				
1	44 (28)	38 (32)	6 (17)	
2	48 (31)	35 (29)	13 (37)	
3	18 (12)	10 (8)	8 (23)	
4	45 (29)	37 (31)	8 (23)	0.17
Breslow median (IQR)	2.10 (1.40 - 3.25)	2.20 (1.45 - 3.55)	1.90 (1.15 - 2.80)	0.11
Tumor stage				
T1	14 (9)	8 (7)	6 (17)	
T2	45 (29)	33 (28)	12 (34)	
T3	44 (28)	37 (31)	7 (20)	
T4	23 (15)	19 (16)	4 (11)	
Unknown primary	9 (6)	7 (6)	2 (6)	
Missing	20 (13)	16 (13)	4 (11)	0.38
Ulceration				
Absent	90 (58)	67 (56)	23 (66)	
Present	44 (28)	36 (31)	8 (23)	
Missing	21 (14)	17 (13)	4 (11)	0.34
Clark level**				
II	1 (0.6)	0 (-)	1 (3)	
III	25 (16)	20 (17)	5 (14)	
IV	65 (42)	49 (41)	16 (46)	
V	11 (7)	11 (9)	0 (-)	
Missing	53 (34)	40 (33)	13 (37)	0.070
Location				
Leg	118 (76)	93 (78)	25 (71)	
Trunk	23 (15)	16 (13)	7 (20)	
Unknown primary	9 (6)	7 (6)	2 (6)	
Missing	5 (3)	4 (3)	1 (3)	0.81
Histology				
SSM	52 (34)	40 (33)	5 (14)	
NM	31 (20)	26 (22)	12 (34)	
Other	10 (6)	8 (7)	2 (6)	
Unknown primary	9 (6)	7 (6)	2 (6)	

Table 3. Baseline Characteristics for patients with negative preoperative imaging. (continued)

Characteristic	Total (n=155)	Pelvic nodes – (n= 120)	Pelvic nodes + (n= 35)	P
	n (%)	n (%)	n (%)	
Missing	53 (34)	39 (32)	14 (40)	0.86
Nº nodes, median (IQR)				
Total	17 (13 - 21)	17 (13 - 21)	17 (14 - 22)	0.42
Inguinal	10 (8 - 12)	10 (8 - 13)	9 (8 - 12)	0.69
Deep	6 (4 - 9)	6 (4 - 9)	7 (4 - 11)	0.15
Nº positive nodes, median (IQR)				
Total	2 (1 - 4)	1 (1 - 2)	5 (3 - 6)	<0.001*
Inguinal	1 (1 - 3)	1 (1 - 2)	3 (1 - 4)	<0.001*
Deep	0 (0)	0 (0)	2 (1 - 2)	<0.001*
LNR, median (IQR)	0.17 (0.11 - 0.31)	0.21 (0.10 - 0.25)	0.33 (0.13 - 0.50)	0.001*
ECE inguinal				
No	96 (62)	79 (66)	17 (49)	
Yes	59 (38)	41 (34)	18 (51)	0.075

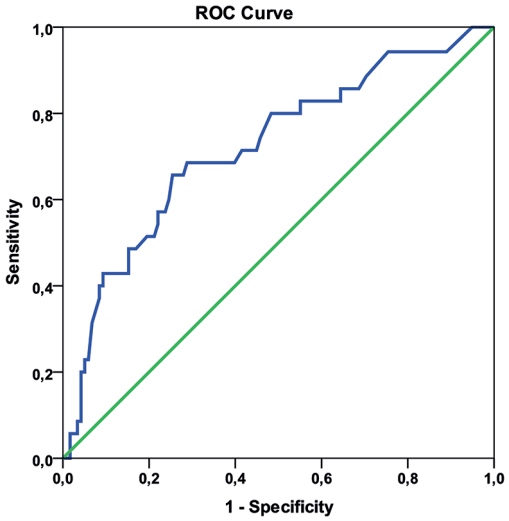
n, number of patients; P, p-value; ; yrs, years; IQR, inter quartile range; T1, Breslow <1.00mm; T2, Breslow 1.01-2.00mm; T3, Breslow 2.01-4.00mm; T4, Breslow >4.00mm; Nº, number of; LNR, inguinal lymph node ratio; ECE, extracapsular extension.

*, significant (p<0.05) ** for Chi-Square test Clark II & III were combined

significant variables assumed to be predictive for deep pelvic nodal status; number of positive inguinal nodes, LNR and ECE status. Evident multicollinearity was observed.

To overcome this problem a predictive Ridge logistic regression analysis was performed. Only LNR remained as significant independent predictor for positive deep pelvic nodes (p = 0.014). Number of positive inguinal lymph nodes and ECE were chosen to remain in the model as contributing covariates as these were thought to be of substantial additional clinical relevance. A receiver operating characteristic (ROC) curve of the predicted probabilities for positive deep pelvic nodes was created, displaying a fair AUC of 0.72 (AUC values range between 0 and 1, where high scores are indicative of high accuracy) (**Figure 1**).

The optimum cut-off value for the predicted probability of the model (i.e. the probability at which the model outcome correctly identifies an observed positive patient as positive) was chosen based on high specificity, in order to minimize false negative outcomes. Corresponding probability cut-off value and sensitivity were deduced from the ROC curve. For a specificity of 90%, the cut-off value for a positive test outcome was a probability for positive deep pelvic nodes of 32% or more. Sensitivity was 43%, PPV was 50%, NPV 84%, and overall accuracy of this model was 77%.



Area Under the Curve

Test Result Variable: Probability Deep Positive Nodes

AUC	SE[a]	Asymptotic Sig.[b]	Asymptotic 95% CI Lower Bound	Asymptotic 95% CI Upper Bound
,722	,051	,000	,622	,821

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5
AUC, area under the curve; SE, standard error; Sig., significance; 95% CI, 95% confidence interval.

Figure 1. ROC curve for prediction model probability positive deep nodes.
ROC; receiver operator characteristics.

Discussion

In this CGD cohort 35% of all patients have deep pelvic nodal involvement, which is in line with the literature[10, 11, 13-15]. This study analyses risk factors to identify deep pelvic nodal involvement. Imaging is a strong predictor. Our prediction model might lower the rate of CGD without positive pelvic nodes and minimizes the number of false negative outcomes after imaging.

Imaging

The imaging modalities used in this study are fair in correctly predicting positive deep pelvic nodes. Still a considerable number of patients have false positive imaging (20-32%). We can only speculate on the possible causes of false positive imaging. This might be partially explained by a small group of patients undergoing diagnostic excision biopsy of the palpable lymph node prior to imaging. This might cause a lymph node enlargement in the pelvic area. Another cause may be the inevitable interobserver vari-

ability in radiology. Improvement of imaging techniques over time may have altered the number of false positive lymph nodes detected during the present study period.

NPVs of the preoperative imaging techniques performed in the current study range between 70% and 83%, leaving a substantial proportion of 23% (17-30%) of patients to be falsely diagnosed with negative deep pelvic nodes. Several studies have reported on NPV of CT, and although high NPVs have been described by Allan et al, and Van der Ploeg et al., overall reported values range considerably [2, 3, 6, 17, 18]. Ongoing development of the newest imaging techniques such as use of a melanoma specific PET tracer ([18F] ICF01006) may enhance the accuracy of imaging and subsequently decrease the FN rate[23].

Predictive factors

Predictive factors for deep pelvic nodal involvement found in the current study are inguinal nodal status as defined by the number of positive inguinal nodes and LNR, inguinal ECE, and suspicious deep pelvic nodes on preoperative imaging, which is concordant with the literature [2, 7, 11, 15, 17-21]. These risk factors may be applied to select patients for SGD in addition to imaging without suspicious deep pelvic nodes. A hypothetical two stage approach would be: when preoperative imaging is negative, patients first undergo solely an SGD. The pathology results can then be used to determine the risk of occult positive deep pelvic nodes, and a decision can be made on whether to perform an additional DGD or not. The fact that patients must undergo two separate operations is a drawback, but this way, a DGD can be spared in 126 out of all patients (60%).

Patient Selection

Standard CGD for palpable stage III melanoma shows that 135 out of 209 deep pelvic groin dissections (65%) have been performed in the absence of pelvic nodal metastases.

Use of pre-operative imaging alone for selection between CGD and SGD would reduce the number of CGDs from 209 to 54. The remaining 155 patients would undergo SGD alone. Thirty-five of these 155 patients undergoing SGD alone are false negative (FN rate 23%), and would be possibly undertreated (i.e. undergoing no DGD).

Better patient selection is necessary in the negative imaging group, as potential decrease in the number of false negatives will make patient selection safer. This formed the rationale for the prediction model, which is based on 153 patients* (155 - 2 patients, *missing data) with negative imaging. Using this model 124 out of 153 patients would undergo SGD alone, and FN rates would be reduced to 20 out of 124 patients (FN rate 16%).

Concluding, this model forms an adjunct to the use of preoperative imaging as selection tool for SGD or CGD, both drastically minimizing the number of patients without

affected pelvic nodes undergoing a DGD, and controlling the number of patients with affected pelvic nodes potentially being undertreated by not undergoing a DGD.

