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RESEARCH ARTICLE

Tumor infiltrating lymphocytes in acral lentiginous melanoma: a study of a large cohort of cases from Latin America

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Abstract

Purpose Acral lentiginous melanoma (ALM) is a poor prognosis subtype and is the most prevalent in non-Caucasian populations. The presence of tumor infiltrating lymphocytes (TILs) has been associated with poor prognosis in melanoma. A large cohort of ALM cases was studied to determine status of TIL and its association with outcome.

Methods All patients with cutaneous melanoma presenting from 2005 to 2012 at Instituto Nacional de Enfermedades Neoplasicas in Peru were retrospectively identified. Clinicopathological information was obtained from the medical charts. A prospective evaluation of TIL was performed. Analysis of association between ALM and clinicopathological features including TIL as well as survival analysis compared the outcome of ALM to whole group and extremity NALM was performed.

Results 537 ALM from a total of 824 cutaneous melanoma cases were studied. Older age ($p = 0.022$), higher Breslow ($p = 0.008$) and ulceration ($p < 0.001$) were found to be more frequent in ALM. Acral had worse overall survival (OS) compared with the whole group ($p = 0.04$). Clinical

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stage (CS) I–II patients had a median OS of 5.3 (95% CI 4.3–6.2) for ALM and 9.2 (95% CI 5.0–7.0) for extremity NALM ($p = 0.016$). Grade 0 (absence of TIL), I, II and III were found in 7.5, 34.5, 32.1, and 25.9%, respectively. Lower TIL grade was associated with larger tumor size ($p = 0.003$), higher Breslow ($p = 0.001$), higher Clark level ($p = 0.007$), higher CS ($p = 0.002$), extremity location ($p = 0.048$), histological subtype ALM ($p = 0.024$) and better OS ($p = 0.001$).

Conclusions ALM is highly prevalent in Peru and carries poor outcome. Lower TIL levels were associated with poor outcome and ALM.

Keywords Melanoma · Lymphocytes · Survival · Acral

Background

Melanoma is the most lethal form of skin cancer. Histologically, four major subtypes are recognized: superficial spreading, nodular, lentigo maligna and acral lentiginous. Acral lentiginous melanoma (ALM) occurs on the palms, soles and subungueal (SU) areas. ALM is the least common subtype in Caucasian populations but appears to be the most prevalent in Hispanic patients in population-based cancer registries and some recent series from South America [1–7]. Acral lentiginous melanoma has been associated with a worse prognosis, and some authors have hypothesized that the lower survival is primarily attributable to a delay in diagnosis and more advanced stage at presentation in ALM [2, 8–15]. However, some studies have reported that ALM harbors shorter survival than cutaneous non-acral lentiginous melanoma (NALM) when controlled for stage. Moreover, some recent reports suggest that ALM carries some biologically aggressive markers that could be responsible for the poor outcome seen even in early stages of these tumors [2, 16–21].

The presence of tumor infiltrating lymphocytes (TILs) is associated with regression in melanoma and was a surrogate indicator of the immune host response. Regression is categorized by Clark et al. [22] into three categories (absent, no-brisk and brisk) based on the presence and location of TIL among melanoma cells. Multiple studies have described favorable prognostic value of TIL in melanoma [23–31]; however, some studies did not confirm this association [28, 30, 32–34].

One of the reasons for discrepant results regarding TIL and melanoma prognosis may be the lack of standard evaluation of TIL. Recently, Azimi et al. described a four-tier system TIL grading (0–3) that takes into account TIL distribution and density. They found a significant correlation between TIL grade and other melanoma features like thickness, mitotic rate and SLN status as well as survival. Patients with grade 3 TIL tumors had 100% survival at 5-year [31].

Only small case series of ALM from South America have been published to date, and evaluation of the relationship between TIL and ALM has not been reported [4–7]. Herein, we present a single-institution series of Peruvian patients with cutaneous melanoma, enriched with high numbers of ALM, in which we explore the role of TIL in the ALM subgroup.

Materials and methods

All patients with cutaneous melanoma diagnosis at Instituto Nacional de Enfermedades Neoplasicas, Lima-Peru, from 2005 to 2012 were included in the study.

