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Diagnostic and Prognostic Value of the Tracer-Count-Rate in Sentinel-Nodes in Melanoma – A Cohort Study

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Abstract

Background and objectives: The variability between individual count rates and blue staining in sentinel lymph nodes raises the question whether these patterns are of diagnostic or prognostic relevance.

Patients and methods: Out of a total of 168 melanoma patients who underwent surgery during 2008 to 2011, the sentinel lymph node could be identified and removed in 162 patients. To answer the questions for the diagnostic or prognostic value of the *ex vivo* measured tracer count rate the data of these 162 patients were retrospectively statistically analysed.

Results: Blue staining of SLNs was associated with a higher tracer count rate (*p=0.0055). The count rate was inversely proportional to patients age (p=1.48×10-5). Micro-metastases were found in 21 SLN, macro-metastases in 6. The count rate did not correlate with either tumor thickness or the presence of metastases. At the end of follow-up, 95 patients had stable disease, 37 had disease progression, including 16 mortalities. 32 patients were lost to follow-up. The count rate did not correlate with disease progression.

Conclusion: Radio-labelling of sentinel lymph nodes appears age-dependent, potentially indicating reduced lymphatic transport due to aging. However, tracer count rate did not reveal any prognostic value.

Keywords: Tracer count rate; Sentinel lymph node; Malignant melanoma

Introduction

Sentinel lymph node biopsy (SLNB) is an integral part of the surgical management of primary malignant melanoma and is recommended in actual guidelines [1], in the absence of loco-regional or distant metastases, when the tumor thickness exceeds 1 mm [1]. Indeed, SLNB may be indicated when the tumor thickness is less than 1 mm in certain cases [2-4].

In order to accurately identify the sentinel lymph node, the radiotracer ^{99m}Tc nano-colloid (100 MBq) is administered subcutaneously in advance the surgical procedure. After 20-24 h, the radiotracer can be detected and localized with a hand-held gamma probe facilitating identification of the sentinel lymph node. Additionally, blue dye is frequently subcutaneously administered in order to aid sentinel lymph node identification [5].

Following sentinel lymph node extirpation, the *ex vivo* count rate is routinely measured (counts per second) and recorded. Interestingly, while the application and dosage of the radiotracer is standardised, there are often substantial intra- and inter-individual differences in the count rates which are measured. Therefore, the question arose as to whether these differences were of diagnostic and/or prognostic value.

Materials and Methods

Study design and participants

The operation logs of all patients who underwent surgery in the Clinic of Dermatology at the University Hospital of Schleswig Holstein, Luebeck campus, between 2008 and 2011 were analysed. Utilizing the clinical and the ICD Code C43** (malignant melanoma) together with the OPS Code 5-401** (resection of lymph nodes identified via radiotracer and/or blue dye) 168 patients were identified who underwent SLNB-procedure in malignant melanoma.

In 162 out of the 168 patients a single (and no more) SLN could be identified successfully by radiolabeling and/or blue staining.

All patient specific data of these 162 subjects was anonymised and corresponding tumor specific data, in particular the histological characteristics of the primary melanoma and the sentinel node was retrospectively collated and analysed.

Furthermore, the methods (radiotracer or blue dye labeling) used to identify the sentinel lymph nodes were recorded and, in order to determine the prognostic value of the SLN count rate, disease progression during follow-up (until March 2015) was documented and analysed.

Pre-operative mapping of the sentinel lymph node: Patients were administered the radiotracer ^{99m}Tc nanocolloid (100 MBq),

approximately 20-22 h prior to surgery, The ^{99m}Tc nanocolloid was administered intracutaneously via 4 injections, spaced 0.5 cm from the melanoma or, in the case of stage-dependent re-excision, 0.5 cm from the primary excision scar.

In addition, 153 of the 162 patients were administered blue dye intradermally, 2 hours prior to surgery following the same pattern as described above for the radiotracer. Unfortunately, due to a technical problem with the gamma probe, the sentinel lymph node in one patient was identified using pre-operative blue dye only.

Ethical approval

The study was approved by the ethics commission of the University of Luebeck, registered as study 11-226A. The retrospective analysis of the data was performed according to the declaration of Helsinki.

