THE PROGNOSTIC SIGNIFICANCE OF SENTINEL LYMPH NODE BIOPSY IN MALIGNANT MELANOMA

Ph.D. dissertation

by

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RELATED PUBLICATIONS

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ABBREVIATIONS

ALM:	acrolentiginous melanoma
bp:	base pairs
CLND:	complete lymph node dissection
ELND:	elective lymph node dissection
LM	lymphatic mapping
LMM:	lentigo maligna melanoma
mRNA:	messenger RNA
MSLT:	Multicenter Selective Lymphadenectomy Trial
NM:	nodular melanoma
RT-PCR:	reverse transcriptase polymerase chain reaction
SLN:	sentinel lymph node
SLNB:	sentinel lymph node biopsy
SSM:	superficial spreading melanoma
WHO:	World Health Organization

ABSTRACT

The sentinel lymph node technique renders the regional tumour stage determination possible. In malignant melanoma this technique helps select those patients for whom the performance of regional block dissection is justified. The tumour involvement of the regional lymph nodes is one of the most important prognostic factors, not directly dependent on the parameters of the primary tumour. Considering the controversial prognostic significance of spontaneous regression of the primary tumours and the uncertainty in determining the tumour thickness, we think that the result of the sentinel lymph node biopsy is a step forward in settling the problem. S-100 protein and circulating tumour cells are important markers of the progression. In theory, they may predict a positive sentinel lymph node, but the related information available in the literature is rather infrequent.

In period of November 1997 to June 2005, we performed sentinel lymph node biopsy with the double labelling technique on 450 patients, with following results: the successful rate 99.4%, the sentinel lymph node positivity 15.5%, the false negative rate 14.8% The independent predictors of the sentinel lymph node status the Breslow thickness and the ulceration of the primary tumour have been found, in harmony with the literary data.

S-100 concentration was measured by the luminescence immunoassay with 301 patients.

The sentinel node negative and positive groups did not differ from each other concerning the average values (p=0.18). The percentage difference was also minimal. Thus the serum S-100 concentrations do not predict the presence of micrometastasis in sentinel nodes.

269 patients with sentinel lymph node biopsy have been selected for histological analysis regarding the intermediate regressing signs of the primary tumour. Signs of intermediate regression were detected by histology in 27.9% of the patients.

Regressive tumours were localized predominantly on the trunk (p=0.006), were significantly thinner (p=0.00001) and less frequently ulcerated (p=0.003) than tumours without regression. Moreover, the majority of regressive melanomas were of the superficial spreading type (p=0.00001) and their sentinel node status was more favourable (p=0.026). By multivariate analysis, however, the Breslow thickness and ulceration of the primary tumour were

predictors of the sentinel lymph node status, in agreement with literature data. A partial intermediate level of regression did not affect unfavourably the sentinel lymph node status.

We failed to demonstrate a significant relationship between the presence of circulating tumour cells and either primary tumour regression or the sentinel lymph node status.

The presence of circulating tumour cells was studied preoperatively in 94 patients by reverse transcription-polymerase chain reaction for tyrosinase messenger RNA.The sentinel node negative and positive tumours yielded nearly identical percentage values for positive tyrosinase mRNA: 10 of 70 (14.3%), and 3 of 24 (12.5%) ($p=0.81$), respectively. We demonstrated them in 5 of 26 (19.2%) regressive and 20 of 68 (29.4%) non-regressive tumours. The difference was not significant ($p=0.32$).

With a mean follow-up period of 40.8 months with 233 patients, the estimated 5-year survival in the sentinel node negative group was 90%, in contrast to the sentinel node positive group where it was only 51% ($p=0.00001$). The corresponding values of patients with regressing tumours were 90% and 73%, respectively ($p=0.032$).

By multivariate Cox hazard regression analysis, the Breslow thickness ($p=0.00001$), the ulceration of the primary tumour ($p=0.00001$) and the sentinel lymph node status ($p=0.048$) have been found to be the independent prognostic factors for the survival. Thus the multivariate analysis did not demonstrate significant difference between the regressing and non-regressing tumours.

1. INTRODUCTION

The surgical removal of sentinel lymph node in malignant melanoma developed by *Morton* is one of the most noteworthy innovations of surgical oncology of the past almost 15 years (1). In addition to melanoma, the technique is also used in breast cancers and other tumours of epithelial origin.

The technique is based on the hypothesis that each lymph region has its own one or more lymph nodes which primarily take the tumour cells up (2,3). If this sentinel lymph node does not contain any tumour cell other lymph nodes in this region are also likely to be intact. This minimally invasive surgical technique, devoid of the adverse complications of elective block dissection, renders the regional tumour stage determination possible and helps select those patients with whom the performance of regional block dissection is justified (4). The tumour involvement of the regional lymph nodes is one of the most significant prognostic factors (5,6). In case of positive nodes the regional lymph node block dissection is of therapeutic significance and loco-regional control of the disease although some authors claim that it has no impact on survival (7,5,6).

S-100 protein is another important marker of melanoma. Its value correlates with the activity and the stage of the disease and is able to predict its progression (8). In theory, it may predict a positive sentinel lymph node as well, but the relevant information available in the literature is rather sparse.

The sentinel lymph node biopsy yields information independent of the parameters of the primary tumour in relation to the prognosis. Considering the controversial prognostic significance of the spontaneous regression of the primary tumours and the uncertainty in determining the tumour thickness according to Breslow and the invasion depth according to Clark, we think, that the result of the sentinel lymph node biopsy is a step forward in settling the problem. The immune response of the host organism may induce the regression of the primary tumour. This phenomenon occurs about 5-6 times more frequently in malignant melanomas than in other tumour types (9). Clinically it appears as decreasing pigmentation, multicoloration, fragmentation of the primary lesion and finally in scarring and, occasionally, the complete disappearance of the tumour. At histological level, in early regression the tumour is accompanied by lymphocytic infiltration, in partial intermediate level regression by

the accumulation of fibroblasts and appearance of macrophages and capillaries, while in complete regression the disappearance of all tumour cells is the characteristic feature. The regression rate reported in literature ranges from 10% to 35% (10).

Though regression is a rather frequent phenomenon, its prognostic significance is still a controversial issue. Regression may reflect a good immune response of the host organism but during this process aggressive clones may also be selected that may give rise to metastases. Many authors (11,12,13,14,15) claim that regression has no adverse effect on the clinical course of the disease whereas others regard it as a sign of poor prognosis (16,17,18). The sentinel lymph node method may help to evaluate the relationship between the spontaneous regression potential of primary cutaneous malignant melanoma and the prognosis of the disease.

Primary metastases of melanoma may develop not only via the lymphatics but, in 20% of the cases, through the blood vessels. Circulating melanoma cells in the blood can be demonstrated by detecting positive signal for tyrosinase messenger RNA by reverse transcription-polymerase chain reaction (RT-PCR) technique. This may indicate the haematogenic metastasizing capacity of the tumour, with a prognostic value independent of the prognostic power of the sentinel lymph node status. Although results reported in literature range between rather wide limits, probably due to variability in the techniques applied, a positive correlation can be found between the results of this technique and the clinical stage of the disease. For instance a positive rate of 0-76% was described for tumours in clinical stages I-II, 0-86% for those in clinical stage III and 6-100% for those in clinical stage IV (19).

In this study, attempts were made to reveal relationships between either the sentinel lymph node status or the characteristics of the primary malignant melanoma in term of regression and the presence of circulating melanoma cells.

Objectives

1. **Introduction of the sentinel lymph node biopsy with double labelling technique in malignant melanoma.**
2. **Correlation between the preoperative S-100 protein tumour marker values and the sentinel lymph node status.**

3. **The prognostic significance of the spontaneous regression of the primary melanoma from the point of view of sentinel lymph node status and circulating tumour cells (tyrosinase mRNA).**
4. **Patients' follow up. The significance of the sentinel lymph node status and the spontaneous regression of the primary tumour in term of disease-free and overall survival.**

HISTORICAL BACKGROUND

The management of clinically negative lymph regions in malignant melanoma has been a controversial matter till now (20,21). In fact, for decades two methods have been applied; there were advocates of elective regional block dissection (ELND) (22,23,24,25), in spite of the fact that in nearly 80% of the removed lymph nodes no metastasis was identified. This means that patients were superfluously exposed to the potential complications of this intervention. Others, however, adopted the "wait and watch" policy. They only performed the intervention if the clinical manifestation of the disease in the region was evident. It was followed by us in the National Institute of Oncology. Four prospective randomized studies were launched to settle the problem. The study conducted by the WHO Melanoma Group with nearly 600 patients, failed to identify any advantage of ELND over the excision of the primary tumour with wide margins in itself (26,27). The team of the Mayo Clinic got similar results, though with fewer patients (28). The WHO Melanoma Programme performed a prospective, randomized study (1982-1989) with melanomas of >1.5 mm thickness, localised to the trunk (29). The elective block dissection did not modify survival significantly. However, when the survival rates of patients with clinically occult metastases and those undergone later block dissection for manifest lymph node involvement were compared, the differences were significant. In the results of the Intergroup Melanoma Surgical Trial (30,25) the patients under 60 years with 1-2 mm thick melanoma had longer survival after elective block dissection.

The minimally invasive surgical technique of Morton put an end to this dispute lasting for decades. This new technique rendered selective intervention possible. The basic method was improved by Alex et al., in 1993 (31) and later, in1995 by Krag et al. by using ^{99m}Tc labelled colloid. The diagnostic success rate increased to 95-99% (32).

In Hungary Török and his co-workers were the first in 1997 to make sentinel lymph node biopsy with blue staining (33), they were followed by our team (National Institute of Oncology). We used the combined method right from the beginning.

