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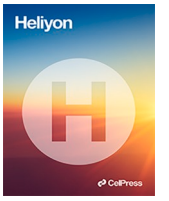

Research article

Role of tumor-infiltrating lymphocytes in melanoma prognosis and treatment strategies: A systematic review and meta-analysis

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ABSTRACT

Purpose: Numerous studies underscore the relevance of tumor-infiltrating-lymphocytes (TILs) as important prognostic factors for melanoma. This meta-analysis aims to provide a comprehensive literature overview elucidating their role in predicting patient outcomes, specifically investigating the association between TIL density and prognosis.
Methods: From an initial pool of 6094 records, 16 met the eligibility criteria, encompassing a collective cohort of 16021 patients. Data on TIL counts, clinical characteristics, and survival metrics (5-year overall survival [5yOS], 10-year overall survival [10yOS], and 5-year melanoma-specific survival [5yMSS]) were extracted from each study and expressed as proportions. Results were graphically presented using forest plots, reporting the estimates from individual studies, summary estimates, and corresponding 95 % confidence intervals (CI).
Results: Analysis revealed a statistically significant difference in 5yOS concerning subgroup differences However, 10yOS and 5yMSS did not exhibit statistical significance. Nonetheless, a consistent trend emerged indicating a higher survival rate corresponding to increased immune cell density, ranging from absent TILs to brisk levels.
Conclusions: TILs present potential as a readily applicable prognostic factor. Yet, further investigations into their density and phenotypic subpopulation characteristics could enhance our understanding of their predictive value in tailoring optimal patient-specific therapies.

1. Introduction

Among skin cancers, melanoma is the third most common form [1]. It originates from the malignant transformation of melanocytes [2]. The annual incidence of malignant melanoma in Europe ranges from 5 to 12/100′000 in Mediterranean countries to 12–35/100′000 in Nordic countries, while it can exceed 50/100′000 in Australia or New Zealand [3,4]. While the average age at diagnosis is 65 years old, this cancer can occur in individuals of any age [5]. Histologically, melanoma can be classified into different subtypes, with common forms encompassing Superficial Spreading Melanoma, Nodular Melanoma, Lentigo Maligna Melanoma, and

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Acral Lentiginous Melanoma [3,6–9].

Understanding the role of tumor-infiltrating lymphocytes (TILs) in melanoma helps to elucidate the mechanisms behind the local immune response against the tumor and its potential implications for disease progression and treatment outcomes. The activation of TILs and their ability to directly kill cancer cells or release immune-stimulatory molecules actively contribute to the suppression and control of the tumor growth [10–12]. TILs are primarily composed of various types of immune cells, including effector and suppressor T cells, B cells, natural killer cells, macrophages, dendritic cells, and myeloid-derived suppressor cells [13,14]. Their infiltration into the tumor microenvironment initiates a sophisticated interplay with the malignant cells, influencing the dynamics of the disease [13–17].

Different methods of classification for the immune infiltrate have been described. According to standard Clark's system, TILs are classified as "brisk" when observed throughout the substance of the vertical growth phase (diffuse) or when present and infiltrating across the entire base of the vertical growth phase (peripheral). If TILs are noted in one or more foci of the vertical growth phase, they are categorized as "non brisk", while "absent" is assigned when lymphocytes are entirely lacking or when present but fail to infiltrate the melanoma [18]. The 1989 Clark classification remains the primary method in use. However, the Melanoma Institute Australia (MIA) has introduced an innovative approach to categorize these immune cells, establishing a four-level scoring system ranging from 0 to 3. This method is determined by assessing both the density (categorized as mild, moderate, or marked) and distribution (described as focal, multifocal, or diffuse across the entire tumor extent) of lymphocytes infiltrating and disrupting tumor nests and/or in direct contact with tumor cells. TILs grades were then defined as follows: grade 0 indicates the absence of TILs, grade 1 means either a mild or moderate focal infiltrate or a mild multifocal TILs presence, grade 2 encompasses a marked focal infiltrate, a moderate or marked multifocal presence, or a mild diffuse TILs infiltrate, and grade 3 denotes a moderate or marked diffuse TILs infiltrate [17,19,20].