Acral Lentiginous Melanoma was defined by anatomic location as palmar, plantar or subungual. Clinicopathological parameters included in the analysis were: age, sex, anatomic location, tumor (Breslow) thickness, Clark level, ulceration, regression, mitoses, perineural infiltration (PNI), perivascular infiltration (PVI), margin status, sentinel lymph node biopsy (SLNB) or lymph node dissection procedure, and the presence of positive lymph nodes. Additionally, we calculated the probability to find sentinel node involvement with the Risk of Melanoma Sentinel Lymph Node Metastasis (RMSLNM) Nomogram [35]. The score includes the following variables: age, thickness, Clark level and melanoma location [36]. Tumor infiltrating lymphocytes were prospectively evaluated by Institute pathologists (SCS, JSS and LCI) through the methodology described by Azimi et al. [31]. TILs were defined as lymphocytes infiltrating and disrupting tumor nests and/or in direct contact with tumor cells in H&E staining. The cases were classified into four grades (0–3) according to TIL density (mild, moderate, or marked) and distribution (focal, multifocal, or diffuse across the entire extent of the tumor) (Fig. 1).

Association analysis between clinicopathological features and ALM was performed using the Student's t and Chi square tests.

Clinical follow-up was obtained from patient files at the Institute Archives, and survival status was obtained from Peruvian government web page (RENIEC) when there was not an appropriate follow-up. Overall survival (OS) was defined as the time from pathological diagnosis to the time of death or last follow-up. A survival analysis of ALM and NALM was performed in the whole population. A stage-matched (stages I–IV) survival analysis was additionally performed in the subset of malignant melanoma in the extremities (upper and lower limbs location) [37].

Univariate Cox regression model was used to examine the association of clinical and pathologic variables with

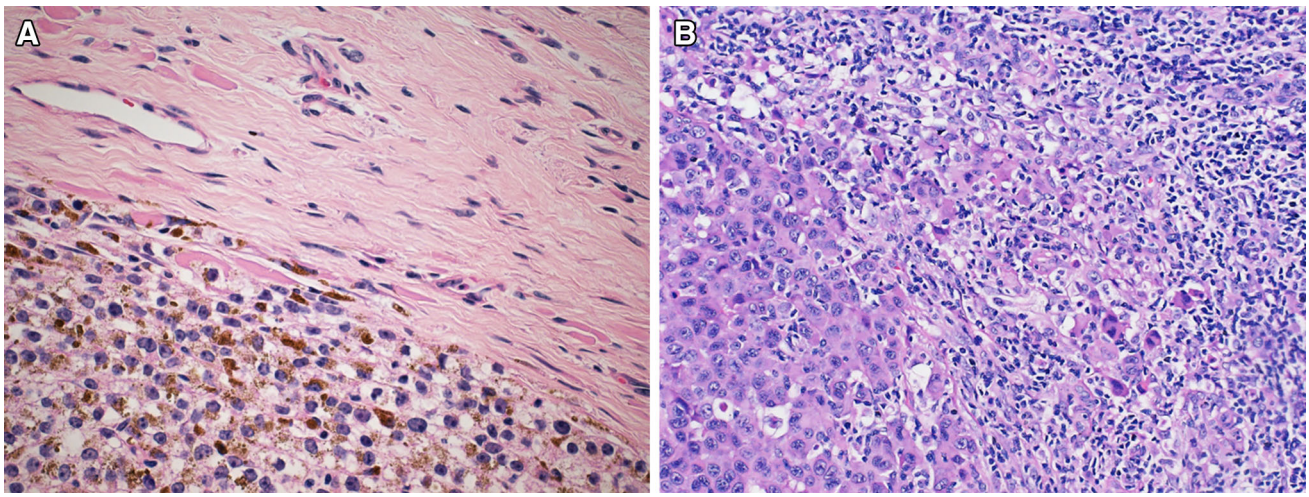

Fig. 1 Infiltrating lymphocytes in acral melanoma (H&E, ×200): **a** melanoma with absence of tumor infiltrating lymphocytes. **b** Melanoma cells with brisk tumor infiltrating lymphocytes (grade III TIL)

OS. Those features with significant association on univariate analysis ($p \leq 0.05$) were entered into a multivariate Cox proportional hazards model. Statistical analysis was performed using SPSSvs23. This study was approved by the institutional review board and received the code number 047-2015-CRP-DI-DICON/INEN. Personal and filiation data including identity of every patient were protected with an added code in the excel table. It is a retrospective case series that does not have any activity or contact with the patients.