2. PATIENTS AND METHODS

2.1. Sentinel lymph node biopsy with double labelling technique in malignant melanoma

From November 1997 to June 2005 we performed sentinel lymph node biopsy with the double labelling technique in 450 patients. The clinical details of these patients are represented in the **Table 1.** The intervention was made either simultaneously with or 2-8 weeks after primary tumour removal. Preceding the new staging system of the American Joint Committee on Cancer (2002) all primary melanoma patients without regional or distant metastasis underwent SLNB, after this publication of the new staging however only patients complying with the criteria of eligibility: tumour thickness between 1.01 and 4 mm; tumour thickness ≤1 mm, presence of ulceration, regression or Clark level IV or V; tumour thickness >4 mm without ulceration entered the study.

The double labelling technique complemented with lymphoscintigraphy

On the previous day before the operation the patients are given ^{99m}Tc labelled colloid. The colloidal human serum albumin of 200-600 particle size is administered in maximum 30 MBq dose of maximum 0.6 ml volume distributed into 2-4 portions injected intracutaneously around the tumour or its earlier scar. The lymphoscintigraphy is a dynamic technique. It is an indispensable tool of identifying the draining lymphatic vessels (regions) and eventual in-transit lymph nodes. (The measurements were made with a TOSHIBA GCA 7100 digital gamma camera). Except some rare body cavity localisation, e.g. paraaortic lymph nodes, the sentinel lymph nodes are removed. In the morning of the very day of the surgery, antero-posterior and lateral static pictures are taken. The sites of the sentinel lymph nodes are marked on the skin. The evaluation requires the consideration of both dynamic and static pictures. Five to ten minutes before starting the operation patent blue dye (Patentblau V, Byk Gulden,

Konstanz) in 0.5-2 ml volume distributed to 2-4 portions is injected intracutaneously around the lesion or the scar. In the region the projection of the sentinel lymph node on the skin is precisely determined with the aid of a gamma probe.

Surgical technique

Above the identified lymph node a small incision is made in the skin so that its direction falls in line with that of an eventual block dissection. The gamma probe is to be used continually while searching for the sentinel lymph node(s). The preparation is significantly facilitated by the finding and following the lymph vessel(s) staining blue up to the lymph node(s) which also stain blue. If the dye does not reach the lymph nodes of the region, it is the gamma probe that guides the preparation. Should the injection site be very close to the lymph region it is advised to excise first the primary lesion to reduce the disturbing background activity. The identified lymph node is removed and sent with separate marking for histology. In our practice the patients with positive sentinel lymph node are referred to therapeutic block dissection within 2-3 weeks.

Histology

Frozen-section investigation was not performed. The sentinel nodes were formalin-fixated, bisected, paraffin-embedded, and cut at a minimum of six levels at 50- to 150 micrometer intervals. Pathologic evaluation included haematoxylin and eosin and immune-histochemical staining (S-100 and HMB-45)

2.2. Correlation between the preoperative S-100 protein values and sentinel lymph node status

S-100 tumour marker determination from blood

This laboratory assay was performed with 301 patients from February 2000 to June 2005. The blood samples were taken 24-48 hours before the sentinel lymph node surgery.

The S-100 concentration was measured by the luminescence immunoassay in the Byk Laboratory in our Institute. The LIA-mat Sangtec-100 is a monoclonal two-site immuno-luminetric assay (sandwich principle). Antibody-coated polystyrene tubes serve as solid phase. Sangtec-100 discriminates between the A1 and B subunit. Sangtec 100 measures the

B-subunit of the protein S-100 as defined by the three monoclonal antibodies SMST 12, SMSK 25 and SMSK 28.The coated antibody reacts with the S-100 present in patient samples or standards during the first incubation. Unbound material is removed by washing step. During the second incubation, the tracer antibody binds to the immobilised S-100. Non-reacting tracer is removed by a second washing step. The anti S-100 tracer conjugate consists of an antibody and a covalently bound isoluminol derivate. The tracer-S-100 complex bound to the tube wall in the immunological reaction is detected by a light reaction. The light signal measured in relative light units is detected proportional to the amount of S-100 present in standard and sample.

Assay procedure: a serum sample of 100 µl volume is transferred into a test tube. By adding 100 µl of diluent it is incubated for one hour at room temperature then it is washed with 3x2 ml wash solution. Thereafter 200 µl anti S-100 tracer conjugate is added to it. The mixture is again incubated at room temperature for two hours. After repeated washing the luminometric measurement is performed that takes 5 seconds. The normal range in our study was between 0.000-0.15 µg/l. The specimens were stored at -20 C°.

2.3. The prognostic significance of the spontaneous regression of the primary melanoma from the point of view of sentinel lymph node status and circulating tumour cells (tyrosinase mRNA)

269 patients who underwent surgery for malignant melanoma at the Department of Dermatology, National Institute of Oncology, Budapest from November 1997 to May 2004 without regional or distant metastasis have been selected.With regard to the parameters of the primary tumour, preceding the publication of the new staging system of the AJCC 2002, patients having malignant melanoma, excepted in situ tumours, who were referred to our Institute have been selected for the investigation. Subsequently, patients with tumours listed below have been chosen:

- tumour thickness between 1.01 and 4 mm
- tumour thickness of 1 mm or less, having ulceration, regression or Clark level IV-V
- tumour thickness of more than 4 mm without ulceration

Sentinel lymph node biopsy with double labelling technique was performed either simultaneously with the primary tumour removal or 2-8 weeks later. The following clinical

and pathological details of 269 patients were available for analysis: distribution of patients according to age and sex, localisation and histological features of the primary tumour, Breslow thickness, invasion depth according to Clark, histological type, presence or absence of regression of intermediate intensity, and the sentinel lymph node status.

Histological evaluation of sentinel lymph nodes was performed in serial sections stained with haematoxylin and eosin. In addition, HMB45 and S-100 immunohistochemical reactions were also performed. Regression of intermediate intensity was classified as an intermediate state that showed definite histological features of regression in extended areas (at least 30% of the total). Less extensive regression was regarded as focal (34,35), and tumours with this type of regression were considered as non-regressive. The evaluation included both the regressive and the non-regressive forms. We found the regression of intermediate intensity to be characterised by the extension of the papillary dermis, initial fibrosis appearing as fibroplasia, moderate lymphocytic and plasmacytic infiltrates with drop-out tumour cells, tumour cell foci, vertically running blood vessels without dilation, accumulation of melanophages and attenuated epithelial pegs (10). The histological examinations were performed by two independent pathologists well skilled in dermatopathology (Z.O. and V.P.). In case of disagreement, a meeting has been organized to reach a consensus.

Blood samples from 94 patients taken 24-48 hours before the sentinel lymph node biopsy procedure were submitted for RT-PCR tyrosinase messenger RNA examination to determine the presence of circulating melanoma cells. Nineteen patients showing locoregional progression and 35 with disseminated melanoma served as controls.

Detections of tyrosinase mRNA

Ten ml of blood were collected in EDTA from each patient. The mononuclear cell fraction of peripheral blood was isolated by Ficoll gradient. Total RNA was isolated from the mononuclear cell fraction by Trizol reagent (Sigma).

For analysis of tyrosinase mRNA, RT-PCR was performed as previously described by Smith et al. in 1991 (36). Blood mRNA integrity was checked by RT with random hexamer primers and consecutive PCR with primers for human β-actin (5’ ATG GAT GAT GAT ATC GCC GCG and 3’TCT CCA TGT CGT CCC AGT TG) (94ºC 45 sec; 60ºC 30 sec; 72ºC 90 sec; 30 cycles of PCR), producing a fragment of 248 base pairs (bp). For PCR analysis of tyrosinase

transcripts, two sets of primers were devised from the sequences as follows: HTYR 1 (5' TTG GCA GAT TGT CTG TAG CC) and HYTR 2 (3' AGG CAT TGT GCA TGC TGC TT) (94ºC 45 sec; 55ºC 30 sec; 72ºC 90 sec; 30 cycles of PCR), which yield a PCR product of 284 bp, and HYTR 3 (5' GTC TTT ATG CAA TGG AAC GC) and HYTR 4 (3' GCT ATC CCA GTA AGT GGA CT) (94ºC for 45 sec; 55ºC for 30 sec; 72ºC for 90 sec; 30 cycles of PCR), which yield a PCR product of 207 bp.

2.4. Patients follow-up (269 patients with regressing and non-regressing tumours). The significance of the sentinel lymph node status and the spontaneous regression of the primary tumour in term of disease free and overall survival.

The follow-up period has been started at the time of the sentinel lymph node biopsy. The disease-free and the overall survival were studied. In term of survival, the event was death, and in that of disease-free survival, the event was disease recurrence or disease recurrence and death. The minimal follow-up time was 18 months. Mean follow-up period: 40.8 months; median follow-up period: 36.0 months; range: 3-88 months.

The criteria of follow-up were met by 233 patients.

STATISTICS

The significance between the different groups was controlled by the χ^2 test. From the point of view of each variable we have determined the Kaplan-Meier survival curves and their significance using the Mantel-Cox test as a control. Two types of multivariate analyses were applied.The joint effect of factors responsible for the sentinel lymph node status and the survival was determined by stepwise discriminance analysis.The F values entered as measures of responsibility and the correct classification rates per group attainable by the discriminance function are also given. In the other multivariate analysis survival was studied with the aid of the Cox proportional hazard regression model. The statistical significance of S-100 protein concentrations was assessed by the Mann-Whitney test. Cut points: Breslow: 2 mm, 4 mm (categories ≤2 mm; 2.01-4 mm; >4 mm). Age: 50 years. Groups: histological type: NM-SSM; regressing –non-regressing tumours; localisation: trunk-extremities; progression: haematogenous-lymphogenous. All analyses were performed with a BMDP statistical software package at a significance level of 0.05.

