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Prognostic Value of Tumor-Infiltrating Lymphocytes in Sinonasal Mucosal Melanoma

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Objectives/Hypothesis: Tumor-infiltrating lymphocytes (TILs) predict better outcome in several types of cancers. However, the prognostic value of TILs in sinonasal mucosal melanoma (SNMM) is uncertain. Here, we investigated whether TILs can be used as a prognostic indicator for survival in SNMM.

Study Design: Retrospective cohort study.

Methods: Patient history and histologic specimens from 27 patients with primary SNMM were retrospectively analyzed. TIL grade was determined and associations between TILs and AJCC tumor stage, overall survival, and recurrence-free survival were analyzed.

Results: Patients with TILs in the primary tumor classified as brisk or non-brisk survived significantly longer than patients with SNMMs lacking lymphocyte infiltrates. Brisk TILs were associated with the lower T3 stage and increased recurrence-free and 5-year survival.

Conclusion: Our results indicate that TIL density is a strong prognostic factor for better survival in SNMM. Prospective studies with larger case numbers are warranted to determine whether TILs should be included in future AJCC staging guidelines.

Key Words: Tumor-infiltrating lymphocytes, melanoma, sinonasal, mucosa, prognosis.

Level of Evidence: 3

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INTRODUCTION

Tumor-infiltrating lymphocytes (TILs) reflect the local antitumor immune response. The degree of lymphocyte infiltration is used as a histopathological prognostic marker in various tumor entities.[1,2] Two recent meta-analyses published in 2019 and 2020 confirmed the prognostic significance of TILs in cutaneous malignant melanoma (CM) and suggested to routinely include the TIL grade in the histopathologic assessment.[3,4]

In a rare but aggressive type of malignant melanoma, the sinonasal mucosal melanoma (SNMM), the significance of the TIL grade as a prognostic factor has not yet been investigated. SNMM differs from primary CM in its genetic, epidemiologic, and pathophysiological characteristics.[5–7] A frequently delayed diagnosis, the aggressive local and often multilocular growth, and a high risk for lymphogenic and hematogenic metastasis contribute to the markedly poor prognosis. Reported 5-year survival rates range between 0% and 45%.[8–10]

Due to the rarity of the disease, small case series and case reports predominate in the literature. To date, little is known about the risk factors, pathogenesis, and prognostic factors of SNMM.[11] There is also a lack of histopathologic assessment standards and established, evidence-based therapeutic guidelines.[11–14] The selection of the appropriate therapeutic and follow-up strategy is a major challenge, especially in view of the usually fatal course of the disease. The identification of reliable prognostic factors could significantly facilitate individual treatment planning. In our study, we investigated whether the TIL grade could serve as a prognostic indicator for recurrence-free and overall survival in patients diagnosed with SNMM.

MATERIALS AND METHODS

The retrospective analysis of anonymized patient records was approved by the medical ethics committee of Ludwig Maximilian University (LMU) and the department's data protection official. The study was conducted in accordance with the Declaration of Helsinki.

Patients

The study group consisted of 27 patients who underwent surgery for sinonasal melanoma at the Department of Otorhinolaryngology, Head and Neck Surgery (LMU Munich) between 2002 and 2015. Staging was performed according to the

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TNM/AJCC classification for upper aerodigestive tract mucosal melanomas and retrospectively adjusted for cases prior to 2016.[15] Surgery was performed with curative intent. Patients with extensive malignant infiltration of vital structures as the brain, the skull base, carotid artery, or prevertebral space were excluded. Other exclusion criteria included malignant melanomas at other sites, distant metastases at the time of initial diagnosis, and a history of chemotherapy or immunotherapy due to other malignant diseases.

All patients received adjuvant radiotherapy at varying doses after surgery. Chemotherapy or immunotherapy was administered inconsistently during the further course of the disease.