Moreover, recent advancements in immunotherapy have highlighted the importance of the immune system in controlling cancer, leading to the investigation of both the prognostic and therapeutic utility of TILs in melanoma [21–25]. Immunotherapeutic approaches, such as immune checkpoint inhibitors, adoptive cell transfer therapies, and cytokine-based therapies, aimed to enhance the activity of TILs, have exhibited promising results in melanoma treatment. These therapies work by blocking inhibitory pathways or by reintroducing expanded populations of TILs into the patient to bolster the immune response against tumor cells [26].

Recognizing the importance of TILs extends beyond their involvement in the local immune response to the tumor; as they also serve as cost-effective predictive biomarkers. With this understanding, we conducted a meta-analysis to investigate the prognostic value of TILs. Establishing the relationship between TILs' density within the tumor and its potential as prognostic and predictive marker is crucial for selecting tailored therapies and enhancing the management of melanoma patients through personalized care.

2. Materials and methods

2.1. Search strategy and selection criteria

The Medline (PubMed) database was used to search for pertinent articles published in peer-reviewed journals, with the latest search update conducted on October 31, 2023. A systematic search was undertaken to identify published studies exploring the potential relationship between density of TILs in primary malignant melanoma and histopathological criteria possibly associated with survival. Only English articles were considered by submitting the following query: "("tumor-infiltrating lymphocytes" OR "tumor infiltrating lymphocytes" OR "TILS" OR "TILs" OR "TIL" OR "lymphocytes") AND melanoma".

The selection criteria for studies included: (a) Observational studies (retrospective or prospective) or clinical trials (randomized or non-randomized), (b) focusing on melanoma, (c) studies with a sample size of at least 14 patients, and (d) providing information on TILs obtained from the initial biopsy/surgery. Reviews, systematic reviews, meta-analysis, case reports, and case series were excluded. In Studies that utilized classifications different from that of Clark [18] were also excluded. The retrieved articles were screened and selected by two independent authors (R.B. and M.G.) according to the inclusion and exclusion criteria. This selection process yielded 16 eligible records.

For each included study, the risk of bias was assessed according to the Newcastle-Ottawa Scale.

2.2. Data extraction

The parameters considered for inclusion in the study encompassed: author, year of publication, study design, total number of patients and categorization by Breslow thickness, 5-year overall survival (5yOS), 10-year overall survival (10yOS), 5-year disease-free-survival (5yDFS), and 5-year melanoma specific survival (5yMSS). Additionally, details regarding disease-free-survival, histological subtype, ulceration, positive sentinel lymph node, lymphatic infiltration, and vascular invasion can be found in the supplementary materials.

2.3. Statistical analysis

The number of cases by TILs and clinical characteristics were extracted from each study, as well as 5yOS, 10yOS, and 5yMSS expressed as proportions. Summary estimates of these proportions, encompassing survival outcomes, were computed along with their corresponding 95 % confidence intervals (CI) utilizing random-effects models of DerSimonian and Laird [27]. These models integrate both within-study and between-study variabilities, assigning weights to each study proportional to its precision. The statistical heterogeneity among studies was assessed using the I^2 and t^2 statistics [27]. To evaluate publication bias, a funnel plot analysis was

conducted [28]. The results of the meta-analysis were graphically represented through forest plots, delineating estimates from individual studies, summary estimates, and their corresponding 95 % CI. Statistical significance was established for p-values less than 0.05.

3. Results

Based on PubMed publications, our research identified 6094 records, of which 16 met the eligibility criteria, totalling 16021 samples [12,13,29–42](Fig. 1). The primary study characteristics were outlined in Table 1. Notably, all studies exhibited a low risk of bias, scoring ≥6 out of 8 on the Newcastle-Ottawa scale.

3.1. Overall survival

Data on 5-year overall survival (Fig. 2) were available for five studies [12,30,33,39,42]. Patients exhibited an 84 % survival rate with brisk TILs (95 % CI: 71–91 %), 68 % (95 % CI: 57–78 %) with non-brisk TILs, and 61 % (95 % CI: 48–72 %) with absent TILs. While subgroup differences reached statistical significance ($p = 0.02$), significant heterogeneity ($p < 0.01$) was observed across all examined groups.