Statistical analysis

For statistical analysis and graphical representation, R version 2.15 statistical software (URL: https://www.r-project.org/, last accessed 7/13/2015) with the front-end RStudio version 0.98.507 (URL: https://www.rstudio.com/, last accessed 7/13/2015) for Microsoft Windows 7 was used together with public packages "car" for improved calculation of regression model statistics.

For estimation of the overall study power, using a one-sided Students' t test for comparison between groups, the number of patients with follow-up and progress (N=37) and no progress (N=89) were used in a resampling approach, together with the observed log-normally distributed noise from the tracer count rates (from the patients with no progress). A power of 0.8 was considered as sufficient.

Results

Out of a total of 168 melanoma cases, in 162 subjects only one sentinel lymph node could be identified and removed. To answer the questions for the diagnostic or prognostic value of the *ex vivo* measured tracer count rate the data of these 162 patients were statistically analysed.

Given patient numbers and observed variation of tracer count rates, the power of the study was sufficient to detect an at least 2,1-fold increase of tracer count rates (effect size: 1.4) in patients with later disease progression.

Melanoma characteristics of the total 168 cases: The tumor thickness ranged from 1.0-4.0 mm in 122 malignant melanomas. 25 malignant melanomas measured less than 1.0 mm thick and 21 had a tumor thickness exceeding 4.0 mm (Table 1). Ulceration of the primary melanoma was present in 32 out of 168 cases. The average tumor thickness was 4.3 mm in ulcerated melanomas compared to 2.1 mm in non-ulcerated tumors. There was a significant correlation between the presence of tumor ulceration and overall tumor thickness ($p<0.005$). The description of the site of the primary melanoma is shown in Table 2.

Sentinel lymph node identification in 162 subjects: 116 sentinel lymph nodes were identified on the basis of both the radioactive tracer and blue dye. 36 sentinel nodes were identified on the basis of the radioactive tracer (i.e. in the absence of blue dye uptake) and 10 sentinel nodes were detected only by blue dye. In 9 of these 10 blue SLNs the distance between the site of the melanoma to the lymph basin was short therefore the use of the gamma probe was not helpful to identify the sentinel intraoperative. The sentinel could be confirmed *ex vivo* taking the count rate. In one case only blue dye uptake was used to identify the sentinel node.

Sentinel lymph node characteristics: Sentinel lymph nodes which demonstrated blue dye uptake exhibited a significantly increased count rate when compared to those which did not (median 1018 *vs.* 497 counts, $p=0.0055$) (Figure 1). The majority of sentinel lymph nodes which showed uptake of blue dye were located in the inguinal region. Comparing the lymph node basins, there was a decline in the average count from sentinel nodes from the inguinal *vs.* axillary *vs.* cervical regions (Table 2). In contrast, sentinel nodes associated with acral melanomas exhibited significantly increased count rates.

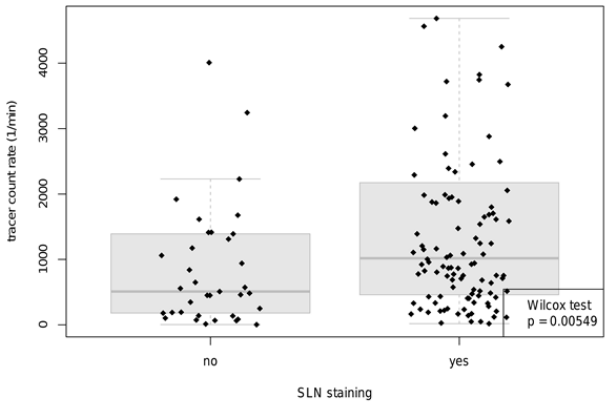

Figure 1: Positive staining of lymph nodes during surgery correlates with a higher tracer count rate. Mixed scatter & box plot of tracer counts by positive staining of the sentinel lymph node during surgery. Each dot represents a single patient case. The Wilcoxon rank sum test reveals a significantly ($p=0.00549$) increased count rate in lymph nodes with positive staining.

