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Clinical and histopathological risk factors to predict sentinel lymph node positivity, disease-free and overall survival in clinical stages I–II AJCC skin melanoma: Outcome analysis from a single-institution prospectively collected database

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This paper is dedicated to Massimo Foglieni and all our patients who, every day, throught their suffering, teach us the difficult art of medicine.

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ABSTRACT

Background: To investigate if the tumour infiltrating lymphocytes (TILs) are able to predict the sentinel lymph node (SLN) positivity, the disease-free survival (DFS) and overall survival (OS) in clinical stages I-II AJCC primary cutaneous melanoma (PCM).

Methods: The study included consecutive patients with PCM, all diagnosed, treated and followed up prospectively. Logistic regression was used to investigate the association between DFS, OS, SLN positivity and Breslow thickness, Clark level, TIL, ulceration, lesion site, gender, regression and age.

Results: From November 1998 to October 2008, 1251 consecutive patients with PCM were evaluated. Median age was 51 (range 15–96) with 32.2% ($N = 393$) of them older than 60; 44.8% of them were males. Of the whole series, a total of 404 patients with primary vertical growth phase (VGP) melanoma and no clinical evidence of metastatic disease underwent SLN biopsy. Of these, 74 (18.8%) had a positive SLN. In a multivariate analysis, primary melanoma on the extremities versus that on the axial locations (truncal and head/neck) (OR 0.49, 95% CI 0.25–0.98, p 0.04) and TILs (TILs versus no TILs) (OR 0.47, 95%CI 0.25–0.90, p 0.02) were predictive for lower probability of SLN involvement, while thickness (>4 mm versus 0–1 mm) (OR 24, 19, 95% CI 4.91–119.13, $p < .001$) was predictive for higher risk of SLN positivity. A multivariate stepwise analysis confirmed these results. The histological status of the SLN was the most significant predictor of DFS and OS. Patients with a negative SLN had a 5-year DFS of 75.9%, compared with 35.2% in patients with a positive SLN ($p < .0001$) and a 5-year OS of 88.7% versus 42.9%, respectively ($p < .0001$).

Conclusions: Our study demonstrates that the absence of TILs predicts SLN metastasis, in multivariate analysis the SLN positivity predicts DFS and OS.

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1. Introduction

Sentinel lymph node (SLN) biopsy has become a standard procedure in the staging and treatment of primary melanoma.[1] The aim of this procedure is to detect micrometastatic lymphatic disease for selective lymphadenectomy, which has been beneficial at least in subsets of melanoma patients. Several studies have evaluated clinical and pathological features predictive of SLN status.[2–5] Many of these results derive from retrospective studies, from national registries or from data collected in academic centres.

Histopathological thickness has consistently been shown to predict SLN status.[6,7] Other factors, such as ulceration, Clark level IV/V, vertical growth phase (VGP), mitotic rate, blood and lymphatic vessel invasion, axial location, the presence of tumour infiltrating lymphocytes (TILs) and younger age have been identified with conflicting results.[8–10]

We intend to investigate the clinical relevance of these pathological characteristics in a prospective database including all consecutive melanoma patients referred to a large volume population-based hospital.

At the Ospedali Riuniti in Bergamo, we have been performing on a routine basis the SLN procedure in melanoma patients from 1998, prospectively collecting all clinical and pathological data of all consecutive primary cutaneous melanoma (PCM) patients in a database. The aim of the present research study was to determine the role of clinical and histopathological variables of the primary cutaneous melanoma in predicting the histological status of the SLN. Our research hypothesis aimed at investigating whether and to what extent the presence of TILs is able to predict the SLN status, and its importance in relation to several other traditional prognostic factors, such as Breslow thickness, Clark invasion level, ulceration, lymphatic invasion, location, TIL, age and gender.

2. Materials and methods

2.1. *Patients' sample*

The study included all consecutive patients with PCM, diagnosed, treated and followed up prospectively by a multidisciplinary team between November 1998 and October 2008. Data have been prospectively collected into a database (Outcome Research in Oncology Project ORO Study) with information on demographics, previous medical history, diagnosis, surgical procedures, pathological features, systemic therapies and follow-up.

