Influence of regression, its extent and tumor-infiltrating lymphocytes on sentinel node status, relapse, and survival in a 10-year retrospective study of melanoma patients

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This case-control study seeks to investigate the influence of histological findings, specifically regression, its extent and tumor-infiltrating lymphocyte (TILs), on result of sentinel lymph node (SLN) biopsy, 5-year melanoma-specific survival (MSS), and relapse-free survival (RFS). We included all patients with cutaneous melanoma who underwent SLN biopsy at the Melanoma Center of the University of Brescia, following the Italian Association of Medical Oncology National guidelines from January 2008 to August 2018. Regression and its extent (<75 or ≥75%) and the presence of TILs were reevaluated by a trained dermatopathologist, adhering to the 2017 College of American Pathologists Cancer Protocol for Skin Melanoma. These patients were followed up for 5 years. Our study uncovered significant associations between regression and male sex ($P < 0.05$), melanoma location on the trunk, upper limbs, and back ($P = 0.001$), ulceration ($P < 0.05$), lower Breslow thickness ($P = 0.001$), and the presence of lymphocytic infiltration (both brisk and nonbrisk) ($P < 0.001$). Regression and its extent, however, did not appear to affect SLN positivity ($P = 0.315$). Similarly, our data did not reveal a correlation between TILs and result of SLN biopsy ($P = 0.256$). When analyzing MSS and RFS in relation to the presence or absence of regression and TILs, no statistically significant differences were observed, thus precluding the need for logistic regression and Kaplan–Meier curve analysis. This study's findings underscore that regression and TILs do not appear to exert an influence on sentinel lymph node status, MSS, or RFS in our cohort of patients. *Melanoma Res* 34: 343–349 Copyright © 2024 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

Melanoma, originating from melanocytes, constitutes only 1% of skin cancers but is responsible for 80% of skin cancer-related deaths [1]. Treatment options are based on disease stage and are influenced by some histological characteristics.

The most critical histopathological factor predicting melanoma outcomes is tumor vertical growth, measured by Breslow thickness. Based on the 2018 recommendations of the American Joint Committee on Cancer (AJCC), the biopsy of the sentinel lymph node (SLN) is recommended for melanomas with ulceration or a thickness of 0.8 mm or more (stage pT1b) [2]. Additionally, although not included in the AJCC guidelines, extensive histologic regression (≥75%) should be considered an indication for sentinel node biopsy in so-called thin melanomas (Breslow thickness ≤ 1 mm) [3].

Histologic regression in melanoma refers to an area within the tumor where neoplastic cells have disappeared or decreased, replaced by fibrosis, melanophages, neovascularization, and inflammatory cells. Regression can occur partially or completely and is observed in approximately 10–35% of cases, reaching up to 58% in thin melanomas [1]. The significance of regression, however, remains debated.

On the one hand, some cases have shown cutaneous or lymph node metastases in patients with completely regressed primary melanoma, suggesting regression may be a negative prognostic factor, possibly leading to an underestimation of Breslow thickness and an increased risk of metastases. On the other hand, regression may indicate a lower likelihood of a positive sentinel node biopsy, as it could reflect the immune system's ability to combat cancer. The precise implications of regression in melanoma prognosis are still under investigation.

Tumor-infiltrating lymphocytes (TILs) are a diverse group of T lymphocytes that have migrated from the bloodstream into the tumor tissue. They are a

critical component of the immune response against cancer, attracted by various signals, including recognition of tumor-specific antigens or other immune signals in the tumor microenvironment. TILs are categorized into three ranks by the Clark grading system: absent, nonbrisk, or brisk. 'Absent' describes a complete lack of tumor infiltration by TILs. 'Nonbrisk' indicates the focal presence of TILs at tumor edges or specific sections of the tumor. On the other hand, 'brisk' signifies the abundant presence of TILs at all tumor edges or diffusely throughout the tumor.

The primary objective of this study is to determine the prognostic significance of the presence and type of immune response in primary cutaneous melanoma. Additionally, the study aims to analyze demographics and histologic features to establish any correlations with regression or SLN biopsy status.

Data collection

In this retrospective study, all patients newly diagnosed with primary cutaneous melanoma and underwent SLN biopsy based on the Italian Association of Medical Oncology (AIOM) National guidelines were included between January 2008 and August 2018. Encoded and anonymous data were obtained from the Melanocytic Neoplasia Research Register of the outpatient clinic of the Dermatology department at the University Hospital of Brescia.