The 16% FN rate of this model is still considerable. Although surgery forms the cornerstone of melanoma treatment, one may question the role of DGD in the current era of upcoming effective systemic treatments. On one hand, the majority of patients undergoing standard CGD for palpable groin metastases have negative deep pelvic nodes. On the other hand, there is evidence to assume that positive deep pelvic nodes may merely be a biomarker for stage IV disease, as survival rates depend on deep pelvic nodal status rather than extent of surgery [6, 8, 11, 12, 15]. Khosrotehrani and Van der Ploeg presented a nomogram for prediction of prognosis in stage III B/C melanoma patients, using pathology results and age[24]. Application of this nomogram could further aid in selecting patients for SGD alone. Another preoperative aid besides the presented model could be use of the biomarker S-100B. As Kruijff et al. have shown, high serum levels of S-100B are associated with a significantly lower DFS and a trend towards worse melanoma specific survival (MSS), indicating its potential as a biomarker for clinically occult stage IV disease [25, 26]. Patients with low risk of deep pelvic nodal involvement and low S-100B could then undergo SGD alone, with regular control visits to detect early signs of deep pelvic nodal involvement (suspicious nodes on imaging/elevated S-100B). Bearing this in mind, the 16% FN rate of the presented prediction model may be allowable.

Limitations

This study is retrospective and is spread over a long timeframe. This entails inevitable alterations and improvement of imaging techniques and clinical practice over time, affecting our results. The prediction model designed for the current study has not been validated internally, due to a small sample of patients with positive deep pelvic nodes. It has to be pointed out that this model in its current state is not suited for clinical use, as there is still much to be gained from further development and testing. A prospective multicenter registration study is planned to be performed, enabling adequate data collection on all patients undergoing CGD for palpable groin metastases within a relatively small time frame. Cross validation of the presented prediction model will be performed, and its role in future clinical practice will be further defined. With the proposed prospective study, accuracy of imaging techniques can be determined more adequately.

Concerning the possible additional morbidity of a DGD; although to date no prospective randomized controlled trial (RCT) has been performed to address this, evidence exists that the additional morbidity of DGD in a CGD might be more limited than has been described in the past.[6, 22] The recently opened Australia and New Zealand Melanoma Trials Group 01.12 EAGLE FM Trial (clinicaltrials.gov identifier: NCT02166788) will hopefully provide an answer to this question. This multicenter RCT compares SGD and CGD for melanoma patients with groin metastases and no suspicious PET/CT scan.

As operating time is generally longer in a CGD, there is a potentially higher risk of surgical site infections. In a large retrospective series of Glarner et al. the number of surgical site infections is indeed significantly higher for CGDs, with an adjusted odds ratio of 2.6[27]. Once again, to gain more insight in the actual differences in morbidity between SGD and CGD, we will have to await results from the EAGLE FM Trial.

Concluding, high LNR, high number of positive inguinal nodes and inguinal ECE are risk factors for positive deep pelvic nodes in patients with palpable groin metastases of cutaneous melanoma. To date, accurate prediction of deep pelvic nodal status is suboptimal still, hence reliable selection of patients who can be spared a DGD remains difficult. Combined use of preoperative imaging and a preliminary prediction model based on histopathology results of the inguinal (superficial) part of CGD could accurately predict negative deep pelvic nodes in up to 84%. Thereby potentially identifying a group of low risk patients, in whom the extent of surgery might safely be minimized. The risk factors and the prediction model will be further investigated in a prospective multicenter registry trial for CGDs.

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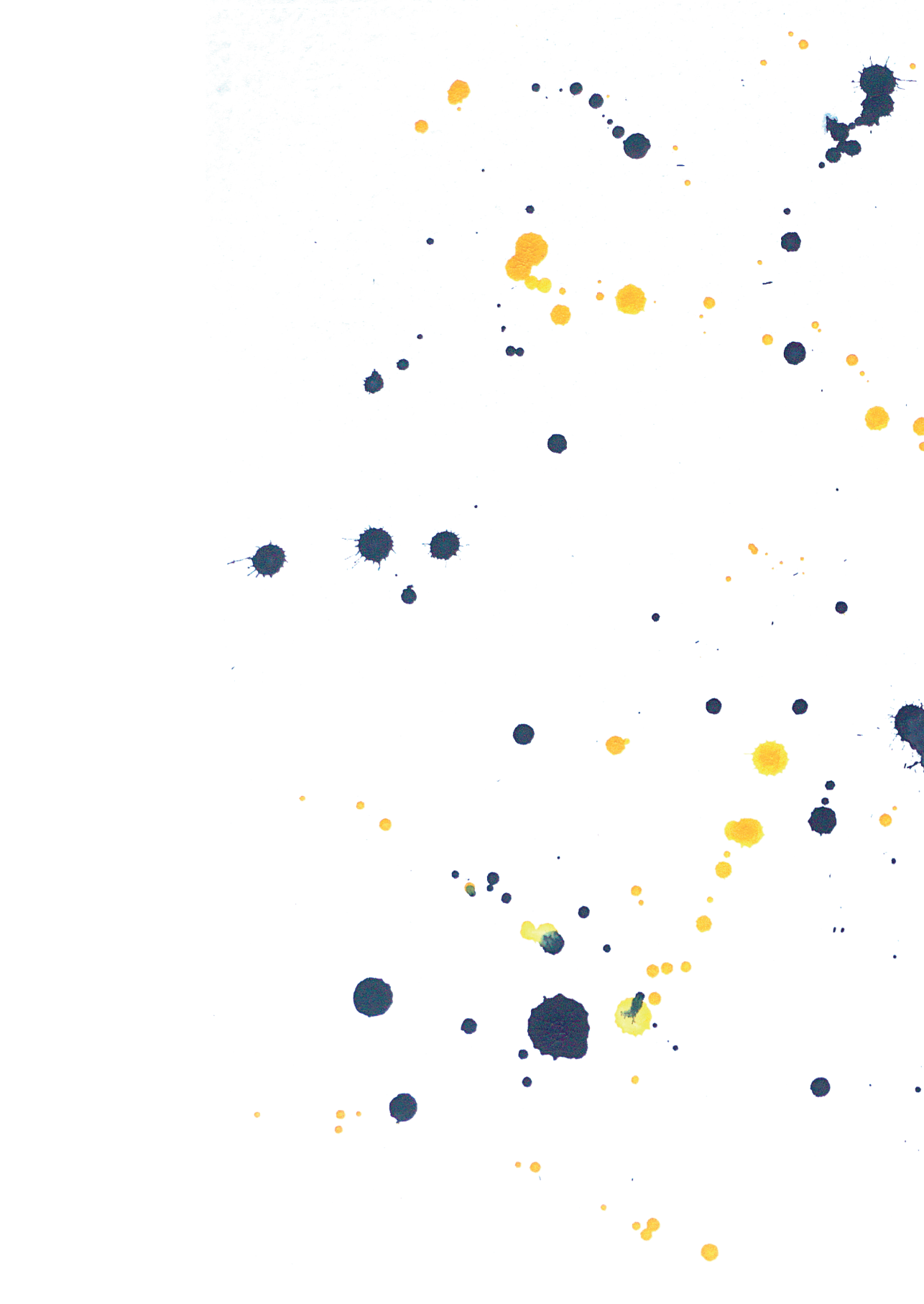
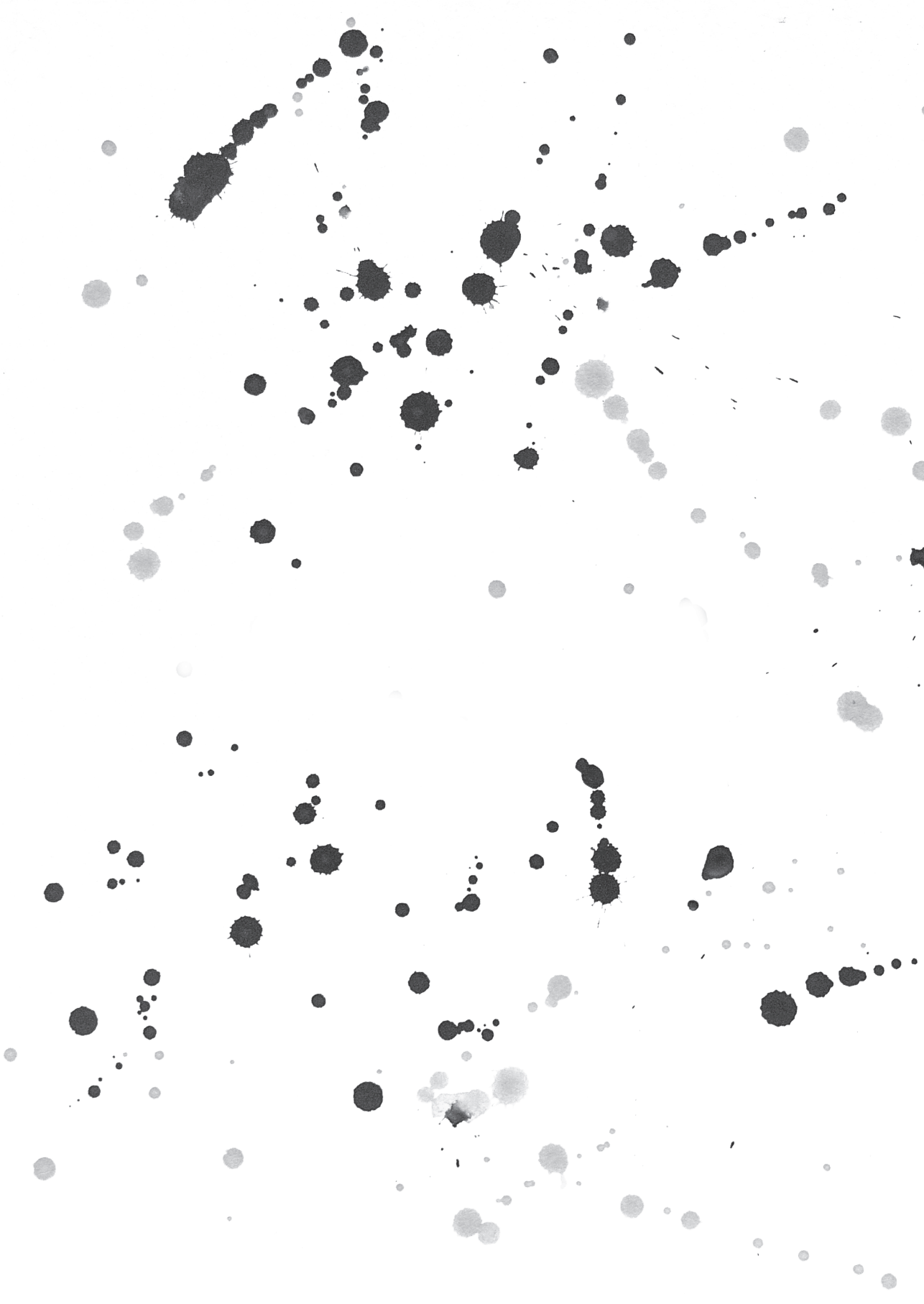


