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Clinical significance of overall assessment of tumor-infiltrating lymphocytes in oropharyngeal cancer: A meta-analysis

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ABSTRACT

Background: The overall assessment of tumor-infiltrating lymphocytes (TILs) evaluated using hematoxylin and eosin (HE) staining has been recently studied in oropharyngeal squamous cell carcinoma (OPSCC).
Methods: We conducted a systematic review of Scopus, Ovid Medline, PubMed, Web of Science, and Cochrane Library to retrieve studies assessing TILs in HE-stained sections of OPSCC. We used fixed-effect models and random-effect models to estimate the pooled hazard ratios (HRs) and confidence intervals (CIs) for disease-free survival (DFS), overall survival (OS) and disease-specific survival (DSS).
Results: Eleven studies were identified that had analyzed the prognostic significance of TILs in OPSCC using HE-stained specimens. Our meta-analyses showed that a high infiltration of TILs was significantly associated with improved DFS (HR 0.39, 95%CI 0.24–0.65, $P = 0.0003$), OS (HR 0.38, 95%CI 0.29–0.50, $P < 0.0001$), and DSS (HR 0.32, 95%CI 0.19–0.53, $P < 0.0001$).
Conclusion: Findings of our meta-analysis support a growing body of evidence indicating that assessment of TILs in OPSCC using HE-stained sections has reliable prognostic value. The clinical significance of such assessment of TILs has been reported repeatedly in many studies on OPSCC. The assessment is cost-effective, feasible, easy to transfer from lab to clinic, and therefore can be incorporated in daily practice.

1. Introduction

Oropharyngeal squamous cell carcinoma (OPSCC) is a common malignancy of the head and neck and with rising incidence of the human papillomavirus (HPV)-related form [1–3]. Survival for a selected group of patients with OPSCC is relatively good and a de-intensification of the treatment has thus been introduced in recent clinical trials [4,5]. De-ESCALaTE HPV and RTOG 1016 were two major trials to introduce de-escalation in HPV+ OPSCC treated with primary radiochemotherapy. The goal was to reduce treatment-related toxicity by replacing cisplatin with cetuximab, an anti-EGFR antibody, while maintaining the high survival rates. Both trials revealed inferior tumor control rates and reduced 5-year overall survival in patients treated with primary radiotherapy and cetuximab compared to patients who received concomitant

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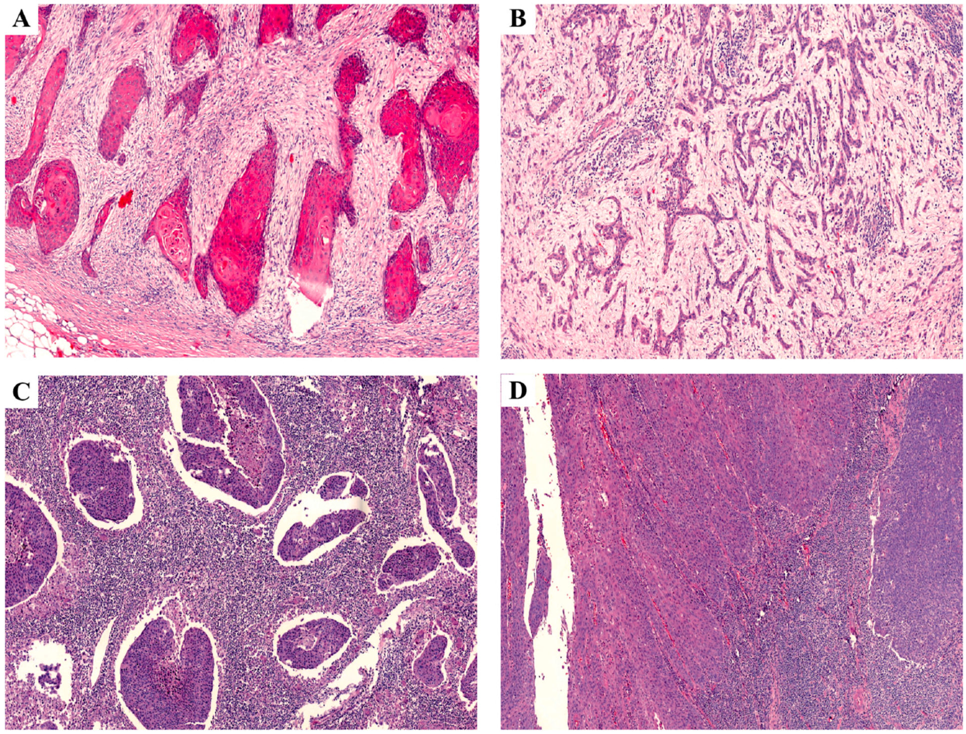