In terms of tumor-specific parameters, there was no correlation between count rate and tumor thickness (Table 1). Despite this, ulcerated tumors were associated with a higher count rate than non-ulcerated tumors.

tumor thickness (mm)	NPat	NSLN found	Ulcer	No metastasis in the SLN Tracercountrate			Micro-metastasís in the SLN Tracercountrate			Macro-metastasis In the SLN Tracercountrate		
				n	MW	Median	n	MW	Median	n	MW	Median

<1.0	25	25	2	24	3.088	1.560	1	431	431	0	.	.
1.01-2.0	74	71	6	65	1.465	775	6	851	414	0		
2.01-4.0	48	46	12	34	1.342	871	11	1.423	995	1	707	707
>4.0	21	20	12	12	1.985	1.174	3	1.055	778	5	4.136	1.889

Table 1: Melanoma characteristics, histology and tracer count rates of the SLN according to tumor thickness

Furthermore there was no significant correlation between sentinel lymph node histology (tumor free, with micro- or with macro-metastases) and count rate (Table 1), although this appeared slightly increased in case of macro-metastases (Figure 2).

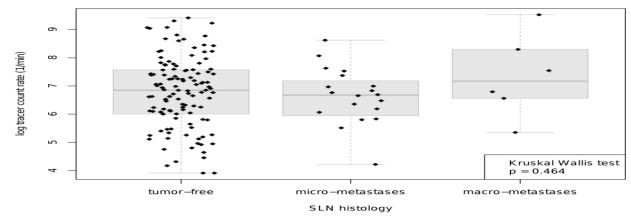

Figure 2: The tracer count rate does not correspond to the sentinel node status. Mixed scatter & box plot of tracer counts by sentinel node status by histological evaluation. The Kruskal-Wallis test procedure (p=0.464) does not reveal a significant relationship between the tracer count rate and the histological status of the sentinel lymph node.

There was however a significant correlation between patients'age and SLN count rate (ANOVA $p=1.34 \times 10^{-5}$) (Figure 3). The count rate decreased by 3.2% per year of age (95% confidence interval: 1.8-4.5%).

Follow up period (until the end of March 2015): 95 patients had stable disease, whilst 37 showed evidence of disease progression, including 16 patients who died from melanoma during the follow-up period.

Site of the melanoma	n	Basin	N of SLN	Counts (MW)
Head	6	Cervical	6	376
Upper limbs total	37	Axillary	87	1.493
Hand	4 out of 37			
Trunc	61			
Lower limbs total	64	Inguinal	69	2.194
Foot	19 out of 64			

Table 2: Sites of the primary melanoma combined with the lymph node basins due to the tracercountrate.

Unfortunately follow-up data for 32 patients was unavailable. There was found a significant correlation between the tumor thickness and the progression of the disease (Figure 4) but no correlation between count rate and progression of the disease.

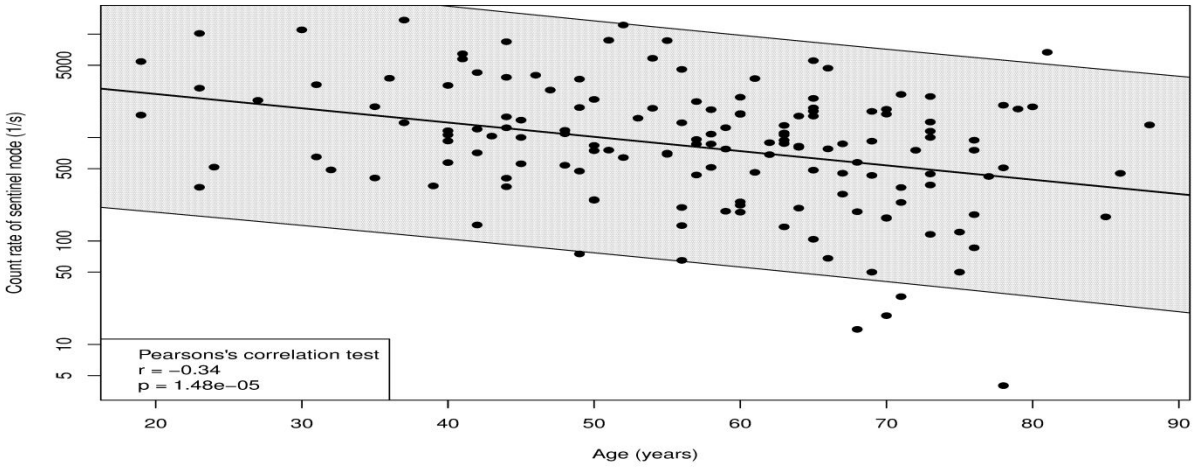

Figure 3: The tracer count rate correlates partially with the age of patients. Scatter plot showing individual tracer count rates ordered by age of the patient. The line indicates the log-linear regression, including 95% confidence bands shown as shaded areas. The correlation coefficient of 0.34 indicates a substantial dependence between age and tracer count rate (p=0.000148).