Results

Clinicopathological features

Demographic, clinical and pathological features of this melanoma cohort ($n = 824$) are shown in Table 1. The median age at diagnosis was 62 (range 3–98) years and with a slight male prevalence (50.4%). Most frequent subtype was ALM ($n = 537$, 61.2%). There were 287 NALM cases and 84 (29.2%) of them were located in extremities NALM. The most frequent ALM location was lower limb (89.4%). The subungual location represented 17.7% and was more commonly located on the toes (57.9%). The most frequent CS were II (37.7%) and III (36.4%). The median Breslow was 4.0 mm (range 0.1–95.0 mm) and node involvement was found in 40.4% of 710 cases with evaluated regional lymph nodes (Table 1).

A large proportion of patients in this series lacked documentation of the presence of regression; however, in cases with available information, tumors were ulcerated in 62.2% (423/680), mitotic index >1 in 65.2% (353/542) (median mitotic index = 3), PNI in 20.4% (118/578), had LVI in 25.4% (157/617) and positive margin status in 18.3% (132/720) of patients (Table 1).

Sentinel lymph node biopsy (SLNB) was performed in 365 (41.6%) cases and 118 (32.3%) were positive. We evaluated the probability to find sentinel node involvement with the RMSLNM-nomogram in the 365 cases and we found a significant correlation ($p < 0.0001$).

Compared to NALM, ALM cases were associated with older age (62.5 vs 57.2 years, $p = 0.022$), thicker depth (Breslow 4.3 vs 4 mm, $p = 0.008$), T3–4 stage (77.6 vs 68.9%, $p = 0.002$), advanced CS (III–IV) (48.8 vs 39.5%, $p = 0.001$), lower rates of involved margins (83.9 vs 77.4%, $p = 0.049$), more frequent ulceration (68.3 vs 47.3%; $p < 0.001$), higher rates of PNI (23.8 vs 14.5%; $p = 0.009$), higher rates of LVI (28 vs 20.4%; $p = 0.047$) and higher Clark Level (IV–V) (80.3 vs 62.7%; $p < 0.001$) (Table 1).

When ALM ($n = 537$) was compared with NALM occurring on extremities ($n = 84$), ALM cases were more frequent in females ($p = 0.003$), presented at older age (62.5 vs 52.5 years, $p < 0.001$), thicker (Breslow 4.3 vs 4 mm, $p = 0.001$), ulceration ($p = 0.014$) and had higher rates of PNI ($p = 0.033$) than NALM. No differences regarding CS ($p = 0.200$), Clark level ($p = 0.341$), regression ($p = 0.505$), mitosis ($p = 0.379$), positive margin status ($p = 0.363$), microsatellitosis ($p = 0.891$), LVI ($p = 0.840$), lymph node involvement ($p = 0.189$) and TIL levels ($p = 0.351$) were found.

Lymphocyte infiltration in acral lentiginous melanoma vs non-acral lentiginous melanoma

Tumor infiltrating lymphocytes were prospectively evaluated in 536 cases, and grade 0 (absence of TIL), grade I, II and III TIL were found in 7.5, 34.5, 32.1 and 25.9%,

Table 1 Comparison of characteristics between acral and non-acral cutaneous melanomas