3. RESULTS

3.1. Sentinel lymph node biopsy with double labelling technique in malignant melanoma

The sentinel lymph node biopsy was successful in 447 patients and unsuccessful in 3, that corresponds to 99.4% successfulness rate. Positive sentinel lymph node was found in 69 patients (15.5%).

Lymphatic mapping was performed in 525 lymph node basins inguinal n=175, axillary n=330, supraclavicular n=18, retroperitoneal n=2 and in-transit sentinel lymph nodes n=12.

The involved lymph nodes were localised in one lymph node basin in 363 cases (80.7%), in two regions in 75 cases (16.7%), in 3 regions in 8 cases (1.8%) and in four regions in one single case (0.2%).

Lymphoscintigraphy displayed 818 sentinel lymph nodes, however, surgery identified 957 lymph nodes that accumulated the isotope and/or stained blue. This means a 1.82 sentinel lymph node per lymph region ratio (957/525).

In 53/69 patients with positive sentinel lymph node we detected one positive sentinel node (76.8); in 13/69 patients two positive (18.8%), in 2/69 patients three positive (2.8%) and in 1/69 patient four positive sentinel lymph nodes (1.4%).

The positive sentinel lymph nodes in 71 lymph regions showed the following anatomical distribution: inguinal n=30, axillary n=40, supraclavicular n=1. This corresponds to a 1.08 positive sentinel lymph node per patient ratio.

In 69 patients the positive result of the sentinel lymph node affected one lymph region (93%). In one patient it involved 2 regions (1.4%). In addition to the involvement of 2 regions in one patient in-transit micrometastasis was also detected (1.4%). In two patients one involved region was associated by an in-transit micrometastasis (2.8%). Finally, in one patient only one in-transit positive sentinel lymph node was identified (1.4%).

Therapeutic block dissection was made in 57 patients with positive sentinel lymph nodes (82.6%). Based on detailed patient information about the expected benefits and potential adverse effects 12 patients refused surgery.

The block dissection revealed tumour involvement of lymph nodes in further 8 patients (14.0%).

The χ^2 –test yielded the following p values for the sentinel lymph node status: sex (p=0.91), age (p=0.62), site of primary melanoma (p=0.45), Breslow thickness (p=0.00001); invasion depth according to Clark (p=0.0005); ulceration (p=0.00001); histological types (p=0.0008). The univariate analysis yielded the highest significance for the Breslow values and the ulceration of the tumours but significant values were obtained also for the correlation between the Clark's invasion depth, the histological type and the sentinel lymph node status, respectively.

The percentage of sentinel lymph node positivity increased parallel with the tumour thickness and the ulceration of the tumours. While it was only 0.9% with tumours of ≤1 mm thickness, it was 41.9% with those of >4 mm thickness. **Table 2., Fig. 1.**

The percentage of the ulceration of the primary tumour in the sentinel negative group was 16.7%; in the other group was 49.2%.

By means of the multivariate discriminance analysis, it was found that the tumour thickness according to Breslow and the ulceration of the primary tumour showed 74.3% correct classification rate (in the negative sentinel lymph node group 76.5% and in the positive sentinel lymph node group 61.4%). F values: Breslow: 62.9%; ulceration: 11.1%.

3.2. Correlation between the preoperative S-100 protein values and sentinel lymph node status

S-100 protein determination was made with in 301 patients of whom one had unsuccessful sentinel lymph node biopsy (99.6% successfulness rate) and 36 patients showed positive sentinel lymph node result (12.0%). The clinical details of the remaining 300 patients are given in the **Table 3.**

In 300 patients the mean S-100 value was 0.089 μg/l (median: 0:08; range: 0.0-0.8)

The 262 sentinel lymph node negative patients' mean S-100 protein value was 0.088 μg/l (median: 0.08; range: 0.0-0.8).

In the 38 sentinel lymph node positive patients the mean was 0.094 μg/l (median: 0.089; range: 0.01-0.22).The values were nearly identical, p=0.18.

Similar results were obtained if not the mean values but those over the normal range were compared with each other (p=0.48). (We found normal S-100 levels in 237 (90.5%) patients,

elevated in 25 (9.5%) in the node negative group; normal levels in the node positive group in 33 (86.8%), elevated in 5 (13.2%) patients).

3.3. The prognostic significance of the spontaneous regression of the primary melanoma from the point of view of sentinel lymph node status and circulating tumour cells (tyrosinase mRNA)

Overall, the clinical details of 269 patients (119 men (44.2%) and 150 women (55.8%)) were analysed.The mean age was 52.8 years (range: 21-80 years).

The tumour localisation in order of frequency was: trunk – 132 cases (49.1%), upper extremities – 48 cases (17.8%), lower extremities – 89 cases (33.1%). Tumours classified according to the Clark depth of invasion as follows: 33 (12.2%) belonged to Clark level II; 83 (30.9%) to level III; 142 (52.8%) to level IV; and 7 (2.6%) to level V. Classification was not feasible in 4 cases (1.5%) because of highly pronounced regression signs. The Breslow thickness ranged from 0.1 to 13 mm (mean 2.09 mm). The histological types were as follows: superficially spreading melanoma (SSM), 160 cases (59.5%); nodular melanoma (NM), 95 cases (35.3%); acrolentiginous melanoma (ALM), 5 cases (1.8%); desmoplastic, 4 cases (1.5%); lentigo maligna melanoma (LMM), 1 case (0.4%); indefinable, 4 cases (1.5%). Ulceration was found in 66 cases (24.5%).

The sentinel lymph node surgery was successful in 266 patients (98.7%). A positive sentinel lymph node was identified in 43 patients (16.2%). Signs of intermediate regression were detected by histology in 75 primary melanomas (27.9%).

A positive signal for tyrosinase messenger RNA by RT-PCR was observed in 25 of 94 patients (26.5%). In the control groups 13 of 19 patients (68%) with locoregional progression and in 26 of 35 (74%) with disseminated tumours proved positive.

The clinical details of 75 patients with regressive primary tumours and 194 patients whose tumours did not show signs of regression are presented in the **Table 4**. The χ^2-test failed to reveal significant differences between the two groups according to age and sex. The regressive tumours were localised significantly more frequently on the trunk than on the extremities ($p=0.006$). The two groups showed highly significant differences in tumour thickness ($p=0.00001$). The mean tumour thickness (1.24 mm) was also less in the regressing tumours than in the others (2.41 mm). Although 50% of regressive melanomas belonged to

thickness category ≤1 mm, this proportion was only 18% for non-regressive melanomas. The results of invasion depth according to Clark corresponded well to those obtained for tumour thickness (p=0.0002). Ulceration was less frequent in the regressive tumours (p=0.003). In regressive tumours, the occurrence of SSM was significantly higher than that of NM when these two dominant histological types were considered (p=0.00001). The sentinel lymph node status was more favourable in the group of regressive tumours (p=0.023). RT-PCR assay for the detection of tyrosinase messenger RNA was positive in 5 of the 26 examined regressive tumours (19.2%), and in 20 of 68 non-regressive ones (29.4%). The difference was not significant (p=0.32).

Univariate analysis of the factors responsible for the sentinel lymph node status showed that patient's age and sex, and the location of the primary tumour did not influence the results (p=0.8; p=0.9; and p=0.89, respectively). A highly significant relationship was found between the sentinel lymph node involvement and Breslow thickness (p=0.00001), Clark's level (p=0.008), ulceration (p=0.00001) and histological type (p=0.01). Only 1 of 73 tumours ≤1 mm in thickness was positive for sentinel lymph node involvement (1.4%). This tumour was a Clark IV one and showed signs of focal regression.

The occurrence of positive sentinel lymph nodes was significantly lower in tumours exhibiting regression features (p=0.026).

The sentinel node negative and positive tumours yielded nearly identical percentage values for positive tyrosinase mRNA: 10 of 70 (14.3%) and 3 of 24 (12.5%) (p=0.81), respectively.

Multivariate analysis of the effect of sex, age, tumour location, Breslow and Clark measurements, ulceration and regression features on sentinel lymph node status yielded the following F values: sex: 0.20; age: 0.08; localisation: 0.74; Clark: 18.51; Breslow: 42.08; ulceration: 17.88 and regression features: 4.04. In the discriminance analysis, the tumour thickness according to Breslow and ulceration of the primary tumour yielded a joint 72.4% correct classification rate, 74.4% in the negative group and 61.9% in the positive group.

3.4. Patients follow-up (269 patients with regressing and non-regressing tumours). The significance of the sentinel lymph node status and the spontaneous regression of the primary tumour in term of disease-free and overall survival.

The criteria of follow-up were met by 233 patients whose details are represented in the **Table 5.** Thirty-five patients were excluded because of too short observation period and unsuccessful intervention in 3 patients. The successfulness rate was: 98.3%. Forty patients showed positive sentinel lymph node (17.4%).

Table 6. represents the clinical details of patients of the sentinel lymph node negative and positive group, respectively. In addition, the corresponding p values of the χ^2 –test performed to check their differences are also given. These significance values are in harmony with those described in our earlier studies.

Similarly to the conclusions drawn after the 450 sentinel node biopsies, the results of the multivariate discriminance analysis also suggest that the primary predictors of the sentinel lymph node status are the thickness of the primary tumour and the presence or absence of ulceration. Together they yielded 72.6% correct classification rate*;* in the sentinel node negative group 76.3% and in the positive: 55.0%.