Study population

The inclusion criteria for this study were patients aged ≥14 years with slides and formalin-fixed and paraffin-embedded primary tumors preserved in the Anatomical Pathology Unit of the University Hospital of Brescia archives and available for reevaluation. Exclusion criteria included patients with head and neck primary cutaneous melanoma (to exclude possible false negative in sentinel node biopsy), in situ melanoma, partially excised primary cutaneous melanoma, extracutaneous melanoma, and completely regressed melanoma. The categorization of patients was carried out in accordance with the 8th edition AJCC Staging Manual, which was implemented as of January 1, 2018.

We reclassified all melanomas diagnosed before this date according to the 8th edition AJCC classification. The follow-up implemented for these patients adhered to the AIOM guidelines in effect at the time of diagnosis.

AIOM guidelines until the December 31, 2014 recommended sentinel node biopsy for melanoma with Breslow thickness ≥ 1 mm or with Breslow thickness < 1 mm melanomas with ulceration and ≥1/mm^2 mitotic rate. From January 1, 2015 to December 31, 2017 sentinel node biopsy was performed for melanoma with Breslow thickness > 0.76 mm and considered for Breslow thickness ≤ 0.75 melanomas with ulceration and ≥1/mm^2 mitotic rate. From January 1, 2018 we recommended this surgical act for melanoma with Breslow thickness ≥ 0.8 mm or Breslow thickness < 0.8 mm and ulceration; AIOM guidelines confirmed the indication of sentinel biopsy for melanoma with Breslow thickness < 0.8 melanomas and extensive histologic regression (≥75%).

The regression of melanoma is by definition mainly characterized by dermal fibrosis with telangiectasias and an inflammatory infiltrate of lymphocytes admixed with pigment-laden macrophages; the overlying epidermis is thinned or atrophic with flattened reticular ridges. In our study, we considered regression according to the criteria provided by Massi & LeBoit [4], including both an inflammatory phase (called regressive melanoma) where the lymphocytic infiltrate predominates and fibrosis is minimal or absent with residual melanoma nests in the dermis and epidermis, and a consolidated phase (called regressed melanoma) when cicatricial fibrosis is predominant and the lymphocyte population is significantly reduced, as is the melanoma component.

The extent of regression was assessed as <75 or ≥75% according to College of American Pathologists (CAP) Cancer Protocol for Skin Melanoma (2021) [5].

This cutoff inevitably means that the first group is particularly large, including even at the lower end those cases in which the regression was minimal and focal. The TILs were define as nonbrisks or brisks according to CAP Cancer Protocol for Skin Melanoma (2017) [5]. Cases in which the tumor lymphocytic infiltrate was brisk and marked, were carefully reviewed and regression was assigned only when, with the same lymphocytic infiltrate, the melanoma component was significantly reduced together with initial fibrosis.

The presence of regression and its extent (<75 or ≥75%) were reassessed firstly by a trained dermatopathologist, reviewing all hematoxylin-and-eosin-stained histological sections of every melanoma from the Anatomical Pathology operating unit of the University Hospital of Brescia. In case of discrepancies, a further evaluation by a second pathologist was carried out to resolve the doubts. For each patient, demographics collected through a specific questionnaire included age, sex, age at diagnosis, sun exposure (intermittent and continuous), solar lamp use (absent, <50 or ≥50), episodes of sunburns during childhood/adolescence (absent or present). A dermatologist reported for each patient Fitzpatrick skin type (from I to IV), number of nevi (<50 or ≥50), family history of melanoma diagnosis (1st or 2nd degree relatives), family history for associated-melanoma malignancies (1st or 2nd degree relatives with breast, pancreas, kidney, or brain tumor), location of the melanoma (trunk, back, upper, or lower limbs), and date and type of first recurrence, if present (local, regional, in transit, or distant). Pathologic data

included melanoma subtype (superficial spreading, nodular, lentigo maligna, acral lentiginous, undetermined, other types), Breslow thickness (mm), vertical growth phase (present or absent), ulceration (present or absent), regression (absent or partial <75% or extensive ≥75%), microsatellites (present or absent), mitotic rate (<1 or ≥1/mm^2), lymphocytic infiltrate (absent, brisk or nonbrisk), lymphovascular invasion (present or absent), nevus-associated melanoma (absent, congenital, common, or dysplastic), and 8th edition AJCC Tumor-Nodes-Metastasis (TNM) staging system.