Characteristics	Total $n = 824$ (%)	ALM $n = 537$ (%)	NALM $n = 287$ (%)	p
Age				0.022
Median (range)	62.0 [3–98]	62.5 [11–98]	57.2 [3–93]	
Sex				0.531
Female	435 (49.6)	271 (50.5)	138 (48.1)	
Male	442 (50.4)	266 (49.5)	149 (51.9)	
Localization				<0.001
Head or neck	118 (14.3)	0 (0)	118 (41.1)	
Trunk	85 (10.3)	0 (0)	85 (29.6)	
Upper limb	89 (10.8)	58 (10.8)	32 (11.1)	
Lower limb	532 (64.6)	480 (89.2)	52 (18.1)	
T stage				0.002
T1–2	181 (26.5)	100 (22.4)	66 (31.1)	
T3	166 (24.3)	114 (25.6)	49 (23.1)	
T4	336 (49.2)	232 (52.0)	97 (45.8)	
Nodes				0.051
N0	423 (59.6)	256 (56.4)	165 (69.0)	
N1	89 (12.5)	63 (13.9)	19 (7.9)	
N2–N3	198 (27.9)	135 (29.7)	55 (23.0)	
Metastases				0.691
M1	74 (9.7)	34 (7.1)	21 (8.4)	
Clinical stage				0.001
I	124 (16.2)	60 (12.5)	53 (21.5)	
II	289 (37.7)	186 (38.7)	96 (39.0)	
III	279 (36.4)	194 (40.3)	72 (29.3)	
IV	74 (9.7)	41 (8.5)	25 (10.2)	
Breslow thickness				0.008
Median (range)	4.0 [0.1–95]	4.3 [0.2–95]	4.0 [0.1–50]	
Clark level				<0.001
I–II	51 (8.6)	22 (5.6)	25 (14.4)	
III	106 (17.9)	56 (14.1)	40 (23.0)	
IV	285 (48.1)	204 (51.5)	77 (44.3)	
V	150 (25.3)	114 (28.8)	32 (18.4)	
Regression				0.595
No	437 (96)	300 (96.5)	125 (94.7)	
Yes	18 (4.0)	11 (3.5)	7 (5.3)	
Mitosis				0.203
Median (range)	3.0 [0–81]	3.0 [0–81]	2.5 [0–45]	
≤1	189 (34.9)	124 (32.8)	65 (39.6)	
>1	353 (65.1)	254 (67.2)	99 (60.4)	
Ulceration				<0.001
No	257 (37.8)	147 (31.7)	107 (52.7)	
Yes	423 (62.2)	317 (68.3)	96 (47.3)	
Margin status				0.049
Negative	588 (81.7)	380 (83.9)	181 (77.4)	
Positive	132 (18.3)	73 (16.1)	53 (22.6)	
Microsatellitosis				0.642
No	424 (87.8)	294 (88.6)	116 (85.9)	
Yes	59 (12.2)	38 (11.4)	19 (14.1)	

Table 1 continued

Characteristics	Total $n = 824$ (%)	ALM $n = 537$ (%)	NALM $n = 287$ (%)	p
LVI				0.047
No	460 (74.6)	295 (72.0)	144 (79.6)	
Yes	157 (25.4)	115 (28.0)	37 (20.4)	
PNI				0.009
No	460 (79.6)	295 (76.2)	142 (85.5)	
Yes	118 (20.4)	92 (23.8)	24 (14.5)	
Resection of primary				0.423
No	133 (15.6)	92 (17.3)	71 (24.7)	
Yes	720 (84.4)	441 (82.7)	216 (75.3)	
SLNB				0.175
Negative	247 (67.7)	176 (65.7)	71 (73.2)	
Positive	118 (32.3)	92 (34.39)	26 (26.8)	
RMSLNM-nomogram				0.411
<10	70 (19.2)	48 (17.9)	22 (22.7)	
10–20	43 (11.8)	32 (11.9)	11 (11.3)	
20–30	72 (19.7)	58 (21.6)	14 (14.4)	
>30	180 (49.3)	130 (48.5)	50 (51.5)	
TIL levels				0.033
Absent	40 (7.5)	27 (7.3)	13 (7.8)	
G-I	185 (34.5)	140 (37.7)	45 (27.3)	
G-II	172 (32.1)	120 (32.3)	52 (31.5)	
G-III	139 (25.9)	84 (22.6)	55 (33.3)	

respectively. Grade III TILs were more frequent in NALM than in ALM (33.3 vs 22.6%, $p = 0.033$) (Table 1). Higher TIL grade was also associated with location on extremities ($p = 0.048$), histological subtype ($p = 0.024$), lower Breslow ($p = 0.001$), lower Clark level ($p = 0.007$), lower rates of T3–4 ($p = 0.003$), higher rates of CS I–II ($p = 0.002$) and lower RMSLNM-nomogram score ($p = 0.036$) (Table 2).

