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Prognostic factors of cutaneous melanoma and a new staging system proposed by the American Joint Committee on Cancer (AJCC): validation in a cohort of 1284 patients

S. Retsas[a,*], K. Henry[b], M.Q. Mohammed[a], K. MacRae[c]

[a]*Melanoma Unit, Directorate of Cancer Services and Haematology, Hammersmith Hospitals NHS Trust, Charing Cross Hospital Campus, London W6 8RF, UK*

[b]*Department of Histopathology, Imperial College School of Medicine, Charing Cross Hospital, London, UK*

[c]*Department of Epidemiology, University of Surrey, UK*

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Abstract

This study, involving a cohort of 1284 evaluable patients, validates the American Joint Committee on Cancer (AJCC) proposal for the introduction of ulceration of primary cutaneous melanoma as an independent prognostic factor of survival. In univariate analyses, ulceration (Hazard Ratio (HR) 1.983; $P<0.0001$; 95% Confidence Intervals (CI) 1.692–2.325) was a predictor of worse overall survival. In multivariate analyses, ulceration (HR 1.302; $P=0.022$; (95% CI: 1.039–1.633) retained its prognostic significance for survival independent of tumour thickness (HR 1.101; $P<0.0001$; 95% CI: 1.055–1.150); mitotic activity (HR 1.039; $P=0.005$; 95% CI: 1.012–1.067); and age (HR 1.009; $P=0.006$; 95% CI: 1.003–1.016). Ulceration lost its significance in a subgroup analysis of 256 patients with clinically apparent regional lymph node metastases to the number of lymph nodes involved (HR 1.15; $P=0.004$; 95% CI:1.047–1.263). Ulceration is prognostically significant in the tumour but not the nodal classification of melanoma, with mitotic activity the second most important prognostic factor after tumour thickness.

Keywords: Melanoma; Ulceration; Mitotic activity; Lymph nodes; Prognosis; Treatment; Survival

1. Introduction

The Melanoma Staging Committee of the American Joint Committee on Cancer (AJCC) propose major revisions of the melanoma TNM and stage grouping criteria, for a new staging system of cutaneous melanoma [1]. One recommendation among these proposals is that the thickness as well as the presence of ulceration of the primary lesion—as determined by microscopic histopathological examination—should be used in the T classification [1]. In the N classification, the Committee propose that the number of lymph node metastases is the dominant and independent predictor of outcome and should be used instead of the gross size (dimensions) of nodal metastases. Furthermore, it is proposed that ulceration of the primary lesion should be retained as an independent prognostic factor in the classification of any clinically palpable nodal disease [1].

The Committee invite data analyses relevant to the proposed new melanoma staging for validation of the system before its general acceptance [1].

In 1994, we reported a series of 169 patients with malignant melanoma who, during the course of their disease, developed clinically apparent regional lymph node metastases [2]. Prognostic factors for relapse and survival in this cohort, evaluated by Cox multivariate regression analyses, showed that Breslow thickness was the dominant factor predicting recurrence and overall survival [2]. After Breslow thickness, the anatomical location of the primary site (axial versus extremity) and the presence of ulceration of the primary tumour as determined by microscopic histopathological examination, were the most important prognostic factors predicting relapse in regional lymph nodes in these

* Corresponding author. Tel.: +44-20-8746-8295; fax: +44-20-8746-8293.

E-mail address: s.retsas@ic.ac.uk (S. Retsas).

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patients, but not overall survival [2]. The number of histologically involved regional lymph nodes was an independent prognostic factor for survival [2]. In univariate and multivariate analyses adjuvant chemotherapy with vindesine administered for 2 years after therapeutic dissection of clinically apparent metastatic regional lymph nodes, was the most important factor determining survival [2].

In this communication, we present data from a cohort of 1284 evaluable patients, registered with the Melanoma Unit at Charing Cross Hospital, in which we validate ulceration of primary cutaneous melanoma as an independent prognostic factor for overall survival, in conjunction with other known prognostic variables.

In relation to the presence of ulceration, we also studied the impact on survival of reported bleeding from the offending primary lesion prior to its excision. Evaluable data on the number of histologically involved lymph nodes, following a therapeutic dissection for clinically apparent regional lymph node metastases, were available for 256 patients from this cohort. This subgroup of patients was included in a subsidiary analysis in order to evaluate the impact on survival of the number of metastatic regional lymph nodes and adjuvant chemotherapy, relative to the presence or absence of ulceration of the primary lesion and the other clinicopathological variables studied in this cohort.

