IJC
International Journal of Cancer

Molecular markers to complement sentinel node status in predicting survival in patients with high-risk locally invasive melanoma

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Sentinel lymph node status is a major prognostic marker in locally invasive cutaneous melanoma. However, this procedure is not always feasible, requires advanced logistics and carries rare but significant morbidity. Previous studies have linked markers of tumour biology to patient survival. In this study, we aimed to combine the predictive value of established biomarkers in addition to clinical parameters as indicators of survival in addition to or instead of sentinel node biopsy in a cohort of high-risk melanoma patients. Patients with locally invasive melanomas undergoing sentinel lymph node biopsy were ascertained and prospectively followed. Information on mortality was validated through the National Death Index. Immunohistochemistry was used to analyse proteins previously reported to be associated with melanoma survival, namely Ki67, p16 and CD163. Evaluation and multivariate analyses according to REMARK criteria were used to generate models to predict disease-free and melanoma-specific survival. A total of 189 patients with available archival material of their primary tumour were analysed. Our study sample was representative of the entire cohort ($N = 559$). Average Breslow thickness was 2.5 mm. Thirty-two (17%) patients in the study sample died from melanoma during the follow-up period. A prognostic score was developed and was strongly predictive of survival, independent of sentinel node status. The score allowed classification of risk of melanoma death in sentinel node-negative patients. Combining clinicopathological factors and established biomarkers allows prediction of outcome in locally invasive melanoma and might be implemented in addition to or in cases when sentinel node biopsy cannot be performed.

Cutaneous melanoma is the deadliest form of skin malignancy with an incidence that is still rising in many age groups.[1,2] The large majority (>80%) of melanomas are currently diagnosed at a stage of local disease. Overall survival for these patients is good, especially for those with thin (<1 mm) melanomas.[3] However, for thicker lesions, or those with ulceration and detectable mitoses, there is a broad range of possible outcomes, with 10-year survival ranging from 86% for American Joint Committee on Cancer (AJCC) stage IB to 39% for AJCC stage IIC.[3] While the current staging for cutaneous melanoma, incorporating Breslow thickness, ulceration and mitotic index, predicts broad probabilities of disease progression for patients with locally invasive disease,[3] there remains a significant level of variability in terms of prognosis amongst patients diagnosed within the same stage.

Sentinel lymph node (SLN) biopsy was introduced as an additional recommended staging procedure to address this issue for patients with tumours >1 mm in thickness and clinically

Key words: biomarker, prognosis, melanoma, sentinel node, CD163
Additional Supporting Information may be found in the online version of this article.
Conflicts of interest: Nothing to report
Grant sponsor: Royal Brisbane and Women's Hospital Foundation; **Grant sponsor:** University of Queensland Diamantina Institute (Australia); **Grant sponsor:** NHMRC Program; **Grant number:** 552429; **Grant sponsor:** NHMRC Fellowship; **Grant number:** 1023371
DOI: 10.1002/ijc.30085
History: Received 16 Oct 2015; Accepted 29 Feb 2016; Online 14 Mar 2016
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What's new?
Sentinel lymph node status is a major prognostic marker in locally invasive cutaneous melanoma. However, this procedure is not always feasible, requires advanced logistics, and carries rare but significant morbidity. In a study based on a cohort of melanoma patients with locally advanced disease, the authors show that the use of clinicopathological factors and established routine and accessible immunohistochemical markers can be combined in a score that is highly predictive of melanoma prognosis. This score remained highly predictive in sentinel lymph node negative patients, suggesting its potential to be applied in a clinical setting immediately.

uninvolved nodes, as well as selected patients with tumours $\leq$1 mm thick with ulceration or a high mitotic count (>1/mm^2).[3] Despite its significant prognostic power, a proportion of patients (15% in MSLT1 trial) with a negative SLN status will still progress to metastases and death.[4,5] Similarly, others with a positive sentinel node may have no further disease progression (62% in MSLT1 trial).[4,5] In a small group of patients, SLN biopsy incurs additional complications including seroma formation, haematoma, infection and lymphoedema, with a significant further increase in risk in patients who proceed to complete lymphadenectomy.[6,7] Additionally, the procedure is associated with extra healthcare costs.[8] This procedure can be technically difficult, particularly if performed for melanomas on the head and neck, and may not be offered to patients with comorbidities or who are frail. Thus overall, there is a strong incentive to identify and validate novel parameters that would distinguish patients at risk of progression, in addition to, or even as a non-invasive alternative to SLN biopsy.