Tumor-Infiltrating Lymphocyte Scoring

TILs were scored according to the grading system described by Clark et al.,[16] which is routinely used to classify lymphocyte infiltration in malignant melanoma of the skin. Accordingly, TILs were defined as lymphocytes that were in direct contact with melanoma cells and located intratumorally. Noninfiltrating or peritumoral lymphocytes were not counted as TILs. Based on their number and intratumoral distribution, three TIL grades were defined: If no tumor-infiltrating lymphocytes were present, TILs were classified as "absent." A "brisk" TIL grade corresponds to lymphocytes infiltrating the whole tumor base or diffusely infiltrating the entire tumor substance. All other intratumoral distribution patterns of TILs (multifocal, segmental, isolated) were defined as "non-brisk." Perivascular lymphocytes were not considered as TILs.[17] Analysis and classification of TILs were performed on hematoxylin and eosin (H&E)-stained sections of formalin-fixed paraffin-embedded (FFPE) tissue (Fig. 1).

Staging, resection status after primary surgery, occurrence of lymph node or distant metastases, recurrence-free survival time, and overall survival time were documented and analyzed for association with TIL density.

Statistical Analyses

Statistical analyses were performed with SigmaPlot 12.5 software (Systat Software Inc., San Jose, CA). Overall survival was defined as the time between primary surgery and death. Patients who were alive at the end of follow-up were censored. Recurrence-free survival refers to the time between primary surgery and relapse. Patients were censored for recurrence-free survival if metastasis was absent at the end of follow-up or at the time of death. Survival curves were constructed using the Kaplan-Meier method and compared by the log-rank test. Associations between TIL grade and demographic, clinicopathological, or survival parameters were analyzed using Student's t test for continuous and Fisher's exact test for categorical variables. P values < .05 were considered statistically significant.

RESULTS

Demographics and Clinical Characteristics

Demographic and tumor characteristics are shown in Table I. A total of 27 patients were included in the study. Tumors were classified as either stage T3, T4a, or T4b. All patients were operated with curative intent and received adjuvant radiotherapy. Chemotherapy or immunotherapy was given to five (18.5%) patients during the further course of the disease. During follow up, regional lymph node metastases were diagnosed in 11.1% and distant metastases in 40.7% of the patients. Intense lymphocyte infiltration (brisk TIL grade) was present in 25.9% and intermediate infiltration (non-brisk TIL grade) in 40.7% of SNMMs. Infiltrating lymphocytes were absent in 33.3% SNMMs.

We examined relationships between TIL grade and demographic parameters or tumor stage, comparing brisk or non-brisk TIL grade to absent TILs (Table II). There were no significant relations between age or sex and TIL grade. Although mucosal melanomas are always advanced by definition (stage T3 or T4), brisk, but not non-brisk TIL grade was more likely among tumors of the lower T3 stage category ($P = .01$). In fact, brisk TIL grade was not observed in any of the tumors classified as T4a or T4b.

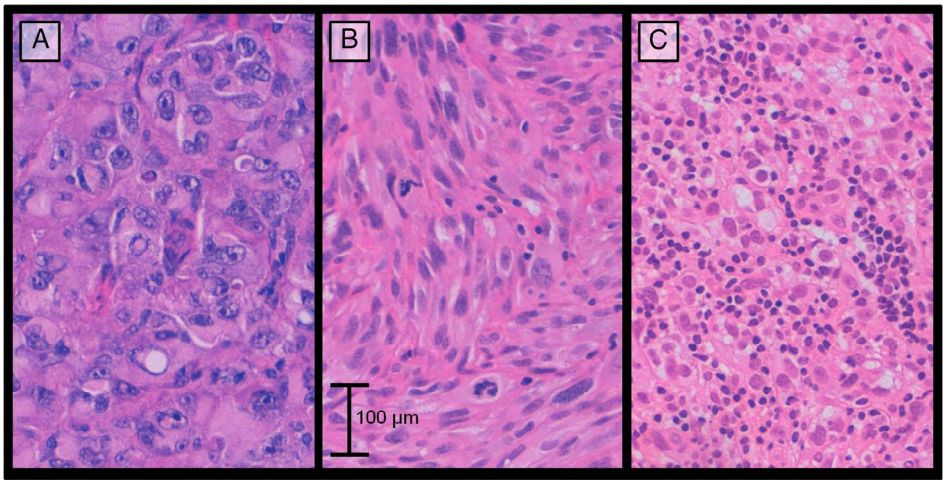

Fig. 1. Grades of tumor-infiltrating lymphocytes (TIL). Representative images from sinonasal mucosal melanoma samples with absent (a), non-brisk (b), and brisk (c) TILs are shown (H&E staining; ×20; scale bar 100 µm).