Primary clinical outcome measures included result of the sentinel node biopsy, melanoma-specific survival (MSS), and relapse-free survival (RFS). We specifically defined RFS as the time from the diagnosis of the primary melanoma to the occurrence of any type of recurrence (local, regional, or distant) or death.

Statistical analysis

The database was formatted using Microsoft-Excel TM software (Redmond, Washington, USA) and subsequently imported from IBM-SPSS (Version 28.0.1; IBM, Armonk, New York, USA) and STATA (Version 18; StataCorp LLC, College Station, Texas, USA) to analyze the data.

For the statistical analysis, the continuously expressed variables were subjected to the Kolmogorov–Smirnov test to evaluate their normal distribution. Continuous variables were summarized as means ± SD; frequency and percentages were the descriptive analyses performed on the categorical variables. Differences in proportions and means were analyzed using χ^2 and Mann–Whitney U tests, respectively. All results were analyzed with an α significance level of 5%.

Results

Tables 1 and 2 display demographics and clinicopathological features of the patients with and without regression or with positive or negative sentinel node biopsy: in total, we included 713 patients (376 males and 337 females) in this study. The average age at diagnosis was 54.8 ± 14.8 (range: 14–92 years old); the highest location prevalence of the melanoma was the back, with 270 cases (37.9%), followed by 183 cases on the lower limbs (25.6%), 149 melanomas on the trunk, and at last 111 on the upper limbs (15.6%). The more frequent subtype was superficial spreading melanoma, diagnosed in 395 patients (55.4%) and the Breslow thickness mean ± SD was 1.8 ± 1.6 mm; primary cutaneous melanomas without histological regression occurred in 542 patients, 273

Table 1 Relationship between regression and sentinel lymph node status with different clinical variables

	Regression			Sentinel lymph node biopsy			
Variables	Absent ($n=542$)	Present ($n=171$)	*P*-value	Negative ($n=565$)	Positive ($n=148$)	*P*-value	Total ($N=713$)
Gender, *n* (%)			<0.05*			<0.05*	
Male	273 (50.4)	103 (60.2)		283 (50.1)	93 (62.8)		376 (52.7)
Female	269 (49.6)	68 (39.8)		282 (49.9)	55 (37.2)		337 (47.3)
Age (years) at diagnosis, mean (DS)	54 (15.6)	57.1 (13.6)	0.210	54.9 (14.7)	54.5 (15.2)	0.316	54.8 (14.8)
Fitzpatrick skin type, *n* (%)			0.138			0.211	
I	16 (2.9)	2 (1.2)		13 (2.3)	5 (3.4)		18 (2.5)
II	415 (76.6)	144 (84.2)		444 (78.6)	115 (77.7)		559 (78.4)
III	108 (19.9)	25 (14.6)		107 (19)	26 (17.57)		133 (18.7)
IV	3 (0.6)	0 (0)		1 (0.1)	2 11.4)		3 (0.4)
Sunburn, *n* (%)			0.315			0.746	
No	290 (53.5)	99 (57.9)		255 (45.1)	69 (46.6)		389 (54.6)
Yes	252 (46.5)	72 (42.1)		310 (54.9)	79 (53.4)		324 (45.4)
Solar lamps, *n* (%)			0.602			0.788	
No	461 (85)	148 (86.5)		483 (85.5)	126 (85.1)		609 (85.4)
Yes, <50	72 (13.3)	18 (10.5)		70 (12.4)	20 (13.5)		90 (12.6)
Yes, ≥50	9 (1.7)	5 (2.9)		12 (2.1)	2 (1.4)		14 (2)
Familiarity for melanoma, *n* (%)			0.230			0.595	
No	480 (88.6)	157 (91.8)		503 (89)	134 (90.5)		637 (89.3)
Yes	62 (11.4)	14 (8.2)		62 (11)	14 (9.5)		76 (10.7)
Familiarity for associated-melanoma malignancies			0.408			0.307	
No	463 (85.4)	149 (87.1)		480 (85)	132 (89.2)		612 (85.8)
Breast	50 (9.2)	13 (7.6)		56 (9.9)	7 (4.7)		63 (8.8)
Pancreas	11 (2)	7 (4.1)		14 (2.5)	4 (2.6)		18 (2.5)
Brain	9 (1.7)	1 (0.6)		9 (1.6)	1 (0.7)		10 (1.5)
Kidney	1 (0.2)	0 (0)		1 (0.2)	0 (0)		1 (0.1)
Breast and pancreas	6 (1.1)	0 (0)		4 (0.6)	2 (1.4)		6 (0.9)
Breast and brain	2 (0.4)	1 (0.6)		1 (0.2)	2 (1.4)		3 (0.4)
Nevi, *n* (%)			0.059			0.293	
<50	485 (89.5)	142 (83)		492 (87.1)	135 (91.2)		627 (87.9)
≥50	57 (10.5)	29 (17)		73 (12.9)	13 (8.8)		86 (12.1)
Sun exposure, *n* (%)			0.602			0.372	
No response	46 (8.5)	16 (9.4)		45 (8)	17 (11.49)		62 (8.7)
Intermittent	454 (83.8)	138 (80.7)		474 (83.9)	118 (79.73)		592 (83)
Continuous	42 (7.7)	17 (9.9)		46 (8.1)	13 (8.78)		59 (8.3)