Part IV – General Discussion and Summary



Chapter 10

General Discussion and Future Perspectives

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General Discussion

Breslow thickness is the most powerful prognostic feature for primary cutaneous melanoma's, closely followed by ulceration[1]. Presence of nodal metastases is another major discriminator in the American Joint Committee on Cancer (AJCC) staging system, dividing patients between stage I or II (no nodal involvement) and stage III (regional nodal involvement or in transit metastases). Finally, stage IV is defined by the presence of distant nodal involvement and/or visceral metastases[1].

Nodal Staging

There is abundant evidence that nodal metastases in melanoma patients equal poor survival[1, 2]. In clinically node negative patients, sentinel node biopsy (SNB) has proven to be a highly discriminative nodal staging tool to further differentiate between patients with a good prognosis (sentinel node (SN) negative) and patients with a less favorable prognosis (SN positive)[3, 4]. As there is no proven therapeutic effect for survival by performing a SNB, it is important to critically reassess the potentially negative aspects of this minimally invasive, albeit invasive nonetheless, surgical staging procedure.

While universally recommended as a staging procedure, not all eligible melanoma patients undergo SNB. This is unlike the practice in other malignancies such as breast cancer. There are several explanations for this discrepancy between guideline recommendations and clinical practice, namely the absence of solid evidence regarding therapeutic effect on survival[5], discrepancies in local health care reimbursements[6, 7], and socio-economic status[8].

In the following paragraphs the indication for SNB is discussed, as well as potential minimally invasive alternatives.

First, recommendations on the indication for SNB will be re-evaluated here. SNB is generally advised for intermediate and thick melanomas: i.e. >1.00mm – 4.00mm or >4.00mm with or without ulceration[9, 10]. In melanomas <1.00mm the risk of a positive SN is minimal, thus standard SNB is not recommended[9]. Although melanoma survival for thin melanomas (Breslow thickness <1mm, pT1) is excellent, it does not equal 100%. This is due to a minority of patients who will develop metastases over time and ultimately die due to melanoma[1, 11]. As the majority of currently diagnosed new cutaneous melanomas consists of thin melanomas (Breslow thickness <1mm, pT1) without clinically evident lymph node metastases[1, 12, 13], additional risk factors have been investigated in order to select those patients who have a high risk of developing nodal metastases, in order to consider SNB for this subgroup as well. As described in detail in **Chapter 2**, several primary tumor features have served as high risk feature, being ulceration (AJCC 6th and 7th edition) high Clark level (IV or V, AJCC 6th edition), or mitotic rate of ≥1 mitosis/mm^2 (AJCC 7th edition)[1, 14]. Since the implementation of the 7th edition AJCC staging system virtually

all melanoma guidelines adopted the recommendation to consider SNB for high risk thin (pT1b) melanomas[9, 15, 16]. **Chapter 2** reports on the effects of these changes in SNB recommendation in the Dutch pT1 melanoma population. No increase in SN positivity rate occurred in this group of patients, remaining <10%. It is questionable whether a surgical staging procedure is the optimal way to go to confirm that 90% of pT1b patients are node negative.

Thick melanomas (pT4) pose a different challenge. Since Breslow thickness and ulceration are the main prognostic features in primary melanoma, and ulceration occurs more often in thicker melanomas, patients with a pT4 melanoma have an a priori worse prognosis than intermediate thickness melanomas, regardless of their nodal status. They form the grey zone between stage II and stage III as was illustrated by Balch et al. in the 7th edition of the AJCC staging system[1]. Thus it is questioned whether nodal staging with SNB adds any substantial information on prognosis in pT4 patients. Nevertheless, several studies have shown that SNB is an accurate discriminator for prognosis in this group as well[3, 17, 18].

Next to reassessment of the indication for SNB, minimally invasive alternatives deserve further attention. **Chapter 3, 4**, and **5** provide varying methods of combined ultrasound (US) and FNAC for non-operative examination of the SN. In **Chapter 3** a new US morphology criterion is presented; the echo free island (EFI). While an infrequent finding in US assessment of the SN, it is associated with presence of peripheral perfusion (PP), another US criterion. Five-year melanoma specific survival was worse for patients with EFI: 80% versus 92% when absent. EFI is found to be a discriminatory US morphology sign which can be useful for early identification of SN metastases in melanoma patients. **Chapter 4** describes the long-term survival results of combined US and FNAC prior to SNB in 1,000 patients. Survival analyses demonstrated that patients with positive US-FNAC had poor survival. Patients with suspicious US and negative FNAC and patients with normal US had comparable survival. A step-wise approach to melanoma patients is supported by these results: in case of a positive FNAC and/or clearly malignant US finding patients can be spared a SNB and be offered a lymphadenectomy instead. In case of suspicious US and negative FNAC, patients could be offered continued US surveillance or SNB for higher risk primary tumors. Completely US-FNAC negative patients might only require follow-up and no SN staging, with continued US surveillance as addendum for high risk T3/4 and/or ulcerated primaries. In **Chapter 5** an overview of the literature on ultrasound assessment of the SN is presented, as well as a pilot and study protocol for Gamma probe and ULtrasound guided Fine needle aspiration cytology of the sentinel node Trial (GULF trial). The literature on pre-operative assessment of regional lymph nodes with US in clinically N0 melanoma patients is disparate. Targeted US-FNAC or other new techniques have the potential to replace SNB in the future, however, the reported findings need to be replicated in prospective clinical trials. A pilot

with gamma probe guided US-FNAC shows accurate SN identification in up to 90% of patients. The presented GULF trial study protocol may provide potential improvement to the reported US-FNAC techniques, and ultimately may become a possible replacement of the SNB. While none of the abovementioned procedures have reached the accuracy of the surgical SNB yet, further tailoring of these techniques may change that situation and lead to minimally invasive assessment of SN status. Simultaneously, other minimally invasive techniques have been developed and are currently being tested in clinical trial setting; for instance sonoelastography[19, 20] and multispectral optoacoustic tomography[21], addressed in **Chapter 5**. Based on the currently available evidence, nodal staging in clinically node negative patients is worthwhile for patients with a Breslow thickness of >1mm, considering that below this Breslow thickness the risk of a positive SN is less than 10%. Since all SN positive patients may potentially benefit from adjuvant immunotherapy, performing SNB in thick melanomas (>4mm) can be justified as well.

Thirdly, the approach of nodal staging in daily clinical practice requires re-evaluation. The decision on whether there is an indication for SNB should be made by a well informed and experienced doctor, preferably a melanoma surgeon or - dermatologist. The next question is when to perform SNB. Ideally this is done as soon as possible, in order to provide information on prognosis to the patient in a short period of time after the initial melanoma diagnosis. The current Dutch melanoma guideline even poses a strict time limit for SNB to be performed within 6 weeks, suggesting a potential detrimental effect if not complied with, without evidence to support this cut-off[22].

Considering the fact that there is a global increase in melanomas, and general practitioners and dermatologists increasingly tend to perform high urgency referrals, increased pressure on wait lists can be expected. Potential effects of SNB timing, and subsequently completion lymph node dissection (CLND) timing in case of positive SNs were investigated in **Part II** of this thesis. No difference in recurrence free survival or melanoma specific survival was found for SN positive patients (**Chapter 6**), or SN negative patients (**Chapter 7**), nor a difference in SN positivity rates (**Chapter 7**). Timing of CLND was also not relevant for survival in the cohort investigated in **Chapter 8**. While these are all retrospective studies, they provide evidence that a small variation in timing of SNB or CLND is not detrimental for survival, which can be used in shared decision making. One potential explanation for these findings could be that the time interval investigated is too narrow for any time dependent effect to become apparent. For instance if a time interval of > 1 year was compared with instant SNB or CLND, there might have been a difference in survival. This was the subject of MSLT I and DeCOG respectively. Neither of these studies showed a significant difference in survival for immediate SNB (and CLND in case of positive SN) versus nodal observation (MSLT I) or immediate CLND versus nodal observation (DeCOG)[3, 23]. Another explanation may be that lymphatic metastases occur already at a very early point in melanoma development and

progression; and that lymphatic metastases may be present for years prior to melanoma diagnosis, but are growing very slowly due to suppression by the immune system[24]. In this case, variation in time interval between primary melanoma excision and SNB and/or CLND of a few weeks may be irrelevant compared to the previous years of melanoma development.