2. Patients and methods

2.1. Patient population

Patients with a histological diagnosis of malignant melanoma at all primary anatomical sites and stages are registered consecutively with the Melanoma Unit at Charing Cross Hospital (previously at Westminster Hospital) in a prospectively maintained electronic database. Referrals to the Unit originate largely from London, but also from the rest of the UK and from overseas. From June 1977 to August 2000, the time of registration of the last subject in the database, there were 2368 patient records.

2.2. Data collection

The electronic database incorporates detailed demographic data, dates of initial diagnosis of melanoma, stages of evolution of the disease, treatment, date of last follow-up and date of death. The last follow-up, date of death and cause of death are ascertained from the hospital records or by correspondence with the patient's family physician or referring hospital. Follow-up of these patients is constantly updated.

The histopathology of the primary lesion is routinely reviewed in-house from histological material obtained, when available, from the referring centre and is reported in a standard format. This, among others, includes the histogenetic type in terms of nodular, superficial spreading, acral lentiginous and lentigo maligna melanoma [3]. The thickness of the primary lesion and whether it is in the horizontal or vertical growth phase [4]. The level of invasion. The presence or absence of ulceration on microscopic examination of the primary lesion. Ulceration is defined as 'the absence of an intact epidermis overlying a portion of the primary melanoma based on pathological microscopic observation of the histological sections'. The mitotic activity, assessed by the number of mitoses counted per mm^2 and the completeness of the excision. Also registered in the database is the prognostic index calculated by multiplying the number of mitoses/mm^2 with the Breslow thickness [5].

The electronic database also incorporates data on the history of bleeding from the offending primary lesion prior to its excision. This and additional information on the cardinal manifestations of the primary lesion is routinely obtained at the initial interview of the patient's first clinical visit to the Unit. This information is registered in appropriate forms with a standard questionnaire and is subsequently transcribed into the electronic database. Bleeding is the most alarming and memorable symptom to a patient from a suspect lesion and often provokes action for advice. Bleeding has been reported in the past as an independent prognostic factor for relapse and survival [6,7].

2.3. Statistical analyses

In the analyses, the following clinico-pathological variables known for their prognostic significance were studied:

- Gender
- Age at initial diagnosis (as a continuous variable)
- Anatomical location of the primary lesion as a categorical variable (axial versus extremity)
- Tumour thickness (as a continuous variable)
- Level of invasion
- The presence or absence of ulceration as a categorical variable (Yes or No)
- Mitotic activity/mm^2 (as a continuous variable)
- Prognostic index (as a continuous variable)
- History of bleeding from the offending primary lesion as a categorical variable (Yes or No)

In the subgroup analysis of 256 patients with clinically apparent regional lymph node metastases, additional variables studied were time from initial diagnosis to lymph node metastases, number of lymph nodes involved and the effect of adjuvant chemotherapy. Chemotherapy protocols following therapeutic dissec-

tion of clinically apparent metastatic regional lymph nodes were administered for 2 years and were vindesine-based, alone [2] or in combination with dacarbazine [8] and more recently with low-dose Interferon-α. The Cox regression model was applied in univariate and multivariate analyses using the STATA software (Release 6.0, Intercooled Version, Stata Corporation, College Station, TX, USA).

Survival from the offending primary lesion according to the presence or absence of ulceration and reported history of bleeding was compared in simple analyses, unadjusted for covariates, with the Kaplan–Meier method.

If death was due to causes other than melanoma, patients were censored at the time of death.

3. Results

Of the 2368 patients registered with the Unit, there were 1284 records in the electronic database with categorical information on the presence or absence of ulceration of the primary lesion (flagged Yes or No). This information was missing if patients had no identifiable primary site, if they had non-cutaneous primary lesions, if the primary lesion had been curetted, or if the histopathology report of the referring centre did not include a statement on ulceration and histological material of the primary lesion was not available for in-house review.

The records of these 1284 evaluable patients, 673 of whom had an ulcerated primary lesion constitute the basis of this report. Patient characteristics of this cohort are detailed in Table 1. The median follow-up of these 1284 patients from initial diagnosis was 3.8 years with a mean of 5.5 years.

In univariate analyses, gender, age, anatomical location of the primary lesion (axial versus extremity), tumour thickness, ulceration, level of invasion, mitotic activity and prognostic index, were all highly statistically significant predictors of survival from initial diagnosis. Hazard ratios, P values and Confidence intervals are shown in Table 2.