Assuming that the observed variability in melanoma survival is a consequence of tumour and host biology, there have been substantial attempts to identify and validate biomarkers of melanoma progression that could be interrogated in the primary tumour at diagnosis.[9–11] In recent systematic reviews, a number of candidate proteins have been found to be associated with survival, including p16/INK4A (p16) and Ki67,[9,12] representing tumour cell cycle, as well as markers of the host immune response such as CD163, reflecting the suppressed immunity of the host through tumour-associated macrophages.[13] These three markers, more than others, are routinely used for other indications in most anatomical pathology laboratory settings, potentially facilitating their widespread implementation.

An important consideration that has prevented translation of these findings into clinical practice is the lack of exploration into their applicability in a clinical setting. We therefore aimed to use these previously reported molecular prognostic markers in a cohort of melanoma patients, complying with the REMARK criteria.[14] We further assessed their combined predictive powers using a nomogram and explored whether these markers could substitute for, or add to, the predictive value of SLN biopsy.

Material and Methods

Ethics statement

This project received ethics approval through the Metro South Health Human Research Ethics Committee (HREC Reference Number: HREC/09/QPAH/217). All patients gave written informed consent to participate in this study.

Cohort

All patients with locally invasive primary cutaneous melanoma, referred to the multidisciplinary melanoma clinic at the Princess Alexandra Hospital (PAH) and related clinics, who proceeded to a SLN biopsy procedure between 1994 and 2011 were considered for inclusion. Patient demographics, date of diagnosis and clinicopathological characteristics of the primary melanoma for each patient were prospectively recorded in a hospital database through the PAH surgical clinic. Follow-up clinical data were collected prospectively until February 2013 for information on recurrence and survival. Additional information on death, especially the cause of death, was obtained retrospectively through the National Death Index (NDI).

Patients diagnosed after December 31, 2007 were excluded to enable a minimum follow-up period of 5 years. Those patients with accessible primary melanoma tissue samples in the form of formalin-fixed, paraffin-embedded tissue blocks or preprepared whole-section slides were included in the study. For patients with subsequent primary melanomas diagnosed during the follow-up period, only the primary tumour sample justifying the SLN biopsy was included for analysis. Tissue blocks or slides were retrieved from pathology laboratories where the diagnosis of the primary melanoma was established and representative sections were cut from the blocks and prepared on slides. Haematoxylin and eosin-stained slides for each melanoma were assessed by a pathologist (DL) to confirm diagnosis.

Given the selected, hospital-based nature of our melanoma cohort, we compared it to the corresponding source population of melanoma patients in the general population. Information on age and sex was obtained from the Queensland Cancer Registry (QCR) of all patients in the general population diagnosed with AJCC Stage IB and II cutaneous melanoma (who therefore had a potential indication for SLN biopsy) between January 1, 1994 and December 31, 2007.

Immunohistochemical staining

Melanoma tissue samples were prepared according to standardised immunohistochemistry (IHC) protocols and antibody staining techniques for each of the three different primary

antibodies. Control samples for each of the different primary antibodies were used to determine positive and negative staining, as well as staining quality in each batch.

The sections were stained with primary monoclonal antibodies against Ki67 (Dako purified mouse anti-Ki67, Clone: MIBI, dilution 1:120), p16 (Santa Cruz purified mouse anti-p16 IgG1, Clone: JC8, dilution 1:250) and CD163 (Abcam mouse anti-human CD163 IgG1, Clone: RM3/1, dilution 1:200). Full details of staining protocol and pretreatment are detailed in Supporting Information methods.

Evaluation of staining

IHC scoring was performed blinded to patient outcome. Positive and negative staining was confirmed through comparison to a positive control for each antibody. Scoring was performed independently by multiple scorers (CR, DL and FT, dermatopathologist) as reported previously (Supporting Information Methods).[15] The study pathologists (FT and DL) agreed on a standardised evaluation. Discrepant scoring was resolved by consensus during simultaneous evaluation. Each lesion was assigned a binary score as detailed in Supporting Information methods, representing high or low/negative expression. Intra-rater variability was calculated using a kappa analysis for a random sample ($n = 29$) of Ki67 and p16 scores, which were scored for a second time by CR, with comparison of the binary scores according to the allocated cut-offs for each protein.