Out of the 230 successful SLNB patients 184 (80.0%) are alive without symptoms, 15 (6.5%) with symptoms, and 31 (13.4%) died. Progression was observed in 46 cases (20.0%). The first metastasis appeared as local recurrence in 3 patients (6.5%), in-transit metastasis in 9 patients (19.5%) (joint percentage of them, when projected to 230 patients, 5.2%), lymph node metastasis in 12 patients (26.0%), haematogenic metastasis in 22 patients (47.8%).

Out of the sentinel lymph node negative patients 162 (85.2%) are living without symptom, 10 (5.3%) with symptoms and 18 (9.5%) died. Progression was detected in 28 (14.7%) cases. The first site of recurrence was in the sentinel lymph node negative patients: local recurrence: 1 case (3.6%); in-transit: 6 cases (21.4%) (joint percentage of local and in-transit metastasis to 190 patients: 3.6%.); lymph node metastasis: 7 cases (25.0%) in the previously negative lymph node basin, that means a false negative rate of 7/47 (14.8%). Haematogenic metastasis in 14 patients (50.0%) developed.

Of the node positive group 22 (55.0%) patients are living without symptoms, with symptoms 5 (12.5%) and 13 (32.5%) died. 18 of 40 patients (45.0%) progression were observed. Local recurrence was detected in 2 cases (11.1%) and in transit metastasis in 3

(16.7%) in relation to the 40 patients it means a 12.5% metastasis formation rate (local and in-transit). 5 patients (27.8%) developed lymph node metastasis. Haematogenic metastases were observed in 8 patients (44.5%).

There were 62 (26.9%) patients whose primary tumour displayed intermediate regression features. Fifty-five of them (88.7%) are living symptom-free, 4 with symptoms (6.4%) and 3 (4.8%) died. Distribution of the first metastases: lymph node metastasis: 2 (28.6%); in-transit metastasis: 1 (14.3%); haematogenic metastasis: 4 (57.1%).

The first site of recurrence (haematogenous or lymphogenous) of the sentinel lymph node negative and positive, ulcerated and non ulcerated primary tumours and the different Breslow thickness categories are described in the **Table 7.** As to the mode of metastasis formation, they did not differ from one another at a significant level.

With tumours of ≤1 mm thickness no progression occurred.

Table 8. represents the p values of our analysis using the Kaplan-Meier test. We focused on the relationships of overall and symptom-free survival with the patients' sex and age, localisation of the primary, tumour thickness according to Breslow, Clark level, presence of ulceration, histological type, regression, and the sentinel lymph node status.

The tumour thickness according to Breslow, the ulceration of the primary tumour and the sentinel lymph node status proved to exert the most significant effect on survival. However, the Clark level, the histological type of the primary and its localisation and the intermediate regression were likewise significant correlating factors from the point of view of both symptom-free and overall survival **(Fig. 2.,3.,4.,5.).**

The 5-year overall survival rate in the sentinel node negative group was 90% in contrast to the sentinel node positive group where it was only 51%. **(Fig. 2.)** The corresponding values of patients with regressing tumours were 90% and 73%, respectively.

As to the effects of the above factors on overall survival assessed by the multivariate discriminance analysis, the tumour thickness according to Breslow and the ulceration of the primary yielded 80.5% correct classification rate (in the group of patients alive 82.6% and in that of those died 67.7%).

The strong correlation of the sentinel lymph node status with the tumour thickness is well demonstrated by the fact that when the effects of the various factors on survival were examined separately, without considering the Breslow thickness, then the correct

classification rate of primary tumour ulceration and sentinel lymph node status was 76.1% (in the group of patients alive 78.4% and in that of those died 61.3%).
In the former case the F values were 73.8 for Breslow and 4.8 for ulceration. In the latter case: 27.4 for ulceration and 5.6 for sentinel lymph node biopsy finding.
By means of the Cox regression analysis, the primary tumour ulceration (p=0.00001), the Breslow thickness (p=0.00001) and the sentinel lymph node status (p=0.048) were found the exert significant effect on survival. Relative risk: ulceration: 3.09 Breslow value: 2.3; sentinel lymph node biopsy finding: 2.15.
The results were fairly similar in relation to disease-free survival, too. The tumour thickness and the ulceration of the primary yielded a correct classification rate of 77% (in the group of patients with symptoms 64.4%, and in that of those without symptoms 80.1%). Without considering the Breslow thickness, the correct classification rate of the primary tumour ulceration and the sentinel lymph node status was the same, 77.0% (in the group of patients with symptoms: 79.3%, and in that of patients without symptoms 67.4%). F values: Breslow: 62.0; ulceration: 8.65. Without considering the Breslow: the F values: ulceration: 28.9; sentinel lymph node result: 12.7.In the Cox' regression analysis the p values of the primary tumour ulceration and the Breslow thickness were 0.00001 and of the sentinel lymph node status 0.017; respectively. Relative risk: ulceration: 2.87; sentinel lymph node status: 2.13; Breslow: 2.04.

In 6/40 sentinel lymph node positive patients no block dissection was performed. The reasons were the same as those described earlier. (The patients refused the intervention.)
Among the 34 patients undergone block dissection further 5 were found to have positive lymph node (14.7%). In the remaining 29 patients (85.3%), however, histology excluded the presence of metastasis in the lymph nodes.

Two of the 5 block dissection positive patients are living symptom-free (40%), and 3 died of haematogenic dissemination of the disease (60%).
Concerning the other, the block dissection negative group: 16 are alive and symptom-free (55.2%), 5 have symptoms (14.7%) and 8 had died (27.6%). With those showing progression

the first recurrence was local in 2 patients (15.4%), in-transit in 3 patients (23.1%), lymph node in 4 patients (30.8%), and haematogenic in 4 patients (30.8%).
No significant difference between the overall survival rates of the two groups was demonstrated by the Kaplan-Meier function (p=0.065). This seems to confirm the favourable effect of the block dissection. However, the 3-yr overall survival of the block dissection positive patients was 60% and that of the block dissection negatives 82.7%.
We did not find any difference between the block dissection negative and positive groups in the symptom-free survival. The p value of the symptom-free survival was 0.46. The 3-yr symptom-free survival rates: 61.6% (block dissection positive group) versus 60.0% (block dissection negative group).

Of the 6 patients who did not undergo block dissection 4 are alive (66.7%), and 2 died (33.3%). With 1 patient the first metastasis appeared in a lymph node, in another a distant haematogenic metastasis occurred.
In the block dissection group 19/34 (55.8%) patients are alive and symptom-free 4 have symptoms (11.7%) and 11 died (32.5%). The first site of recurrence showed the following pattern: local recurrence 2 (12.5%), in-transit 3 (18.8%), lymph node 4 (26.0%) and haematogenic metastasis 7 (43.8%).
The overall survival rates did not show significant differences, p=0.82. The probability of the 3-yr overall survival was 83.3% with 6 patients and 79.4% with the others.
The symptom-free survival of patients yielded p=0.70. The probability of 3-yr disease free survival was 66.6% with 6 patients and 70.2% with the others.

DISCUSSION

Over many decades the elective block dissection in primary melanoma patients without palpable lymph node has been a controversial matter. In 1992, however, the sentinel lymph node biopsy proposed by Morton seemed to settle this problem. This minimally invasive procedure provided accurate assessment of the regional node status in the melanoma patients.

Thus, the complete regional dissection in the sentinel node negative patients could be avoided, that is in 80% of the patients. Three years after Morton's publication the diagnostic accuracy of the new method was confirmed in the USA (37), and in Australia (38). The true accuracy of the method can be determined when it is already used in clinical practice by the identification rate of the sentinel lymph nodes and by the false negative rate (nodal recurrence in a tumour-negative dissected SN basin).

With the aid of the double labelling technique the identification rate rose to 95-99% (39,40,41). In our 450 patients we performed successful sentinel lymph node biopsy in 99.4%. Two of the three unsuccessful interventions happened during the learning phase of the techniques (20 biopsies).The false negative rate in the related publications ranges from 4-32%. (42,43,44,45,46,47) that raises some questions concerning the sensitivity of the method (48,49). The 14.8% false negative rate in our material is relatively high, in 7 patients we detected isolated lymph node metastasis during the follow up after previously established diagnosis of negative sentinel lymph node status. The number of false negative results may be attributed to the learning phase, note that the surgeon, the nuclear physician and the pathologist, too were also not familiar with the technique. Estourgie (50) for instance described half of the false negative cases with the first 20 patients. In our study 2 (28.5%) false negative patients were operated on during the learning phase. The second reason lies in the non adequate performance of the technique. After many years clinical experiences it can be concluded that the best method for the identification of the sentinel lymph nodes is the lymphoscintigraphy with gamma ray detection probe and patent blue staining. Histological processing may also have inherent source of errors. In addition to serial sections stained by H.E. there is a need for the performance of immune-histochemical reactions. Conditions for this technique were given in our Institute right from the beginning. Pathological revision might confirmed the presence of metastasis in 80% of the cases (45,51). However, one of our patients qualified positively following the pathological revisions. The third possible cause of a false negative status is the obstruction of lymphatics, i.e. when the lymph vessels between the primary tumour and the sentinel lymph node are occluded by a tumour embolus or the sentinel lymph node is so heavily packed by the tumour that it is unable to take up the contrast medium. It is also an alternative explanation that simultaneously with the primary tumour removal the tumour cells have not reached the sentinel lymph node, yet (47,52). Based on this

hypothesis the delayed SLNB (some weeks after primary surgery) seems to be justified. In almost half of our study population we performed this delayed type SLNB and still we got relatively high false negative rate.