*Statistically significant.
DS, standard deviation.

Table 2 Assessment of the association between various histological variables and sentinel lymph node status, TILs, and regression

	Regression			Sentinel lymph node biopsy			
Variables	Absent ($n=542$)	Present ($n=171$)	*P*-value	Negative ($n=565$)	Positive ($n=148$)	*P*-value	Total ($N=713$)
Subtype, *n* (%)			0.083			<0.001*	
SSM	291 (53.7)	104 (60.8)		328 (58)	67 (45.3)		395 (55.4)
LMM	7 (1.3)	3 (1.8)		9 (1.6)	1 (0.7)		10 (1.4)
NM	69 (12.7)	13 (7.6)		48 (8.5)	34 (23)		82 (11.5)
ALM	5 (0.9)	2 (1.2)		5 (0.9)	2 (1.4)		7 (1)
Undetermined	136 (25.1)	46 (26.8)		142 (25.1)	40 (27)		182 (25.5)
Other	34 (6.3)	3 (1.8)		33 (5.8)	4 (2.7)		37 (5.2)
Primary site, *n* (%)			0.001*			0.261	
Trunk	111 (20.5)	38 (22.2)		114 (20.2)	35 (23.6)		149 (20.9)
Back	189 (34.9)	81 (47.4)		208 (36.8)	62 (41.9)		270 (37.9)
Upper limbs	84 (15.5)	27 (15.8)		94 (16.6)	17 (11.5)		111 (15.6)
Lower limbs	158 (29.1)	25 (14.6)		149 (26.4)	34 (23)		183 (25.6)
Breslow thickness (mm), mean (DS)	1.8 (1.6)	1.6 (1.7)	0.001*	1.5 (1.3)	2.8 (2.1)	<0.001*	1.8 (1.6)
Breslow thickness, *n* (%)			0.001*			<0.001*	
<0.8 mm	77 (14.2)	36 (21.1)		107 (18.9)	6 (4)		113 (15.8)
0.8–1.0 mm	115 (21.2)	48 (28.1)		153 (27.1)	10 (6.6)		163 (22.9)
1.01–2.0 mm	189 (34.9)	55 (32.2)		189 (33.5)	55 (37.2)		244 (34.2)
≥2.01 mm	161 (29.7)	32 (18.7)		116 (20.5)	77 (52.2)		193 (27.1)
Ulceration, *n* (%)			<0.05*			<0.001*	
No	442 (81.5)	151 (88.3)		502 (88.8)	91 (61.5)		593 (83.2)
Yes	100 (18.5)	20 (11.7)		63 (11.1)	57 (38.5)		120 (16.8)
Mitotic rate/mm^2, *n* (%)			0.791			<0.001*	
No	61 (11.3)	18 (10.5)		67 (11.9)	12 (8.1)		79 (11.1)
Yes	481 (88.7)	153 (89.5)		498 (88.1)	136 (91.9)		634 (88.9)
Vertical growth phase, *n* (%)			0.721			<0.05*	
No	52 (9.6)	18 (10.5)		62 (11)	8 (5.4)		70 (9.8)
Yes	490 (90.4)	153 (89.5)		503 (89)	140 (94.6)		643 (90.2)
Lymphocytic infiltration, *n* (%)			<0.001*			0.256	
No	247 (45.6)	50 (29.2)		227 (40.2)	70 (47.3)		297 (41.6)
Yes, brisk	113 (20.8)	53 (31)		137 (24.2)	29 (19.6)		166 (23.3)
Yes, nonbrisk	182 (33.6)	68 (39.8)		201 (35.6)	49 (33.1)		250 (35.1))
Lymphovascular invasion, *n* (%)			0.494			<0.001*	
No	502 (92.6)	161 (94.2)		545 (96.5)	118 (79.7)		663 (93)
Yes	40 (7.4)	10 (5.8)		20 (3.5)	30 (20.3)		50 (7))
Microsatellites, *n* (%)			0.464			<0.001*	
No	531 (98)	169 (98.8)		564 (99.8)	136 (91.9)		700 (98.2)
Yes	11 (2)	2 (1.2)		1 (0.2)	12 (8.1)		13 (1.8)
Associated nevus, *n* (%)			0.692			0.730	
None	383 (70.7)	116 (67.8)		391 (69.2)	108 (73.1)		499 (70)
Congenital	109 (20.1)	36 (21.1)		117 (20.7)	28 (18.9)		145 (20.3)
Common	38 (7)	13 (7.6)		41 (7.3)	10 (6.6)		51 (7.2)
Dysplastic	12 (2.2)	6 (3.5)		16 (2.8)	2 (1.4)		18 (2.5)
Dimension (cm), mean (DS)	1.1 (0.6)	1.4 (0.8)	0.589	1.1 (0.6)	1.4 (0.9)	0.540	1.2 (0.7)
SNL biopsy, *n* (%)			0.160			NA	
Negative	423 (78)	142 (83)		NA	NA		565 (79.4)
Positive	119 (22)	29 (17)		NA	NA		148 (20.76)
Regression, *n* (%)			NA				
No	NA	NA		423 (74.9)	119 (80.4)		542 (76)
Yes, <75%	NA	NA		115 (20.4)	22 (14.9)		137 (19.2)
Yes, ≥75%	NA	NA		27 (4.8)	7 (4.7)		34 (4.8)

