Journal of
Clinical & Medical Surgery

www.jclinmedsurgery.com

ISSN 2833-5465
Open Access
Volume 4

Research Article

Absence of Tils in Biopsy is Associated with Worse Progression Free Survival in Early Stage Melanoma

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Article Information

Received: Jan 03, 2024
Accepted: Jan 23, 2024
Published: Jan 30, 2024
Archived: www.jclinmedsurgery.com
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Abstract

Background: Recent data suggest that a subset of early stage melanoma patients may benefit from adjuvant immunotherapy.

Objective: To identify findings in biopsy or surgical pathology reports associated with poor prognosis in patients diagnosed with stage I and II cutaneous melanoma.

Methods: This was a retrospective cohort study of 68 stage I and II cutaneous melanoma patients treated at our institution from 2010 to 2022. Inclusion criteria were patients with stage I or II melanoma with both a biopsy and surgical pathology report. The primary outcome of the study was the prognostic value of biopsy findings on progression free survival (PFS). Kaplan-Meier and Cox-proportional hazards models were used to evaluate risk factors for progression.

Results: Patients with ulceration on biopsy had a 16.1x greater risk of progression than those without ulceration [HR 16.1, 95% CI: 2.60-99.5]. Patients with absent lymphocytes on biopsy had a 9.5x greater risk of progressing compared to those with lymphocytes present [HR 9.45, 95% CI: 1.99-44.8]. Ulceration or absent TILs were associated with significantly worse PFS.

Conclusions: Ulceration and absence of TILs on biopsy are associated with increased risk of progression in stage I and II melanoma patients.

Introduction

Melanoma is a highly aggressive form of skin cancer characterized by a propensity for early metastasis, resulting in the highest mortality rate among skin cancers (2.7 deaths per 100,000) [1,2]. With its incidence reaching 22.0 per 100,000 in 2018 and an average increase of 1.2% per year since 2009, reducing mortality in patients with melanoma has become a top priority [2,3]. The advent of immune checkpoint inhibitors has transformed adjuvant therapy for patients with metastatic melanoma. PD-1 inhibitor therapy with nivolumab or pembrolizumab has become the standard adjuvant treatment for patients with resected stage III and stage IV melanoma [4,5]. However, the decision to initiate adjuvant care for patients with low-stage (I-II) melanoma is more complex. Despite lower staging, stage IIA-IIC melanomas still have a 12-25% 10-year mortality rate [6]. Standard of care for these patients consists of a wide local

excision with margins according to lesion Breslow depth, with sentinel lymph node biopsy being offered to all medically suitable candidates with intermediate thickness melanomas or low thickness melanomas with ulceration and/or high mitotic figures [7]. Some patients are then considered candidates for adjuvant therapy to prevent recurrence. Recently the Keynote 716 trial demonstrated that in resected stage IIB or IIC melanoma, pembrolizumab treatment decreased rates of recurrence from 24% to 15% compared to placebo [8]. However, immune checkpoint inhibitors carry the risk of serious side effects including pneumonitis, pancreatitis, myelitis, colitis, thyroiditis, and severe skin reactions [8,9]. In the same trial, 16% of patients receiving pembrolizumab had a Grade [3] or greater adverse reaction, compared to 4% in the placebo arm [8]. Additionally, most patients on placebo did not see a recurrence in their melanoma without treatment, indicating if the entire IIB-IIC population were to be treated in the adjuvant setting we would be overtreating roughly 70% of patients. Given the risk of serious adverse events and the relatively small reduction in recurrence risk, it remains unclear which stage II melanoma patients should receive adjuvant anti-PD1 therapy. Identifying a subpopulation of early stage melanoma patients at higher risk for progression could spur future trials testing immunotherapy or other interventions specifically within this group. Several histopathological prognostic predictors for low-stage melanoma are currently utilized including lesion ulceration, thickness, mitotic rate, neutrophil count, microsatellites, and tumor-infiltrating lymphocyte (TIL) status [10,13]. Ulceration is an independent risk factor for decreased overall survival and is included in staging [10]. Non-histopathological prognostic factors may also be used to inform decisions to start adjuvant therapy. Molecular analyses, like the Decision Dx-Melanoma assay, have been increasingly utilized; however, their prognostic ability varies by stage and is not reliable in predicting recurrence for low-stage melanoma patients [14]**.** This test additionally is limited by lack of ability to monitor for recurrence longitudinally and may incur significant costs for the patient, highlighting the need for alternative predictors to stratify low-stage patients. Circulating tumor-DNA is another tool being investigated to assess prognosis in metastatic melanoma, but its use in deciding initial treatment remains limited [15]. In this retrospective chart review, we analyzed patients with stage I-II melanoma at our institution between 2010-2022 to determine independent variables associated with progression-free survival within readily available pathology reports. We hypothesized that absent TIL status would be associated with worse progression-free survival since the adaptive immune response is critical for anti-tumor response in melanoma [9,16]. Evidence already exists that TIL may be a useful tool for assessing risk in thicker tumors, but there are ambiguous results when thin and radial growth melanomas are included [17]. Herein, we evaluate the prognostic value of commonly reported histopathologic findings in biopsy and surgical pathology specimens for stage I-II melanoma patients to identify higher-risk patients that may benefit from closer follow-up or early intervention.