Survival analysis

The median follow-up was 5 years. Relationship between clinical and pathological features and overall survival (OS) in the entire melanoma cohort ($n = 824$) is shown in Table 3. Clinicopathological features associated with shorter OS on univariate analysis were: older age ($p = 0.001$), male gender ($p < 0.001$), Breslow >4 ($p < 0.001$), Clark level V ($p < 0.001$), T3–4 stage ($p < 0.001$), N2–3 stage ($p < 0.001$), CS IV ($p < 0.001$), ulceration ($p < 0.001$), mitotic index >1 ($p < 0.001$), positive margin status ($p < 0.001$), positive sentinel lymph node ($p < 0.001$) and RMSLNM-nomogram ($p = 0.001$). The ALM subtype was associated with shorter OS in the whole population (35 vs 46% at 5-year, $p = 0.04$) (Table 3). High-grade TIL was generally associated with longer OS (grade 0, I, II, III: 46.3, 26.0, 31.5, 39.8% at 5 years, $p = 0.000$). High-grade TILs were associated with longer OS in the ALM (grade 0, I, II and III: 46.8, 22.4, 28.5 and 37.0% at 5 years, $p = 0.002$) but not in the cutaneous NALM (grade 0, I, II and III: 27.1, 38.6, 38.8 and 44.2% at 5 year, $p = 0.180$) subsets (Table 3; Fig. 2).

Older age ($p = 0.016$), male gender ($p < 0.001$), CS III–IV ($p < 0.001$), ulceration ($p = 0.001$), positive margin status ($p = 0.019$) and lower TIL levels ($p = 0.031$) remained significantly associated with shorter OS on Cox proportional hazard model (Table 3).

The ALM subtype demonstrated a significantly reduced OS when compared with the extremity NALM cohort (34.7 vs 59.4% at 5 years, $p = 0.001$) (Fig. 2). There was also a shorter OS median for ALM than NALM occurring on extremities cases (5.3 years, 95% CI 4.3–6.2 vs. 9.2 years, 95% CI 5.0–7.0) when only CS I–II was evaluated ($p = 0.016$).

Clinicopathological features associated with reduced progression-free survival (PFS) on univariate analysis were: older age ($p = 0.003$), male gender ($p < 0.001$), Breslow >4 ($p < 0.001$), Clark level V ($p < 0.001$), T3–4 stage ($p < 0.001$), N2–3 stage ($p < 0.001$), CS IV ($p < 0.001$), ulceration ($p < 0.001$), mitotic index >1 ($p < 0.001$), positive margin status ($p < 0.001$) and positive sentinel lymph node ($p < 0.001$). The ALM subtype was associated with reduced PFS in the whole population