The database was used to identify all patients who presented to our attention with histologically confirmed cutaneous melanoma, clinically negative lymph nodes and no evidence of distant disease. In general, SLN biopsy was offered to patients with lesions that were >1.0 mm in thickness or to patients with VGP lesions that were 1.0 mm or less in thickness if adverse histopathological features of the primary tumour were present, such as ulceration or Clark level IV/V. Demographic factors including age at diagnosis, gender and site of primary tumour were prospectively collected in all patients. All patients underwent wide local excision with free margins of at least 1 cm for melanomas with a Breslow depth of 1 mm or less, of 1–2 cm for a Breslow depth of 1–2 mm and of at least 2 cm for a Breslow depth of 2 mm or more. Patients with positive SLNs were eventually offered radical lymphadenectomy. Systemic adjuvant therapy was considered for selected patients with nodal involvement.

Postoperative follow-up consisted of physical examination, abdominal ultrasound, chest X-ray and determinations of lactate dehydrogenase levels. Further investigations, including computed tomography, magnetic resonance imaging and/or PET, were also selectively performed to investigate abnormal clinical findings suspicious for metastatic melanoma. Routine surveillance was planned every 4 months for the first 2 years, every 6 months for years 3–5 and annually thereafter.

Clinical factors evaluated were the age of the patient, gender and site of the primary lesion. The anatomical site was classified as axial (comprising truncal, head/neck, volar and subungual lesions) or extremity. We excluded in situ melanoma and radial growth phase lesions because they are apparently incapable of metastasis.[5] Candidate histopathological prognostic factors were the Breslow thickness (measured in millimetres), Clark level invasion, brisk and non-brisk lymphocyte infiltrate, ulceration and regression. Approval to conduct this study was obtained from Local Ethical Committee of our Hospital.

2.2. *Pathological features*

All the pathological specimens were assessed by an expert dermatopathologist and by a clinical dermatologist. Features routinely examined included the Breslow thickness (measured in millimetres), Clark level, the presence or absence of ulceration, regression and histological subtype. Tumours were routinely assessed for lymphocytic infiltration in the VGP and were classified as brisk, non-brisk and absent according to the criteria initially formulated by Clark et al.[7] and more recently they were classified according to Clemente et al.[9] into three categories: brisk, non-brisk and absent. We used a semiquantitative method.

TILs in the cutaneous melanoma were classified as follows:

(1) 'brisk', if the lymphocytes were present throughout the substance of the vertical growth phase or were present and infiltrating across the entire base of the vertical growth phase,
(2) 'non-brisk', if the lymphocytes were in one focus or more foci of the vertical growth phase, either dispersed throughout or situated focally in the periphery,
(3) 'absent', if there were no lymphocytes or if the lymphocytes were present but did not infiltrate the melanoma.

Histological regression is characterised by the absence of melanoma cells in a focal region of horizontal growth phase melanomas in which lymphocytes, no matter how dense, are arrayed as a band around the vertical growth phase, with-

out infiltration of the tumour cells. This phase is followed by the appearance of melanophages, fibrosis and vascular changes.[11]

2.3. SLN biopsy technique

Patients underwent lymphatic mapping and SLN biopsy as follows: lymphoscintigraphy was performed by intradermal injection of technetium-99 m-sulphur colloid adjacent to the tumour or biopsy site to identify draining lymphatic basins by gamma imaging. Intradermal injection of metilen blue was similarly performed during surgery. Blue-stained and/or radioactive lymph nodes were removed and considered SLNs, fixed in 10% neutral-buffered formalin and embedded in paraffin. Serial 4-μm thick sections (average: 10 levels) were analysed by haematoxylin and eosin (H&E) staining, and immunohistochemistry (S-100, HMB-45) if the H&E did not reveal evidence of the metastatic disease.

2.4. Statistical methods

Baseline characteristics of the patients according to TILs were reported as percentages and mean ± SD or median (range) and were compared with Pearson chi-square test and Kruskal–Wallis non-parametric one-way ANOVA for categorical and continuous variables, respectively.