Statistical analysis

Melanoma-specific survival was calculated from the date of histological diagnosis to either date of death due to melanoma or date of last follow-up (May 1, 2013). Disease-free survival was calculated from the date of histological diagnosis to either date of recurrence or date of last follow-up. Patients who died during the follow-up period due to causes other than melanoma were censored at the time of death. Age at diagnosis, sex and melanoma tumour characteristics were tested for association with melanoma-specific survival and recurrence status using χ^2 test for categorical variables and *t*-test for continuous variables.

To identify biomarkers and other predictors of melanoma-specific survival we first performed univariate Cox proportional hazards regression models and Kaplan–Meier survival analyses on the three biomarkers (p16, Ki67 and CD163), in addition to classical prognostic clinicopathological factors (age at diagnosis, sex, Breslow thickness, ulceration and SLN status). All factors reaching a significance of $p < 0.3$ on univariate Cox regression analysis and showing a trend for association with survival as confirmed on Kaplan–Meier analysis were included in a multivariate model to identify independent predictors. SLN status was not included as a variable in the model in order to find variables of prognostic significance that may substitute for this procedure.

Coefficients from the multivariate predictive model were used to generate nomogram scores (details in the Supporting Information methods). Scores were modified according to the following formula: $(x + 1.454) \times 100/3.833$ to result in a score of 0 to 100 and was divided into four equal categories based on the range of the score. Survival across the four categories was compared using Kaplan–Meier curves and log-rank test. Proportions of patients who died within 5 years and Kaplan–Meier 5-year survival rates in each category were also calculated. A multivariate analysis was performed with two variables, nomogram score categories and SLN status in order to compare the predictive value of each. The C-index for failure time models and the area under the receiver operating characteristic curve (AUC) were generated as indications of predictive power as previously.[16] The C-index was calculated using the rms and epi packages in R version 3.0.1. Survival analyses were performed in patients according to SLN status to explore prognostic capabilities of the nomogram among low- and high-risk groups separately. We also explored whether SLN status provided any additional prognostic value when applied to each of the four nomogram categories generated, separately. A final analysis was performed to determine whether the nomogram provided further prognostic information when patients' melanomas were adjusted for AJCC stage using Kaplan–Meier analysis.

A sensitivity analysis was performed comparing the nomogram with and without sex as an included variable.

Cox proportional hazards regression was performed using SPSS Statistics 22.0 software,[17] while the assess and resample options in the proc phreg command in SAS v9.2[18] were used to assess adherence to the assumption of proportional hazards. Analysis was also performed with disease-free survival as an outcome (Supporting Information Methods).

Results

Of 559 patients with locally invasive primary cutaneous melanoma diagnosed between 1994 and 2007 who underwent SLN biopsy and consented to the study, 189 with available tissue samples and confirmed diagnoses upon pathology review were included in analysis (Table 1). Baseline clinicopathological and demographic characteristics of these 189 patients compared to the rest of the cohort showed that they were representative of the entire cohort (Supporting Information Table 1), with only age at diagnosis differing between groups. Compared to melanomas of stage IB and II in the general Queensland population, the cohort was not significantly different in terms of sex distribution in either staging groups. However, our study cohort of patients that underwent SLN biopsy was significantly younger than the QCR source population of all stage IB and II patients (Supporting Information Fig. 1 and Supporting Information Table 2).

The study sample included 55% males and the mean age at diagnosis was 51 years. Mean Breslow thickness was 2.5 mm $\pm$ 0.14 (SE). Median follow-up time for recurrence assessment was 5.6 $\pm$ 2.9 years and for death assessment was 7.4 $\pm$ 3.4 years. Sentinel node status was available for all patients (17.5% had a positive SLN). Fifty-two (27.5%)