Table 2 Comparison of TIL levels with clinical and pathological features

Characteristics	Absent—TIL-I (%)	TIL-II (%)	TIL-III (%)	*p*
Age				0.217
<40	28 (12.3)	17 (9.7)	12 (8.5)	
40–60	74 (32.6)	49 (27.8)	42 (29.8)	
>60	125 (55.1)	110 (62.5)	87 (61.7)	
Sex				0.834
Female	112 (49.3)	87 (49.4)	76 (53.9)	
Male	115 (50.7)	89 (50.6)	65 (46.1)	
Localization				0.048
Head or neck	16 (7.0)	22 (12.5)	27 (19.1)	
Trunk	18 (7.9)	16 (9.1)	13 (9.2)	
Upper limb	25 (11.0)	16 (9.1)	15 (10.6)	
Lower limb	168 (74.0)	122 (69.3)	86 (61.0)	
Histological subtype				0.024
Cutaneous NALM	58 (25.6)	52 (29.5)	56 (39.7)	
ALM	169 (74.4)	124 (70.5)	85 (60.3)	
T stage				0.003
T1–2	32 (17.1)	17 (11.0)	31 (24.6)	
T3	41 (21.9)	38 (24.5)	40 (31.7)	
T4	114 (61.0)	100 (64.5)	55 (43.7)	
Clinical stage				0.002
I	27 (12.9)	15 (8.9)	23 (16.8)	
II	88 (42.1)	75 (44.6)	59 (43.1)	
III	80 (38.3)	73 (43.5)	47 (34.3)	
IV	14 (6.7)	5 (3.0)	8 (5.8)	
Breslow thickness				0.001
<1.0	13 (6.6)	9 (5.6)	11 (8.1)	
1.0–2.0	24 (12.2)	13 (8.0)	20 (14.8)	
2.0–4.0	34 (17.3)	33 (20.4)	37 (27.4)	
>4.0	125 (63.8)	107 (66.0)	67 (49.6)	
Clark level				0.007
I–II	14 (8.0)	6 (4.4)	10 (8.3)	
III	21 (12.0)	15 (11.0)	21 (17.5)	
IV	90 (51.4)	68 (50.0)	70 (58.3)	
V	50 (28.6)	47 (34.6)	19 (15.8)	
Regression				0.525
No	220 (98.2)	172 (98.3)	135 (95.7)	
Yes	4 (1.8)	3 (1.7)	6 (4.3)	
Mitosis				0.526
≤1	18 (10.5)	21 (15.3)	13 (10.9)	
>1	153 (89.5)	116 (84.7)	106 (89.1)	
Ulceration				0.091
No	85 (37.4)	66 (37.5)	62 (44.0)	
Yes	142 (62.6)	110 (62.5)	79 (56.0)	
Margin status				0.939
Negative	15 (88.2)	4 (100)	6 (85.7)	
Positive	2 (11.8)	0 (0.0)	1 (14.3)	
Microsatellitosis				0.895
No	208 (92.0)	159 (91.9)	129 (91.5)	
Yes	18 (8.0)	14 (8.1)	12 (8.5)	

Table 2 continued

Characteristics	Absent—TIL-I (%)	TIL-II (%)	TIL-III (%)	*p*
LVI				0.171
No	129 (70.5)	94 (65.7)	94 (75.2)	
Yes	54 (29.5)	49 (34.3)	31 (24.8)	
PNI				0.203
No	132 (74.6)	102 (75.0)	97 (84.3)	
Yes	45 (25.4)	34 (25.0)	18 (15.7)	
SLNB				0.083
Negative	71 (67.0)	57 (58.2)	17 (24.3)	
Positive	35 (33.0)	41 (41.8)	53 (75.7)	
RMSLNM-nomogram				0.036
<10	21 (19.8)	9 (9.2)	14 (20.0)	
10–20	9 (8.5)	11 (11.2)	13 (18.6)	
20–30	22 (20.8)	22 (22.4)	16 (22.9)	
>30	54 (50.9)	56 (57.1)	27 (38.6)	

(37.7 vs 25.5% at 5-year, $p = 0.037$). Lower TIL grade was associated with longer PFS ($p = 0.02$).

Gender ($p < 0.001$), histological sub-type ($p = 0.047$), CS ($p < 0.001$), ulceration ($p = 0.001$) and TIL levels ($p = 0.002$) remained significantly associated with PFS on Cox proportional hazard model.

Discussion

In our series, ALM was the most frequent melanoma subtype (61.2%) and demonstrated a shorter overall survival (5 year—overall survival 35.0%) [4–7]. A population-based study with more than 8000 ALM cases as well as a surveillance, epidemiology, and end results (SEER) Program analysis of 17 population-based cancer registries with 1413 ALM cases similarly describes that American Hispanics have higher incidence rates of ALM than Caucasian population, and ALM survival is lower in Hispanic than Caucasian population (5-year melanoma-specific survival rates of 82.6 vs 72.8%) [2, 38]. Lino-Silva et al. recently published a retrospective series of more than 1200 mexican patients with cutaneous melanoma and found that ALM was the most frequent (44.1%) and was associated with shorter disease-specific survival in univariate ($p = 0.001$) and multivariate ($p = 0.004$) analysis [4].

Most of our ALM cases, including those of subungual location, arose on the feet. This finding is similar to what is described in previous studies and could be related to a constant sole exposure to trauma, irritation and maceration [8, 13–15, 39].