Methods

Study design: This was a retrospective cohort study aimed to determine the prognostic value of biopsy findings for progression free survival in early stage cutaneous melanoma. Patients with an initial stage I and II melanoma diagnosis between 2010-2022, defined using the American Joint Committee on Cancer's clinical prognostic grouping criteria, with available biopsy and surgical pathology reports were included in the study. Patients before 2010 were excluded because our institution implemented the electronic health record in 2010, so records prior to 2010 were either not available or were formatted significantly differently. Exclusion criteria included melanoma in situ, stage III or IV melanoma, and non-cutaneous melanoma.

Patient population: The medical record number of patients were initially identified using the following billing codes: ICD-9 172, ICD-10 C43, and CPT 11600-11606, 11620-11624, or 11640-11646 (Table 1). Our institution's Integrated Data Repository identified 5291 patients meeting these criteria and provided medical record numbers. Each patient's chart was reviewed by study members to determine if the patient had at least one confirmed diagnosis of melanoma, regardless of stage, along with both a biopsy pathology report and a surgical pathology report. Patients whose medical records were incomplete or did not have both pathologic reports were excluded. Of the initial 5291 patients, 236 patients had both biopsy and surgical pathology reports available. After excluding patients with a diagnosis prior to 2010, 227 patients remained. This group was further stratified by stage, including only Stages I (IA and IB) and 2 (II2A, IIB, and IIC) according to American Joint Committee on Cancer's 8th edition on clinical prognostic grouping for melanoma, resulting in 69 patients [18,19]. Additionally, 1 patient did not have recurrence information or death status available, so this patient was excluded yielding a final study population of 68. All patients in this cohort were managed with wide local excision of their tumor. Patients were only treated with systemic therapy if they progressed and systemic therapy became indicated.

Variables collected: Patient demographics, biopsy pathologic findings, surgical pathologic features, and other clinical characteristics were collected from each record. This data was recorded and stored on the our institution's REDCap, a secure web platform for building and managing online databases. Tumor infiltrating lymphocyte (TIL) status at biopsy was reported as preset-brisk, present-non brisk, and absent. We analyzed this variable considering all 3 categories and also as present (both brisk and non-brisk) vs absent where indicated in the Results section. Mitotic rate was recorded as >1 or <1. The primary outcome was progression-free survival (PFS). Progression was defined as either melanoma recurrence or death by any cause. Patients were censored after their last documented encounter, including either phone call or office visit with any provider, in the medical record.

Statistical analysis: Descriptive statistics such as mean and standard deviation (SD) and range, were reported for continuous variables, and frequency (%) for categorical variables. Two group comparisons were conducted using Wilcoxon rank sum exact tests for continuous data and Fisher's exact tests for categorical data. A Firth's logistic regression model was used to determine biopsy variables associated with residual melanoma at the time of surgical excision. We employed a backward selection approach to create a parsimonious model and applied Firth's logistic regression to mitigate bias in estimates, given the limited sample size. Kaplan-Meier progression-free survival curves and log-rank tests were used to evaluate ulceration and presence of tumor-infiltrating lymphocyte (TIL) status at biopsy.