A categorical statement on the history of bleeding (Yes or No) was available for 1102 of the 1284 patients, 655 of whom gave a positive history of bleeding from the primary lesion prior to excision. In univariate analyses, bleeding from the primary lesion was predictive of survival (Table 2). Kaplan–Meier survival graphs according to ulceration and bleeding are shown in Figs. 1 and 2, respectively. In these graphs, a history of bleeding from the primary lesion prior to excision has a similar negative impact on survival as the presence of ulceration (Figs. 1 and 2).

In multivariate analyses including all the above variables, only tumour thickness ($P<0.0001$); mitotic activity ($P=0.005$); age ($P=0.006$); and ulceration, ($P=0.022$) were statistically significant predictors of survival (Table 2). Gender ($P=0.051$) and prognostic index ($P=0.059$) were just short of statistical significance. Bleeding did not achieve a statistical significance in multivariate analyses (Table 2).

In the subsidiary analysis of 256 patients with evaluable data on the number of clinically apparent regional lymph node metastases, the variables that influenced survival from development of lymph node metastases were, the number of lymph nodes histologically involved and adjuvant treatment—versus no treat-

Table 1
Patient characteristics of the entire cohort ($n=1284$) patients

Gender[a]	Male ($n=643$)		Female ($n=641$)		Cohort ($n=1284$)	
Presence of ulceration in primary melanoma[a]	Yes	No	Yes	No		
	376	267	297	344	1284	
	29%	21%	23%	27%	100%	
History of bleeding from primary before excision[a]	Yes	No	Yes	No	Known (yes or no)	Unknown
	348	205	307	242	1102	182
	27%	16%	24%	19%	86%	14%
Anatomical location of primary lesion[a]	Axial	Extremity	Axial	Extremity		
	430	213	200	441	1284	
	33%	17%	16%	34%	100%	
Age (year)[b]	50 (11–86)		51 (17–95)		50 (11–95)	
Thickness (mm)[b]	3.0 (0.2–50.0)		2.5 (0.2–55.0)		2.9 (0.2–55)	
Mitotic activity (mm^2)[b]	5.0 (0.1–50.0)		4.0 (0.1–40.0)		4.0 (0.1–50.0)	
Prognostic index[b]	15.0 (0.07–476.0)		10.0 (0.03–1100.0)		12.0 (0.03–1100.0)	
Level of invasion[b]	IV (I–V)		IV (I–V)		IV (I–V)	

[a] Categorical variables, values shown are number of patients and% of total cohort.
[b] Continuous variables, values shown as median (range).

Table 2
Univariate and multivariate analyses of prognostic factors influencing survival from initial diagnosis of primary cutaneous melanoma ($n=1284$)

Variables	Univariate analyses			Multivariate analyses		
	Hazard Ratio	$P>\|z\|$	95% Confidence Intervals	Hazard Ratio	$P>\|z\|$	95% Confidence Intervals
Thickness	1.073	0.0001	1.062–1.084	1.101	0.0001	1.055–1.150
Mitotic activity	1.039	0.0001	1.027–1.050	1.039	0.005	1.012–1.067
Age	1.017	0.0001	1.011–1.022	1.009	0.006	1.003–1.016
Ulceration	1.983	0.0001	1.692–2.325	1.302	0.022	1.039–1.633
Gender	1.356	0.0001	1.162–1.582	1.228	0.051	0.999–1.511
Prognostic index	1.003	0.0001	1.002–1.004	0.996	0.059	0.991–1.000
Clarks's level of invasion	1.631	0.0001	1.410–1.887	1.182	0.112	0.962–1.452
Bleeding from primary lesion	1.829	0.0001	1.517–2.205	1.187	0.147	0.941–1.496
Primary location axial/extremity	1.392	0.001	1.140–1.550	1.138	0.231	0.921–1.405

ment—following therapeutic dissection (Table 3). For overall survival from initial diagnosis, the variables predicting survival were the number of lymph nodes involved, the time interval from primary diagnosis to regional lymph node involvement and adjuvant versus no adjuvant treatment following therapeutic dissection (Table 3).

4. Discussion

All studied variables in this cohort influenced overall survival in the univariate analyses (Table 2).