The prognostic value of SN tumor burden poses a paradox here, as maximum SN tumor diameter is clearly associated to survival[25, 26]. Potentially our immune system is capable to contain growth of very small micrometastases (i.e. <0.1mm), but once a certain threshold size has been reached, the proliferative and invasive nature of the lymphatic metastases may overrule the suppressing capabilities of the immune system[24], leading to further growth and perhaps to simultaneous accelerated growth of micrometastases at distant sites as well. Kakavand et al. have found that patients with tumoral PD-L1 expression in the sentinel node had a median larger maximum SN tumor burden, which may be an explanation for acquired anti-tumor immunity evasion by the tumor[27]. The threshold at which anti-tumor immunity fails may be size dependent, time dependent, age and gender dependent, and probably dependent on many other patient and tumor characteristics; but what is mainly important is that in our daily clinical practice nodal (staging) surgery does not need to be performed in a fortnight after diagnosis.

Lymphadenectomy for microscopic stage III– necessary?

As mentioned in **Chapter 1** the MSLT 1 did not show an overall survival benefit for SNB (plus CLND in case of a positive SN) compared to patients who underwent WLE alone[3].

Following the results of MSLT 1, the MSLT 2 investigates whether omission of a CLND in SNB positive patients causes any difference in survival outcomes[28]. While final results from the MSLT 2 are still awaited, recently Leiter et al. published the first results of the DECOG trial, in which SN positive patients either underwent CLND or nodal observation with repeated ultrasound imaging[23]. This study showed no survival benefit for CLND at 3 year follow-up. While the study was underpowered due to lower than expected accrual rate, and patients with low SN tumor burden were overrepresented, the fact that there was absolutely no survival difference at 3 years suggests that a survival benefit from CLND is unlikely to be expected. Final results from the MSLT 2 will have to be awaited, as this study has included a larger number of patients with longer follow-up, and thus will be able to provide more information on the possible therapeutic value of CLND.

Meanwhile, daily clinical practice already differs substantially from guideline recommendations. Despite that CLND is still ubiquitously recommended for SN positive patients pending the final study results on its therapeutic value[9, 15, 16, 28], not all patients actually undergo CLND. Bilimoria et al reported that only 50% of the SN positive melanoma patients in the United States of America had a CLND[29], which is in line with results from the worldwide survey performed by Pasquali et al[30]. It is not known whether the

decision to not undergo CLND is generally patient driven or physician driven, but these studies clearly demonstrate that there can be a significant disparity between guideline recommendations and actual daily clinical practice.

Extent of surgery for macroscopic stage III (lymphadenectomy)

Finally, surgery for clinically nodal positive patients is again a different story altogether. The role of lymph node dissection is threefold in these patients: to achieve regional control; to provide more detailed prognostic information based on the number of involved lymph nodes and presence of extracapsular extension, and to achieve curation in a certain proportion of patients. It is universally recommended as standard procedure[9, 15, 16]. An aggressive surgical approach may seem appropriate to achieve maximal regional control and potentially therapeutic benefit, but risk of potentially significant surgical morbidity is increased due to the presence of enlarged or even giant bulky or matted nodes, which may increase surgery time and risk of hemorrhage and infections[31, 32]. In a prospective morbidity analysis of the MSLT I no significant differences in short term morbidity were found between CLND and delayed LND, although there was a higher percentage of wound separation, seroma/hematoma, and hemorrhage in the delayed group[33]. Another consequence of radical lymph node dissection is the frequent development of chronic lymph edema; this occurred significantly more often in delayed LNDs than in CLND (20% vs. 12%)[33]. Less extensive surgery may limit these negative effects. This is relevant especially in patients with positive groin lymph nodes, which have a higher complication rate than patients with axillary or head and neck lymph node involvement[31, 33]. There is no uniform approach to patients with groin lymph node metastases; either a combined superficial and deep lymph node dissection is performed removing all inguinal, iliac and obturator nodes; or a superficial inguinal lymph node dissection is performed removing only inguinal lymph nodes. Since only 30% of removed pelvic (iliac and obturator) nodes are positive after a combined groin dissection, this approach may be too radical, as there is no uniform evidence that standard removal of pelvic nodes improves survival[34-39]. **Chapter 9** provides a two-step approach for patients with palpable groin lymph node metastases in order to safely minimize the number of negative pelvic lymph node dissections. Considering the low OS rates for patients with pelvic nodal involvement[34], patients with a high risk of pelvic nodal involvement may ultimately be spared an additional pelvic groin dissection as well. Instead they can be offered systemic targeted therapy or immunotherapy, since this has shown to improve survival in irresectable stage III and stage IV melanomas[2].

Systemic Therapy and Future Perspectives

Adjuvant treatment of high risk stage II/III disease with anticancer vaccines were not effective or even harmful[40], and interferon alfa has shown to only have a marginal effect

on relapse free survival, but not on overall survival in the entire group[41-43]. Trials with pegylated interferon alfa did show a survival benefit, but only for microscopic stage III disease in ulcerated primaries[44-46].

Recently, immunotherapy has led to a breakthrough in the adjuvant treatment of stage III melanoma. Ipilimumab, a selective CTLA4 checkpoint inhibitor, can inhibit immune tolerance and thus might cause regression of tumor cells, as was reflected in improved stage IV survival[47]. In the adjuvant ipilimumab trial by the EORTC (EORTC 18071) a significant survival benefit was demonstrated (hazard ratio 0.76, 95% confidence interval 0.64-0.89, $p<0.001$)[48]. Grade III to IV immune related adverse events occurred in 41.6% in the ipilimumab group, and five patients (1.1%) died due to immune related adverse events. These results will have to be validated in order to adequately value the costs of these side effects versus the gains in terms of recurrence free and melanoma specific survival, but so far, results are promising. This trial has opened the gateway for other studies investigating checkpoint blockade treatment in the adjuvant setting for melanoma and other types of cancer. The results of the EORTC 1325, which investigates pembrolizumab (PD-1 checkpoint inhibitor) are currently awaited, as full accrual was reached in October 2016. Molecular targeted therapy is currently studied as adjuvant treatment as well, for instance in the COMBI-AD trial, which compares simultaneous use of dabrafenib (BRAF inhibitor) and trametinib (MEK inhibitor) versus placebo for high risk BRAF V600 positive melanoma patients. It has reached full accrual in December 2014 and is awaiting analyses.

In the near future, minimally invasive alternatives to the SNB such as US or MSOT[21] guided FNAC will be implemented in standard care, and ultimately will replace surgical SNB as we know it. Nevertheless, nodal staging has become increasingly important in the light of adjuvant systemic therapy with checkpoint inhibitors or combined targeted therapy. Thus an initial increase in the number of performed SNBs can be expected in the coming years. The same may be true for CLND; depending on entry criteria for upcoming adjuvant trials. A next step would be to randomize between SNB only and adjuvant therapy versus SNB plus CLND and adjuvant therapy; considering the fact that only 20% of SN positive patients have additional positive non-SNs. The role of melanoma surgery thus may become more limited in stage III disease. At the other hand patients with previously irresectable stage III or IV disease may become suitable candidates for surgery after successful treatment with either checkpoint inhibitors or BRAF- and/or MEK-inhibitors, as is currently being investigated in a phase-II setting in the REDUCTOR trial[49].

Melanoma has claimed many lives and will sadly continue to do so, but finally time seems to be on our side. When once aggressive radical surgery was the only available option to slow disease progression and achieve local control, over the years better insight into melanoma biology has taught us which factors can determine the prognosis

of each melanoma patient. Treatment options can be tailored based on this. Minimal invasive staging procedures continue to be developed, and reconsideration of the extent of nodal surgery is in place in the light of limited therapeutic effect and promising adjuvant therapies.