When analyzing the 10-year overall survival (based on only two available studies [12,30]; see Fig. 3), patients exhibited a 79 % survival rate (95 % CI: 26–98 %) with brisk infiltrates, 51 % (95 % CI: 39–64 %) with non-brisk infiltrates, and 40 % (95 % CI: 17–69 %) with absent infiltrates. Subgroup differences did not reach statistical significance ($p = 0.43$), primarily due to sparse data. Moreover, significant heterogeneity ($p < 0.01$) was observed among the three considered groups.

3.2. Melanoma Specific Survival

The MSS according to TILs category is shown in Fig. 4. Patients with brisk TILs exhibited a 94 % MSS rate (95 % CI: 88–97 %), those with non-brisk TILs showed a 90 % MSS rate (95 % CI: 82–94 %), and patients with absent TILs experienced an 88 % MSS rate (95 % CI: 77–94 %). However, subgroup differences did not reach statistical significance ($p = 0.34$), and significant heterogeneity ($p < 0.01$) was evident across all three groups.

3.3. Breslow thickness

Breslow I (i.e., T ≤ 1 mm) was more frequently observed among patients with brisk TILs (52 %; 95 % CI: 26–78 %), followed by those with non-brisk TILs (47 %; 95 % CI: 21–74 %) and patients with absent TILs (43 %; 95 % CI: 20–69 %; Fig. 5). A similar trend was noticed for Breslow II (1.0–2.0 mm), which was more prevalent in brisk TILs (26 %; 95 % CI:14–45 %) compared to non-brisk TILs (20 %; 95 % CI: 10–36 %) and absent TILs (19 %; 95 % CI: 11–30 %).

Conversely, an inversion of this trend emerged concerning Breslow III (2–4 mm) and Breslow IV (T > 4 mm). Only 11 % (95 % CI: 6–20 %) of patients with brisk TILs, 15 % (95 % CI: 9–24 %) with non-brisk TILs, and 17 % (95 % CI: 10–26 %) with absent TILs presented with Breslow III thickness. Moreover, 4 % (95 % CI: 1–15 %) of patients with brisk TILs, 10 % (95 % CI: 6–18 %) with non-

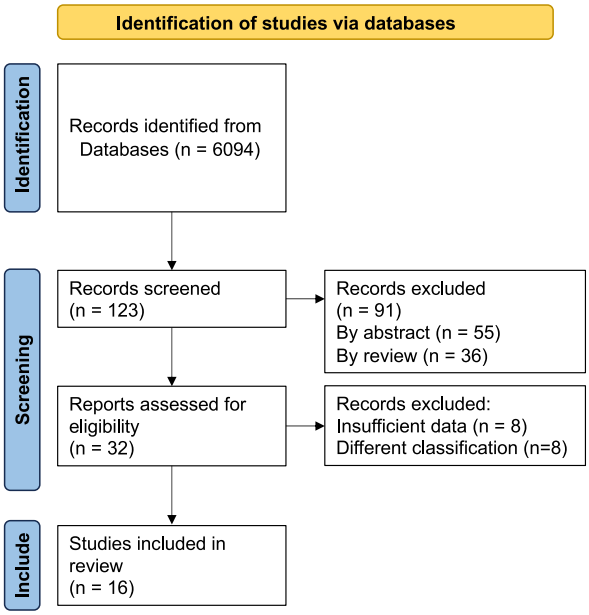

Fig. 1. PRISMA flow chart of study inclusion process [43].

Table 1
Samples enrolled in the meta-analysis.

Publication	Country	Period	Patients				NOS score[a]
			Total	Brisk	Non-brisk	Absent	
Vita et al.	Romania	2023	79	26	47	6	6
Morrison et al.	USA	2022	3201	691	1691	745	7
Straker et al.	USA	2022	1017	87	759	171	7
Zaladonis et al.	USA	2021	669	51	359	259	6
Yang et al.	USA	2021	2624	274	1916	434	6
Gata et al.	Romania	2020	114	68	46	0	6
Saldanha et al.	UK	2017	655	161	464	30	6
Weiss et al.	China	2016	1241	523	330	388	6
Dionizy et al.	Poland	2015	104	52	34	18	6
Thomas et al.	USA	2013	2827	509	2108	690	6
Burton et al.	USA	2011	515	100	415	0	7
Rao et al.	USA	2010	293	40	156	97	6
Mandalà et al.	Italy	2009	1251	114	436	701	6
Taylor et al.	USA	2007	887	51	641	195	7
Tuthill et al.	USA	2002	259	30	98	131	7
Clemente et al.	Italy	1996	285	47	133	105	8

[a] NOS: Newcaste-Ottawa Scale.