TIL Grade and Recurrence-Free Survival

Median recurrence-free survival of all patients was 15.7 (range: 1.5–124.8) months. A lower number of tumor-infiltrating lymphocytes was associated with a shorter

TABLE I.
Demographics and Clinical Characteristics.

Age		
Mean (yr)	68	
Range (yr)	33–94	
	No. of Patients	%
Sex		
Female	12	44.4
Male	15	55.6
Tumor stage		
T3	14	51.9
T4a	9	33.3
T4b	4	14.8
Local recurrence	16	59.3
Metastasis		
Regional lymph node	3	11.1
Distant	11	40.7
Site of metastasis		
Lung	3	11.1
Skin	1	3.7
Brain	1	3.7
Liver	1	3.7
Bone	1	3.7
Multiple sites	4	14.8
TIL grade		
Absent	9	33.3
Non-brisk	11	40.7
Brisk	7	25.9

TIL = tumor infiltrating lymphocytes.

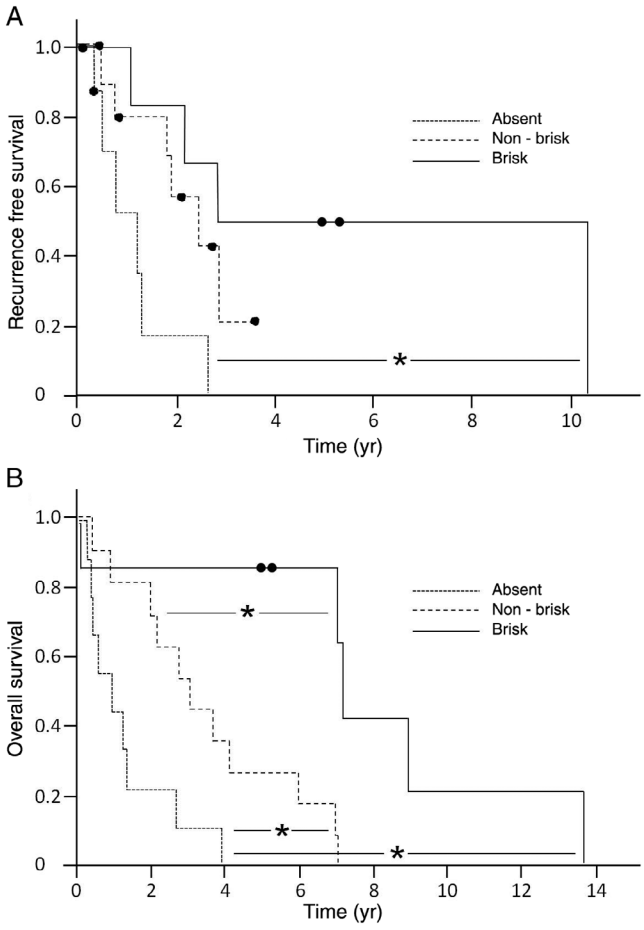

Fig. 2. Absence of tumor-infiltrating lymphocytes (TIL) is associated with shorter recurrence-free and overall survival in sinonasal mucosal melanoma (SNMM) patients. Recurrence-free survival (a) and overall survival (b) of SNMM patients diagnosed with absent (n = 9), non-brisk (n = 11), or brisk (n = 7) TIL grade were analyzed using the Kaplan-Meier method and the log-rank test. *$P < .05$.

TABLE II.
Relationship Between TIL Grade and Demographic or Clinicopathological Parameters.