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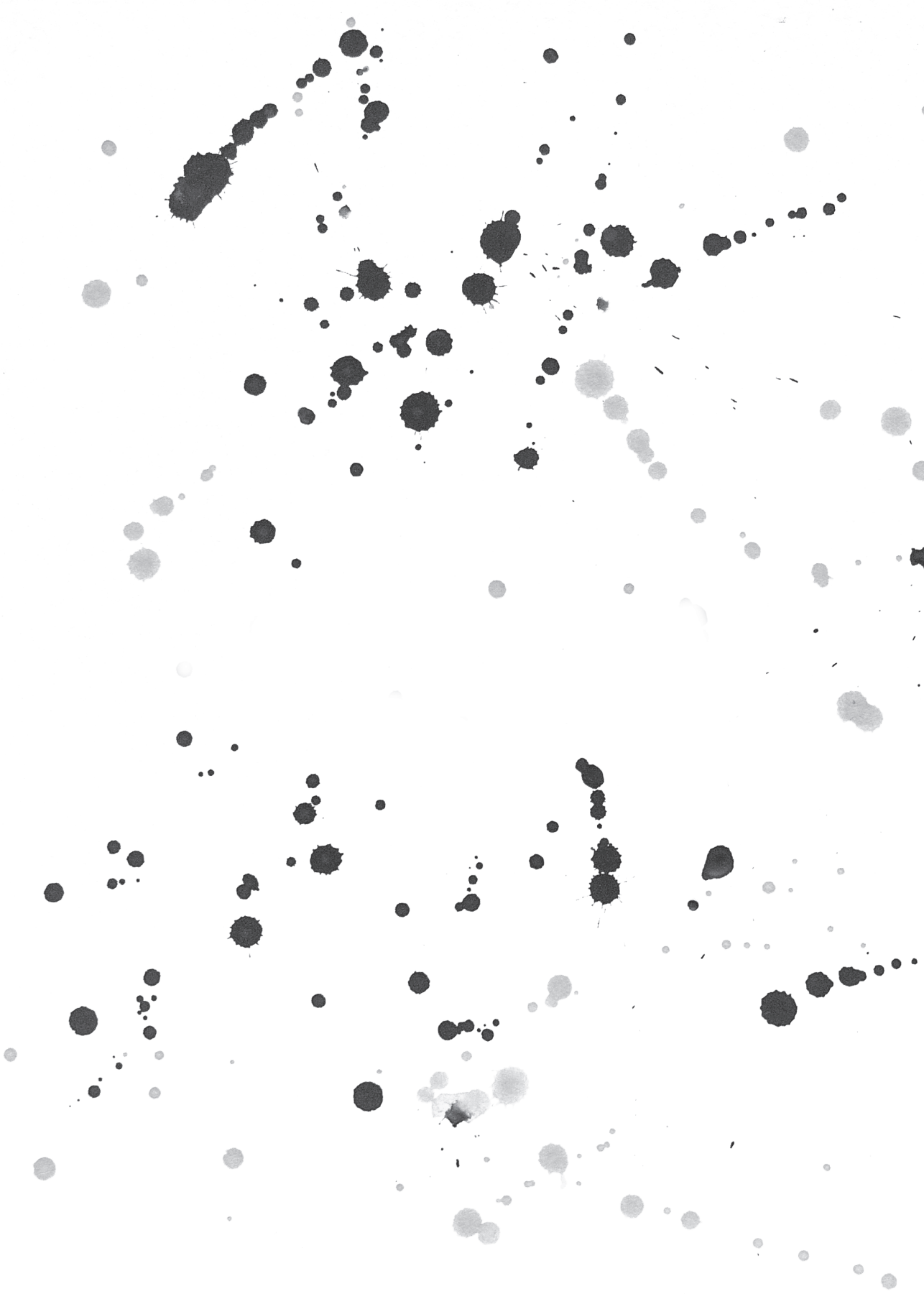


Chapter 11

Summary

Charlotte M.C. Oude Ophuis

Summary

This thesis aims to give insight in the current approach to nodal staging of melanoma patients in the light of continuing lack of evidence of a therapeutic effect from the sentinel node biopsy (SNB). This includes reassessment of guideline recommendations on SNB regarding thin melanomas, a subgroup at low risk of nodal involvement; and discussion of potential minimally invasive alternatives for the SNB, including a prospective trial protocol (**part I**). Current practice regarding the timing of elective nodal staging surgery is evaluated in the light of a stressed referral system and lack of evidence for a highly urgent approach (**part II**); and finally a two-step approach to therapeutic groin dissections is presented in order to minimize the number of patients who will undergo extensive groin lymph node dissection without having pelvic nodal metastases (**part III**).

Part I – Nodal Staging

In **Chapter 2** the effects of implementation of the Dutch Melanoma Guideline 2.0 on nodal staging of thin melanomas were investigated, with in particular the new recommendation to consider SNB for high risk thin melanomas, namely pT1B melanomas. For this study all newly diagnosed thin (pT1) melanomas between 2003 and 2014 were selected from the Netherlands Cancer Registry, in total this concerned near 30,000 patients. Main findings were that next to a general increase in pT1 melanomas, introduction of the mitotic rate criterion for pT1b substaging and the recommendation to perform SNB for pT1b melanoma has led to a proportional increase in pT1b melanomas, and an increase in performed SNBs. Sentinel node (SN) positivity rate has not increased and survival remained stable for pT1b melanomas, indicating that mitotic rate alone as criterion for pT1b has not improved selection of high risk pT1 patients for optional further (nodal) staging. Based on the results of this study, recommendations on SNB for pT1b melanomas might be reconsidered.

Chapter 3 reports on an ultrasound morphology criterion of the SN, which may add to the increased sensitivity and specificity of preoperative ultrasound assessment of clinically nodal negative melanoma patients. In this study 1,000 melanoma patients who underwent ultrasound of the SN area prior to a planned SNB at the Charité Universitätsmedizin Berlin, Berlin, Germany were examined to investigate a new ultrasound morphology criterion predictive of SN tumor involvement: "Echo free island" (EFI). Of the 953 patients in which EFI information was available, EFI was present in 40 patients (4%). EFI sensitivity and specificity were 10.8% and 97.6%, positive and negative predictive value were 50% and 80.2%. Presence of EFI was significantly correlated to presence of peripheral perfusion, another ultrasound criterion. Five-year melanoma specific survival (MSS) was worse for patients with EFI: 80% versus 92% when absent. EFI is found to be a

discriminatory ultrasound morphology sign which can be useful for early identification of sentinel node metastases in melanoma patients. It is an early sign of involvement and thus associated with a decreased survival.

Chapter 4 addresses the long-term results of a potentially minimally invasive alternative for the SNB. This approach consisting of ultrasound (US) assessment using the Berlin morphology criteria and use of fine needle aspiration cytology (FNAC) in case of suspicious or malignant US findings was investigated in 1,000 melanoma patients scheduled for SNB at the Charité Universitätsmedizin Berlin, Berlin, Germany. Survival analyses demonstrated that patients with positive US-FNAC had poor survival. After adjustment for SN status and other known prognostic features, patients with positive US-FNAC had worse survival than patients with normal US. Patients with suspicious US and negative FNAC and patients with normal US had comparable survival. A step-wise approach to melanoma patients is supported by these results: in case of a positive FNAC and/or clearly malignant US finding patients can be spared a SNB and be offered a lymphadenectomy instead. In case of suspicious US and negative FNAC, patients could be offered continue US surveillance or SNB for higher risk primary tumors. Completely US-FNAC negative patients might only require follow-up and no SN staging, with continue US surveillance as addendum for high risk T3/4 and/or ulcerated primaries.

In **Chapter 5** an overview of the literature regarding nodal staging of clinically node negative melanoma patients is given, as well as a presentation of a pilot and consequent study protocol for a minimally invasive alternative to the SNB. The literature on pre-operative assessment of regional lymph nodes with US in clinically N0 melanoma patients is disparate. Targeted US of the SN area in combination with FNAC or other new techniques has potential to become a minimally invasive alternative for the SNB, however, findings need to be replicated in prospective clinical trials first. A pilot with gamma probe guided US-FNAC shows that accurate SN identification in up to 90% of patients is feasible. The presented study protocol of the Gamma probe and ULtrasound guided Fine needle aspiration cytology of the sentinel node Trial (GULF trial) may provide potential improvement to the reported US-FNAC techniques and ultimately even a possible replacement of the SNB.

Part II – Timing of SNB and CLND

In **Chapter 6** a retrospective series of 1,015 SN positive patients from 9 European Organization for Research and Treatment of Cancer Melanoma Group Centers is investigated. Timing of a SNB after melanoma diagnosis is not associated with 5-year disease free survival (DFS) and MSS outcomes. Patients who underwent SNB after a longer time interval had a slightly larger SN tumor burden, and although this may have implications for prognosis this study did not detect any difference in survival. These findings indicate

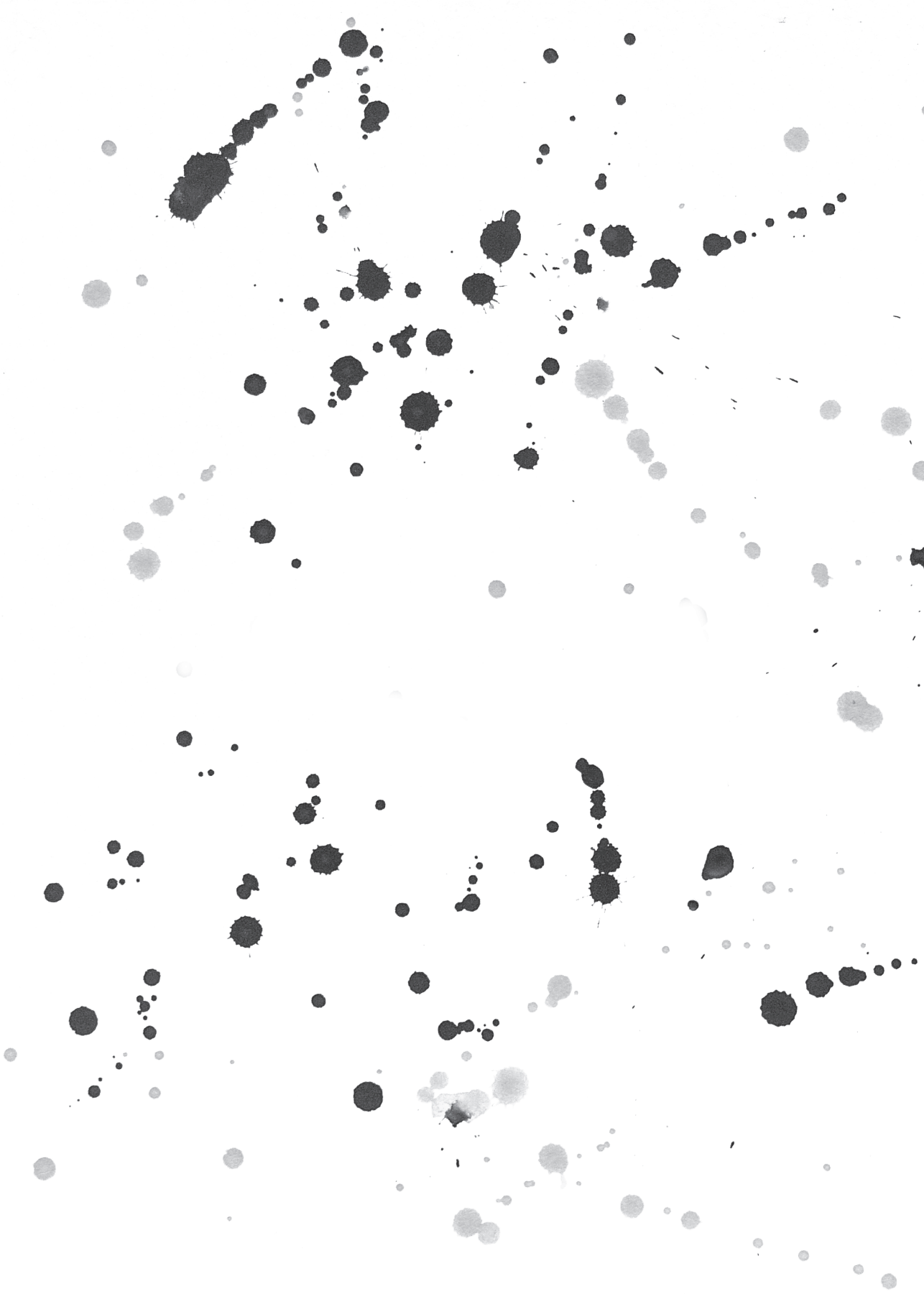
that it is safe and equally informative to perform SNB after a prolonged interval of >9 weeks. This information can be used to counsel patients.