Table 1. Cohort characteristics

Characteristic	Total ($n = 189$)	Recurrence ($n = 52$)	p values[1,2]	Melanoma-specific death ($n = 32$)	p values[1,2]
	n (%)[1]	n (%)[1]		n (%)[1]	
Sex					
Male	104 (55.0)	29 (55.8)	0.899[3]	21 (65.6)	0.186[3]
Female	85 (45.0)	23 (44.2)		11 (34.4)	
Age at diagnosis					
0–40	37 (19.6)	8 (15.4)	0.619[4]	6 (18.8)	0.710[4]
41–60	104 (55.0)	31 (59.6)	0.857[3]	20 (62.5)	0.724[3]
61–80	48 (25.4)	13 (25)		6 (18.8)	
Mean ± SD	51.1 ± 13.6	51.8 ± 12.9		50.3 ± 12.4	
Site of primary melanoma					
Head and neck	14 (7.4)	6 (11.5)	0.263[3]	5 (15.6)	0.066[3]
Upper limb	45 (23.8)	8 (15.4)		7 (21.9)	
Lower limb	58 (30.7)	17 (32.7)		5 (15.6)	
Trunk	72 (38.1)	21 (40.4)		15 (46.9)	
Breslow thickness					
T1: 0–1.0 mm	14 (7.4)	2 (3.8)	0.001[4]	0	0.015[4]
T2: 1.0–2.0	97 (51.3)	17 (32.7)	<0.001[3]	10 (31.3)	0.004[3]
T3: 2.0–4.0	53 (28.0)	19 (36.5)		16 (50.0)	
T4: >4.0	25 (13.2)	14 (26.9)		6 (18.8)	
Mean (mm) ± SD	2.5 ± 1.95	3.4 ± 2.3		3.3 ± 2.3	
Histology[1]					
SSM	111 (59.0)	31 (59.6)	0.373[3]	19 (59.4)	0.399[3]
NM	54 (28.7)	17 (32.7)		12 (37.5)	
LMM	5 (2.7)	0		0	
Desmoplastic	8 (4.3)	3 (5.8)		0	
Other	10 (5.3)	1 (1.9)		1 (3.1)	
Unknown	1	0		0	
Ulceration					
No	140 (74.1)	33 (63.5)	0.040[3]	20 (62.5)	0.101[3]
Yes	49 (25.9)	19 (36.5)		12 (37.5)	
SLN status					
Positive	33 (17.5)	21 (40.4)	<0.001[3]	14 (43.8)	<0.001[3]
Negative	156 (82.5)	31 (59.6)		18 (56.2)	
Biomarker positivity[5]					
Ki67	28 (15.2)	13 (25.5)	0.016[3]	5 (15.6)	0.944[3]
p16	90 (48.1)	26 (50)	0.751[3]	12 (37.5)	0.186[3]
CD163	73 (40.6)	23 (44.2)	0.522[3]	17 (53.1)	0.110[3]

[1]Percentages and p-values calculated based on known data.
[2]p-values for age and Breslow thickness calculated on continuous data.
[3]p-values calculated by Pearson χ^2 test.
[4]p-values calculated by t-test.
[5]IHC data were unavailable for five samples for Ki67, two samples for p16 and nine samples for CD163.
Abbreviations: n: number; SD: standard deviation; SSM: superficial spreading melanoma; NM: nodular melanoma; LMM: lentigo maligna melanoma.

patients had a recurrence/metastasis (subcutaneous, nodal or distant) and 32 (16.9%) patients died from melanoma during follow-up.

High Ki67 was scored in 15% of patients, p16 was scored as high in 48% and CD163 was scored as high in 41% of the cohort (Table 1). Kappa scores for Ki67 and p16 intra-rater variability were 0.707 (SE = 0.153, $p < 0.001$) and 0.861 (SE = 0.094, $p < 0.001$) respectively.

Associations with melanoma outcome

On univariate analysis, Breslow thickness (HR 2.2 [1.3–3.7], $p = 0.002$) and SLN status (HR 4.5 [2.2–9.0], $p < 0.001$) were significantly associated with increased risk of death from melanoma. Of the biomarkers, high CD163 presence was associated with increased risk of death from melanoma (HR 1.9 [0.935–3.765], $p = 0.077$] but not significantly with $p > 0.05$ (Supporting Information Fig. 2). Sex, ulceration status and p16 status were associated with survival at a significance level of $p < 0.3$ and were included in the multivariate model (Table 2). Kaplan–Meier analysis confirmed a trend for association with survival for these included biomarkers (Supporting Information Fig. 2). Breslow thickness and CD163 were found to be significantly and independently predictive of survival on multivariate analysis (Table 2). Similar findings regarding Breslow thickness, Ki67 and CD163 were seen for the risk of recurrence (Supporting Information Results).