Multivariable Cox proportional hazards model in combination with backward elimination method on preselected variables, was used to determine variables associated with progression-free survival.

Results

Patient characteristics: Characteristics and descriptive statistics of the final study population are shown in Table 2. Overall there were 13 (19.1%) patients that progressed and 55 (80.9%) that did not. The average age of patients at diagnosis was 64 years with a standard deviation of 14.1 years. There was a trend towards increased age in the progression group but this did not reach statistical significance (68.2 vs 62.6, p=0.305). There were 44 (64.7%) males and 24 (35.3%) females in the study, and these proportions were consistent between the group that progressed and did not (p=0.355). The overall distribution of TIL status was 10 (16.1%) absent, 7 (11.3%) present-brisk, and 45 (72.6%) present-non brisk. TIL status did not significantly differ between the 2 groups (p=0.132). There was no significant difference in the percentage of involvement of deep margins (83.3% vs 64.8%, p=0.311) and peripheral margins on biopsy (63.6% vs 48.1%, p=0.509) between patients that did and did not progress. Of note, all patients that progressed did not have tumor regression identified on biopsy pathology (100% vs 81.6%, p=0.332) and a mitotic rate >1 (100% vs 72%, p=0.052) but these did not meet the threshold of statistical significance. Mean depth of tumor on biopsy was significantly increased in the progression group (4.2 vs 2.2, p=0.008). Tumor ulceration was significantly increased in the progression group (83.3% vs 35.2%, p=0.003). Only 2 patients with type of biopsy reported had a punch biopsy, so the effect of biopsy type could not be evaluated (p>0.999).

Biopsy findings associated with residual melanoma on wide local excision: We first sought to identify variables of interest from the biopsy pathology report associated with whether there would be residual melanoma at the time of wide local excision. Of the 67 patients whose surgical pathology report identified presence or absence of residual melanoma, 31 (46.3%) did not have residual melanoma and 36 (53.7%) did. The 10 variables listed in Table 3 were used as predictors in a univariable Firth logistic regression model. Of these, only involvement of the deep margins at biopsy was a significant predictor of residual melanoma at the time of surgery (Odds ratio 4.12, p=0.009). This finding coincided with the results in a backward elimination multivariable Firth logistic model.

Ulceration is associated with worse progression free survival: There is extensive literature demonstrating that ulceration carries a poor prognosis in cutaneous melanoma. We generated a Kaplan-Meier progression free survival (PFS) curve stratified by ulceration to verify that our study population recapitulates this finding. As expected, our results indicated that the presence of ulceration at biopsy was associated with significantly worse PFS (p=0.002) than the lack of ulceration (Figure 1). The 5-year PFS for non-ulcerated patients was 72.7% while only 47.9% for ulcerated patients.

Absent tumor infiltrating lymphocytes is associated with worse progression free survival: Next, we sought to investigate the prognostic value of TIL status on PFS in stage I and II melanoma patients. We initially generated a Kaplan-Meier PFS curve stratifying patients by TILs present (both brisk and non-brisk) and TILs absent (Figure 2). Absence of TILs was associated with significantly worse PFS (p=0.044). 5-year PFS was 78.2% for the TILs present group and only 33.3% for the TILs absent group. It should be noted that only 10 patients in our study had absent TILs.

We then performed a similar analysis but stratified TIL status by present-brisk, present-non brisk, and absent (supplementary information). Because the majority of patients had present-non brisk TILs (n=45, 72.6%), we could not identify a significant difference between these groups. Univariable Cox-proportional hazard models were generated for the biopsy variables listed in Table 4. Tumor depth and ulceration were the only statistically significant predictors with hazard ratios of 1.12 (95% CI [1.00-1.25], p=0.050) and 7.43 (95% CI [1.62-34.0], p=0.010) respectively. While absence of lymphocytes had a trend towards increased hazard ratio (3.44 95% CI [0.98-12.1]) it did not meet the threshold for statistical significance in a univariable model (p=0.054). To confirm that presence/absence of TILs was an independent prognostic factor for PFS, we generated a multivariable Cox-proportional hazards model using ulceration, tumor thickness, and absence of TILs as predictors (Table 4). Ulceration and absence of TILs were the only variables associated with significantly worse PFS. In this model, the hazard ratio for ulceration was 16.1 (95% CI [2.60-99.5], p=0.003). For absence of TILs the hazard ratio was 9.45 (95% CI [1.99-44.8], p=0.005). This indicates that patients with an ulcerated lesion at biopsy had a 16.1x greater risk of disease progression. Patients with absent TILs at biopsy had a 9.5x greater risk of disease progression compared to those with TILs present.