*Statistically significant.

ALM, acral lentiginous melanoma; DS, standard deviation; LMM, lentigo maligna melanoma; NA, not applicable; NM, nodular melanoma; SMM, superficial spreading melanoma; SNL, sentinel lymph node; TIL, tumor-infiltrating lymphocyte.

of whom were males (50.4%), whereas the presence of regression was found in 171 cases (23.9%), more prevalent in men than women (103 vs 68) with an average age of 57.1 ± 13.6 years old.

The presence of regression was significantly associated with the male sex, location on the trunk, the upper limbs and the back, presence of ulceration, a lower Breslow thickness, and the presence of lymphocytic infiltration (brisk and nonbrisk). There were no significant differences between regression and the remaining clinical characteristics (age at diagnosis, sun exposure, solar lamps use, episodes of sunburns during childhood/adolescence, Fitzpatrick skin type, number of nevi, family history of melanoma diagnosis and family history for diagnosis of associated-melanoma malignancies) and pathological features (mitotic rate, vertical growth phase, lymphovascular invasion, microsatellites, and associated nevus).

Positive sentinel node biopsy was detected in 148 patients (20.7%), 93 of whom were males (62.8%), with an average age of 54.5 ± 15.2 years old. Considering demographics, positive sentinel node were found to be associated just with the male sex; conversely, the results

shown in Table 1 demonstrated a significant association with higher Breslow thickness, nodular and superficial spreading melanoma, mitotic rate, ulceration, vertical growth phase, microsatellites, and lymphovascular invasion. In contrast, no differences were identified regarding age at diagnosis, sun exposure, solar lamps use, episodes of sunburns during childhood/adolescence, Fitzpatrick skin type, number of nevi, family history of melanoma diagnosis and family history for associated-melanoma malignancies, associated nevus, and lymphocytic infiltration.