Discussion

Based upon clinical experience we hypothesized that the *ex vivo* tracer count rate recorded directly after extirpation of the SLN could be associated with the presence of meta states and may have additional diagnostic and prognostic value. The high inter-individual variability of tracer count rate poses a challenge for the study design, we assumed that our clinical experience would correlate to at least a twice as high tracer count rate in patients with later progress than in patients with no progression.

Therefore, we retrospectively collected data from operation logs of 168 patients in total with melanoma who underwent sentinel lymph node surgery between 2008 and 2011 in a tertiary referral setting. Only patients (no 162) in which a single SLN was detected and removed were included in the study. The power of this study was sufficient to detect the assumed twofold increase in tracer count rates between patients with or without later disease progression (Table 3).

Progress	N	Average TCR1	95% CI2	Median TCR	2.5% – 97.5% Quantiles
Yes	37	772	562 - 1060	941	21 - 8419
No	94	677	436 - 1051	707	70 - 6124

Table 3: Tracer count rate *vs.* progress of melanoma disease: (1) Geometric mean of TCR (2). 95% confidence interval for geometric mean TCR).

Sentinel node identification was based on the uptake of the radioactive tracer ^{99m}Tc (100 MBq), administered 20-22 hours pre-operatively. The half-life of ^{99m}Tc is approximately 6 hours; therefore the concentration present in the sentinel lymph node at the time of excision is minimal. Given the standardized administration and detection of the radiotracer, the variability in the count could neither be explained by differences in the intracutaneous administration of the tracer nor by variation in the time between administration and detection.

The central focus of the study was the potential correlation between the SLN count rates and known prognostic factors, for example tumor thickness and the presence of ulceration, providing evidence of the clinical utility of the count rate as a prognostic and/or diagnostic factor in malignant melanoma.