Chapter 7 describes the largest SNB population to date to report on the effects of SNB timing on SN positivity rate and survival. A total of 3,546 patients from 4 European Organization for Research and Treatment of Cancer Melanoma Group Centers undergoing SNB from 1997 – 2013 were selected. No differences in survival were found for different time interval cut-offs. As expected, a short change in time interval (up to 1 month) has no obvious impact on SN positivity rate and prognosis. Whether intervals longer than 3 months may have an effect cannot be determined by this study. This reassuring information supports the removal of strict time intervals for wide local excision (WLE) and SNB from melanoma guidelines and can be used in daily clinical practice to counsel patients and reduce the number of high urgency referrals.

Chapter 8 shows that in a selection of 784 SN positive melanoma patients from the cohort described in **Chapter 6** with updated follow-up, non-SN positivity and survival were not associated with completion lymphadenectomy (CLND) timing. Which indicates that it is safe to wait for at least 3 months after diagnosis, as there is no need to perform CLND as soon as possible. This information can be used to counsel patients and referring physicians and can potentially relieve pressure on the wait list.

Part III – Extent of Lymph Node Dissections in the Groin Area

Chapter 9 provides an accurate two-step approach to predict presence of pelvic nodal metastases in patients undergoing therapeutic superficial groin dissection. A total of 209 therapeutic combined groin dissection (CGD) patients from four tertiary centers in The Netherlands (1992–2013) were selected based on complete preoperative imaging and pathology reports. Combined use of preoperative imaging and a preliminary prediction model based on histopathology results of the inguinal (superficial) part of CGD could accurately predict negative deep pelvic nodes in up to 84 % of patients, thereby potentially identifying a group of low-risk patients in whom the extent of surgery might safely be minimized. The risk factors and the prediction model will be further investigated in a prospective, multicenter registry trial for CGDs.



Chapter 12

Samenvatting

Charlotte M.C. Oude Ophuis

Samenvatting

Deze thesis bestaat uit 3 delen. In **Deel I** van deze thesis wordt gepoogd inzicht te geven in de benadering van lymfklier stadiering bij melanoompatiënten in het huidige tijdperk, gezien het continuerende gebrek aan bewijs voor een therapeutische waarde van de schildwachtklier (SWK) procedure. Hieronder valt het herevalueren van aanbevelingen in de Nederlandse Melanoom richtlijn ten aanzien van het al dan niet uitvoeren van een SWK procedure voor dunne melanomen, een subgroep van patiënten met een laag risico op lymfklieruitzaaiingen. Tevens worden potentiele minimaal invasieve alternatieven voor de SWK procedure besproken, waaronder ook een prospectief trial protocol. In **Deel II** worden resultaten uit de huidige klinische praktijk ten aanzien van de timing van een SWK procedure geëvalueerd in het kader van overbelaste verwijzingspaden en de afwezigheid van wetenschappelijk bewijs voor een hoog urgente aanpak. **Deel III** omvat de presentatie van een 2-staps benadering voor therapeutische liesklierdissecties in patiënten met palpabele lymfkliermetastasen in de liesregio ter minimalisatie van het aantal negatieve pelviene lymfklierdissecties (afwezigheid van lymfkliermetastasen).

Deel I – Lymfklierstadiering

In **Hoofdstuk 2** zijn de effecten van implementatie van de Nederlandse Richtlijn Melanoom 2.0 inclusief het gebruik van de 7e editie van het American Joint Committee on Cancer Staging System voor dunne melanomen onderzocht. Specifiek werd hierbij gelet op de toegevoegde aanbeveling om een SWK te overwegen bij hoog risico dunne melanomen (pT1b). Voor deze studie werden alle nieuw gediagnosticeerde pT1 melanomen tussen 2003 en 2014 geselecteerd uit de Nederlandse Kankerregistratie, in totaal betrof het bijna 30,000 patiënten. De voornaamste bevindingen waren dat, naast een algemene stijging van het aantal pT1 melanomen, het aantal pT1b melanomen proportioneel steeg na introductie van het mitose index criterium voor de pT1b classificatie in de nieuwe richtlijn. Tevens steeg het aantal uitgevoerde SWK procedures. De proportie positieve SWK's is niet gestegen, en overleving is stabiel gebleven voor pT1b melanomen. Dit wijst uit dat het mitose index criterium voor de classificatie als pT1b melanoom geen verbeterde selectie van hoog risico pT1 melanomen voor eventuele verdere (lymfklier) stadiering teweeg heeft gebracht. Aanbevelingen over een SWK procedure voor pT1b melanomen zouden kunnen worden herzien op basis van de resultaten van deze studie.

Hoofdstuk 3 beschrijft een nieuw echografie morfologie criterium voor de SWK, het "echo free island" (EFI) welke mogelijk kan bijdragen aan een verhoogde sensitiviteit en specificiteit van preoperatieve echografie bij klinisch lymfklier negatieve melanoompatiënten. In deze studie zijn 1.000 melanoompatiënten geïncludeerd die een preoperatieve echografie van het SWK gebied ondergingen voorafgaand aan een SWK procedure in het Charité Universitätsmedizin Berlin, Berlijn, Duitsland. EFI was aanwezig

in 40 patiënten (4%) van de 953 patiënten waarbij EFI informatie beschikbaar was. EFI sensitiviteit en specificiteit voor aanwezigheid van SWK metastasen waren 10.8% en 97.6%, positief en negatief voorspellende waarde waren 50% en 80.2%. De aanwezigheid van EFI was significant gecorreleerd met aanwezigheid van perifere perfusie, een ander echografie criterium. Vijf-jaars melanoom specifieke overleving was slechter voor patiënten met EFI: 80% versus 92% in afwezigheid van EFI. Concluderend is EFI een goed te onderscheiden echografie morfologiekenmerk dat behulpzaam kan zijn voor vroege identificatie van SWK metastasen in melanoompatiënten. Het is een vroeg teken van SWK betrokkenheid en daarmee geassocieerd met een lager overlevingspercentage.

Hoofdstuk 4 spitst zich toe op de lange termijn resultaten van een potentieel minimaal invasief alternatief voor de SWK procedure. Het gaat hierbij om echografie met gebruik van de Berlin morfologie criteria en het gebruik van een dunne naald cytologische punctie (fine needle aspiration cytology, FNAC) in het geval van een verdacht of evident maligne ogende echobevinding van de SWK. Deze procedure is onderzocht in meer dan 1.000 melanoompatiënten die een geplande SWK procedure ondergingen het Charité Universitätsmedizin Berlin, Berlijn, Duitsland. Analyse van de overlevingsdata toonde dat patiënten met positieve echo en/of FNAC een slechte overleving hadden. Na correctie voor SWK status en andere bekende prognostische kenmerken hadden patiënten met positieve echo/FNAC nog steeds een slechtere overleving dan patiënten met een normale echo uitslag. Patiënten met een verdachte echo uitslag en negatieve FNAC en patiënten met een normale echo uitslag hadden een vergelijkbare overleving. Deze resultaten ondersteunen een stapsgewijze aanpak voor melanoompatiënten: bij een positieve FNAC en/of duidelijk maligne echo uitslag kan een SWK procedure achterwege blijven en direct een complete lymfklierdissectie worden aangeboden. In het geval van een verdachte echo uitslag en negatieve FNAC kan echografische follow-up of een SWK procedure worden aangeboden voor hoog risico melanomen. Bij echo en FNAC volledig negatieve patiënten kan overwogen worden om alleen follow-up uit te voeren zonder SWK stagering, met als aanvulling regelmatige echografie bij hoog risico T3/T4 of ge-ulcereerde melanomen.