For univariate analysis, the following variables were considered: sex, Breslow thickness (0–1 mm versus 1.1–2.0 mm versus 2.1–4.0 mm versus > 4.0 mm), regression (presence versus absence), TILs (Absent versus Brisk versus Non-Brisk), age (<60 versus >60), anatomical site (Extremities versus Axial) and Clark level (<3 versus >4). Multivariate logistic regression was performed to identify clinical and pathological features predictive of SLN metastases. Multivariate models were constructed using stepwise regression methods using the same variables tested in the univariate analysis.

Disease-free survival (DFS) and overall survival (OS) were calculated from the date of SLN biopsy to the date of the first recurrence, death or last follow-up.

Plots of the Kaplan–Meier estimates of survival distribution for OS and DFS according to the TILs status and for TILs status separated by SLN status are presented along with the results of the log-rank tests.

The Cox proportional hazard model was used to estimate the size of differences in survival rates for OS and DFS adjusted for age, sex, Breslow thickness, presence of ulceration and anatomical site. Adjusted hazard ratio (HR) with 95% confidence intervals (CIs) was reported, all statistical tests were two-sided and p-values < 0.05 were considered significant.

Statistical analyses were performed using the SAS software package (SAS® Software Release 9.1, SAS Institute Inc., Cary, NC).

3. Results

3.1. Clinical and pathological characteristics

A total of 1251 patients were included in the analysis. The clinical characteristics of the patients grouped by TILs (brisk, non-brisk and absent) are summarised in Table 1.

The median age of all the patients was 51 years, 55.2% of them were females, and the most common site of melanoma was axial (55.4%). Regarding these clinical characteristics, only age was significantly correlated with TIL status ($p < 0.001$).

The pathological characteristics of the primary melanoma according to TIL status are summarised in Table 1.

The mean Breslow depth was 1.3 (±2.1) mm, and only 25.2% were of Clark level IV.

Ulceration was present in 233 patients (19.6%) and was significantly correlated with tumour thickness. In particular, as tumour depth increases, the proportion of patients with ulcerated tumours increases (3.4% in pts with a Breslow thickness <1.0 mm; 37.8% in pts with a Breslow thickness between 1.0 and 2.0 mm; 61.1% in pts with a Breslow thickness between 2.0 and 4.0 mm and 82.3% in pts with a Breslow thickness >4.0 mm). Regression was present in 375 patients (31.9%) and was correlated with TIL status. TILs were present in 550 patients (44%), whereas 114 patients (20.7%) had 'brisk' TILs.

The TIL status of the lesion was correlated with the Breslow thickness, Clark level and regression. There was no difference in other variables evaluated, including SLN status or the presence of ulceration, among patients with brisk, non-brisk and absent TILs (Table 1).

There was no difference in other variables evaluated, including SLN status or the presence of ulceration, among patients with brisk, non-brisk and absent TILs (Table 1).

The Breslow thickness ($p = 0.0005$), Clark level ($p < 0.0001$), regression ($p = 0.009$) and ulceration ($p = 0.04$) were significantly correlated with TIL status (positive versus negative). In our series, we did not find any significant difference between patients with brisk melanoma and patients with non-brisk melanoma.

Regarding the location as a potential predictor of SLN positivity, the melanoma located on the extremity had a lower probability for lymph node metastases ($p = 0.016$).

No significant associations were detected between anatomical site (trunk versus extremities) and Breslow thickness ($p = 0.61$), TIL status ($p = 0.47$), Clark level ($p = 0.25$), regression ($p = 0.07$) and ulceration ($p = 0.07$).

However, female gender ($p < 0.001$) and the younger patients ($p = 0.002$) had a higher probability of having a melanoma localised at the extremity.

3.2. SLN status

A total 404 patients underwent sentinel lymph node mapping, and a SLN was identified in 394 patients (97.5%). At least one SLN was positive in 74 patients (18.8%). The characteristics of the patients with positive and negative SLNs are summarised in Table 2.