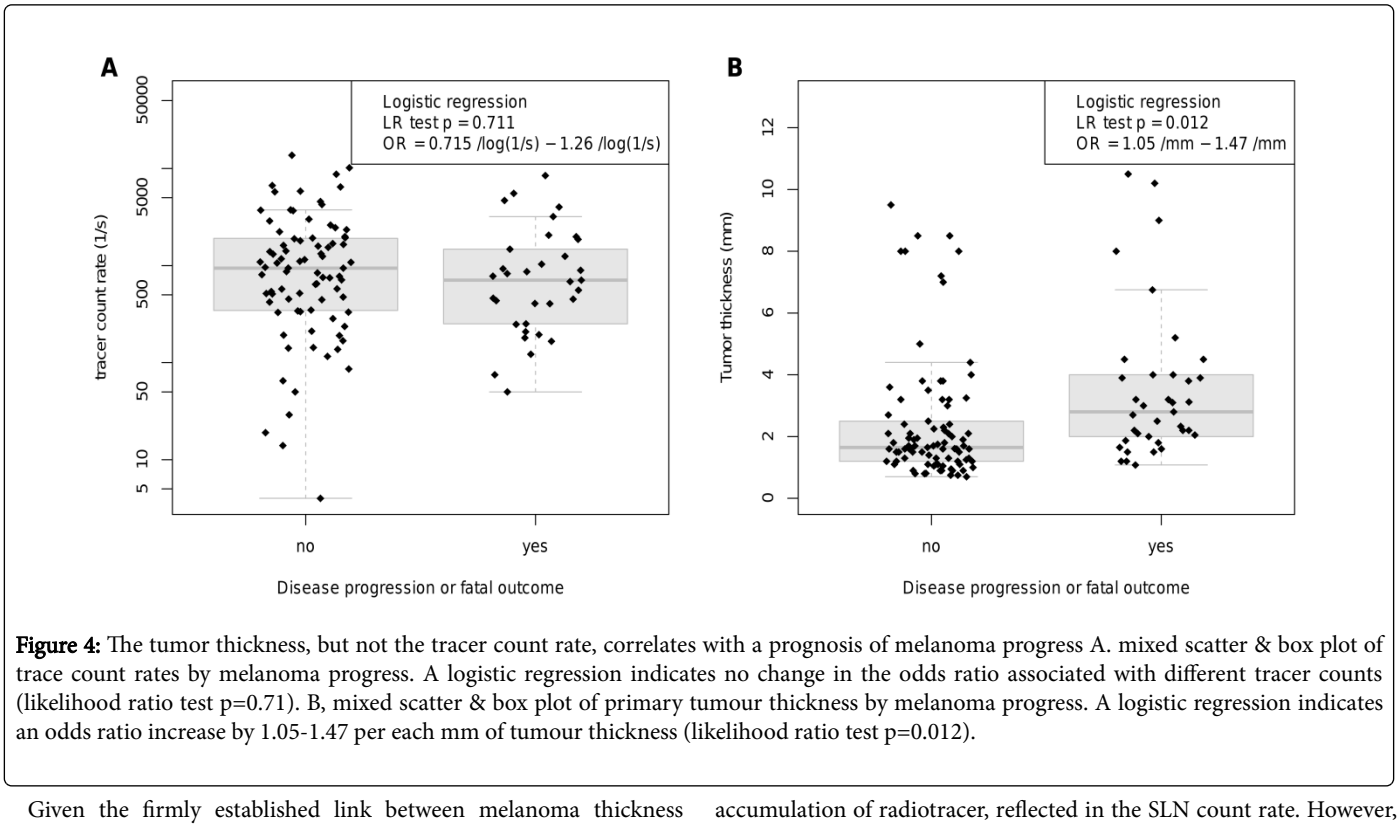

Figure 4: The tumor thickness, but not the tracer count rate, correlates with a prognosis of melanoma progress A. mixed scatter & box plot of trace count rates by melanoma progress. A logistic regression indicates no change in the odds ratio associated with different tracer counts (likelihood ratio test p=0.71). B, mixed scatter & box plot of primary tumour thickness by melanoma progress. A logistic regression indicates an odds ratio increase by 1.05-1.47 per each mm of tumour thickness (likelihood ratio test p=0.012).

Given the firmly established link between melanoma thickness (Breslow thickness) and the risk of metastasis to the sentinel lymph node [6-10], it was conceivable that increased tumor thickness would correlate with sentinel node metastasis and therefore increased accumulation of radiotracer, reflected in the SLN count rate. However, such a correlation between tumor thickness and sentinel lymph node count rate could not be substantiated (Table 4).

Stadium[1]	N	Average TCR[2]	95% CI[3]	Median TCR	2.5%-97.5% Quantiles
IA	18	1468	803-2685	242	1369-10905
IB	56	754	471-1208	15.125	766-8264
IIA	39	623	411-944	50	867-5970
IIB	19	955	532-1714	170.9	1107-4095
IIC	6	506	150-1704	174.7	540-1445
IIIA	17	707	339-1471	105.65	650-7724
IIIB	11	1337	634-2817	335	927-11644
IIIC	1	4008	N.A.	4008	N.A.
IV	0	N.A.	N.A.	N.A.	N.A.

[1]Melanoma stadium, [2]Geometric mean of TCR, [3]95% confidence interval for geometric mean of TCR

Table 4: Tracer count rate *vs.* progress of melanoma disease.

In addition to tumor thickness, the presence of ulceration in the primary melanoma is an independent risk factor pre-existing metastasis to the sentinel node at the time of SLNB [2,10-12]. In the cohort identified, ulceration of the primary tumor was present in 32 out of 168 cases. Although the average count rate was increased in sentinel lymph nodes from patients with ulcerated melanomas, and there was a trend towards an association between the magnitude of the count rate and the presence of ulceration, these differences did not reach statistical significance. It may be speculated that the study was underpowered to identify such an association.