The sentinel lymph node positivity in our patients was 15,5%. In literature one can encounter with 10-40% positivity rates (53,54,55).

In our 447 successful interventions the lymph nodes were situated in 525 lymph node basins. Morton in the MSLT-I (56) examining 1173 patients identified 1419 lymph regions. In our material in 363 cases the sentinel lymph node involved one lymph region (76.8%). Estourgie found the same in 72% (50) and Vuylsteke in 84% (57) of the patients. The lymph nodes were found in two lymph regions in 75 patients (16.7%), in 3 regions in 8 patients (1.8%) and in 4 regions in one single patient (1.4%) in our study.

The positive sentinel lymph node involved one lymph region in 94.2% (n=69).

Therefore, in the majority of the cases only one lymph region surgery was necessary.

The surgical complication rate in the MSLT-I trial (56) was 13.8%. 2 of 1173 patients (0.17%) had allergic reactions to the blue dye administered at the time of LM/SLNB. We found seroma and inflammation in approx. 25% of our patients. In one case we discovered blue dye allergy (l/450=0.22%).

By multivariate analysis, the predictors of sentinel lymph node status with us, as in most of other studies, were the thickness of the primary and its ulceration (58,59). With tumour of ≤1 mm thickness we found 1 positive lymph node (0.9%), while in the range of >4 mm thickness, 13 positive lymph nodes were detected (41.9%).

With the thin tumours sentinel lymph node positivity is very low, therefore attempts are made to find other factors that may predict the prognosis in these cases, as well. They may be the high mitotic activity, ulceration and regression, Clark level higher than III, lymphatic invasion (60). The usefulness of the intervention is doubtful in tumours of >4 mm, too because of the high risk of haemogentic metastatisation of that tumours (61). Currently, SLNB biopsy is recommended and has become widely accepted for patients with clinically localized intermediate-thickness (1-4 mm) melanomas.

The development of in-transit metastases – as the surgical effect of SLNB – though it is a controversial matter. In-transit and local recurrence were found in 5.2% of our patients during the follow up; in the sentinel negative group in 3.6% and in the positive one in 12.5%. The

Netherlands Cancer Institute, found an overall incidence of in-transit metastases of 10.8% (23% in SLN positive patients and 7% in SLN negative patients) (50). Thomas and Clark (62) found the joint rates of local and in-transit metastases to be 5.7% in the SLN negative group, and 20.9 % in the positive. Publications are rather scanty which report on nearly identical rate of in-transit metastases in the sentinel node negative and positive groups (63,64). Most authors have experienced a 2-4 fold increase in the number of local/in-transit metastases in the SLNB positive group (65,66,67,68). Cerovac (69) observed local and in-transit recurrence in 7.9% of 972 patients (17% in the SLN positive group and 5.6% in the negative one). In their study, however, the local/in-transit metastases were strongly associated with other risk factors of recurrence, such as elderly age, thicker primary tumour and positive SLNB. They do not support the idea that SLNB increases the frequency of in-transit metastases. The appearance of in-transit metastases is regarded as markers of biologically aggressive tumours. Their arguments sound very convincing since in the SLN negative group the rate of in-transit metastases is not high although these patients also undergo SLNB. In our own material the proportion of in-transit metastases is not too high, even in the SLNB positive group it is only 12.5%. It is true, however, that it is 4-fold of the 3.6% value found in the SLNB negative group. As discussed in the former chapters in the SLNB positive patients it is not only the lymph node status but the parameters of the primary tumour that may be suggestive of a poorer prognosis. (The mean tumour thickness in the sentinel node negative group was 1.81 mm in the positive group, however, 3.56 mm. Ulceration was present in 20.5% of the sentinel node negative patients and in 47.5% of the sentinel node positives one).

SLNB has become a routine diagnostic method during the past 15 years and the sentinel lymph node status as a parameter has been included in the new staging system of AJCC. This staging system is based on the analysis of the clinical details of 17 600 patients from 13 melanoma treatment centres (70).

The prognostic significance of the sentinel lymph node status has been described by several large sized study. It is generally considered to be an independent prognostic factor (46,47,53,63,71,72,73,74). The results of the Sydney Melanoma Unit (75) are typical, their 5-yr overall survival rate is 56% for SN positive patients (n=145) and 90% for SN negative patients (n=846). In fact, our results are almost identical with theirs, among the sentinel node positive patients (n=40) 51%, and among the negative group (n=190) 90% were the 5-yr

overall survival-rates. In our patients the Cox regression analysis confirmed that the ulceration and the tumour thickness according to Breslow are likewise significant prognostic factors in addition to the sentinel lymph node status. Kim et al. arrived to the same conclusion (76).The discriminance analysis has displayed that the *Breslow thickness* and the *ulceration* or, if disregarding from the thickness of the primary tumour, the *sentinel lymph node status* and the *ulceration* of the primary tumour in themselves, have fairly similar diagnostic accuracy.

This refers to a strong correlation between tumour thickness and sentinel lymph node status.

Although many authors regard the sentinel lymph node status to be the most important prognostic factor, the published data are not in agreement. In the European Organization for Research and Treatment of Cancer Trial (55) the SN status, location and ulceration and high Clark level (V) are considered to exert significant influence on disease-free survival , and on overall survival the SN status and the ulceration of the primary tumour. Estourgie (50) demonstrated a single significant interaction between the overall survival and the ulceration of the primary tumour in the group of sentinel node negative patients. On the other hand, Vuylsteke thought (57) based on the analysis of the details of 209 patients, the sentinel lymph status, the Breslow thickness and the lymphatic invasion to be independent prognostic factors for overall survival.

At present the most controversial issue in relation to sentinel lymph node biopsy the clinical consequences of complete block dissection in positive sentinel node patients. This problem was in the limelight of many publications. The preliminary results of the first Multicenter Selective Lymphadenectomy Trial show that in the SLN positive patients the immediate regional block dissection improves survival (77,78,56). Kretschmer (79) in a retrospective study compared the 3-year survival rates of 314 sentinel lymph node positive patients undergone early performed lymph node dissection with 623 patients who underwent delayed lymph node dissection. In order to avoid lead- time bias, the survival was generally calculated from the excision of the primary tumour. A difference between the two survival curves was statistically significant. He stated that among the positive sentinel lymph node patients the early completing block dissections results in highly significant overall benefit. Opposite to him, Starz (80) at the 4th Biennial International Sentinel Node Congress in 2004 claimed that by multivariate analysis the CLND proved to be independent predictor from the point of view of haematogenic metastases development. They performed the complete regional block

dissection in 50 patients for micrometastases situated not deeper than 1 mm in the subcapsular region and in 43 patients they did not perform the intervention. Nevertheless, without the 3 ongoing large sized (56,81,82), prospective, randomized studies several aspects of SLNB are still to be clarified. Further the practical significance of the molecular pathological examination of the sentinel nodes should also be clarified. Using this sensitive method, the sentinel lymph node positivity can be increased to approximately 60% (83). This assay may be too sensitive for routine clinical use.

In the course of block dissection we evidenced 14.7% metastatic lymph node involvement that corresponds to the relevant literature data. For instance this value was 18% with Mozzillo (84). In Sabel's (85) study 980 patients were included of which 232 (24%) proved to be SLN positive. Further lymph node involvement was detected in 34/232 (14.6%). According to the multivariate analysis the predictors of the positive block dissection findings were: tumour thickness, the presence of extranodal involvement, and three or more sentinel lymph nodes.

Approximately in 85% of the patients with positive sentinel lymph nodes the result of the block dissection is negative. This means that surgery is usually superfluous.

The micrometric classification (S-classification) developed by Starz et al. (86) shows that patients belonging to SI-II groups whose tumour burden is ≤1 mm, have significantly more favourable prognosis than those classified in SIII group for their larger size. Studying the anatomical localisation of micrometastasis in sentinel lymph nodes Dewar concluded in his lecture at the 4th Biennial International Sentinel Node Congress (87) that if the metastasis was in subcapsular localisation, the CLND could have been omitted because if only this type was present in the SLN, the intervention never ever disclosed further positive lymph nodes.

Our 5 patients (14.7%) with positive block dissection finding and the 29 (85.3%) with negative results did not differ significantly by the Kaplan-Meier function either in the symptom-free survival ($p=0.46$) or overall survival ($p=0.065$), though the 3-yr overall survival in the former group was only 60% in contrast to the block dissection negative patients whose value was 82.7%. (The probability of the 3-yr disease-free survival rates of the two groups was fairly identical.)

The non-significant nature of the difference may indicate, in principle, that block dissection has a therapeutic efficiency.

In 6 (6/40=15%) sentinel node positive patients we did not perform therapeutic block dissection. When comparing them to the 34 patients undergone block dissection we failed to demonstrate significant difference in their symptom-free (p=0.70) and overall survival (p=0.82) rates as well as significant percentage difference in the probability of the 3-yr survival. This result does not support the therapeutic efficiency of the block dissection.

In view of the prognostic significance of the sentinel lymph node status one is inclined to consider other well known prognostic factors for comparative purposes. It is worth testing whether they are able to predict sentinel lymph node positivity.

Tumour marker assays with S-100 protein was applied in 300 patients having successful sentinel lymph node biopsy. In fact, the sentinel node negative and positive groups did not differ from each other concerning the average values (p=0.18). The difference expressed in percentage was also minimal. It is true that in the sentinel node positive group the values were somewhat higher.

In the 2002 volume of the British Journal of Dermatology (88) an article reported on the details of 31 patients analysed from similar point of view. They did not observed elevated serum S-100 concentration in any single case. In the sentinel node negative group, however, the mean value was significantly higher than that in the positive.