Univariate analysis showed that the absence of TILs, increasing Breslow thickness, Clark level, the presence of ulceration, anatomical site and male sex showed a significant relationship to SLN positivity (Table 3). In a multivariate logistic regression analysis, increasing Breslow thickness, anatomical site and the absence of TILs were independently associated with the finding of a positive SLN. These finding were confirmed in a multivariate stepwise analysis (Table 3).

Table 1 – The pathological characteristics of the primary melanoma according to TIL status.

	Brisk		Non-brisk		Absent		Total		P
	No	%	No	%	No	%	No	%	
Age, years									
Median	46		48		54		51		<0.0001
Range	15–87		15–88		19–96				
Mean	48.2 ± 15.5		49.7 ± 15.7		53.4 ± 17.1		51.6 ± 16.6		
Sex									
Male	47	41.2	207	47.5	307	43.8	561	44.8	0.78
Female	67	58.8	229	52.5	394	56.2	690	55.2	
Anatomical site									
Axial	69	60.5	233	53.7	387	54.6	689	55.4	0.47
Extremity	45	39.5	201	46.3	309	45.4	555	44.6	
Breslow thickness mm									
0–1	60	52.6	315	72.4	472	69.3	847	68.9	0.018
1.1–2.0	25	21.9	63	14.5	86	12.6	174	14.1	
2.1–4.0	16	14.1	36	8.3	75	11.1	127	10.3	
>4.0	13	11.4	21	4.8	48	7.0	82	6.7	
Clark level									
I	0	–	6	1.4	164	25.0	170	14.2	<0.0001
II	17	14.2	223	52.1	206	31.4	446	37.3	
III	50	41.7	89	20.8	115	17.5	254	21.3	
IV	41	42.5	103	24.1	157	23.9	301	25.2	
V	2	1.7	7	1.6	15	2.3	24	2.0	
SLN status									
Positive	11	17.7	17	12.4	46	22.4	74	18.3	0.06
Negative	51	82.3	120	87.6	159	77.6	330	81.7	
Ulceration									
No	82	72.6	348	81.1	525	81.3	955	80.4	0.10
Yes	31	27.4	81	18.9	121	18.7	233	19.6	
Regression									
No	73	65.8	225	52.0	504	79.6	802	68.1	<0.0001
Yes	38	34.2	208	48.0	129	20.4	375	31.9	

3.3. *Disease-free survival and overall survival*

The histological status of the SLN was the most significant predictor of DFS (Fig. 1) and OS (Fig. 2). Patients with a negative SLN had a 5-year DFS of 75.9%, compared with 35.2% in patients with a positive SLN ($p < .0001$) and a 5-year OS of 88.7% versus 42.9%, respectively ($p < .0001$).

These results were confirmed by the multivariate adjusted Cox proportional hazard model for DFS and OS (HR = 2.10, 95%CI 1.20–3.35 and HR = 3.69, 95%CI 1.57–8.68, respectively).

When SLN status was not considered, the 5-year DFS was marginally better for patients where TILs were present (brisk and non-brisk) (87.6%) in their primary melanoma than for those in whom TILs were absent (82.5%) (p 0.025) (Fig. 3). Furthermore, OS differed between patients in whom TILs were present and those in whom TILs were absent in the primary lesion, with a 5-year OS of 94.8% and 90.4%, respectively (p 0.024) (Fig. 4).

However, these results could not be confirmed by the multivariate adjusted Cox proportional hazard model for DFS and OS in TIL status, where the difference observed did not reach a statistical significance (HR = 0.82, 95%CI 0.54–1.24 and HR = 0.56, 95%CI 0.29–1.12, respectively).

4. Discussion

The most striking result of our study is that the absence of TILs, along with the Breslow thickness and anatomical site, predicts SLN positivity.