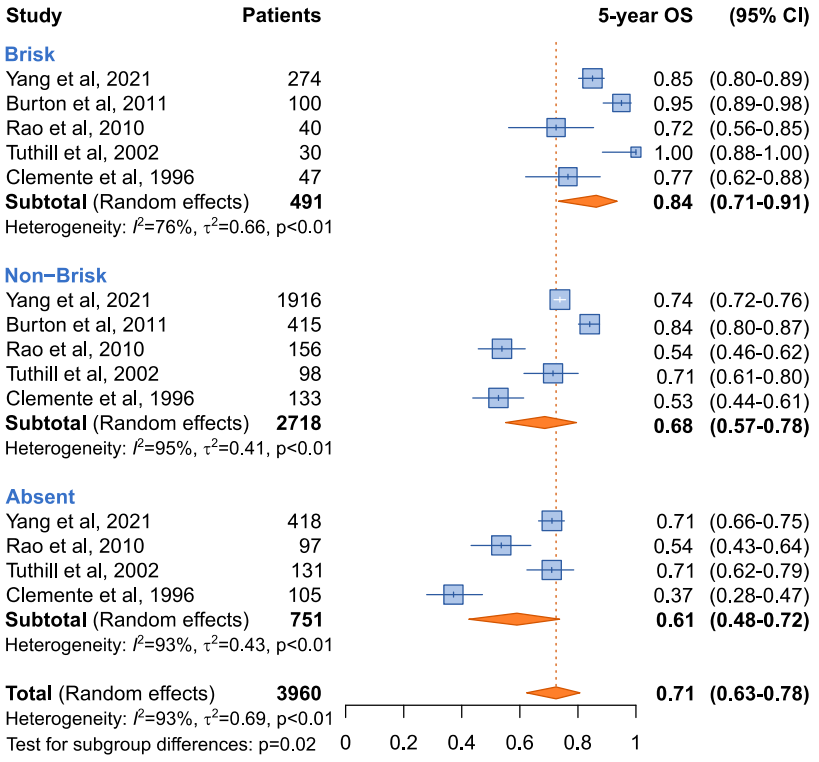

Fig. 2. Forest plot for 5-year overall survival according to TILs category.

brisk TILs, and 14 % (95 % CI: 8–22 %) with absent TILs were classified as stage IV (Fig. 5). Overall, a tendency toward thicker tumors was observed among patients with absent TILs compared to those with brisk TILs, although subgroup differences did not reach statistical significance (p = 0.20).

3.4. Additional findings

The main objective of this meta-analysis is to investigate whether tumor-infiltrating lymphocytes are able to predict survival among patients with cutaneous melanoma. However, the data gathered from existing studies allow for an assessment of the association between TILs grades (classified as brisk, non-brisk, and absent) and other prognostic histopathological and clinical features of the tumor.