In **Hoofdstuk 5** wordt een overzicht van de literatuur gegeven over de lymfklierstadiering van klinisch lymfklier negatieve melanoompatiënten, alsmede een presentatie van een studie pilot en bijbehorend aanvullend studieprotocol voor een minimaal invasief alternatief voor de SWK. De literatuur over preoperatieve beoordeling van regionale lymfklieren met echografie in klinisch lymfklier negatieve melanoompatiënt is uiteenlopend. Gerichte echografie van het SWK gebied in combinatie met FNAC of andere nieuwe technieken heeft potentie om een minimaal invasief alternatief te vormen voor de SWK procedure, echter moeten deze resultaten tot op heden nog gerepliceerd worden in prospectieve klinische trials. De uitgevoerde pilot studie met gamma-probe geleide echografie en FNAC van de SWK laat zien dat hiermee correcte SWK identificatie

in 90% van de patiënten uitvoerbaar is. Het gepresenteerde studieprotocol van de gamma probe en echografisch geleide FNAC van de SWK trial (GULF trial) kan mogelijk bijdragen aan een verbetering van de reeds gerapporteerde echo/FNAC technieken en uiteindelijk eventueel een vervanging zijn voor de SWK procedure.

Deel II – Timing van SWK en completerende lymfklierdissectie

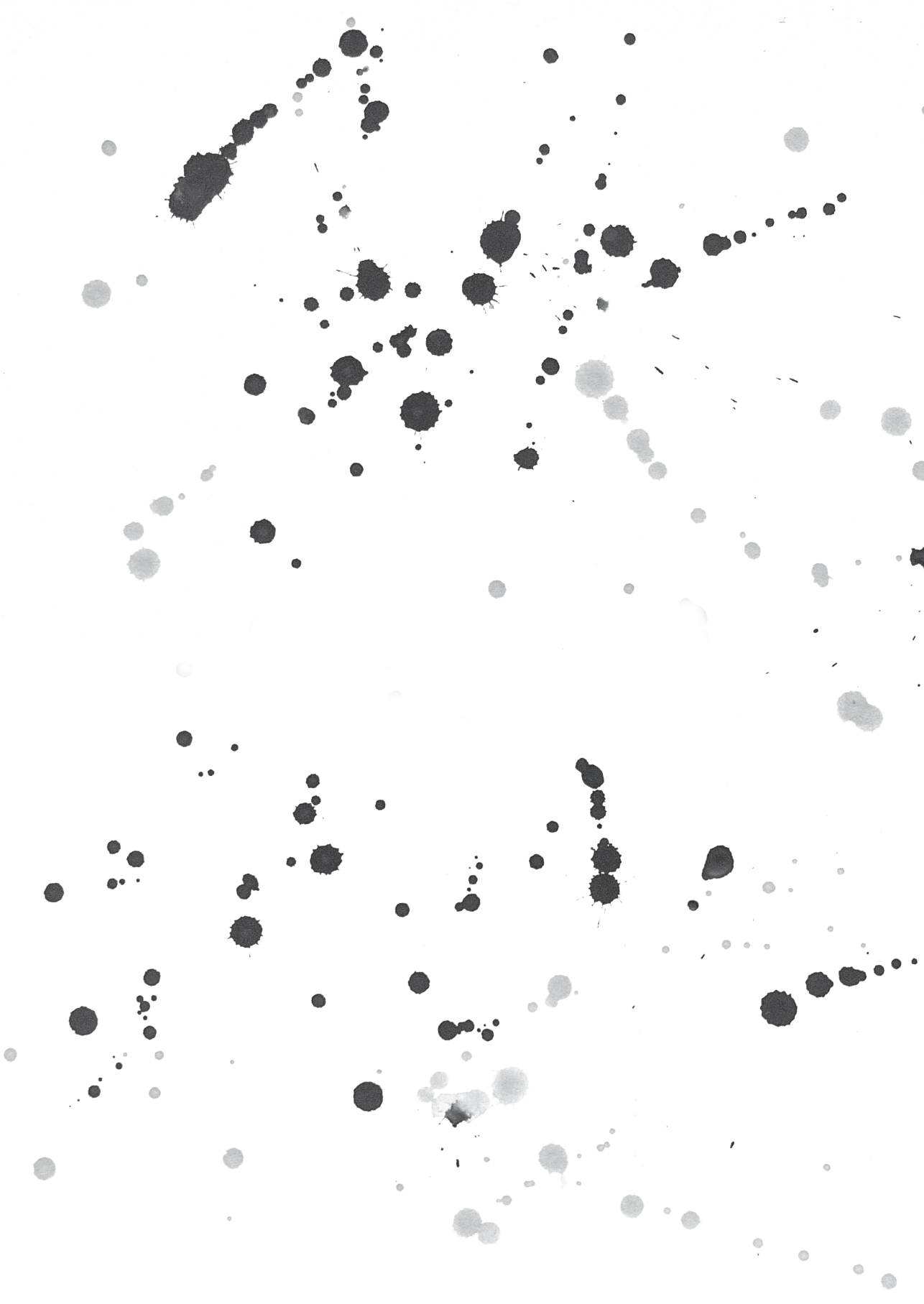
Hoofdstuk 6 beschrijft een retrospectief cohort van 1.015 SWK positieve patiënten uit 9 European Organization for Research and Treatment of Cancer Melanoma Group (EORTC-MG) centra. In dit cohort werden de effecten van timing van de SWK procedure na melanoomdiagnose op ziektevrije en ziekte specifieke overleving onderzocht. De timing van een SWK procedure is niet geassocieerd met 5-jaars ziektevrije of ziekte specifieke overleving. Patiënten die een langer tijdsinterval hadden tussen diagnose en SWK procedure hadden een minimaal hogere SWK tumor grootte, en hoewel dit implicaties kan hebben voor de prognose liet deze studie geen verschil in overleving zien. Deze resultaten indiceren dat het veilig is en even informatief om een SWK procedure te verrichten na een langer tijdsinterval van > 9 weken. Deze informatie kan gebruikt worden om patiënten te adviseren.

Hoofdstuk 7 beschrijft de resultaten van de grootste SWK populatie tot op heden waarbij de effecten van SWK procedure timing op SWK uitslag en overleving zijn onderzocht. In totaal werden 3.546 patiënten geselecteerd die een SWK procedure ondergingen tussen 1997 – 2013 in 4 EORTC-MG centra. Er werden geen verschillen in overleving gevonden voor verschillende tijdsinterval afkapwaarden. Zoals te verwachten heeft een korte variatie in tijdsinterval tussen diagnose en SWK-procedure (tot 1 maand) geen duidelijke invloed op de kans op een positieve SWK en prognose. Of een interval van meer dan 3 maanden een dergelijk effect heeft kan niet worden bepaald op basis van deze studie. Deze geruststellende resultaten ondersteunen de verwijdering van strikte tijdslimieten voor re-excisie en SWK-procedures uit melanoom richtlijnen. Daarnaast kan deze informatie in de dagelijkse praktijk worden toegepast om patiënten te adviseren en het aantal hoog urgente verwijzingen te minimaliseren.

Hoofdstuk 8 laat zien dat in een selectie van 784 SWK positieve patiënten met aangevulde follow-up gegevens uit het cohort beschreven in **Hoofdstuk 6** er geen associatie werd aangetoond tussen timing van een completerende lymfklierdissectie en aanvullende positieve lymfklieren of overleving. Dit indiceert dat het veilig is om minimaal 3 maanden te wachten met een lymfklierdissectie na diagnose van het primaire melanoom, aangezien er in het licht van deze resultaten geen noodzaak is om deze operatie zo snel mogelijk uit te voeren. Deze informatie is bruikbaar voor het adviseren van zowel patiënten als verwijzende artsen, en kan mogelijk zorgen voor een daling van de druk op de wachtlijst.

Deel III – Uitgebreidheid van lymfklierdissecties in de liesregio

Hoofdstuk 9 geeft een accurate twee-staps benadering weer om aanwezigheid van pelviene lymfkliermetastasen te voorspellen in patiënten die een therapeutische inguinale (oppervlakkige) lymfklierdissectie ondergaan. Voor deze studie werden 209 patiënten met een therapeutische ilio-inguinale (oppervlakkige en diepe) lymfklierdissectie tussen 1992-2013 geselecteerd uit 4 tertiaire melanoomcentra in Nederland, gebaseerd op aanwezigheid van preoperatieve beeldvorming en adequate pathologieverslagen. Gecombineerd gebruik van preoperatieve beeldvorming ter uitsluiting van pelviene lymfkliermetastasen en een preliminair predictiemodel gebaseerd op pathologieresultaten van de inguinale lymfklierdissectie leidde tot accurate predictie van negatieve pelviene lymfklieren in 84% van de patiënten. Hiermee kan potentieel een laag risico groep patiënten worden geïdentificeerd waarbij de uitgebreidheid van de lymfklierdissectie veilig geminimaliseerd kan worden. De risicofactoren en het predictiemodel zullen verder onderzocht moeten worden in een prospectieve multicenter registratie trial voor patiënten die een ilio-inguinale lymfklierdissectie ondergaan.