Acral lentiginous melanoma had lower survival rates when compared to cutaneous NALM in the whole population as well as NALM occurring on the extremities. Acral Lentiginous Melanoma patients in CS I and II had a shorter survival than those with extremity NALM (through a paired stage evaluation). Tumor thickness, node involvement, ulceration, mitotic index, positive margin status and older age are recognized prognostic factors for melanoma and we found that all these factors were higher in ALM than in cutaneous NALM. This explains the differences in survival between these two groups, even for patients with similar CS [2, 8, 9, 13–16, 40–48]. Bello et al. [16] evaluated a series of 281 ALM and 843 extremity NALM and also found that ALM had a worse outcome in patients with similar CS. Bradford et al. [2] evaluated the data from 1413 ALM cases from the surveillance epidemiology and end results Program (SEER) and also found that these tumors are associated with worse prognosis than cutaneous NALM in stage-matched analysis.

From the molecular genetics point of view, the reported differences between ALM and NALM cutaneous melanoma, like lower rates of the serine-threonine protein kinase B-RAF (BRAF) mutations, higher rates of *KIT* mutations and higher vitamin D receptor expression, could also explain the poor prognosis of ALM [18, 19, 49–51].

Our analysis of TILs confirmed that a lower TIL grade was associated with poor outcome ($p = 0.001$) [31] and to ALM histopathological type ($p = 0.012$). This latter finding, to our knowledge, has not been previously reported. Therefore, low TIL grade could be added to aggressive features previously described to be more frequent in ALM and could also explain the poor prognosis of this melanoma subtype. Poor prognosis of ALM could be a reflection of a combination of unique biological nature coupled to a poor immune host response. Immune system modulation has become an important strategy in melanoma [23–27, 52–56], and TIL levels have demonstrated to

Table 3 Overall survival according to the clinical and pathological characteristics

Characteristic	Median survival (years)	Survival rate	Log-rank test	Cox model	
		5 years	*p* valor	*p*	HR (IC 95%)
Age			0.001	0.016	1.6 (1.1, 2.4)
<40	6.1	55.0			
40–60	4.1	45.0			
>60	3.0	31.0			
Sex			<0.001	<0.001	2.2 (1.4, 3.2)
Female	4.9	49.0			
Male	2.5	27.0			
Localization			0.171	0.636	–
Head and neck	3.3	39.0			
Trunk	3.5	42.0			
Upper limb	5.4	55.0			
Lower limb	3.3	35.0			
Histological sub-type			0.040	0.089	–
Cutaneous NALM	4.3	46.0			
ALM	3.3	35.0			
Breslow thickness (mm)			<0.001	0.305	–
<1.0	Non achieved	78.0			
1.0–2.0	5.5	61.8			
2.0–4.0	4.6	45.1			
>4.0	2.3	25.3			
Clark level			<0.001	0.881	–
I–II	9.2	75.5			
III	6.0	62.5			
IV	4.0	41.2			
V	2.1	22.1			
T stage			<0.001	0.433	–
T1–T2	9.2	70.0			
T3–T4	3.1	31.6			
N stage			<0.001	0.501	–
N0	5.3	55.1			
N1	3.1	32.1			
N2–N3	1.6	15.7			
Clinical stage			<0.001	<0.001	2.6 (1.8, 3.8)
I	9.2	77.8			
II	4.9	48.4			
III	2.1	21.6			
IV	0.4	3.7			
Ulceration			<0.001	0.001	2.6 (1.7, 4.19)
No	6.0	56.6			
Yes	3.0	29.2			
Mitotic index			<0.001	0.907	–
≤1	5.2	51.1			
>1	3.1	32.9			
Margin status			<0.001	0.019	2.0 (1.1, 3.4)
Negative	4.4	45.1			
Positive	2.0	29.9			