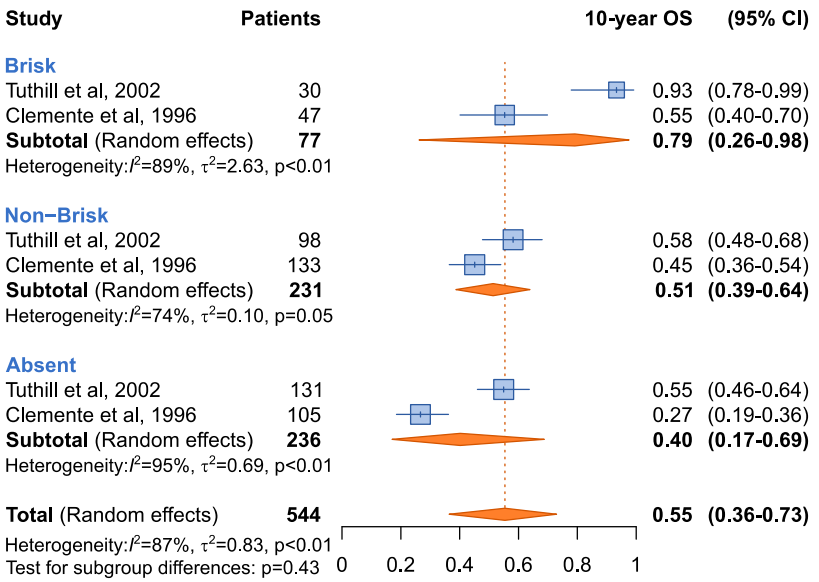

Fig. 3. Forest plot for 10-year overall survival according to TILs category.

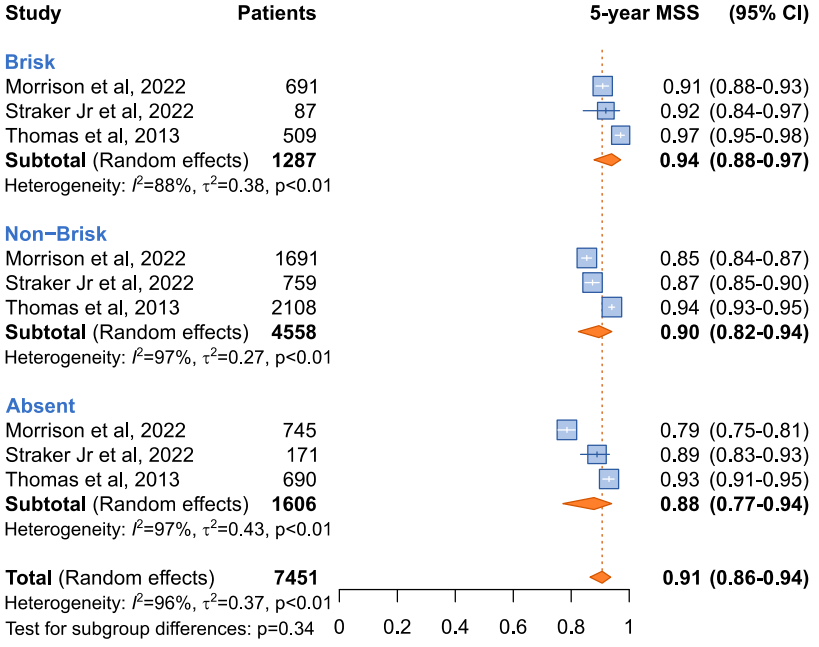

Fig. 4. Forest plot for Melanoma Specific Survival according to TILs category.

Data reporting estimates pertaining to 5-year disease free survival, ulceration, sentinel lymph node involvement, lymphatic invasion, and vascular invasion can be found in Supplementary Materials.

4. Discussion

Melanoma, acknowledged as the most aggressive form skin cancer, is an immunogenic tumor [3,5,10,44–47]. Thus, through the review of studies conducted to date, this meta-analysis aims to assess the association between TILs and patient outcomes, investigating whether their density is related to prognosis.

Accurate prognostic prediction is crucial in selecting appropriate therapies. However, the current dearth of robust biomarkers poses