However in the multivariate analyses, only four of these clinico-pathological variables remained predictors of overall survival. These in order of statistical significance were:

- tumour thickness
- mitotic activity
- age
- ulceration

Tumour thickness of the primary melanoma is universally accepted as the most important predictor of relapse and overall survival [1,9–13]. Mitotic activity is the next most important predictor of survival in this cohort. This variable has been previously reported as a significant predictor of relapse and survival for cutaneous melanoma [3,7,12,14–18]. When mitotic rate was included in multivariate regression analyses in the study of Ostmeier and colleagues, its prognostic significance was retained at the expense of ulceration [14]. In our study, the powerful statistical significance of mitotic activity may emanate from the large sample of this cohort and the fact that in virtually all histopathological material, assessment has been consistently made by one observer, both initially at Westminster and currently at Charing Cross Hospital. Our observer reports the number of mitoses per mm^2 rather than their number per 10 high power fields. This standardised method of counting allows direct comparison between cases which is not so with counting per high power fields since the area covered in 10 high power fields varies between microscopes according to the magnification of the eye pieces and the high power lenses used.

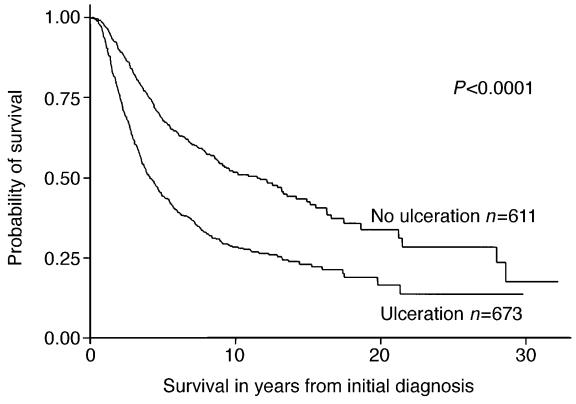

Fig. 1. Survival according to the presence or absence of ulceration of primary cutaneous melanoma. Ulceration: $n=673$. No ulceration: $n=611$.

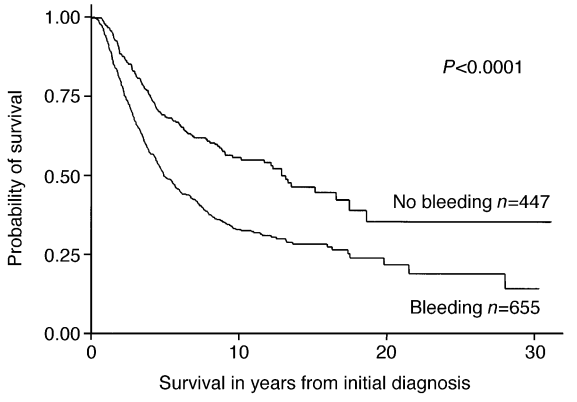

Fig. 2. Survival according to the history of bleeding from primary cutaneous melanoma prior to excision. Bleeding: $n=655$. No bleeding: $n=447$.

Table 3
Multivariate analyses of prognostic factors influencing survival on development of clinically apparent regional lymph node metastases ($n = 256$)

Variables	Analysis from lymph node involvement			Analysis from initial diagnosis		
	Hazard Ratio	$P > \|z\|$	95% Confidence Intervals	Hazard Ratio	$P > \|z\|$	95% Confidence Intervals
Number of lymph nodes	1.150	0.004	1.047–1.263	1.163	0.002	1.056–1.280
Adjuvant versus no adjuvant treatment after therapeutic lymph node dissection	0.733	0.004	0.595–0.903	0.719	0.003	0.580–0.891
Time interval from primary diagnosis to lymph node involvement	1.000	0.739	0.9998–1.0002	0.99925	0.016	0.999–0.9995
Thickness	1.015	0.365	0.983–1.047	1.025	0.117	0.994–1.058
Ulceration	0.834	0.346	0.573–1.216	0.914	0.644	0.625–1.338
Gender	0.839	0.357	0.578–1.218	0.783	0.204	0.537–1.142
Primary location axial/extremity	0.861	0.448	0.586–1.266	0.881	0.526	0.595–1.305
Mitotic activity	1.003	0.862	0.967–1.041	1.010	0.605	0.972–1.049
Age at nodal involvement	1.001	0.897	0.988–1.014	–	–	–
Age at initial diagnosis	–	–	–	1.001	0.831	0.988–1.015

The prognostic index is short of statistical significance in the multivariate analyses probably because mitotic activity was available for study in only 1083 patients of this cohort, whereas all 1284 patients had evaluable data for Breslow thickness.

Age has remained an independent predictor of survival in the multivariate analyses of our study. It has been identified as an independent predictor of survival both as a categorical and continuous variable in univariate and multivariate analyses in previous studies; with older age having an adverse effect on survival [11,13,14,19–22].