In our study the presence of TILs is strongly inversely correlated with the Breslow thickness, Clark level and regression. Our results are consistent with previous studies reporting that the presence of TILs is more frequently seen in thin lesions.[9,10] Although, in our study TILs were more commonly found in thinner lesions, the impact of TILs is not solely related to its association with the Breslow depth. In fact, in a multivariate analysis TILs resulted an independent predictor for SLN positivity. Furthermore these results have been confirmed in the stepwise analysis. One may argue that categorisation of TILs may be difficult to perform in clinical practice, basically due interobserver variability. However Busam et al. demonstrated that the categorisation of TILs can be easily taught and can be applied with an acceptable level of reproducibility in routine diagnostic practice.[12]

Regression has long been considered as an important feature of melanomas, with implications of an adverse effect on prognosis by some. In 146 patients subjected to the SLNB Kaur

Table 2 – The characteristics of the patients with positive and negative SLNs.

Clinical and pathological characteristics	LFN positive N = 74		LFN negative N = 320		p
	No	%	No	%	
Age, years					
Median	54.5		51.0		0.001
Range	29–85		19–86		
Mean	55.7 ± 15.2		50.4 ± 16.2		
Sex					
Male	44	59.5	168	59.5	0.28
Female	30	40.5	152	40.5	
Anatomical site					
Axial	49	66.2	162	50.6	
Extremity	25	33.8	158	49.4	0.02
Breslow thickness mm					
<1.0	3	4.2	82	25.8	<0.0001
>1.0 and <2.0	18	25.0	128	40.3	
>2.0 and <4.0	22	30.5	79	24.8	
>4.0	29	40.3	29	9.1	
Clark level					
I–II–III	10	14.1	113	37.0	0.0002
IV–V	61	85.9	192	63.0	
Ulceration					
No	29	40.3	187	59.9	0.003
Yes	43	59.7	125	40.1	
Regression					
No	53	75.7	209	70.8	0.4
Yes	17	24.3	86	29.2	
TIL					
Absent	46	62.1	154	48.1	0.03
Brisk + Non-brisk	28	37.9	166	51.9	

et al. observed that a statistically significant greater proportion of individuals without regression showed SLN positivity compared with those who did show regression.[13] Testori et al. suggested that regression should be considered a protective factor for SLN metastasis.[14] We did not observe any correlation between regression and SLN positivity. Our data are consistent with the data reported by Morris et al., who found that the presence of histological regression in a primary melanoma predicted neither SLN positivity when stratified by the Breslow depth nor increased risk of recurrence when compared with melanomas with no regression.[15]

However in the present study regression did not imply the absence of tumour; since the definition of regression may be different in several studies, this consideration should be taken into account to better understand the conflicting data in the literature.[14,15]

Furthermore, although ulceration has frequently been shown to be a predictor for SLN involvement,[16,17] our study did not confirm this finding, and our results are consistent with a well-conducted study by Sondak et al.[18]

In our single-institution study at least one SLN was found in 97.5% of the patients during surgical biopsy. This is comparable with other studies reporting success rates between 98.5% and 100%. In biopsied patients, a 18.8% positivity of SLN was found. These data compare well with other previously reported data in the literature.[16,19,20] Van Akkooi et al. reported a 29.5% positivity of SLN, with a median Breslow thickness of 2.0 mm.[21] However in our cohort the mean Breslow depth was 1.3 (±2.1) mm, and this may partially justify slightly lower rates of SLN positivity.[21]

The present study provides information about the independent influence of clinically relevant prognostic factors on the chances of remaining disease free for 5 years after a SLN procedure. The histological status of the SLN was the most significant predictor of DFS (Fig. 1) and OS (Fig. 2). Patients with a negative SLN had a 5-year DFS of 75.9%, compared with 35.2% in patients with a positive SLN ($p < .0001$) and a 5-year OS of 88.7% versus 42.9%, respectively ($p < .0001$).

When SLN status was not considered, the 5-year DFS was marginally better for patients in whom TILs were present (brisk and non-Brisk) (87.6%) in their primary melanoma than for those in whom TILs were absent (82.5%) (p 0.025) (Fig. 3).

The value of the sentinel node status as a prognostic factor in PCM has been widely reported by several authors.[7,14,21] These results suggest that, in spite of the absence of a survival benefit associated with SLN staging, this safe and highly reliable procedure is extremely useful to better stratify patients in phase III prospective adjuvant trials, in order to compare homogeneous group of patients. This represents an extra advantage of the SLN procedure.