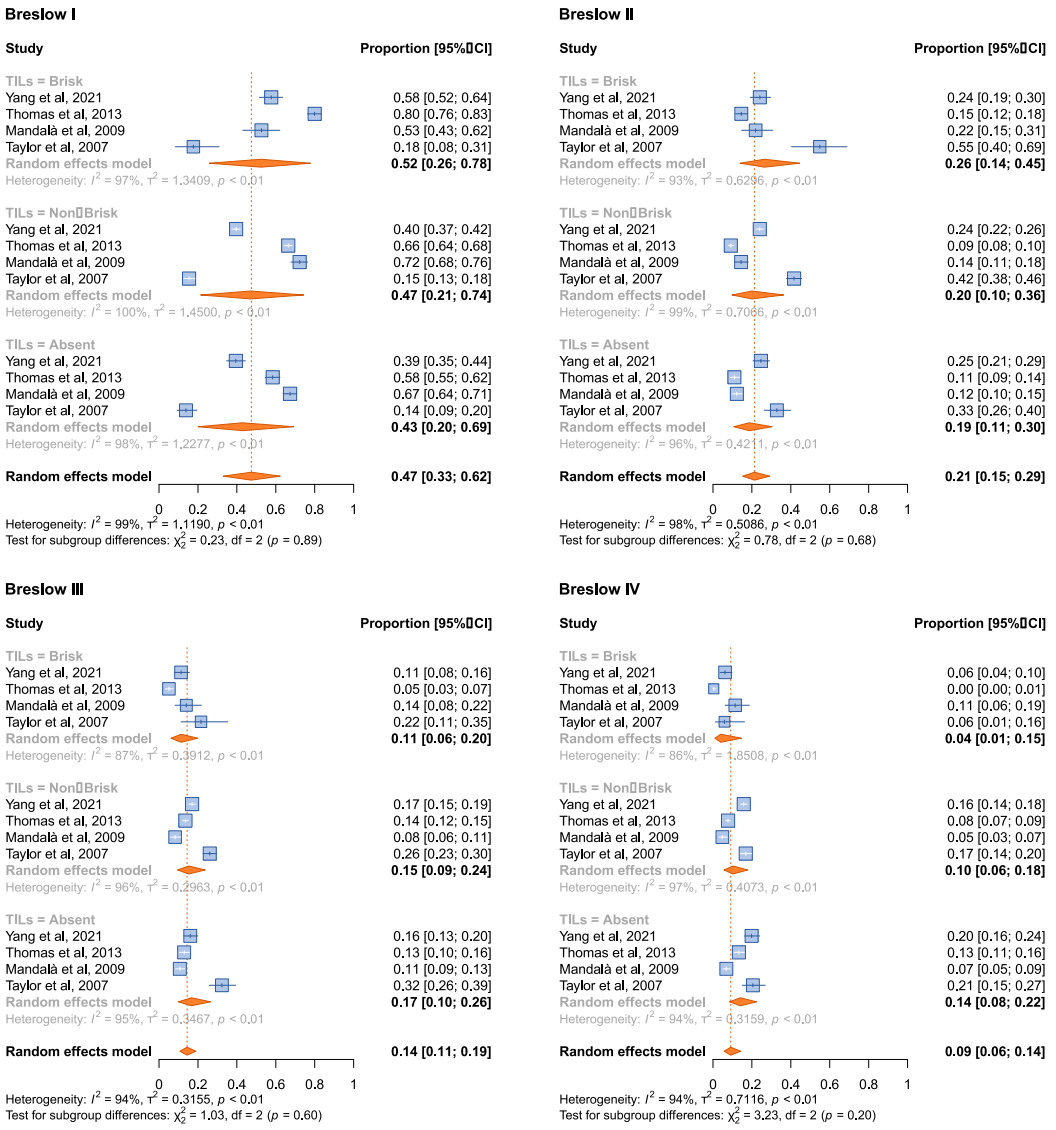

Fig. 5. Forest plot for prevalence of Breslow stage according to TILs category.

a challenge. As our comprehension of tumor biology advances, the staging system has undergone multiple revisions. Currently, the 8th edition American Joint Committee con Cancer (AJCC) melanoma staging system [48] stands as the most widely accepted framework for initial diagnosis. It aids in risk stratification for patients and provides guidance for treatment [48,49]. A comprehensive grasp of prognostic factors and the staging of cutaneous melanoma holds significance for initial case assessment, treatment planning, surveillance strategy formulation, and the design and analysis of clinical trials [48,50]. As a result, there is an ongoing search for new prognostic markers and the development of models aimed at refining the prediction of patient outcomes.

Our meta-analysis specifically included studies categorizing the density and distribution pattern of lymphocytes within the tumor as brisk, non-brisk, or absent, adhering to Clark's classification (1989)(18). The association between tumor-infiltrating-lymphocytes density and prognosis has been consistently observed. Overall survival refers to the period of time from the diagnosis of cancer until the death of the patient from any cause [51]. Regarding 5-year overall survival, several studies claim that the immune cells infiltrate is a statistically significant prognostic factor [12,30,34,39,52]. Instead, in certain instances, the difference in OS is only marginally significant across the three groups [33]. Investigation through a meta-analysis provided a quantitative synthesis of these reports, indicating that TILs present a statistically significant correlation with a favorable prognosis for the OS.