The adverse prognosis on survival of an ulcerated primary cutaneous melanoma has been reported by numerous studies in the past [9–13,15] and is confirmed in the study of this cohort in univariate and multivariate regression analyses.

The survival graphs of ulceration versus no ulceration and bleeding versus no bleeding from the primary lesion are remarkably similar (Figs. 1 and 2). Bleeding has been reported in earlier studies with a smaller number of patients to achieve independent prognostic significance on relapse and survival in univariate and multivariate analyses [6]. Bleeding does not retain statistical significance in multivariate analyses in this study probably because it is evaluated in a smaller sample of patients (1102) compared with the whole cohort. This prognostically important clinical sign should be part of routine clinical enquiry at initial presentation.

In the subsidiary analysis of 256 patients with evaluable data on the number of clinically apparent regional lymph node metastases, the variables that influenced survival from dissection of regional lymph nodes were, the number of lymph nodes involved and adjuvant treatment following therapeutic dissection (Table 3).

In this subgroup, the variables predicting overall survival from initial diagnosis were the number of lymph nodes involved, the time interval from primary diagnosis to regional lymph node involvement and adjuvant *versus* no adjuvant chemotherapy following therapeutic dissection. In patients with clinically apparent regional lymph node metastases, ulceration of the primary lesion lost its prognostic significance both, for overall survival and for survival from dissection of metastatic regional lymph nodes. The new AJCC system proposes the inclusion of ulceration in nodal staging. Our study does not support this proposal at least in the presence of *clinically apparent* regional lymph node metastases.

This is probably because the core data evaluated by the AJCC for the inclusion of ulceration in nodal staging, incorporate studies from the USA and Australia, that address largely clinically *occult* regional lymph node metastases detected at elective lymph node dissections and more recently following sentinel node biopsy. In contrast, our initial [2] and current analyses relate to patients with clinically *apparent* regional lymph node metastases at initial presentation or first relapse.

When mitotic activity is entered into the Cox model of the subgroup of patients with lymph node metastases, the prognostic independence of the time interval from initial diagnosis to nodal involvement, the number of metastatic lymph nodes and adjuvant treatment (versus no treatment) is retained. This observation validates the proposal of the AJCC on the prognostic value of the number of lymph nodes histologically involved. It also lends support to our earlier observations [2,8] on the impact on survival of adjuvant treatment upon the development of clinically apparent regional lymph node metastases, but does not resolve the controversies that surround the adjuvant therapy of melanoma.

A detailed analysis of this subgroup of patients with clinically apparent regional lymph node metastases treated with vindesine-based adjuvant chemotherapy will be the subject of a separate publication.

The aggravated prognosis of ulcerated malignant tumours has been known to physicians in antiquity [23]. Eighteen centuries later, the validity of this empirical observation is—for some tumours such as melanoma—endorsed with sophisticated contemporary statistical methods.

Our study of one of the largest single-institution cohorts reported to date validates the proposal of the AJCC that ulceration of the primary lesion—as determined by microscopic histopathological examination—should be used in the T classification [1]. It does not support its inclusion in nodal staging, at least in the presence of *clinically apparent* regional lymph node metastases.

Histopathological determination of ulceration is technically simpler and less laborious than assessment of mitotic activity, but the latter achieves greater prognostic significance in our study. We therefore recommend that this important variable which reflects the proliferative potential of the tumour at the time of the biopsy should also be included routinely in histopathology reports of primary cutaneous melanoma and standardisation of reporting should be agreed internationally. Furthermore, this mitotic activity reported in conjunction with other established histopathological prognostic variables such as ulceration and tumour thickness may enhance prognostic predictions and obviate invasive staging procedures such as biopsy of the sentinel node [24].