Nomograms to predict 5-year survival

Using the coefficients from the final multivariate predictive model, a nomogram score was developed (detailed in Supporting Information methods): −0.460 × sex (coded 0,1) + 0.859 × log Breslow (continuous variable) + 0.232 × ulceration (coded 0,1) + 843 × CD163 status (coded 0,1) −0.163 × p16 status (coded 0,1). The nomogram score ranged from −1.454 to 2.379 and was readjusted to a score from 0 to 100. Four nomogram categories were defined based on the score values only. Proportions of 5-year deaths from melanoma in each nomogram category and 5-year melanoma death rates rose steadily across the four categories 0/9 and 0% in the lowest category to 10/31 and 32.3% in the highest category (Table 3). Correspondingly, crude Kaplan–Meier melanoma survival curves showed a significant decrease in melanoma-specific survival as nomogram score categories increased ($p = 0.002$) (Fig. 1). Interestingly, the nomogram was able to distinguish risk groups within AJCC stage I or stage II patients considered separately although this did not reach significance (Supporting Information Fig. 4). Additionally, as an indication of predictive power, the C-index for the nomogram was 0.69 (95% CI 0.6–0.78) and the AUC was also 0.69. A similar nomogram was established that significantly predicted recurrence and disease-free survival (Supporting Information Results).

Table 2. Multivariate results for melanoma-specific death

Factor	HR [95% CI]	*p* values
Sex[1]	0.631 [0.300–1.328]	0.225
Log Breslow	2.360 [1.342–4.153]	0.003
Ulceration	1.261 [0.600–2.649]	0.540
p16	0.850 [0.408–1.769]	0.664
CD163	2.323 [1.104–4.888]	0.026

[1]Male as reference.
Abbreviations: HR: hazard ratio; CI: confidence interval.

Biomarkers help predict outcome in SLN-negative patients

Multivariate analysis for melanoma-specific death, incorporating the nomogram score (as a categorical variable) and SLN status as covariates, showed that the nomogram performs similarly to and independently from SLN status with HR 2.07 [95% CI 1.25–3.43] ($p = 0.005$) for the nomogram score and HR 3.05 [95% CI 1.47–6.33] ($p = 0.003$) for SLN status. To further establish the independence of these two variables we assessed the capacity of the nomogram to predict outcome in SLN-positive and SLN-negative patients separately. Expectedly, the nomogram score categories were not associated with survival among SLN-positive patients. However, among SLN-negative patients, nomogram scores clearly identified those at risk of melanoma death (categories 3 and 4) compared to potential survivors (categories 1 and 2). Therefore, the nomogram was able to further subdivide these lower risk melanoma patients into risk groups ($p = 0.02$) (Fig. 2).

Biomarkers may modify the need for SLN biopsy as a prognostic tool

Nomogram categories were associated with SLN positivity. In category 1, none of nine patients had a positive SLN. In category 2 patients only four of 58 (7%) patients had SLN positivity. Finally, the rate of SLN positivity in categories 3 and 4

Table 3. Nomogram categories and associated number of deaths from melanoma at 5 years and 5-year death rates

Nomogram category	Melanoma deaths/ total in 5 years	Percentage death in 5 years	Kaplan–Meier 5-year melanoma death rate	95% Confidence interval
1	0/9	0	0	N/A
2	3/58	5.2	5.1	0.396–10.98
3	19/80	23.8	21.0	11.98–30.41
4	10/31	32.3	28.6	12.72–45.26