				Compared With Absent TIL Grade			
	TIL Grade			Non-Brisk TILs		Brisk TILs	
	Absent n = 9	Non-Brisk n = 11	Brisk n = 7	OR (95% CI)	*P*	OR (95% CI)	*P*
Age at diagnosis					.5*		.3*
Mean (yr)	72	68	63				
Median (yr)	78	70	55				
Range (yr)	33–89	53–76	47–94				
Sex					.4†		.1†
Female (%)	6 (67)	5 (45)	1 (14)	Reference		Reference	
Male (%)	3 (33)	6 (55)	6 (86)	2.4 (0.4–14.9)		12 (0.96–151)	
Tumor stage					1.0†		**.01**†
T3 (%)	3 (33)	4 (36)	7 (100)	Reference		Reference	
T4 (%)	6 (67)	7 (64)	0 (0)	0.9 (0.1–5.6)		0	

*t test.
†Fisher's exact test.
CI = confidence interval; OR = odds ratio; TIL = tumor-infiltrating lymphocytes.
Statistically significant results ($P < .05$) are indicated in bold.

TABLE III.
Relationship Between TIL Grade and Outcome.

	TIL Grade			Compared With Absent TIL Grade			
				Non-brisk TILs		Brisk TILs	
	Absent n = 9	Non-brisk n = 11	Brisk n = 7	OR (95% CI)	P	OR (95% CI)	P
Recurrence-free survival					1.0*		**.04***
<3 yr (%)	8 (89)	10 (91)	2 (29)	Reference		Reference	
>3 yr (%)	1 (11)	1 (9)	5 (71)	0.8 (0.04–15.0)		20.0 (1.4–282.5)	
Overall survival					.2*		**<.001***
<5 yr (%)	9 (100)	8 (73)	1 (14)	Reference		Reference	
>5 yr (%)	0 (0)	3 (27)	6 (86)	+∞		+∞	

*Fisher's exact test.
CI = confidence interval; OR = odds ratio; TIL = tumor-infiltrating lymphocytes.
Statistically significant results ($P < .05$) are indicated in bold.

period of disease-free survival and an increased risk of relapse. The Kaplan-Meier analysis revealed a significant reduction in recurrence-free survival in patients with absent TILs as compared to patients with brisk TIL grade ($P = .04$). Differences between non-brisk and absent or non-brisk and brisk TIL subgroups were not statistically significant (Fig. 2a). In agreement, brisk TIL grade was significantly correlated with relapse-free survival of 3 years or more (71% vs. 11% of patients with brisk and absent TIL grade, respectively), with an odds ratio of 20 (CI 95% 1.4–282.5, $P = .04$). There was no significant relationship between non-brisk TIL grade and 3-year recurrence-free survival when compared to absent TIL grade (Table III).

TIL Grade and Overall Survival

Median survival of all patients after initial surgery was 33.0 (range: 1.5–164.5) months. As shown in the Kaplan-Meier analysis, brisk as well as non-brisk infiltration of lymphocytes into the tumor significantly correlated with longer overall survival (Fig. 2b). Furthermore, there was also a significant difference in overall survival between patients with brisk and non-brisk TIL-grade SNMMs ($P = .01$). The 5-year survival was 0% with absent, 27% with non-brisk, and 86% with brisk TIL grade (Table III). Brisk, but not non-brisk TIL grade was more likely among patients who survived for at least 5 years as compared to absent TIL grade ($P < .001$, Table III).

DISCUSSION

We found a strong positive relationship between TIL grade and outcome in SNMM patients in our study. Lymphocyte infiltration in the primary tumor was associated with prolonged survival. Brisk TIL grade provided additional advantage over intermediate (non-brisk) infiltration and was also correlated with lower AJCC tumor stage and recurrence-free survival.