The role of TILs as prognostic factors has been suggested by several reports, although conflicting data have been

Table 3 – Clinical and pathological characteristics predicting sentinel lymph node positivity.

Variables	Univariate			Multivariate			Multivariate stepwise		
	OR	95%CI	*p*	OR	95%CI	*p*	OR	95%CI	*p*
Breslow thickness, mm									
0–1	1.00			1.00					
1.1–2	3.59	1.79-7.22	<0.001	3.69	1.69–8.03	<0.001	1.00		
2.1–4	7.11	3.49-14.51	<0.001	7.37	3.09–17.59	<0.001	2.82	1.46–5.45	0.004
>4	27.33	7.44-96.54	<0.001	24.19	4.91–119.13	<0.001	10.41	5.21–20.80	<0.001
Sex									
Female	1.00			1.00					
Male	1.33	0.79–2.07	0.472	1.01	0.52–1.94	0.982			
Ulceration									
No	1.00			1.00					
Yes	2.22	1.32–3.74	0.003	1.15	0.59–2.25	0.674			
Regression									
No	1.00			1.00					
Yes	0.78	0.43–1.42	0.417	1.55	0.72–3.22	0.237			
Age									
<60	1.00			1.00					
>60	1.22	0.71–2.07	0.417	0.76	0.40–1.45	0.404			
Anatomical site									
Axial	1.00			1.00			1.00		
Extremity	0.52	0.31–0.88	0.015	0.49	0.25–0.98	0.040	0.50	0.28–0.88	0.037
TIL									
Absent	1.00			1.00			1.00		
Brisk + Non-brisk	0.53	0.34–0.95	0.031	0.47	0.25–0.90	0.021	0.54	0.31–0.9	0.05
Clark level									
I–II–III	1.00			1.00					
IV–V	3.59	1.77–7.29	<0.001	1.18	0.45–3.07	0.733			

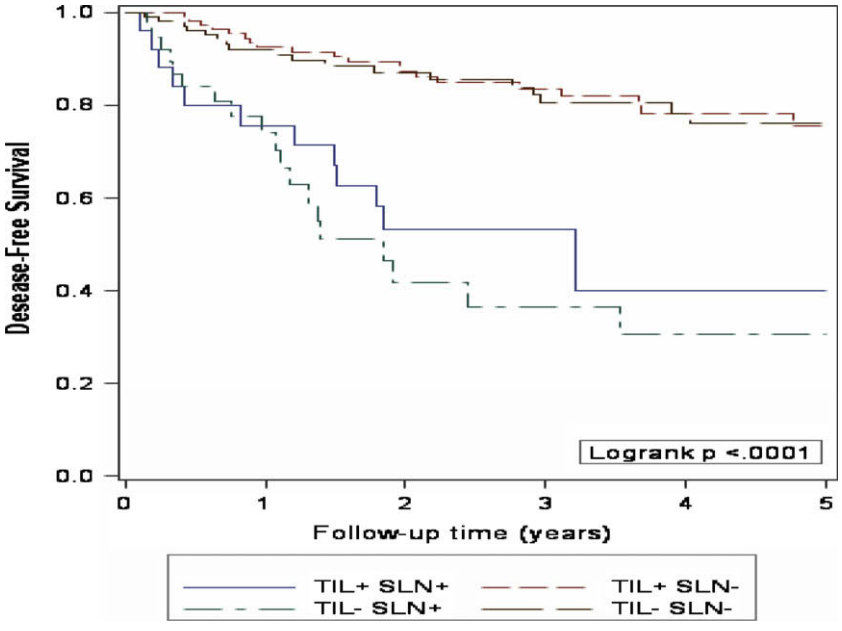

Fig. 1 – Disease-free survival according to TILs (Tumour Infiltrating lymphocytes) and Sentinel lymph node (SLN) status.

reported so far. It has been suggested that studies demonstrating a survival advantage associated with TILs tend to involve a high-risk population of patients, such as patients with thicker melanoma. In a pivotal study, Clark et al.[7] found that TILs predicted survival in patients with PCM with 46% of the patients having a Breslow thickness of 1.7 mm or more.