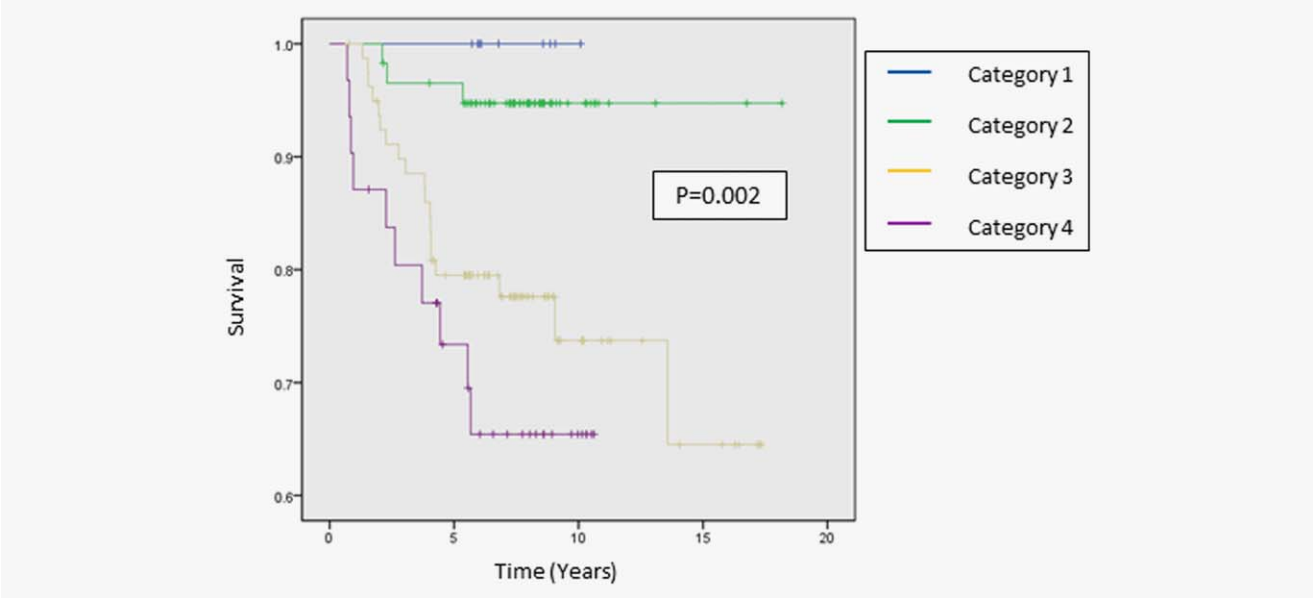

Figure 1. Kaplan–Meier actuarial curves for melanoma-specific death were established to compare the survival of patients according to their score category.

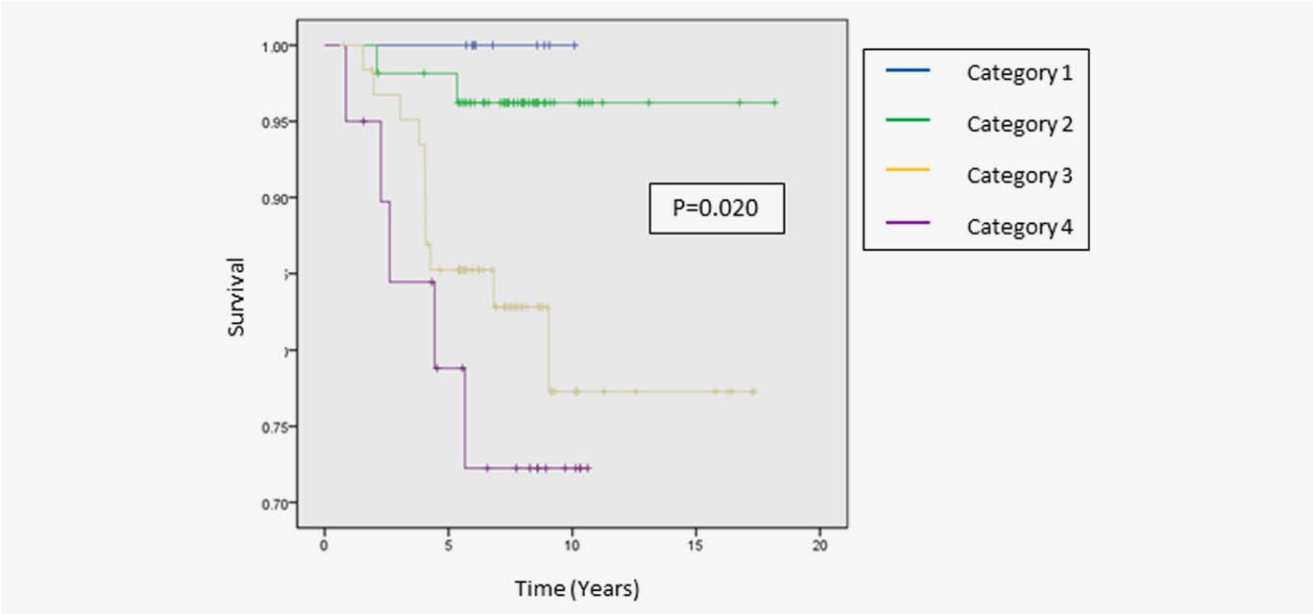

Figure 2. Kaplan–Meier actuarial curves for melanoma-specific death were established to compare the survival of patients with a negative sentinel node according to their score category.

was largely above 20%. Differences in SLN status between categories were highly significant ($p = 0.003$) (Table 4).

We finally assessed in a clinical scenario where the nomogram score could be established immediately upon diagnosis by the pathology laboratory for high-risk locally invasive melanomas whether the SLN status would still be useful as a predictor of outcome. In the intermediate risk categories 2 and 3, SLN status provided additional prognostic information (Fig. 3). Indeed, in these categories SLN-positive patients had significantly poorer outcome than their SLN-negative counterparts. When applied to the highest risk (category 4) group of patients as identified through the nomogram, SLN-positive and -negative patients did not differ in survival ($p = 0.215$) (Fig. 3).