In our material the SLN status and the ulceration of the primary tumour by discriminance analysis yielded nearly identical correct classification rate with the Breslow value and the ulceration. This suggests that in regressing tumours whose Breslow value is rather uncertain the sentinel lymph node status may be a particularly useful prognostic factor. This issue is of current importance since almost 30% of the primary melanomas show intermediary regression signs and the majority of the regressing tumours are thin. Half of our patients had tumours of 1 mm thickness or less. It is doubtful whether it is worth performing sentinel lymph node biopsy with tumours of ≤1 mm thickness. It is hoped that our study has contributed to adopt a more uniform standpoint in this matter. The regression of primary malignant melanomas was first described by Gromet et al. in 1978 (89). Tumours may regress completely and the disease is usually discovered due to the appearance of lymph node or, less often, an internal organ metastasis (90). The duration of the process and the proportion of the tumour involved are important factors. The presence of regression signs encumbers the correct histological evaluation as a massive inflammatory reaction associated with disintegrated tumour cells and

scarring may mimic a thin tumour (91). Sometimes precise Breslow and Clark classifications are not feasible because of marked regression phenomena. Shaw et al. (92) reported on 28 thin tumours with simultaneous lymph node metastasis. All exhibited signs of regression. In contrast, in another study she stated that the evidence of regression in thin lesions had no influence on prognosis (93). Sagebiel (94) claimed that regressive primary tumours do not shorten survival but more often exhibit metastases in the lymph nodes.

The sentinel lymph node resulted in a new prognostic factor not dependent directly on the histological parameters of the primary tumour. The Breslow thickness and the ulceration of the primary tumour are unquestionably the most important predictors of sentinel lymph node status. In our study relationship was sought between sentinel lymph node status and prognostic factors other than those mentioned above that indicate partial histologic regression of the primary tumour. The aim was to determine indirectly their prognostic significance. Three phases of regression are known in malignant melanoma: early, lymphocytic infiltration, that means a favourable prognostic sign; partial and complete regression. Partial regression is what influences tumour thickness and thus prognosis more than anything else. Complete regression of the primary becomes evident only if metastases develop. In this case, sentinel lymph node excision is out of the question. Our results, that are 75 of 269 patients (27.8%) with melanoma showing regression signs, are in line with the figures reported by other authors.

Comparison of the clinical details of patients with primary regressive tumours and their tumour characteristics, with those of patients having tumours without regression did not show a significant difference in sex distribution in contrast with the observations of Shaw et al. (93) and Kelly et al. (14) who found regressive primary melanoma to be predominant in men. In our study, regressive tumours were significantly thinner, 50% being ≤1 mm, compared with 18% of the non-regressive ones. This value compared well with the 58% reported by McGovern et al. (95). The invasion depth of regressive tumours was also lower and ulcerated forms were less frequent than for non-regressive melanomas. In line with Kelly et al. (14) observations, the majority of these tumours were localised on the trunk and were of the SSM type. The sentinel lymph node status was more favourable than that of non- regressive tumours.

The predictors of sentinel lymph node status on univariate analysis were the tumour thickness, Clark level, ulceration of the primary tumour and nodular histological type in agreement with literature data. Regressive tumours were found to develop a significantly smaller number of micrometastases. Multivariate discriminance analysis of our patients confirmed the observations of other authors that the most important predictors of sentinel lymph node status are the thickness of the primary tumour and the presence of ulceration (58,59).

On the basis of the univariate analysis of sentinel lymph node status, regressive primary melanomas seem to have a more favourable prognosis than non-regressive ones, presumably because they are significantly thinner, rarely ulcerated and are predominantly of the SSM type tumours. In patients with tumours ≤1 mm thickness we found only one with a positive sentinel lymph node (1.3%). This tumour was a Clark level IV and exhibited signs of focal regression. Cascinelli et al. (96) detected positive sentinel lymph nodes in 2% of patients with tumours ≤1 mm in thickness. In view of these findings we cannot presume that thin regressive tumours would have poorer clinical course than non-regressive ones.

No significant relationship could be established between a positive signal for tyrosinase messenger RNA by RT-PCR and either the regressive features of the primary tumour or the sentinel lymph node status. The percentage values in the sentinel lymph node positive and negative group were almost identical. Non-regressive tumours, however, yielded a somewhat higher rate for positive values. This phenomenon might be related to the greater tumour thickness. The results of the control tumours, showing locoregional progression and dissemination, correlated with the clinical stage (68% and 74%) and yielded a high ratio for positive values, as expected.

Summing up, we can state, that in accordance with the data in the literature, an intermediate level regression of the primary tumour has no predictive value for the presence of occult regional lymph node involvement (10,15,97). In our study population this statement was also valid for tumours ≤1 mm thick.

We failed to demonstrate significant relationship between the presence of circulating tumour cells and either the regressive features of the primary tumour or the sentinel lymph node status. The lack of association between tyrosinase mRNA expression and SN status may just be a reflection of the small number of patients. Further studies with patients' follow-up are

necessary to determine whether the RT-PCR results may be predictive of haematogenic dissemination.

The univariate analysis of the follow up data of our patients with regressing primary melanoma presumes that the presence of regressing signs refers to more favourable prognosis. In case of symptom-free survival the p value was 0.045 and in the overall survival 0.032. By multivariate Cox hazard regression analysis the Breslow thickness (p=0.00001), the ulceration of primary tumour (p=0.00001) and the sentinel lymph node status (p=0.048) have been found to be the independent prognostic factors for the survival. Thus the multivariate analysis did not demonstrate significant difference between the regressing and non-regressing tumours. Though 50.7% of the regressing tumours (n=38) belonged to the group of tumour thickness ≤1 mm, progression did not occur at all.

Our results, although obtained with modest number of patients, entitle us to believe that with thin regressing tumours one should consider the benefit of the sentinel lymph node biopsy.

CONCLUSION

1. **The sentinel lymph node biopsy has been introduced with double labelling technique in the clinical management of 99.4% successful, 14.8% false negative rate in malignant melanoma.**
2. **Serum S-100 concentrations do not predict the presence of micrometastasis in sentinel nodes in primary cutaneous melanoma.**
3. **The intermediate level of regression of the primary tumour has no predictive value for the presence of occult regional involvement. In our study population, this statement was also valid for tumour thickness of 1 mm or less. No significant relationship could be established between a positive signal for tyrosinase messenger RNA by RT-PCR and either the regressive features of the primary tumour or the sentinel lymph node status.**
4. **The thickness of the primary tumour, its ulceration and the sentinel lymph node status have proven independent predictors of the symptom-free and overall**

survival. Thus the multivariate analysis did not demonstrate significant difference between the regressing and non-regressing tumours. Though half of the regressing tumours belonged to the group of tumour thickness ≤1 mm, progression did not occur at all.

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Table 1.: POPULATION OF ALL PATIENTS

Total no. (%) of cases	450	(100)
Gender [no. (%)]		
male	204	(45.3)
female	246	(54.7)
Age (yr) [mean (range)]	52.2 (17-83)	
Site of primary melanoma [no. (%)]		
trunk	222	(49.3)
upper extremity	89	(19.8)
lower extremity	139	(30.9)
Breslow thickness (mm), mean (range)	2.01 (0.1-13)	
≤ 1[no. (%)]	104	(23.15)
1.01-2.00 [no. (%)]	171	(38.0)
2.01-4.00 [no. (%)]	138	(30.6)
> 4.00 [no. (%)]	31	(6.9)
not identificable [no. (%)]	6	(1.3)
Clark level [no. (%)]		
I	8	(1.8)
II	43	(9.6)
III	150	(33.6)
IV	229	(51.3)
V	10	(2.2)
not identificable	6	(1.3)
no information	4	
Ulceration [no. (%)]		
yes	92	(21.6)
no	334	(78.4)
no information	24	
Histological types [no. (%)]		
in situ	8	(1.9)
SSM	255	(60.4)
NM	136	(32.2)
ALM	10	(2.4)
LMM	1	(0.2)
desmoplastic	6	(1.4)
not identificable	6	(1.4)
no information	28	

Table 2.: RELATIONSHIP BETWEEN THE VARIOUS BRESLOW GROUPS AND SENTINEL LYMPH NODE STATUS

Breslow thickness mm	Negative sentinel lymph node n (%)	Positive sentinel lymph node n (%)
≤ 1	108 (99.1%)	1 (0.9%)
1.01-2.00	148 (87.1%)	22 (12.9%)
2.01-4.00	104 (75.9%)	33 (24.1%)
> 4	18 (58.1%)	13 (41.9%)

Fig. 1.