Fig. 1. Hematoxylin and eosin-stained sections of oropharyngeal cancer: A and B: Tumors with a low infiltration of TILs. C and D: Tumors with a high infiltration of TILs.

radiochemotherapy [6,7]. Therefore, it is of a high clinical importance to identify/predict cases with good prognosis that might be eligible to treatment deintensification in order to avoid suboptimal treatment [8]. In daily practice, Tumor-Node-Metastasis (TNM) is the primary tool for risk stratification. However, tumor heterogeneity within the same stage occurs widely among the cases. One of the major points of discussion about the recent update in the TNM staging was the lack of the contribution of the immune microenvironment. Indeed, the clinical importance of tumor microenvironment has been recently emphasized in many malignancies where it has a major clinical significance [9]. In the

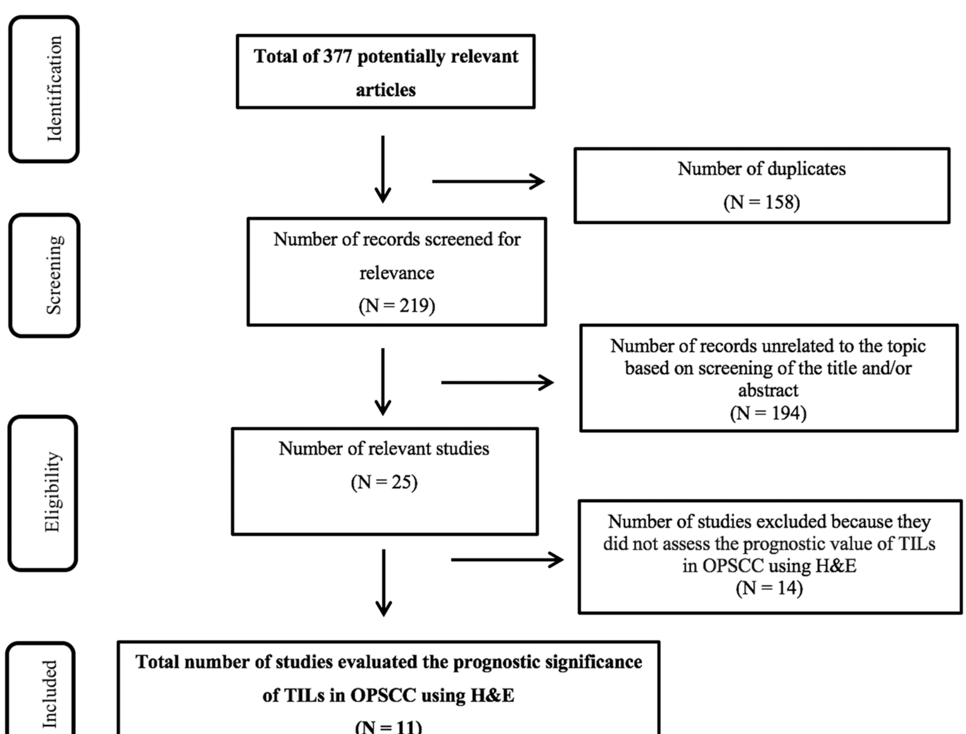

Fig. 2. PRISMA flowchart shows the search strategy for studies that evaluated tumor-infiltrating lymphocytes in oropharyngeal cancer using hematoxylin and eosin staining.