Regrettably, due to the limited dataset regarding 10-year overall survival, reliable data cannot be obtained through statistical analysis [12,30]. Despite this, it is possible to notice a positive trend between survival and immune infiltrate, where a higher density of TILs (absent, non-brisk and brisk) corresponds a better survival rate. Hopefully, additional studies aimed to investigate this relationship will contribute valuable insights.

Nevertheless, we observed a higher 5-year Melanoma Specific Survival when brisk TILs were present (94 % vs 88 %), although statistical significance was not reached. MSS survival refers to the percentage of patients who have not died from melanoma within 5 years. Its link to TILs could potentially play a significant role as part of personalized medicine, as their examination allows for the evaluation of an individual's specific immune response against cancer [34,40,41]. This means that by analyzing their quantity and activity, clinicians could tailor therapies on a personalized basis. Moreover, several studies have suggested a relationship between immune infiltration and tumor thickness. The Breslow classification is used to indicate the depth of melanoma invasion from the surface of the epidermis to the deepest point of tumor penetration into the skin. Thickness is then divided into four stages: T1 $\leq$ 1 mm, T2 1.0–2.0 mm, T3 2–4 mm, T4 $>$ 4 mm [53]. Based on the data available in this review, it seems that when the tumor is small or in its early stages, the immune system still has en effect, as evidenced by the higher presence of TILs in thinner melanoma [31,34,39]. In fact, Fig. 5 shows that Breslow I (T $\leq$ 1) is more frequently found among patients with brisk TILs, while Breslow IV (T $>$ 4 mm) is more prevalent among those with absent TILs. It is established that one of the cancer's hallmarks is its ability to evade the immune system, which conversely endeavors to suppress its growth [54]. These findings further support the notion that when the tumor proliferates, it has found a way to elude the patient's defense response [11,55,56]. Still, this intricate relationship necessitates additional studies to fully comprehend how TILs density influences the development and progression of melanoma. Such insights could offer guidance for more precise therapeutic decisions, potentially enhancing treatment effectiveness and optimizing outcomes for individual cases.

An additional challenge regarding TILs is understanding whether, in addition to their role as prognostic markers, they also serve as predictive markers. Prognostic markers typically predict cancer outcomes, such as survival or disease recurrence, while predictive markers are specifically associated with treatment, identifying which patients are likely to benefit from a particular therapy.

Immunotherapy todays comprises different options: immunomodulatory strategies that enhance the body's natural anti-tumor immune response; vaccination protocols designed to sensitize the immune system against the autologous tumor; and adoptive cell transfer (ACT) which involves ex vivo expanded immune effector populations specifically targeting cancer antigens [11,57–59].

In recent years, the realm of melanoma immunotherapy has witnessed considerable progress, particularly following the unveiling of monoclonal antibodies targeting immune checkpoint inhibitors, notably cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) [22,60,61]. Nonetheless, approximately 60 % of patients fail to derive benefits from immunotherapy [62]. Based on these premises, the identification of predictive biomarkers for the selection of responders is an emerging need. On one hand, there exist expensive markers -such as tumor mutation burden, circulating tumor DNA (ctDNA), and specific mutations-that are not consistently available. On the other hand, less costly markers, like lactate dehydrogenase (LDH) and neutrophil-to-lymphocyte ratio (NLR), can be easily assessed via a blood sample. However, these markers have yet to demonstrate consistent predictive value [63].

Therefore, the importance of investingating whether TILs can contribute to the basis of a personalized medicine becomes evident. In this scenario, artificial intelligence, especially Machine Learning, can play a pivotal role in predicting melanoma prognosis. Its purpose is to scrutinize extensive datasets encompassing various patient information, including biomarkers [64], and build algorithms able of identifying pertinent features associated with melanoma progression, helping the creation of predictive models. However, further studies on the immune cells are necessary, including a more comprehensive investigation of features as density, localization, and phenotype, before definitive statements can be made regarding their role in treatment selection [65].