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References

1. Balch CM, Buzaid AC, Atkins MB, *et al*. A new American Joint Committee on Cancer staging system for cutaneous melanoma. *Cancer* 2000, **88**, 1484–1491.
2. Retsas S, Quigley M, Pectasides D, Macrae K, Henry K. Clinical and histologic involvement of regional lymph nodes in malignant melanoma. Adjuvant vindesine improves survival. *Cancer* 1994, **73**, 2119–2130.
3. McGovern VJ, Shaw HM, Milton GW, Farago GA. Prognostic significance of the histological features of malignant melanoma. *Histopathology* 1979, **3**, 385–393.
4. Elder EE, Murphy GF. *Melanocytic Tumours of the Skin. Atlas of Tumour Pathology*. Washington, DC, Armed Forces Institute of Pathology, 1991.
5. Kopf AW, Gross DF, Rogers GS, *et al*. Prognostic index for malignant melanoma. *Cancer* 1987, **59**, 1236–1241.
6. Karjalainen JM, Kellokoski JK, Eskelinen MJ, Alhava EM, Kosma VM. Downregulation of transcription factor AP-2 predicts poor survival in stage I cutaneous malignant melanoma. *J Clin Oncol* 1998, **16**, 3584–3591.
7. Karjalainen JM, Eskelinen MJ, Nordling S, Lipponen PK, Alhava EM, Kosma VM. Mitotic rate and S-phase fraction as prognostic factors in stage I cutaneous malignant melanoma. *Br J Cancer* 1998, **77**, 1917–1925.
8. Retsas S, Mohith A, Macrae K, Henry K. 1st Interim Analysis of adjuvant Vindesine and DTIC for clinical and histologic involvement of regional lymph nodes in malignant melanoma. *Melanoma Res* 1997, **7**, 159–160.
9. Balch CM. Cutaneous melanoma: prognosis and treatment results worldwide. *Semin Surg Oncol* 1992, **8**, 400–414.
10. Schlagenhauff B, Ellwanger U, Breuninger H, Stroebel W, Rassner G, Garbe C. Prognostic impact of the type of anaesthesia used during the excision of primary cutaneous melanoma. *Melanoma Res* 2000, **10**, 165–169.
11. Cochran AJ, Elashoff D, Morton DL, Elashoff R. Individualized prognosis for melanoma patients. *Hum Pathol* 2000, **31**, 327–331.
12. Sondergaard K, Schou G. Survival with primary cutaneous malignant melanoma, evaluated from 2012 cases. A multivariate regression analysis. *Virchows Arch A Pathol Anat Histopathol* 1985, **406**, 179–195.
13. O'Brien CJ, Coates AS, Petersen-Schaefer K, *et al*. Experience with 998 cutaneous melanomas of the head and neck over 30 years. *Am J Surg* 1991, **162**, 310–314.
14. Ostmeier H, Fuchs B, Otto F, *et al*. Can immunohistochemical markers and mitotic rate improve prognostic precision in patients with primary melanoma? *Cancer* 1999, **85**, 2391–2399.
15. Shaw HM, McCarthy WH, McCarthy SW, Milton GW. Thin malignant melanomas and recurrence potential. *Arch Surg* 1987, **122**, 1147–1150.
16. Barnhill RL, Fine JA, Roush GC, Berwick M. Predicting five-year outcome for patients with cutaneous melanoma in a population-based study (published erratum appears in Cancer 1997 Jan 15;79(2):423). *Cancer* 1996, **78**, 427–432.
17. Smolle J, Hofmann-Wellenhof R, Kofler R, Soyer HP, Kerl H. Tumor cell proliferation and motility estimates are prognostic factors in malignant melanoma. *Anal Cell Pathol* 1993, **5**, 113–124.
18. Massi D, Borgognoni L, Franchi A, Martini L, Reali UM, Santucci M. Thick cutaneous malignant melanoma: a reappraisal of prognostic factors. *Melanoma Res* 2000, **10**, 153–164.
19. Hsueh EC, Lucci A, Qi K, Morton DL. Survival of patients with melanoma of the lower extremity decreases with distance from the trunk. *Cancer* 1999, **85**, 383–388.
20. Masback A, Westerdahl J, Ingvar C, Olsson H, Jonsson N. Cutaneous malignant melanoma in southern Sweden 1965, 1975, and 1985. Prognostic factors and histologic correlations. *Cancer* 1997, **79**, 275–283.
21. Austin PF, Cruse CW, Lyman G, Schroer K, Glass F, Reintgen DS. Age as a prognostic factor in the malignant melanoma population. *Ann Surg Oncol* 1994, **1**, 487–494.
22. Dong XD, Tyler D, Johnson JL, DeMatos P, Seigler HF. Analysis of prognosis and disease progression after local recurrence of melanoma. *Cancer* 2000, **88**, 1063–1071.
23. Retsas S. On the antiquity of cancer from Hippocrates to Galen. In Retsas S, ed. *Palaeo-Oncology: The Antiquity of Cancer*. London, Farrand Press, 1986, 41–58.
24. Gershenwald JE, Thompson W, Mansfield PF, *et al*. Multi-institutional melanoma lymphatic mapping experience: the prognostic value of sentinel lymph node status in 612 stage I or II melanoma patients. *J Clin Oncol* 1999, **17**, 976–983.