Table 1
Summary of studies that examined the prognostic significance of TILs in HE-stained sections of oropharyngeal cancer.

First author et al. Year, (Country)	Number of cases	HPV status	Stage	Treatment options	Cutoff point	% of low TILs	Survival endpoint	Main finding of the study HR (95% CI), *P*
Ward et al. 2014 (United Kingdom)	270	Both HPV+ /-	I-IV	Surgery/ Radiotherapy/ Chemoradiotherapy	< 20% < 20% of tumour/stroma	28.8%	DSS All cases	High TILs 0.21 (0.11–0.41), < 0.001 **High TILs 0.28 (0.13–0.62), 0.002**
	149	HPV+				14.8%	DSS HPV-Pos	Low TILs 5.67 (1.96–16.38) **Low TILs 4.86 (1.34–17.60), 0.02**
	121	HPV-				46.3%	DSS HPV-Neg	Low TILs 1.71 (0.71–4.13) **Low TILs 2.11 (0.73–6.16), 0.17**
De Meulenaere et al. 2017 * (Belgium)	78	Both HPV+ /-	I-IV	Surgery/ Radiotherapy/ Chemoradiotherapy	Median scores	56%	OS	High TILs 0.52 (0.29–0.93), 0.031 **High TILs 1.06 (0.46–2.46), 0.893**
De Meulenaere et al. 2017 * (Belgium)	99	Both HPV+ /-	I-IV	Surgery/ Radiotherapy/ Chemoradiotherapy	Median scores	-	OS	High TILs 0.55 (0.33–0.96), 0.033 **High TILs 1.06 (0.48–2.35), 0.882**
							DFS	High TILs 0.70 (0.32–1.50), 0.354
Ruangritchankul et al. 2019 (United Kingdom)	232	HPV+	I-IV	Radiotherapy or Chemoradiotherapy	< 20% tumor/ stroma	13.4%	OS	**High TILs 0.22 (0.10–0.51), < 0.0001**
							DFS	**High TILs 0.14 (0.05–0.42), < 0.0001**
Faraji et al. 2020 (USA)	115	HPV+	I-II	Surgery/ Radiotherapy/ Chemoradiotherapy	-	33.3%	RFS	High TILs 0.249 (0.085–0.732), 0.012 **High TILs 0.210 (0.061–0.723), 0.013**
Gurin et al. 2020 (Czech Republic)	65	Both HPV+ /-	II-IV	Definitive intensity-modulated radiotherapy	Median scores	60%	OS	High TILs 0.47 (0.23–0.94), 0.0298
							LR	High TILs 0.55 (0.25–1.22), 0.1359
							PFS	High TILs 0.48 (0.22–1.03), 0.0554
Kreinbrink et al. 2021 (USA)	143	Both HPV+ /-	I-IV	Surgery/ Radiotherapy/ Chemoradiotherapy	30%	14.7%	OS	High TILs 0.32 (0.15–0.72), 0.005
							PFS	High TILs 0.57 (0.31–1.05), 0.071
De Keukeleire et al. 2021 * (Belgium)	92	Both HPV+ /-	I-IV	Surgery/ Radiotherapy/ Chemoradiotherapy	30%	-	OS	High TILs 0.33 (0.20–0.53), < 0.0001 **High TILs 0.31 (0.17–0.56), 0.0001**
Corredor et al. 2022 (USA)	439	HPV+	I	Surgery/ Radiotherapy/ Chemoradiotherapy	-	-	OS	Low TILs 2.34 (1.08–5.07), 0.02 High TILs 0.43 (0.19–0.93), 0.02 **Low TILs 2.58 (1.09–5.68), 0.03** **High TILs 0.39 (0.18–0.92), 0.03**
							DFS	Low TILs 2.56 (1.52–4.32), < 0.001 High TILs 0.39 (0.23–0.66), < 0.001 **Low TILs 2.27 (1.32–3.94), 0.003** **High TILs 0.44 (0.25–0.76), 0.003**
Shaban et al. 2022 (United Kingdom)	95	-	I-IV	-	20%	23%	DSS	High TILs 0.20 (0.08–0.49), 0.000159
Almangush et al. 2022 (Finland)	182	Both HPV+ /-	I-IV	Surgery/ Radiotherapy/ Chemoradiotherapy	20%	26.9%	OS	Low TILs 2.27 (1.38–3.75), 0.001 High TILs 0.44 (0.27 −0.72), 0.001 **Low TILs 1.87 (1.11–3.13), 0.018** **High TILs 0.53 (0.32–0–90), 0.018**
							DSS	Low TILs 2.84 (1.58–5.11), < 0.001 High TILs 0.35 (0.19–0.63), < 0.001