Table 1: Table depicting patient selection process.

N	Selection criteria
5,291	UF Integrated Data Repository filtered with ICD-9 172, ICD-10 C43, CPT 11600-11606, 11620-11624, or 11640-11646
236	Corresponding dermatology pathology and surgical pathology reports available
227	Year of diagnosis after 2010
69	Patient diagnosed with Stage I or II malignant cutaneous melanoma
68	Recurrence information or date of death available

Abbreviations: ICD: International classification of diseases; CPT: Current procedural terminology.

Table 2: Table depicting patient selection process.

Characteristic		Overall (n=68)	Progression (n=13)	No Progression (n=55)	p-value
Mean Age at Diagnosis (SD)		63.7 (14.1)	68.2 (14.1)	62.6 (14.0)	0.305
Mean Depth of Tumor, mm (SD)		2.5 (3.1)	4.2 (4.0)	2.2 (2.8)	**0.008**
Sex	Female	24	3 (23.1%)	21 (38.2%)	0.355
	Male	44	10 (76.9%)	34 (61.8%)	
Ulceration	No	37	2 (16.7%)	35 (64.8%)	**0.003**
	Yes	29	10 (83.3%)	19 (35.2%)	
Lymphocyte status	Present-non brisk	45	6 (54.5%)	39 (76.5%)	0.126
	Present-brisk	7	1 (9.1%)	6 (11.8%)	
	Absent	10	4 (36.4%)	6 (11.8%)	
Mitotic Rate	<1	14	0 (0.0%)	14 (28.0%)	0.052
	>1	48	12 (100.0%)	36 (72.0%)	
Tumor Regression	No	48	8 (100.0%)	40 (81.6%)	0.332
	Yes	9	0 (0.0%)	9 (18.4%)	
Deep Margins Involved	No	21	2 (16.7%)	19 (35.2%)	0.311
	Yes	45	10 (83.3%)	35 (64.8%)	
Peripheral Margins Involved	No	31	4 (36.4%)	27 (51.9%)	0.509
	Yes	32	7 (63.6%)	25 (48.1%)	
Type of Biopsy	Punch	2	0 (0.0%)	2 (3.9%)	>0.999
	Shave	62	13 (100.0%)	49 (96.1%)	

Abbreviations: ICD: International classification of diseases; CPT: Current procedural terminology.

Table 3: Variable associated with residual melanoma at surgical excision using a univariable Firth logistic model and a selected multivariable Firth logistic model. Involvement of deep margins was the only statistically significant predictor of residual melanoma at the time of surgery in the univariable model, and it was identified using backward elimination method.

Univariable logistic model					
Characteristic		**N**	**Odds Ratio**	**95% CI**	**p-value**
Age at Diagnosis		67	1.00	0.97, 1.03	0.932
Depth of Tumor (mm)		66	1.05	0.91, 1.28	0.494
Sex	Female	23	-	-	-
	Male	44	1.84	0.68, 5.11	0.230
Ulceration	No	37	-	-	-
	Yes	28	0.85	0.32, 2.25	0.749
Lymphocyte status	Present	51	-	-	-
	Absent	10	0.89	0.24, 3.36	0.862
Mitotic Rate	<1	14	-	-	-
	>1	47	0.87	0.26,2.78	0.810
Tumor Regression	No	47	-	-	-
	Yes	9	1.08	0.27,4.47	0.914
Deep Margins Involved	No	21	-	-	
	Yes	44	4.12	1.42, 13.1	0.009
Peripheral Margins Involved	No	31	-	-	-
	Yes	31	2.14	0.80, 5.92	0.132
Type of Biopsy	Punch	2		-	-
	Shave	61	0,24	0.00, 3.05	0.293
Multivariable logistic model					
Characteristic		**N**	**Odds Ratio**	**95% CI**	**p-value**
Deep Margins Involved	No	21	-	-	
	Yes	44	4.12	1.42, 13.1	0.009

P-values < 0.05 were considered statistically significant and are bolded.