Table 3 continued

Characteristic	Median survival (years)	Survival rate	Log-rank test	Cox model	
		5 years	*p* valor	*p*	HR (IC 95%)
SLNB			<0.001	0.325	–
Negative	8.0	66.0			
Positive	3.3	29.2			
TIL level			<0.001	0.031	
Absent	2.1	46.3			
G-I	1.8	26.3			
G-II	2.4	31.5			
G-III	4.4	39.8			
TIL in ALM			0.002	–	
Absent	2.4	46.8			
G-I	1.9	22.4			
G-II	2.3	28.5			
G-III	4.2	37.0			
TIL in NALM			0.180	–	
Absent	1.5	27.1			
G-I	1.7	38.6			
G-II	2.6	38.8			
G-III	4.9	44.2			
RMSLNM-nomogram			0.001	–	
<10	10.0	68.0			
10–20	6.3	53.0			
20–30	6.4	53.0			
>30	4.0	35.0			

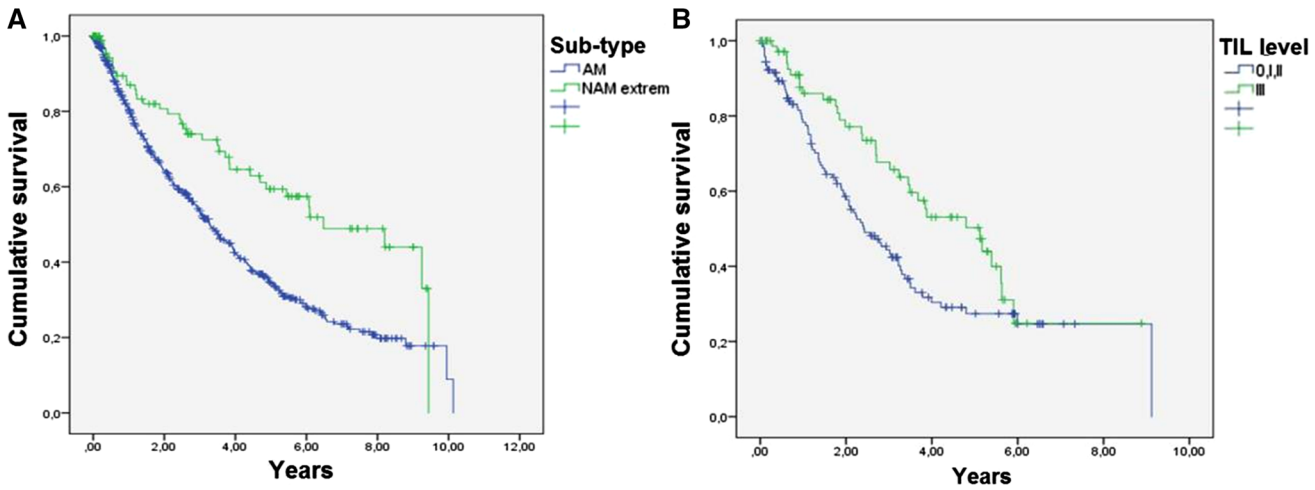

Fig. 2 Overall survival regarding **a** ALM versus NALM in extremity ($p < 0.001$), and regarding **b** grade of tumor infiltrating lymphocyte (TIL) ($p < 0.001$)

behave as a biomarker for chemotherapy and immunotherapy [57, 58].

A potential weakness of our study is its retrospective fashion when recording some pathological features, a few of them not available for evaluation. Evaluation of TIL, however, was performed using a standardized method by more than one pathologist. Using the same methodology of Azimi et al. [31], we found higher rates of grade II–III TIL (58 vs 19.5%) in our series; however, we observed a similar association between lower TIL grade and both higher T stage and shorter survival.

Conclusions

Our study revealed that ALM is the most common subtype in the Peruvian population. Acral lentiginous melanoma was associated with clinicopathological features related to aggressive behavior and had a trend to shorter survival. Low levels of TIL were associated with shorter survival and to ALM. This novel finding suggests that a deficient immune activity would be responsible for ALM poor prognosis.

Authors' contribution CAC, SC and CTC contributed to the conception and design of the study and performed data analysis and interpretation; CAC, SC and MC performed data acquisition, as well as providing administrative, technical, and material support; all authors drafted the article and made critical revisions related to the intellectual content of the manuscript, and approved the final version of the article to be published.

Compliance with ethical standards

Conflict of interests The authors declare that they have no conflict of interests.

Ethics approval and consent to participate The Institutional Review Board of INEN approved the conduct of this survey (#047-2015-CRP-DI-DICON/INEN), and all written informed consent was obtained from patients.

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