The sensitivity analysis comparing the nomogram with and without sex as an included variable showed that both nomograms performed similarly to and independent from SLN status; however, the original nomogram which included sex had a better association with mortality as witnessed by a higher hazard ratio (original nomogram: HR 2.070 [1.248–

Table 4. Nomogram categories predictive of SLN status

Nomogram category	Total	SLN positive, *n* (%)	SLN negative, *n* (%)	*p* values
1	9	0	9 (100)	0.003[1]
2	58	4 (6.9)	54 (93.1)	
3	80	17 (21.3)	63 (78.8)	
4	31	11 (35.5)	20 (64.5)	

[1] *p* values calculated by χ^2.
Abbreviation: *n*: number.

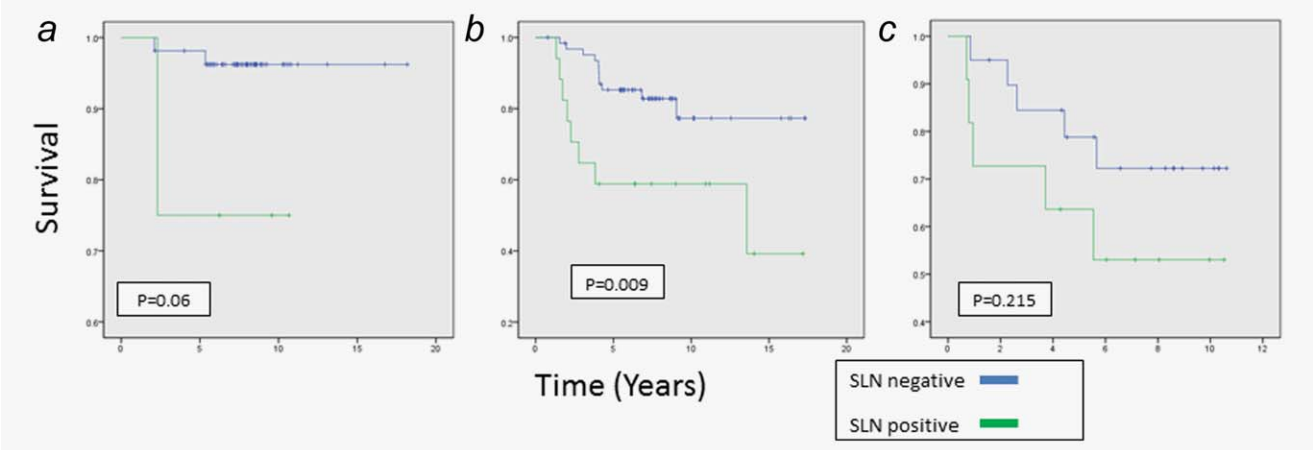

Figure 3. Kaplan–Meier actuarial curves for melanoma-specific death were established to compare the survival of patients with or without a positive sentinel node within each of the score categories. No patient within the first score category had a positive sentinel node biopsy. (*a*) Score category 2, (*b*) score category 3, (*c*) score category 4.

3.433] $p = 0.005$; nomogram without sex as a variable: HR 1.756 [1.030–2.995] $p = 0.039$). In terms of concordance, C-index and AUC/ROC analysis results were very similar for both nomograms (original nomogram: C-index [95% CI] = 0.69 [0.6–0.78], AUC = 0.69; nomogram without sex as a variable: 0.68 [0.59–0.76], AUC = 0.69).

Discussion

SLN biopsy is currently proposed for melanoma patients with high-risk, locally invasive tumours as it offers added prognostic information. However, this additional procedure is best performed by experienced surgeons in advanced or tertiary settings, where there are added costs, a low but measurable incidence of lymphoedema, and there is ongoing debate about its influence on melanoma survival.[4,19] By using a nomogram score which incorporates the prognostic powers of multiple previously established protein markers from the primary melanoma,[20] added to existing clinicopathological factors, we here report survival prediction in a cohort of high-risk melanoma patients without reference to SLN status. In particular, this biomarker nomogram can help predict risk in patients whose SLN biopsy is negative. This is novel in that it permits the development of a prognostic score immediately upon diagnosis.