Table 4: Hazards models. Some patients did not have all variables reported in their biopsy. The multivariable Cox-proportional hazard model identified lymphocyte status and ulceration as significantly associated with PFS while tumor depth was not statistically significant in this model.

Univariable Cox-proportional hazards models for progression-free survival					
Characteristic		**N**	**Hazard Ratio**	**95% CI**	**p-value**
Age at Diagnosis		68	1.02	0.98, 1.07	0.259
Depth of Tumor, mm		67	1.12	1.00, 1.25	0.050
Sex	Female	24	-	-	
	Male	44	1.47	0.40,5.34	0.560
Ulceration	No	37	-	-	
	Yes	29	7.43	1.62,34.0	0.010
Lymphocyte status	Present	52	-	-	
	Absent	10	3.44	0.98, 12.1	0.054
Deep Margins Involved	No	21	-	-	
	Yes	45	2.36	0.52,10.8	0.268
Peripheral Margins Involved	No	31	-	-	
	Yes	32	1.14	0.32, 4.08	0.836
Multivariable Cox-proportional hazards models for progression-free survival					
Characteristic		**N**	**Hazard Ratio**	**95% CI**	**p-value**
Depth of Tumor, mm		62	1.06	0.93, 1.21	0.368
Ulceration	No	36	-	-	
	Yes	26	16.1	2.60, 99.5	0.003
Lymphocyte status	Present	52	-	-	
	Absent	10	9.45	1.99, 44.8	0.005

P-values < 0.05 were considered statistically significant and are bolded.

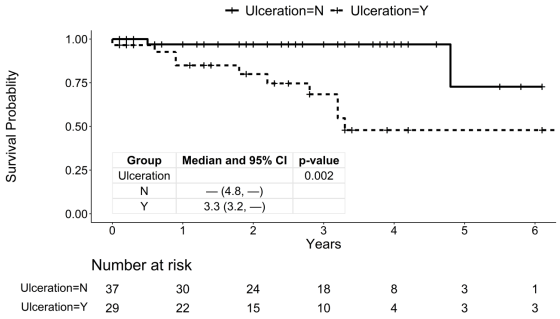


Figure 1: Kaplan-Meier plot of progression free survival stratified by presence (n=29) or absence (n=37) of ulceration on biopsy. Log-rank test identified a statistically significant decrease in survival for patients with ulceration on biopsy (p=0.002).

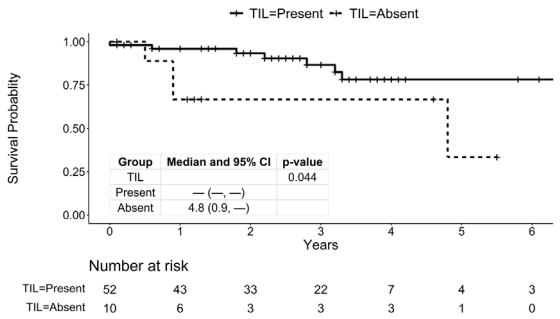
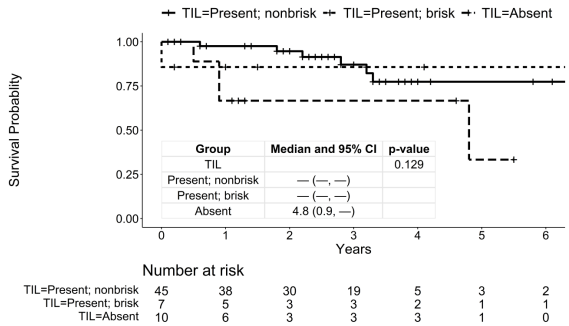


Figure 2: Kaplan-Meier plot of progression free survival stratified by presence (n=52) or absence (n=10) of tumor-infiltrating lymphocytes. Log-rank test identified a statistically significant decrease in survival for patients with absent TILs on biopsy (p=0.044).



Sup Figure 1: Kaplan-Meier plot of progression free survival stratified by present-brisk (n=7), present-non risk (n=45), or absent (n=10) tumor-infiltrating lymphocytes at biopsy. Log-rank did not identify statistically significant differences between the groups (p=0.129).