(*continued on next page*)

Table 1 (*continued*)

First author et al. Year, (Country)	Number of cases	HPV status	Stage	Treatment options	Cutoff point	% of low TILs	Survival endpoint	Main finding of the study HR (95% CI), *P*
								Low TILs 2.13 (1.14–3.96), 0.017 <u>**High TILs 0.47 (0.25–0.88), 0.017**</u>

Notes:
Values in bold are from multivariate analyses.
High TILs values that are <u>underlined</u> were calculated using 1 divided by the hazard ratio (and 1 divided by confidence interval).
% of cases that at high-risk as being having low TILs.
× 20: Refer to × 20 objective lens.
*Overlapped.
Abbreviations: CI: Confidence interval; DFS: Disease-free survival; DSS: Disease-specific survival; HR: Hazard ratio; H-E: Hematoxylin and eosin staining; HPV: Human Papillomavirus; LR, locoregional control; NA: Not available; OS: Overall survival; PFS: Progression free survival; RFS: Recurrence free survival.

era of immunotherapy, many promising immune-related biomarkers have been studied in the microenvironment of head and neck cancers [10]. Among these, tumor-infiltrating lymphocytes (TILs) in hematoxylin and eosin (HE)-stained sections have been widely studied [11]. Remarkably, such assessment has been studied recently in the daily practice of breast cancer where evaluation of TILs in HE-stained slides was recommended for routine pathology [12].

In OPSCCs, however, TILs or any other immune prognosticator is not routinely assessed yet. Of note, recent studies [13,14] have reported promising findings in overall assessment of TILs in HE stained slides of OPSCC (Fig. 1). Guidelines from the International Immuno-oncology Biomarker Working Group (IIBWG) form the mainstay for the assessment of TILs [15,16]. In many studies of OPSCC, TILs have been scored according to the IIBWG protocol. HE-based evaluation of TILs has the advantages of being inexpensive and readily applicable to routine pathology practice. Thus, the assessment of TILs can pave the way to be established as a routine biomarker [17]. In this systematic review and meta-analysis, we sought to analyze the accumulated findings on the significance of TILs in HE-stained specimens of OPSCC.

2. Methods

We used the keywords "tumo(u)r-infiltrating lymphocytes" and "oropharyngeal cancer" to search the databases of Scopus, Ovid Medline, PubMed, Web of Science, and Cochrane Library. Our search included the time period from the inception until 11 February 2022 for articles in the English language only. Further, we manually searched the references of relevant studies to check if there were any articles that were not identified in the database search. Review papers and articles in another language than English were excluded. We followed the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) [18].

Two authors (A.A. and R.A.) independently conducted the database search and the screening of the retrieved records. The inclusion and exclusion criteria were independently checked by the two authors for all relevant studies. To estimate the risk of bias in the relevant studies, the Quality In Prognosis Studies (QUIPS) tool [19] was used.