However, when the histology of the sentinel lymph node was examined, it became evident that the presence of both ulceration of the primary melanoma and micro-metastases in the sentinel lymph node was associated with an increased average count rate (1.952 counts/s) when compared with patients with ulcerated melanoma in the absence of micro-metastases to the sentinel node (1.602 counts/s). Indeed, in the case of macro-metastases, the average count rate increased to an average of 4.698 counts/s. Therefore, ulceration of the primary melanoma as a prognostic risk factor was associated with increased count rates in the presence of micro and/or macro-metastases (p=0.071). However, a statistically significant correlation between ulceration of the primary melanoma and the count rate could not be demonstrated in our cohort.

In addition, long-term follow-up (up to 7 years post sentinel lymph node resection) did not reveal a significant correlation between count rate and disease course, neither in terms of progression nor stability.

Whilst the variability between individual count rates initially raised the question as to whether count rates may have biological significance as diagnostic/prognostic markers in malignant melanoma, our findings could substantiate this hypothesis. However, our study highlights several potentially important factors which deserve further investigation.

Namely, sentinel nodes which demonstrated uptake of blue dye exhibited a significantly higher count rate than those which did not (p=0.0055). This was consistent with the finding that the majority of sentinel nodes which were dyed blue were from the inguinal region; the region associated with the highest average count rates. It could be hypothesized that this might be because these nodes are larger than lymph nodes in other basins. However, we found no correlation between the size of the SLN and the count rate. The lower counts in the cervical and axillary regions may be due to the complexity of the lymphatic draining system in the head, neck and trunk areas when compared with the limbs.

Finally, a significant association could be demonstrated between the magnitude of the count rate and the age of the patient. Specifically, increased patient age was inversely proportional to the magnitude of the count rate. It is likely that the accumulation of radiotracer is not only dependent on the lymphatic anatomy, but also on the presence of functionally intact lymphatic drainage.

Conflict of Interest

The authors have no conflicts of interest. There are no conflicts to disclose.

References

1. Pflugfelder A, Kochs C, Blum A, Capellaro M, Czeschik C, et al. (2013) Malignant Melanoma S3 guideline "diagnosis, treatment and follow-up of melanoma"-short version. J Dtsch Dermatol Ges 11: 563-602.
2. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, et al. (2009) Final Version of 2009 AJCC Melanoma Staging and Classification. J Clin Oncol 27: 6199-6206.
3. Warycha MA, Zakrzewski J, Ni Q, Shapiro RL, Berman RS, et al. (2009) Meta-analysis of sentinel lymph node positivity in thin melanoma (≤1 mm). Cancer 115: 869-879.
4. Wong SL, Balch CM, Hurley P, Agarwala SS, Akhurst TJ, et al. (2012) Sentinel lymph node biopsy for melanoma: American Society of Clinical Oncology and Society of Surgical Oncology joint clinical practice guideline. J Clin Oncol 30: 2912-2918.
5. Kretschmer L, Peeters S, Beckmann I, Thoms KM, Mitteldorf C, et al. (2005) Intraoperative detection of sentinel lymph nodes in cutaneous malignant melanoma-blue dye alone versus blue dye plus gamma detection. J Dtsch Dermatol Ges 3: 615-622.
6. Garbe C, Schadendorf D, Stolz W, Volkenandt M, Reinhold U, et al. (2008) Short German guidelines: malignant melanoma. J Dtsch Dermatol Ges 6: 1: S9-S14.

7. Satzger I, Klein M, Löser C, Möhrle M, Reske S, et al. (2011) Zum Benefit der Patienten: Die Schildwächterlymphknotenentfernung. J Dtsch Dermatol Ges 9: 922-926.
8. Gutzmer R, Satzger I, Thoms KM, Völker B, Mitteldorf C, et al. (2008) Der histologische Status des Sentinel-Lymphknotens ist der wichtigste Prognosefaktor bei Melanomen ≥4 mm Tumordicke. J Dtsch Dermatol Ges 6: 198-203.
9. Meyer T, Möhrle M, Garbe C, Hohenberger W (2004) Sentinel Lymph Node beim Melanom. Der Chirurg 75: 774-780.
10. Balch CM, Soong SJ, Gershenwald JE, Thompson JF, Reintgen DS, et al. (2001) Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. J Clin Oncol 19: 3622-3634.
11. Balch CM, Wilkerson JA, Murad TM, Soong SJ, Ingalls AL, et al. (1980) The prognostic significance of ulceration of cutaneous melanoma. Cancer 45: 3012-3017.