Appendices

PhD Portfolio Summary

List of Publications

Dankwoord

Curriculum Vitae

PhD Portfolio Summary

Summary of PhD training and teaching activities

Name PhD student: Charlotte Maria Catharina Oude Ophuis, MD	**PhD period:** September 2014 – September 2016
Erasmus MC Department: Surgery, Division of Surgical Oncology	**Promotor:** Prof. dr. C. Verhoef, MD PhD
Research School: Medicine	**Supervisors:** Dr. D.J. Grünhagen, MD PhD Dr. A.C.J. van Akkooi, MD PhD

1. PhD training

	Year	Workload (Hours/ECTS)
General academic skills		
- Research Integrity	2015	0.5
- Basiscursus Regelgeving en Organisatie Klinisch Onderzoekers (BROK)	2015	1.5
Research skills		
- Open Clinica Course	2015	0.5
- Systematic Literature Retrieval Course	2015	0.5
Oral Presentations		
- 37th ISDS meeting, Amsterdam, the Netherlands	2016	1
- ISNS 2016 biannual meeting, Milan, Italy	2016	1
- EORTC MG spring meeting, Milan Italy	2016	1
- EORTC MG fall meeting, Berlin, Germany	2016	1
- 18th ECCO – 40th ESMO Congress, Vienna, Austria	2015	1
- 68th SSO meeting, Houston, Texas, USA	2015	1
- EORTC MG fall meeting, Rotterdam, the Netherlands	2015	1
- 34th ESSO-BASO congress, Liverpool, United Kingdom	2014	1
- Annual Melanoma Congress 2014, Paris, France	2014	1
- Chirurgendagen 2015 NVVH, Veldhoven, the Netherlands	2014	1
- EORTC MG spring meeting, London, United Kingdom	2014	1
Poster Presentations		
- 70th SSO meeting, Seattle, Washington, USA	2017	0.5
- ECCO 2017, Amsterdam, the Netherlands	2017	0.5
- 69th SSO meeting, Boston, Massachusetts, USA	2016	0.5
- Annual Melanoma Congress 2014, Paris, France	2014	0.5
- Najaarsdag NVVH, Den Bosch, the Netherlands	2013	0.5
International Conferences		
- ECCO 2017, Amsterdam, the Netherlands	2017	1
- 37th ISDS meeting, Amsterdam, the Netherlands	2016	0.5
- ISNS 2016 biannual meeting, Milan, Italy	2016	1
- EORTC MG spring meeting, Milan Italy	2016	0.5

- EORTC MG fall meeting, Berlin, Germany	2015	1
- 18th ECCO – 40th ESMO Congress, Vienna, Austria	2015	2
- 68th SSO meeting, Houston, Texas, USA	2015	1.5
- EORTC MG fall meeting, Rotterdam, the Netherlands	2014	1
- 34th ESSO-BASO congress, Liverpool, United Kingdom	2014	2
- Annual Melanoma Congress 2014, Paris, France	2014	1
- EORTC MG spring meeting, London, United Kingdom	2014	1

2. Teaching activities

	Year	**Workload (Hours/ ECTS)**
Supervising practicals and excursions		
- Basic Life Support examinator medical students	2015 - 2016	0.5
Supervising Master students		
- Supervision master students extracurricular research	2016	3
Other		
- Supervisor Kennismaking Beroepspraktijk (KBP)	2016	0.5

List of Publications

This thesis:

- **Oude Ophuis CM**, Louwman MW, Grünhagen DJ, Verhoef C, van Akkooi AC
  Implementation of the 7th Edition AJCC Staging System: Effects on Staging and Survival for pT1 Melanoma. A Dutch Population Based Study
  Int J Cancer. 2017 Apr 15;140(8):1802-1808.

- **Oude Ophuis CM**, Koppert LB, de Monyé C, van Deurzen CH, Koljenović S, van Akkooi AC, Verhoef C, Grünhagen DJ
  Gamma Probe and Ultrasound Guided Fine Needle Aspiration Cytology of the Sentinel Node (GULF) Trial - Overview of the Literature, Pilot and Study Protocol
  BMC Cancer. 2017 Apr 12;17(1):258.

- **Oude Ophuis CM**, Verhoef C, Grünhagen DJ, Siegel P, Shoengen A, Röwert-Huber J, Eggermont AM, Voit CA, van Akkooi AC
  Long-term Results of Ultrasound Guided Fine Needle Aspiration Cytology in Conjunction with Sentinel Node Biopsy Support Step-wise Approach in Melanoma. Eur J Surg Oncol. 2017 Aug;43(8):1509-1516

- **Oude Ophuis CM**, van Akkooi AC, Rutkowski P, Powell WE, Robert C, Testori A, van Leeuwen BL, Siegel P, Eggermont AM, Verhoef C, Grünhagen DJ
  Timing of Completion Lymphadenectomy After Positive Sentinel Node in Melanoma Patients.
  Br J Surg. 2017 May;104(6):726-733.

- **Oude Ophuis CM**, van Akkooi AC, Rutkowski P, Voit CA, Stepniak J, Erler NS, Eggermont AM, Wouters MW, Grünhagen DJ, Verhoef C
  Effects of Time Interval Between Primary Melanoma Excision and Sentinel Node Biopsy on Positivity Rate and Survival – a Multicenter Retrospective Cohort Study.
  Eur J Cancer. 2016 Nov;67:164-173.

- **Oude Ophuis CM**, Verhoef C, Rutkowski P, Powell WE, van der Hage JA, van Leeuwen PA, Voit CA, Testori A, Robert C, Hoekstra HJ, Grünhagen DJ, Eggermont AM, van Akkooi AC
  The Interval Between Primary Melanoma Excision and Sentinel Node Biopsy is not associated with survival in Sentinel Node Positive Patients – *An EORTC Melanoma Group study*.
  Eur J Surg Oncol. 2016 Dec;42(12):1906-1913.

- Voit CA, **Oude Ophuis CM**, Ulrich J, van Akkooi AC, Eggermont AM. Ultrasound of the Sentinel Node in Melanoma Patients: Echo Free Island (EFI) is a Discriminatory Morphologic Feature for Node Positivity.
  Melanoma Res. 2016 Jun;26(3):267-71.

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  Ann Surg Oncol. 2015 Dec;22 Suppl 3:1172-80.

Other publications:

- Verver D#, Madu MF#, **Oude Ophuis CM**, Faut M, de Wilt JHW, Bonenkamp HJ, Grünhagen DJ, van Akkooi ACJ, Verhoef C, van Leeuwen B. *#: equal contribution.*
  Timing of Completion Lymphadenectomy after Positive Sentinel Node in Melanoma Patients.
  Br J Surg. 2017 May; accepted, in press.

- Das AM, **Oude Ophuis CM#,** Bolkestein M#, van der Klok T#, Vermeulen CE, Rens JA, Dinjens WN, Atmodimedjo PN, Verhoef C, Koljenović S, Smits R, ten Hagen TL, Eggermont AM *#: equal contribution.*
  Tissue inhibitor of metalloproteinase-3 (TIMP3) expression decreases during melanoma progression and inhibits melanoma cell migration.
  Eur J Cancer. 2016 Oct;66:34-46.

- Das AM, **Oude Ophuis CM#,** Koljenovic S#, van der Klok T, Galjart B, Nigg AL, van Cappellen WA, Hegt VN, Dinjens WN, Atmodimedjo PN, Vermeulen CE, Verhoef C, Eggermont AM, ten Hagen TL *#: equal contribution.*
  Association of TIMP3 expression with vessel density and macrophage infiltration in human malignant melanoma.
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  Oncotherapie. 30 Dec 2015.

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  The value of intraoperative neurophysiological monitoring in tethered cord surgery.
  Childs Nerv Syst. 2011 Sep;27(9):1445-52.

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Curriculum vitae

Charlotte Maria Catharina Oude Ophuis, auteur van dit proefschrift, werd geboren op 9 december 1988 te Wageningen. Aldaar groeide zij op en behaalde in 2006 cum laude het eindexamen Gymnasium aan de Regionale Scholengemeenschap Pantarijn. Hetzelfde jaar begon zij aan de studie Geneeskunde in Groningen. Al tijdens het eerste jaar van haar studie raakte zij betrokken met het doen van wetenschappelijk onderzoek, eerst in het kader van de Junior Scientific Masterclass, en later extracurriculair op de afdeling Neurochirurgie van het Universitair Medisch Centrum Groningen (UMCG). Hier verrichte zij ook haar wetenschappelijke stage, voorafgaand aan haar coschappen in het UMCG en de Isala Klinieken te Zwolle. De laatste 2 maanden van haar coschappen voltooide zij op de afdeling Transplantatiechirurgie van het Mount Sinai Hospital te New York, New York, USA. In maart 2013 behaalde zij cum laude haar artsexamen, en in mei 2013 begon als zij arts-assistent bij de Snijdende Oncologische Groep in de Daniel den Hoed Kliniek. Alhier startte zij onder begeleiding van Prof. dr. C. Verhoef, Dr. D.J. Grünhagen en Dr. A.C.J. van Akkooi een onderzoek naar stadium III melanomen. Hiermee werd de basis gelegd voor meerdere studies welke hebben geleid tot dit proefschrift. Tijdens haar onderzoeksperiode heeft Charlotte meerdere voordrachten gehouden op internationale congressen met multipele nominaties en in 2015 de toekenning Best Abstract op het 18e ECCO – 40e ESMO Congres in Wenen. Tevens heeft zij een prospectieve studie opgezet, de GULF trial, welke momenteel loopt in het Erasmus Medisch Centrum Kankerinstituut (voorheen Daniel den Hoed Kliniek) en het Nederlands Kankerinstituut – Antoni van Leeuwenhoek. Per 1 oktober 2016 is zij gestart als arts-assistent chirurgie in het Maasstad Ziekenhuis te Rotterdam, waar zij per 1 juli 2017 tevens is begonnen met de opleiding Heelkunde. Op 2 juni 2017 is zij getrouwd met Frans Gysolt Smits met wie zij gelukkig samenwoont in Rotterdam.