2.1. Statistical analysis

The statistical software R (version 4.1.3) was used to run both an inverse-variance weighted fixed-effect meta-analysis and a random-effects meta-analysis as implemented in the 'meta' package (version 5.2–0). In addition to the meta-analyzed hazard ratios (HR), we also report the estimated proportion of variation in effect sizes that was due to heterogeneity (I^2) [20] and the between-study variance τ^2. Funnel plots were generated using standard errors on y-axis.

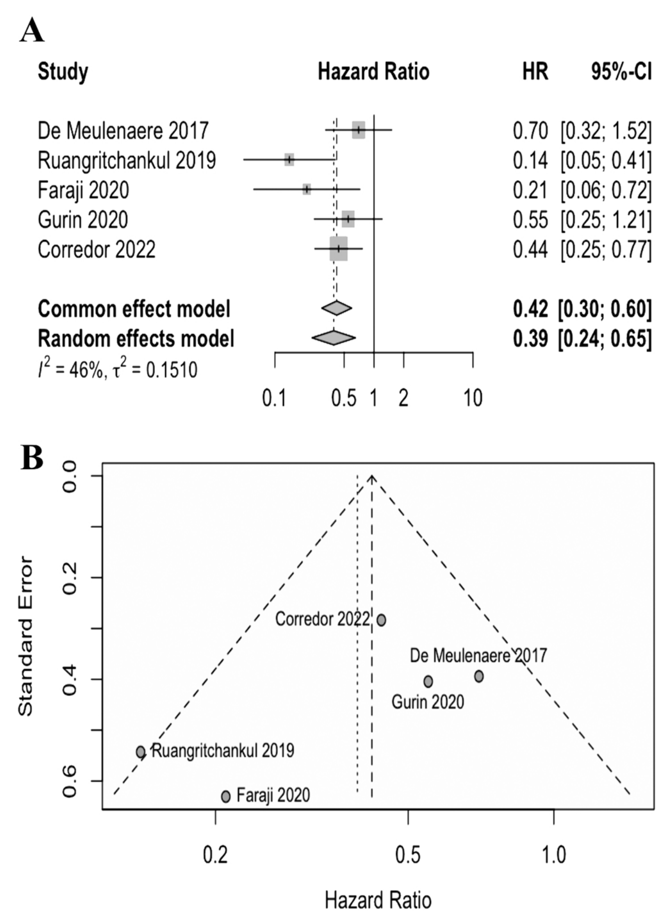

Fig. 3. Disease-free survival forest plots (A) and funnel plots (B) for the meta-analysis of studies evaluated TILs in oropharyngeal cancer.

3. Results

After removing duplicates 219 articles were screened for eligibility (Fig. 2). Studies unrelated to the topic (i.e. not reporting TILs in OPSCC using HE-stained slides) were excluded. Studies on head and neck cancer which included cases of OPSCC and reported significance of TILs but did not provide analysis of TILs in OPSCC separately were also excluded [21–23]. A total of 11 studies (Table 1) were relevant as they analyzed the prognostic value of TILs using HE staining in OPSCC.

The relevant studies were published between 2014 and 2022 (Table 1), and they were from the United Kingdom [24–26], Belgium [14,27,28], USA [29–31], Czech Republic [32] and Finland [13].