Many studies have reported protein biomarkers predicting melanoma outcome. Some of these markers are currently available for routine use in pathology laboratories for other indications. Ki67 and p16 were selected based on the numerous previous reports highlighting their usefulness and their general availability in cancer evaluation. Similarly, CD163 was selected to represent altered immunocompetence, which is a topic of major focus in relation to melanoma progression.[20] Our assessment of the clinical utility of these biomarkers in an independent cohort, following the REMARK criteria,[14] provides additional evidence to support the implementation of their use in routine clinical practice. The IHC tests may be implemented in any pathology setting as they are part of routine pathology procedures and the antibodies are used by most laboratories. The simplicity of these tests would allow them to be routinely performed upon diagnosis of thick, localised melanomas. Thus, implementation would be very different to recently published gene expression tests requiring microdissection of the tumour followed by multiplex polymerase chain reaction amplification.[21]

Our approach has significant potential clinical utility when considering that the vast majority (80%) of patients that undergo SLN biopsy will have a negative result. There remains a risk of mortality amongst this group that is not measurable by any other means. Therefore, the search for alternative prognostic indicators is needed to further subdivide this large group of patients in terms of survival.

This study was based on a hospital series as it focused on high-risk locally invasive melanoma patients who underwent SLN biopsy. As compared to melanomas of stage IB and II in the general population, our cohort was expectedly significantly younger, but had otherwise similar characteristics. In a recent study of patterns of surgical care in a large localised melanoma cohort also derived from our hospital unit, it was found that age was a major determinant of whether patients with stage IB or II melanomas were ultimately subjected to a SLN biopsy, likely in part due to the increasing number of medical comorbidities associated with increasing age, as well as the higher number of head and neck melanomas.[22] This selection bias is acknowledged but was necessary in order to satisfy the study objective of comparing the prognostic ability of the nomogram score with SLN status amongst high-risk melanoma patients. It is notable that the patients with available tumour material had very similar characteristics to the rest of the cohort and did not reflect a convenience sample but a representative population of melanoma patients undergoing SLN biopsy. A limitation was the size of the cohort that made subgroup analyses difficult especially when considering AJCC stages I and II separately. Of note, our sample size of 189 patients is very comparable to other published REMARK compliant studies, with only 14 of 51 studies included in two published systematic reviews having larger cohort size.[9,12] This is likely to reflect the logistical difficulties in completing studies of this sort.

In a routine clinical setting this nomogram could have a number of applications. It could be argued that staining and scoring should be performed only in SLN-negative patients. A major difficulty with such an approach is that only a minority of high-risk melanoma patients (stage IB and II) undergo SLN biopsy (34% in Queensland) for a number of personal or medical reasons.[22] Although limited by our small dataset, our findings seem to indicate that SLN status provides no additional prognostic information when applied to patients identified as lowest risk (category 1) or highest risk (category 4) according to our biomarker nomogram. This might be due to the limited power of our analysis. However, remarkably those patients with the lowest score band of the nomogram were devoid of any SLN invasion, suggesting that they may be excluded from the SLN procedure. Similarly, for patients in the second lowest score band, where only 7% had a positive SLN, the nomogram offers support to avoid the extra operative intervention of SLN biopsy, with its associated small but definite comorbidity. It may be considered that this nomogram, by classifying patients into four risk categories, could limit the number of patients indicated for SLN biopsy procedure, with only those patients in the intermediate risk categories (category 2 and 3) gaining further prognostic information from this procedure. For these reasons, it would be reasonable to use the nomogram at diagnosis when tumour tissue is still easily available to pathologists.

In summary, we have demonstrated the clinical utility of prognostic biomarkers in a cohort of melanoma patients with locally advanced disease, following strict REMARK criteria. The identification of additional prognostic biomarkers independent of the current factors incorporated in the AJCC staging guidelines has the potential to allow for a more personalised management approach, targeting only the high-risk individuals with potentially toxic adjuvant therapies. The use of mathematical models or nomograms to create a combined predictive score incorporating multiple clinicopathological prognostic factors has been applied to assess survival in melanoma patients previously[16,23]; however, the inclusion of biological variables has not yet been explored. Our study shows that a prognostic score incorporating protein markers and clinicopathological factors (excluding SLN status) can predict melanoma-specific outcome equally as effectively as SLN status. The score may better inform about the need to perform this procedure that is invasive and costly.[7,8] Finally, this simple predictive score provides unique prognostic information in SLN-negative patients or among those patients unfit to undergo SLN biopsy. The use of this score may significantly increase the possibility of stratification for melanoma clinical trials.

Acknowledgement

The authors thank Clay Winterford from the QIMR Berghofer histology core services for performing the staining.

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