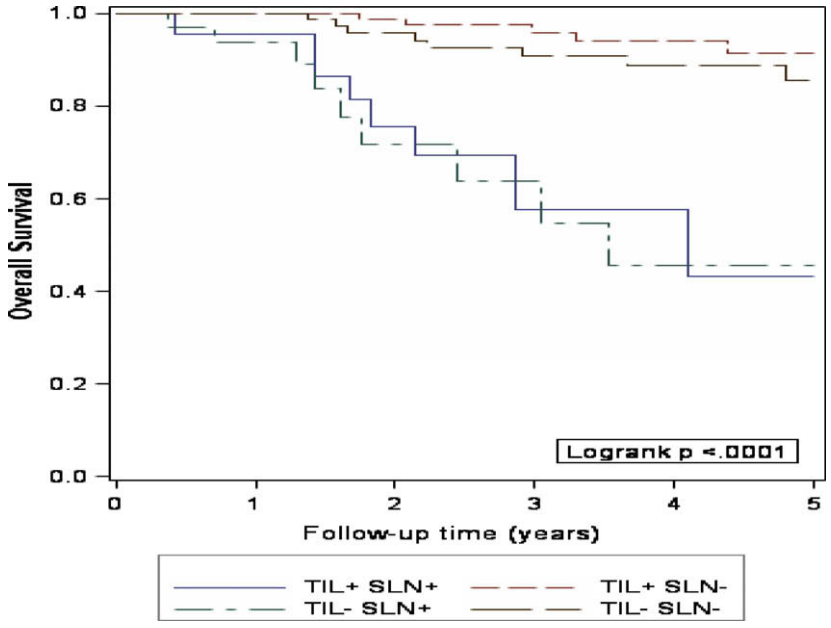

Fig. 2 – Overall survival according to TIL (Tumour Iniltrating lymphocytes) and Sentinel lymph node (SLN) status.

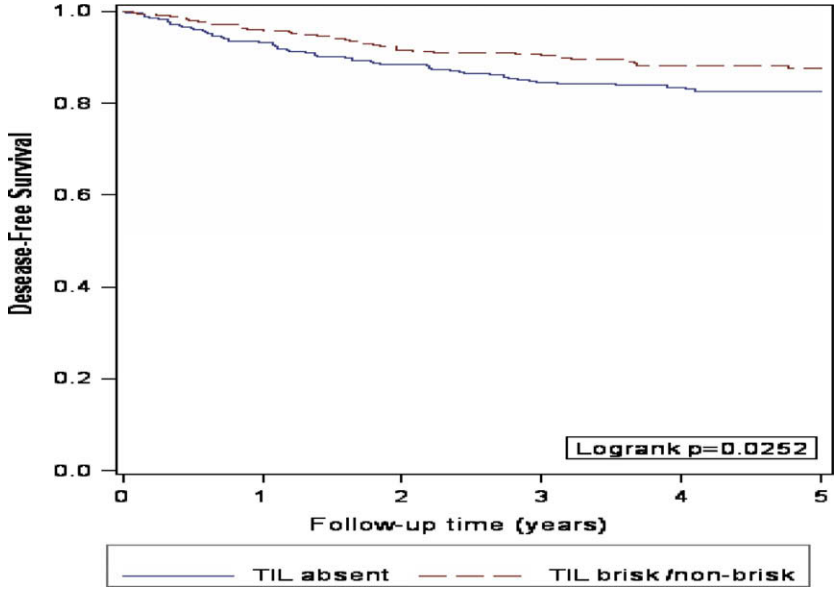

Fig. 3 – Disease-free survival according to TILs (Tumour Infiltrating lymphocytes) status.

Similarly, in two other positive studies the proportions of patients with a lesion thicker than 1.7 mm were 82% and 71%, respectively.[9,22] In our study, we did not observe any effect of TILs on survival, and in our study, only 18% of patients had lesions thicker than 2.0 mm. Similarly to our results Barhill and Taylor did not find any prognostic role for TILs in multivariate analysis.[10,23] It is interesting to underline that in these two studies the majority of patients presented with lesions thinner than 2 mm.