Prognostic significance of regression in melanoma: association with lymph node metastases

Table 3 shows the distribution of included patients stratified by regression and sentinel node status, together for stages according to 8th ed. AJCC Staging Manual: a Chi-square test of independence demonstrated that there was no significant association between the presence of regression and the result of sentinel node biopsy for every considered T-stage. We extended the analysis quantifying the presence of regression in absent or less than or more than 75% without the T-stage, but the results did not find any correlation with sentinel node status.

Table 3 Correlation between sentinel lymph node status and extensive or partial regression according to T-stage

	SNL status, *n*		
T-stage I	Negative	Positive	*P*-value
Regression, *n*			0.936
Absent	73	4	
Present	34	2	
T-stage II			
Regression, *n*			0.616
Absent	108	7	
Present	45	3	
T-stage III			
Regression, *n*			0.642
Absent	146	43	
Present	43	12	
T-stage IV			
Regression, *n*			0.704
Absent	96	65	
Present	20	12	
All T-stages			
Regression, *n*			0.315
Absent	423	119	
Present, <75%	115	22	
Present, ≥75%	27	7	

SNL, sentinel lymph node.

Five-years survival by regression status as an outcome predictor

The analysis was conducted with a five-year follow-up period after the diagnosis, evaluating the outcome measures in terms of MSS and RFS. Among the 713 patients, 70 (9.8%) experienced a melanoma recurrence, and out of those, 33 died due to melanoma-related complications. When considering MSS and RFS stratified for regression (absent, present) or TILs (absent, present), no statistically significant difference was found (Table 4). Based on the results, logistic regression and Kaplan–Meier curves stratified by the presence and type of immune response were not performed in the analysis.

Discussion

Histologic regression is characterized by the disappearance or reduction of neoplastic cells within the tumor, replaced by fibrosis, melanophages, neovascularization, and inflammatory infiltrate. TILs are lymphocytes, that permeate the tumor microenvironment in various types of cancers, including melanoma. These lymphocytes are part of the body's immune response and play a crucial role in recognizing and attacking cancer cells.

These two pathologic aspects are connected in melanoma, as TILs are believed to act as effector cells, leading to the subsequent regression process of the melanocytic lesion [6]. The prognostic significance of these two histological factors in melanoma, however, remains controversial.

Regarding histologic regression, some studies have suggested that regression may be associated with a favorable prognosis and better survival outcomes [7–14].

These data are supported by the idea that regression represents an immune response against the tumor, indicating a more robust antitumor immune reaction. In such cases, patients with melanomas exhibiting significant

Table 4 Interaction between TILs and regression with melanoma-specific survival and relapse-free survival

	5-year death			5-year recurrence		
Variables	No	Yes	*P*-value	No	Yes	*P*-value
Regression, *n* (%)			0.232			0.256
Absent	514 (94.8)	28 (5.2)		485 (89.5)	57 (10.5)	
Present	166 (97.1)	5 (2.9)		158 (92.4)	13 (7.6)	
TILS, *n* (%)			0.241			0.234
Absent	280 (94.3)	17 (5.7)		259 (87.2)	38 (12.8)	
Present	400 (96.2)	16 (3.8)		384 (92.3)	32 (7.7)	

TIL, tumor-infiltrating lymphocyte.

regression may experience a lower risk of recurrence and improved survival rates. Subramanian *et al*. report on a registry of 4790 patients that regression correlates with a lower risk of lymph node metastasis and improved MSS [7].

In a study of El Sharouni *et al*., regression was significantly correlated with improved overall survival (OS) and RFS in two large independent cohorts of stage I and II melanoma patients from the Australian and Dutch populations [8]. Furthermore, a systematic review by Ribero *et al*. appears to confirm the protective role of regression on the SLN positive status [9].

Despite some of these studies coming from large patient cohorts, however, the heterogeneity of data among these works is evident. In addition, there is not a clear stratification of the extent of regression in the studies. Lastly, in certain instances, the stratification of results presents conflicting evidence, as exemplified by the favorable impact of regression. This phenomenon is apparent primarily in thick melanomas, according to certain studies, while in others, it's confined to superficial or intermediate stages [7,8].