To provide a comprehensive understanding of the meta-analysis's reliability, it is crucial to consider the study's limitations. Primarily, the absence of a standardized and universally adopted classification system for TILs could introduce potential heterogeneity across studies. Given our decision to adhere to the Clark method, categorizing immune cells as brisk, non-brisk, and absent, it became necessary to exclude studies that employed different stratifications of TILs, such as the MIA grading system (0–3).

Stratification of the results is also a key limitation. Although the Clark classification is used in the eligible studies for a first layering, some of them later subdivide TILs as "present" (including brisk and non-brisk) and "absent"(40,66–69). To enhance data consistency, studies employing this criterion of subdivision were excluded.

It is important to note that biomarkers might exhibit different prognostic values across distinct histological subtypes and tumor stages. However, many studies did not specify stratification for these groups. Therefore, a more comprehensive categorization could provide deeper insights into distinctions among patients or tumor subgroups. Moreover, our analysis did not encompass treatment modalities due to limited mentions in the studies. Given that different therapies operate through distinct mechanisms, the prognostic significance of TILs is likely influenced by the employed therapy. Therefore, future analyses should incorporate various treatment modalities among patient to better understand the role of TILs. An in-depth description of TILs' phenotype is also crucial. Existing studies did not include stratification of lymphocytes based on their subpopulations, known to be diverse within the microenvironment mainly composed of effector T lymphocytes, regular T lymphocytes, natural killer cells, dendritic cells, and macrophages [10,66].

5. Conclusions

To date, a significant body of evidence suggests that tumor-infiltrating-lymphocytes serve as critical prognostic factors for patients with melanoma, influencing treatment choices, postoperative care, and survival rate. This meta-analysis provides a literature overview about the role of TILs in predicting melanoma outcome. Our analysis, based on key studies, revealed that only the 5-year overall survival reached statistical significance when tested for subgroup differences. Nonetheless, there is a noticeable trend indicating improved survival with increasing immune cells density (from absent TILs to brisk). Additionally, a comprehensive characterization of TILs, coupled with a universal accepted staging system, could serve as a valuable tool for clinicians, aiding them in selecting appropriate treatment strategies and managing melanoma patients with a focus on tailored therapeutic approaches.

Furthermore, machine learning has emerged as a transformative force in medicine due to its ability to analyze vast medical

datasets. Our study provides evidence that TILs perform different functions in melanoma. They could potentially serve as therapeutic targets and aid in predicting and optimizing responses to melanoma immunotherapy. Therefore, integrating TILs into a prognostic and/or predictive algorithm could help develop a model capable of predicting cancer prognosis and response to immunotherapy. However, for further exploration of tumor-infiltrating-lymphocytes as predictive biomarkers in clinical practice, future research is crucial. Establishing a standardized stratification system based on the same TILs classification, which also incorporates TILs subtypes, is essential for advancing this field.

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CRediT authorship contribution statement

Mattia Garutti: Writing – review & editing, Validation, Supervision, Project administration, Methodology, Data curation, Conceptualization. **Rachele Bruno:** Writing – review & editing, Writing – original draft, Investigation, Data curation. **Jerry Polesel:** Writing – review & editing, Visualization, Validation, Supervision, Software, Methodology, Formal analysis, Data curation. **Maria Antonietta Pizzichetta:** Writing – review & editing, Validation, Supervision. **Fabio Puglisi:** Writing – review & editing, Validation, Supervision.

Declaration of competing interest

M.G. reports receipts of honoraria or consultation fees from Novartis, Eli Lilly, PierreFabre, Roche, MSD, Daichii Sankyo, Organon and travel fees from Daichii Sankyo, all outside the submitted work. F.P. reports the receipt of grants/research support from AstraZeneca, Eisai, and Roche, and receipts of honoraria or consultation fees from Amgen, AstraZeneca, Daichii Sankyo, Celgene, Eisai, Eli Lilly, Gilead, GSK, Ipsen, MSD, Novartis, Pierre-Fabre, Pfizer, Roche, Seagen, Takeda, Menarini, and Viatris, all outside the submitted work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32433.

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