The link between the immune system and cancer has been an area of intensive ongoing research. Immune cells are attracted by the hypoxic milieu of the tumor environment and by signaling substances released in the context of cell necrosis and inflammatory processes.[18] Cancer cell surface proteins altered by genetic mutations—so-called neoantigens—can then provoke an immune response that may ultimately lead to cancer cell death.[19] Strength and effectiveness of the antitumor immune response depend on the density and composition of the leukocyte infiltrate.[20] Recent progress in understanding the complex interplay between tumor and immune cells has led to new revolutionary immunotherapeutic concepts in the treatment of oncologic patients, like the use of chimeric antigen receptor (CAR) T cells or checkpoint inhibitors.[21] There is therefore a large interest in investigating the role of TILs in various tumor entities.[22–24]

Our results are consistent with previous reports describing TILs as powerful prognostic biomarkers in a variety of cancers. For example, the density of inflammatory cell infiltrates correlates with better overall and disease-free survival in colorectal carcinoma.[25] Similar results were obtained in studies of gastric, pancreatic, liver, ovarian, and breast carcinoma.[26–30]

The prognostic value of TILs in cutaneous melanoma (CM) has been controversial. Although some studies did not find any association between TILs and outcome,[17,31,32] in other studies, the density of TILs correlated with a better prognosis.[33–37] In recently published meta-analyses, authors showed that brisk TILs in CM were associated with longer overall survival, recurrence-free survival, and disease-specific survival.[3,4] Some studies further investigated the complex composition of TILs and were able to define individual TIL subgroups as prognostic factors.[3,24] However, mucosal melanoma is genetically distinct from CM with a significantly lower mutational burden.[5,7,38] Because the immunogenicity of a tumor is largely determined by the mutational burden and the resulting number of neoantigens, findings from studies conducted in CM cannot be readily applied to mucosal melanoma.[38–40] Other prognostic parameters established in CM, such as Breslow's thickness and Clark's level, are of limited or no use in mucosal melanomas in the head region because of the anatomical differences.[41]

Clinically, SNMM presents as an aggressive tumor with a very poor prognosis.[9,11] Radical surgical resection with a wide safety margin is considered the treatment of choice in the non-metastatic stage.[9,14] Due to the multilocularity of the tumor and its proximity to vital structures like the carotid artery and the brain, complete resection with adequate safety margins is often difficult or impossible or may require mutilating procedures such as (partial) removal of the external nose or eye. Frequently, numerous recurrent procedures become necessary as the disease progresses. Overall, this poses a considerable physical and psychological burden on the patient.[8,42] In recent years, targeted therapies and immunotherapies have been increasingly used, especially in the inoperable or metastatic stage. In addition to the mutation status of the oncogenes BRAF, NRAS, and KIT, the therapy is based on individual patient characteristics.[5,11,14,43] Late-line therapies also include platinum-based chemotherapies.[43–45] Currently available treatment regimens vary considerably with regard to side effects and efficacy and often require compromises between cancer control and therapy-associated morbidity.[46–50] A precise prognostic assessment of the individual disease course is therefore important to avoid overly aggressive treatments.

A limitation of our study is the comparatively small number of cases due to the rarity of the disease. This did not allow multivariate analysis of the data to assess the independent effect of TIL grade in predicting outcome. Nevertheless, our results suggest that TILs are a strong prognostic indicator for survival in SNMM. The density of TILs could be reported as a comparatively straightforward scoring parameter in the histopathological report, similar to the recommendations outlined in the guidelines for the management of CM.[51,52] Including TIL grade as a prognostic parameter may guide a therapeutic approach that is better targeted to the individual needs of the patient. For example, absent TILs could suggest avoiding overly aggressive and debilitating procedures and prioritizing the maintenance of a satisfactory quality of life given the likelihood of a poor prognosis.[3,4] Further (multicenter) studies with larger patient cohorts are needed to confirm our findings and define the composition of TILs as well as the significance of individual lymphocyte subsets for the prognosis of SNMM.

CONCLUSION

Our results indicate that TIL density is a strong prognostic factor for better survival in SNMM. As a good prognostic marker, the TIL grade could facilitate treatment and follow-up planning in SNMM. Therefore, prospective studies with larger case numbers are warranted to determine whether TILs should be included in future AJCC staging guidelines.

AUTHORS' CONTRIBUTIONS

S.L. and G.L. conceived of the study. S.L., J.P., and G.L. collected the data. S.L. analyzed the data with help of all authors. G.L. provided supervisory support. S.L. wrote the manuscript. C.L., J.P., and G.L. reviewed the manuscript. All authors discussed the results and contributed to and approved of the final version of the manuscript.

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