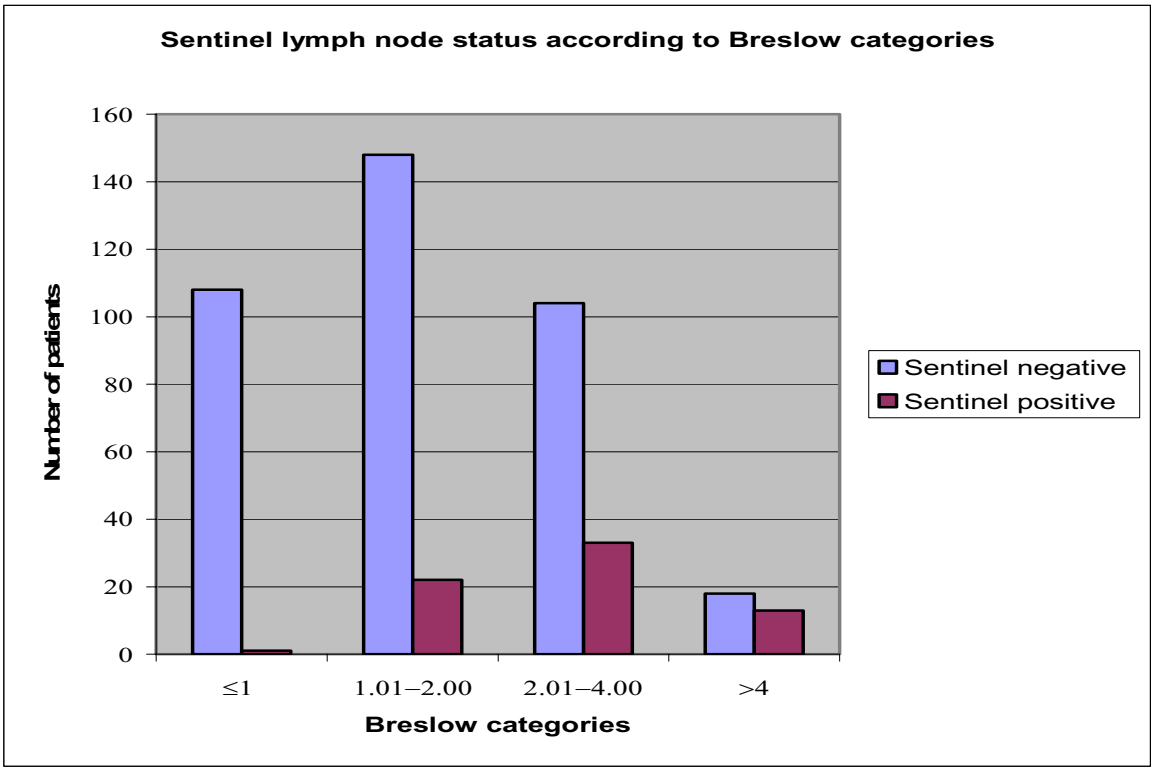

Table 3.: POPULATION OF PATIENTS UNDERWENT S-100 EXAMINATION

Total no. (%) of cases	300	(100)
Gender [no. (%)]		
male	133	(44.3)
female	167	(55.7)
Age (yr) [mean (range)]	51.6 (17-83)	
Site of primary melanoma [no. (%)]		
trunk	156	(52.0)
upper extremity	62	(20.7)
lower extremity	82	(27.3)
Breslow thickness (mm), mean (range)	1.85 (0.1-12)	
≤ 1[no. (%)]	83	(27.7)
1.01-2.00 [no. (%)]	112	(37.3)
2.01-4.00 [no. (%)]	91	(30.3)
> 4.00 [no. (%)]	10	(3.4)
Not identificable	4	(1.3)
Clark level [no. (%)]		
I	3	(1.0)
II	29	(9.7)
III	103	(34.3)
IV	156	(52.0)
V	5	(1.7)
Not identificable	4	(1.3)
Ulceration [no. (%)]		
yes	59	(20.7)
no	267	(87.3)
no information	15	
Histological types [no. (%)]		
in situ	3	(1.1)
SSM	174	(62.3)
NM	91	(32.6)
ALM	5	(1.8)
LMM	1	(0.4)
desmoplastic	5	(1.8)
no information	26	

Table 4.: POPULATION OF PATIENTS ACCORDING TO THE REGRESSION OF THE PRIMARY TUMOUR

		Primary tumours with intermediate level of regression	Tumours without regression	p value
Total no. (%) of cases		75 (27.8)	194 (72.2)	
Gender [no. (%)]	male	34 (45,3)	85 (43.8)	0.82
	female	41(54.7)	109 (56.2)	
Age (yr) [mean (range)]		53.8(28-79)	52.5 (21-80)	0.39
Site of primary melanoma	trunk	48 (64)	84 (43.3)	0.006
[no. (%)]	upper extremity	12 (16)	36 (18.6)	
	lower extremity	15 (20)	74 (38.1)	
Breslow thickness (mm)		1.24 (0.1-4)	2.41 (0.2-13)	0.0000
[mean (range)]	≤ 1[no. (%)]	38 (50.7)	35 (18)	
	1.01-2.00 [no. (%)]	20 (26.7)	73 (37.6)	
	2.01-4.00 [no. (%)]	13 (17.3)	60 (30.9)	
	> 4.00 [no. (%)]	0 (0)	26 (13.4)	
	not identificable [no. (%)]	4 (5.3)	0 (0)	
Clark level [no. (%)]	II	20 (26.7)	13 (6.7)	0.0000
	III	25 (33.3)	58 (30.1)	
	IV	26 (34,6)	116 (59.8)	
	V	0(0)	7 (3.6)	
	not identificable	4 (5.3)	0 (0)	
Ulceration [no. (%)]	yes	9 (12)	57 (29.4)	0.003
	no	66 (88)	137 (70.6)	
Histological types [no. (%)]	SSM	63 (84)	96 (49.5)	0.0000
	NM	8 (10.7)	86 (44.3)	
	ALM	0 (0)	7 (3.6)	
	LMM	0 (0)	1 (0.5)	
	desmoplastic	0 (0)	4 (2.1)	
	not identificable	4 (5.3)	0 (0)	
Sentinel lymph node	positive [no. (%)]	6 (8)	37 (19.1)	0.026
biopsy result	negative [no. (%)]	68 (90.7)	155 (79.9)	
	unsuccessful	1 (1.3)	2 (1)	
Tyrosinase RT-PCR result	negative [no. (%)]	21 (80.8)	48(70.6)	0.32
	positive [no. (%)]	5 (19.2)	20 (29.4)	
	not done [no.]	49	126	

Table 5.: POPULATION OF FOLLOW-UP PATIENTS

Total no. (%) of cases	233	(100)
Gender [no. (%)]		
male	100	(42.9)
female	133	(57.1)
Age (yr) [mean (range)]	53.07 (21-80)	
Site of primary melanoma [no. (%)]		
trunk	112	(48.1)
upper extremity	42	(18.0)
lower extremity	79	(33.9)
Breslow thickness (mm),mean (range)	2.11 (0.1-13)	
≤ 1[no. (%)]	62	(26.6)
1.01-2.00 [no. (%)]	82	(35.2)
2.01-4.00 [no. (%)]	66	(28.3)
> 4.00 [no. (%)]	23	(9.9)
not identificable [no. (%)]	4	
Clark level [no. (%)]		
II	31	(13.3)
III	69	(29.6)
IV	123	(52.8)
V	6	(2.6)
not identificable	4	(1.7)
Ulceration [no. (%)]		
yes	59	(25.3)
no	174	(74.7)
Histological types [no. (%)]		
SSM	137	(58.8)
NM	83	(35.6)
ALM	5	(2.1)
LMM	1	(0.4)
desmoplastic	3	(1.3)
not identificable	4	(1.7)
Sentinel lymph node biopsy result		
positive [no. (%)]	40	(17.4)
negative [no. (%)]	190	(82.6)
unsuccessful	3	(1.3)

Table 6.: FOLLOW-UP PATIENTS ACCORDING TO SLNB RESULTS

	Sentinel neg.	Sentinel pos.	p value
Total no. (%) of cases	190 (82.6)	40 (17.4)	
Gender [no. (%)]			0.57
male	81 (42.6)	19 (47.5)	
female	109 (57.4)	21 (52.5)	
Age (yr) [mean (range)]	53.2 (21-79)	51.1 (29-80)	0.46
Site of primary melanoma [no. (%)]			0.81
trunk	91 (47.9)	20(50)	
upper extremity	37 (19.5)	3 (7.5)	
lower extremity	62 (32.6)	17 (42.5)	
Breslow thickness (mm),mean (range)	1.81 (0.1-6.0)	356 (0.82-13)	0.00001
≤ 1[no. (%)]	60	1 (2.5)	
1.01-2.00 [no. (%)]	67	14 (35.0)	
2.01-4.00 [no. (%)]	47	14 (35.0)	
> 4.00 [no. (%)]	12	11 (27.5)	
not identificable [no. (%)]	4 (2.1)		
Clark level [no. (%)]			0.0028
II	30 (15.8)	0	
III	60 (31.6)	9 (22.5)	
IV	96 (50.5)	25 (62.5)	
V		6 (15.0)	
not identificable	4 (2.1)		
Ulceration [no. (%)]			0.0004
yes	39 (20.5)	19 (47.5)	
no	151 (79.5)	21 (52.5)	
Histological types [no. (%)]			0.098
SSM	117 (61.6)	18 (45)	
NM	64 (33.7)	18 (45)	
ALM	1 (0.5)	4 (10)	
LMM	1 (0.5)		
desmoplastic	3 (1.6)		
not identificable	4 (2.1)		
Regression			0.061
yes	56 (29.5)	6 (15.0)	
No	134 (70.5)	34 (85.0)	

Table 7.: HAEMATOGENOUS AND LYMPHOGENOUS PROGRESSION

	Haematogenous progression	Lymphogenous progression	p value
Sentinel lymph node result			0.71
negative [no. (%)]	14 (50)	14 (50)	
positive [no. (%)]	8 (44.4)	10 (65.6)	
Regression			0.64
yes	4 (57.1)	3 (42.9)	
no	18 (41.0)	21 (49.0)	
Breslow thickness (mm)			0.75
≤ 1[no. (%)]	0	0	
1.01-2.00 [no. (%)]	7 (50)	7 (50)	
2.01-4.00 [no. (%)]	9 (52.9)	8 (47.1)	
> 4.00 [no. (%)]	6 (40)	9 (60)	
Ulceration [no. (%)]			0.87
yes	13 (50)	13 (50)	
no	9 (45.0)	11 (55.0)	