Discussion

In this single institution, retrospective study we identified an association of involvement of deep margins at biopsy with residual melanoma at the time of wide local excision. Ulceration and absence of TILs at biopsy for stage I and II melanoma patients were independently associated with increased risk for disease progression. Notably, the hazard ratio for absence of TILs was [9] using the best Cox-proportional hazards model, suggesting that patients with absent TILs have a 9x increased risk of progression relative to patients with present TILs. That is over half the hazard ratio for ulceration, which is a well-established risk factor incorporated into staging, found by the same model. While the number of absent TIL patients in our study was small, the magnitude of the effect suggests it warrants further study.

The recent Keynote-716 study suggests that some lower stage melanoma patients could benefit from early adjuvant immunotherapy [8]. The identification of absent TILs could be used as a readily available indicator of high-risk disease in stage I-II patients who may be more likely to benefit from early intervention. The caveat is that commonly used immunotherapies rely on lymphocytic infiltration for clinical efficacy [20,22]. Thus, patients with absent TILs may also be resistant to immune checkpoint inhibitors, which in part could explain their worse overall prognosis. An alternative approach is to consider absent TILs indicative of a "cold" melanoma. Though melanoma is conventionally considered a "hot" tumor, patients with absent TILs may benefit from strategies that convert immunologically "cold" tumors to "hot" [23]. Incorporating agents that boost immune infiltration into the tumor are likely essential for these patients to benefit from immunotherapy. Zakharia et al combined indoximod, an indoleamine 2,3-dioxygenase inhibitor (IDO) inhibitor, with pembrolizumab in patients with advanced melanoma, resulting in an objective response rate of 51% [24]. IDO depletes tryptophan from the extracellular environment, and increased expression of IDO has been shown to decrease T cell infiltration in colorectal cancer [25]. The TLR-7/8 agonist imiquimod has been demonstrated to increase T cell infiltration and activation for squamous cell carcinoma of the skin [26]. Imiquimod has also been used for melanoma-in-situ with positive margins with an estimated 95% resolution of residual melanoma-in-situ [27]. Case series suggest that imiquimod stimulates T cell infiltration in the context of melanoma-in-situ [28], raising the possibility that imiquimod could improve the outcomes of patients with absent TILs on biopsy. Imiquimod is well tolerated with few side effects and warrants consideration as neoadjuvant treatment for early stage melanomas [29]. Treatment with agents like imiquimod to enhance T cell tumor infiltration, either as monotherapy or in combination with immunotherapy, may improve outcomes in stage I and II patients with absent TILs. Our study k8 has several limitations. A major limitation is the small sample size (68 patients), which is further compounded by missing data from some biopsy reports (Table 2). Furthermore, the relatively small sample size prevented us from evaluating associations between lymphocyte status and response to immunotherapy in patients that recurred. Despite this limitation, our results suggest absent lymphocytes and ulceration identify early stage melanoma patients at higher risk for progression and should be confirmed in a larger study. To do this, we are expanding the scope of this retrospective study to include all evaluable patients within a clinical research database available at our institution. This larger, multi-institutional study could elucidate whether absent lymphocyte status predicts tumor resistance to immune checkpoint inhibitors in early stage patients. Like all retrospective studies, our analysis identifies associations, but we cannot infer causal relationships. Additionally, this study only analyzes patients seen at the an academic tertiary care center. Cheraghlou et al. demonstrated that treatment for early stage melanoma at hospitals with an academic affiliation or high-volume is associated improved survival compared to those treated at non-academic or low-volume centers [30]. A prospective study treating patients with imiquimod after wide local excision would determine 1) if absent TIL status can be reversed pharmacologically and 2) whether recruitment of TILs improves progression free survival in early stage melanoma patients.

Declarations

Author contributions: KM contributed to conceptualization, study design, data collection, data interpretation, manuscript writing and review. TE, VA, and EM contributed to data collection, data interpretation and manuscript writing. ZG and JHL performed statistical analyses and manuscript review. BD contributed to conceptualization, study design, data interpretation, manuscript writing, and manuscript review. All authors approved the final version of the manuscript for submission.

Data availability: The de-identified datasets generated during and/or analysed during the current study are available from the corresponding author (BD) on reasonable request.

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