Other works, however, suggest that regression may not always serve as a reliable indicator of a better prognosis. In some cases, regression has been associated with worse outcomes [15–19]. The presence of regression within the primary tumor might obscure the true extent of tumor invasion, leading to an underestimation of the Breslow thickness and potential metastatic risk. Consequently, the true depth of tumor infiltration into the skin may not be accurately assessed, leading to misclassification of melanomas and potentially affecting their prognostic evaluation.

Whether a protective role is demonstrated or not, the variability in reported prognostic significance of regression in the literature can be attributed, at least in part, to inconsistent histological criteria used for evaluating and reporting regression. The definition and assessment of regression in melanoma can vary among studies, making it difficult to generalize the obtained data. On the contrary, our study aligns with a body of research that fails to show any significant impact of regression. We have, in fact, established that histological regression in primary cutaneous melanoma is linked to distinct clinical-pathological features (male sex, melanoma location on the trunk, upper limbs, or back, presence of ulceration, lower Breslow thickness, and the presence of brisk/nonbrisk TILs). Regression or its extension, however, could not serve as a reliable predictor of SLN status, whether stratifying melanoma into AJCC stages or not. The same results are evident for impact on the role of regression on clinical outcomes, particularly in predicting better survival or longer relapse-free time at the five-year landmark time points.

The same results were also achieved by Botella-Estrada *et al*., who retrospectively demonstrated no influence on the SLN status in a sample of patients with melanoma >0.75 mm [20].

Some data are also available for melanomas <1 mm, where regression is more frequent. In this case, some authors also emphasize the lack of a clear correlation with the SLN status [21]. It's important, however, to signal that some recent studies in the literature have reported a higher risk of false negatives in SLN analysis in these patients [22]. Conversely, in our literature search, we did not find any other articles that do not show impact of regression on OS or other clinical outcomes.

Similarly, researchers have explored the presence of TILs within the tumor microenvironment as a potential prognostic factor in melanoma. Some studies have shown that a higher density of TILs is associated with improved outcomes, suggesting that an increased immune response may contribute to better disease control [23–26].

On the contrary, the presence of TILs may not always guarantee a better prognosis, as their ability to halt tumor growth is not consistently observed in all cases [27]. In some instances, despite the presence of TILs, tumors may still progress and metastasize, indicating that the immune response is not sufficient to completely eliminate the cancer cells. This phenomenon might be attributed to immune escape mechanisms employed by the tumor, enabling it to evade immune surveillance and continue growing. Moreover, the effectiveness of TILs in controlling tumor growth can vary depending on the type and density of lymphocytes infiltrating the tumor. A diverse and robust TIL population might be more effective in curbing tumor progression, while a less diverse or dysfunctional immune response may not yield the same beneficial outcome. Our research did not identify a clear correlation between the presence of TILs and either improved or worsened patient outcomes. This neutral effect suggests that the immune response mediated by TILs did not distinctly influence the progression or regression of melanoma in the studied patient population.

Our study, however, has some limitations. The first one is related to the retrospective and single-center nature of the work. Additionally, this study aimed to thoroughly analyze data for melanomas below 0.75 mm, but there are few reports concerning these patients, making the final data not easily generalizable. Moreover, in our experience, only cases with a prior diagnosis of regression were reexamined, so it's not possible to rule out the presence of false negatives in the sample of patients who did not show regressions. Lastly, it is noteworthy that fewer than 1% of the slides were deemed unsuitable for revision.

Conversely, one of the key strengths of this study lies in the meticulous reassessment of the presence and extent

of regression, thorough review of histological sections of each melanoma specimen, adhering to the most up-to-date standardized guidelines. To ensure consistency and accuracy, all evaluations were performed by the expert pathologist, minimizing interobserver variability. In case of discrepancies, another pathologist conducted an evaluation to resolve any doubts. By adopting these standardized and contemporary protocols, we ensured a consistent and reliable evaluation of regression and TILS.

In conclusion, in our case series, this study demonstrates no correlation between regression, whether partial or complete, ±TILS, with lymph node sentinel status or other clinical outcomes. Given the limited size of our study and the heterogeneity of data from other studies in the literature, further research with a larger cohort could potentially provide more robust and generalizable results, clarifying definitively the role of both regression and TILs in the pathogenesis and progression of melanoma.

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The patients in this manuscript have given written informed consent to publication of their case details.

Conflicts of interest

There are no conflicts of interest.

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