Table 8.: P VALUES OF OVERALL AND DISEASE FREE SURVIVAL

	P values	
	Overall survival	**Disease free survival**
Gender	0.10	0.10
Age	0.56	0.25
Site of primary melanoma	0.045	0.028
Breslow thickness	0.00001	0.00001
Clark level	0.0078	0.0014
Ulceration	0.00001	0.00001
Histological types	0.0068	0.0045
Regression of primary tumour	0.032	0.045
Sentinel status	0.00001	0.00001

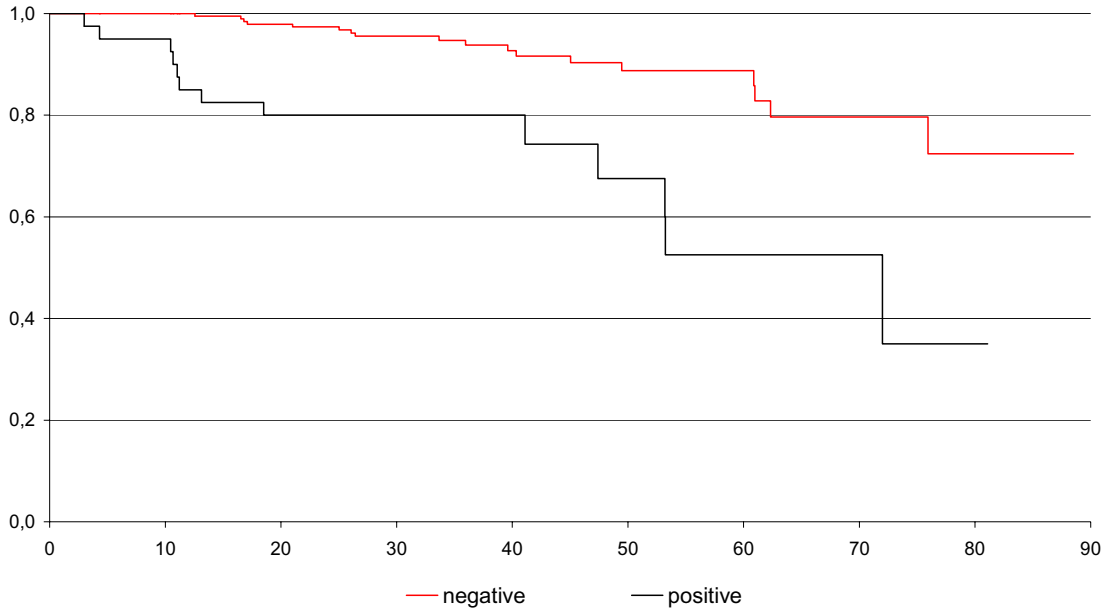

Fig. 2: Kaplan-Meier overall survival curves of SLN negative and positive patients (p=0.00001)

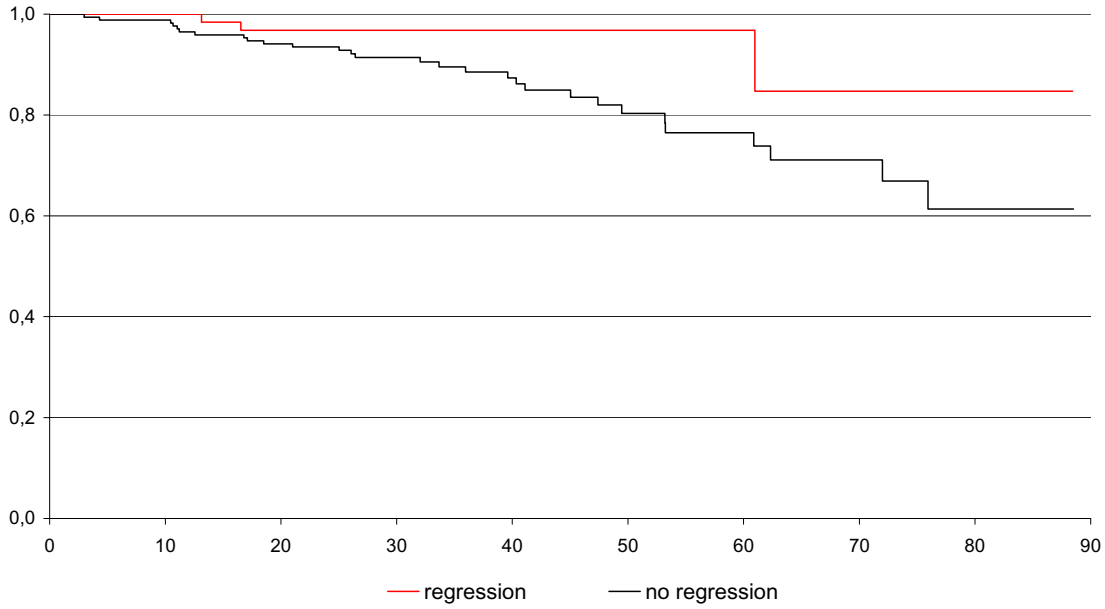

Fig. 3: Kaplan-Meier overall survival curves of the regressing and non-regressing tumours (p=0.032)

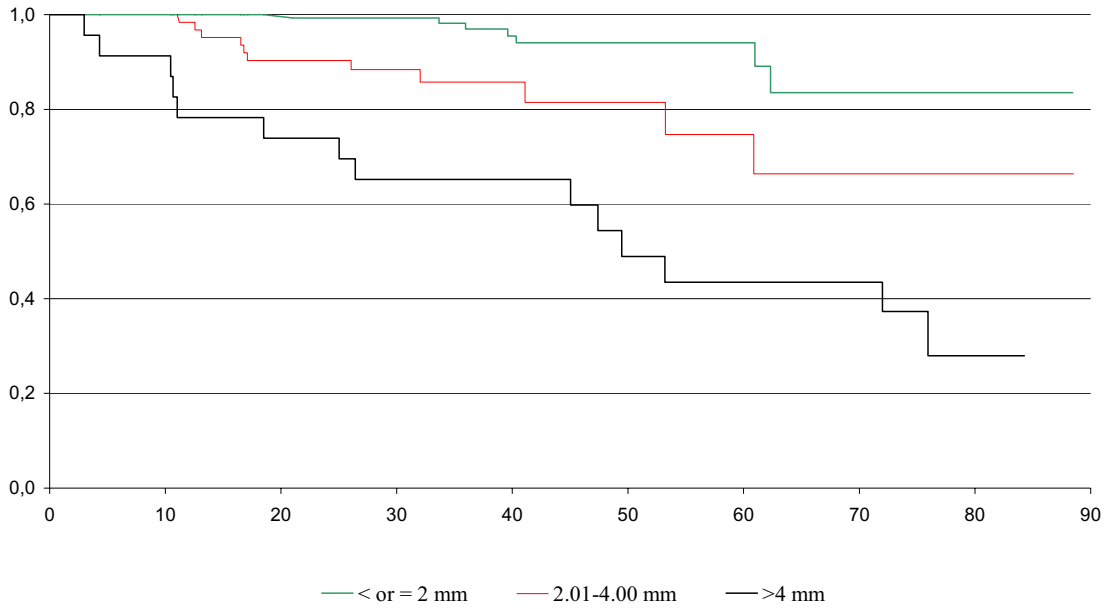

Fig. 4: Kaplan-Meier overall survival curves of the different Breslow categories (p=0.0001)

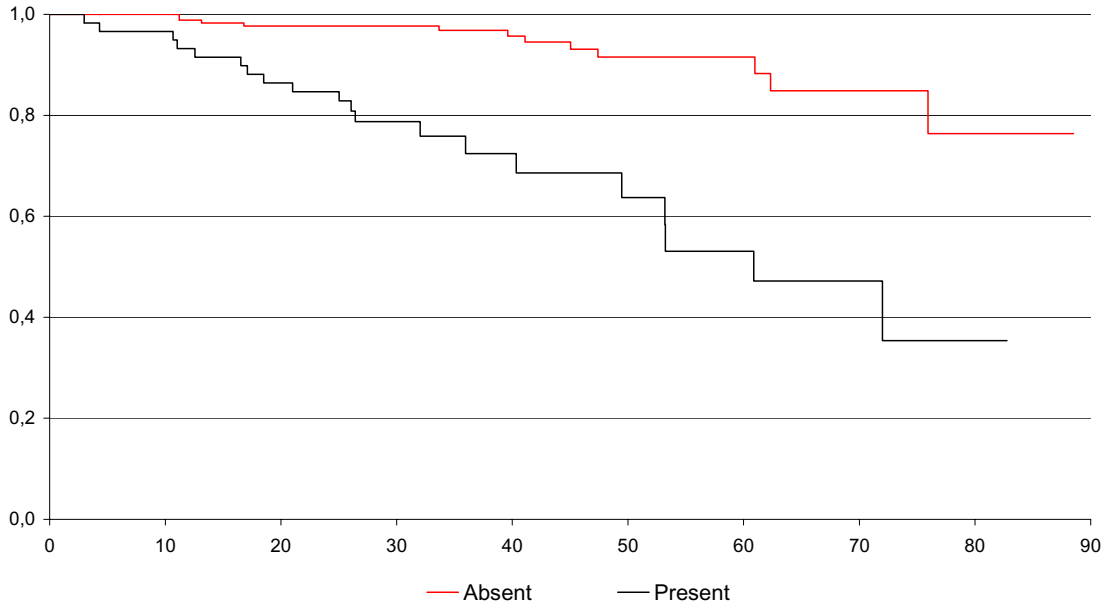

Fig. 5: Kaplan-Meier overall survival curves regarding the ulceration of the primary tumours (p=0.0001)