Among prognostic factors in patients with PCM, mitotic rate has received the most attention in the last years. Several prognostic analyses have been recently published that evaluated the importance of mitotic rate in the stage I/II population.[24–26] It seems that not only the presence or absence of mitoses in the dermal vertical growth component but the number of mitoses is also important.

Furthermore Ki67 is another potential important marker of melanoma aggressiveness. Gimotty et al demonstrated that high dermal Ki67 expression and mitogenicity are independent prognostic factors.[27] Mitotic rate and ki67 could be potentially two important features of melanoma proliferation and aggressiveness. These two markers give different important information because the longer-lasting Ki67 expression identifies potentially mitogenic cases in which a mitosis does

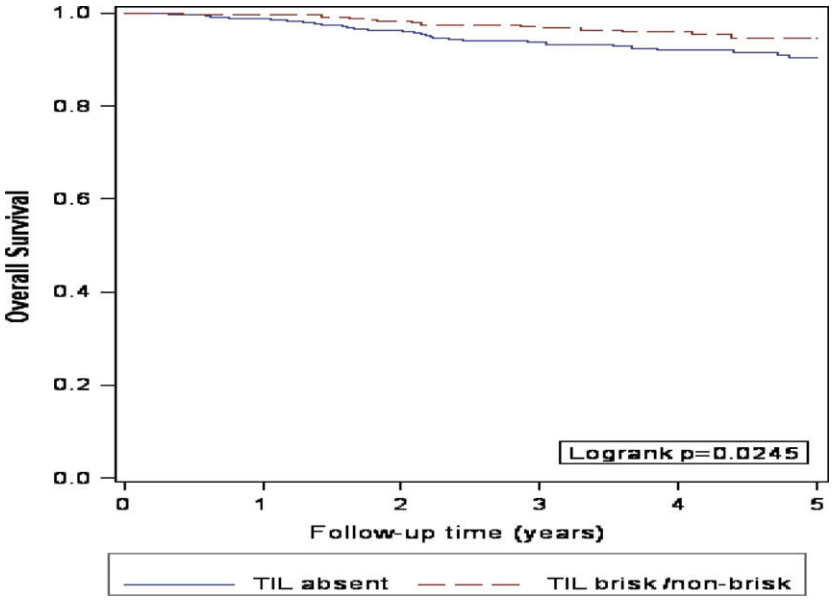

Fig. 4 – Overall survival according to TILs (Tumour Infiltrating lymphocytes).

not happen to have been present at the time of excision, whereas the presence of at least a single mitosis retains prognostic value because it is a more unequivocal demonstration of cell division.

An important limitation of our study is that we did not evaluate specific population of infiltrating lymphocytes. Recently, van Houdt et al. investigated whether the presence of TILs correlates with the expression of MHC class I antigen and MHC class II antigen on tumour cells and/or tumour infiltrating antigen presenting cells.[28] The authors found that a favourable clinical outcome was strongly associated with the presence of GrB^+ and $CD4^+$ TILs, with the expression of MHC class I antigen on tumour cells and with the expression of MHC class II antigen on intratumoural antigen presenting cells. Similarly, Piras et al. evaluated the distribution and density of T lymphocyte subsets, macrophages and dendritic cells in samples of primary cutaneous melanoma from 47 patients with Stages I and II melanoma according to the American Joint Committee on Cancer staging system.[29] The authors found that the presence and the number of infiltrating CD8 lymphocytes as well as the overall occurrence of HLA-DR cells may be considered independent, favourable prognostic factors in melanoma.

In conclusion our study demonstrates that that absence of TILs, along with the Breslow thickness and anatomical site, predicts the SLN positivity in PCM patients. However melanoma progression still occurs in the face of this infiltration. This, in turn, suggests the inability of TILs to mount an effective immune response.

Whether these TILs are functionally defective, incompletely activated or anergic is still open to further investigation. Moreover, a better molecular characterisation of TILs in PCM patients mandates further studies.

Conflicts of interest statement

None declare.

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