Among the relevant studies (Table 1) there were three studies [14,

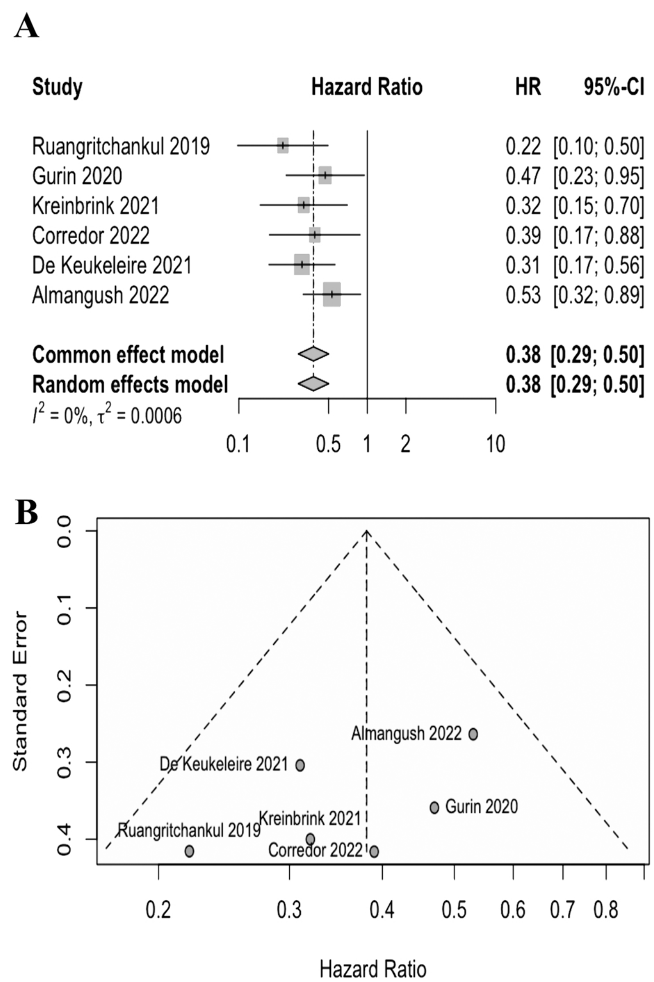

Fig. 4. Overall survival forest plots (A) and funnel plots (B) for the meta-analysis of studies evaluated TILs in oropharyngeal cancer.

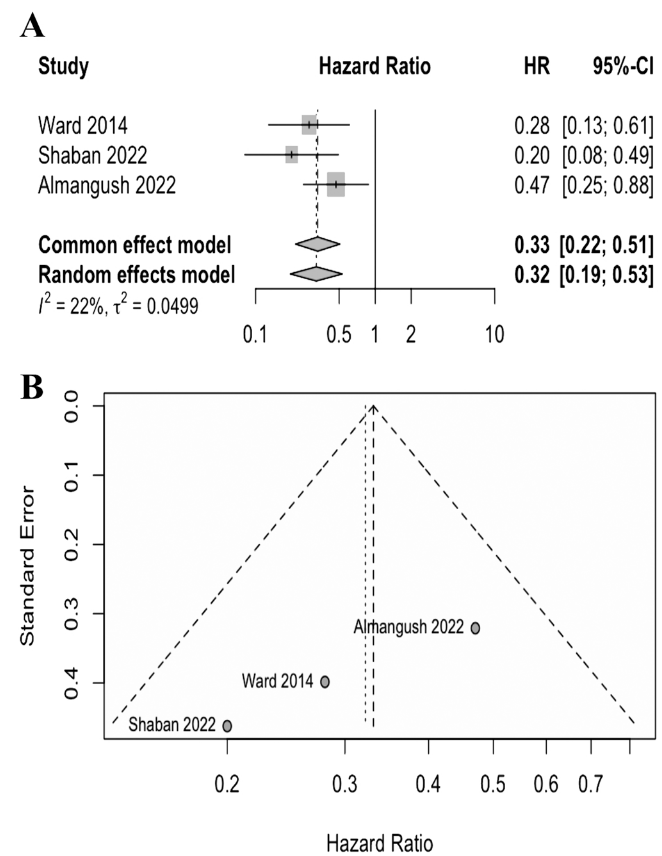

Fig. 5. Disease-specific survival forest plots (A) and funnel plots (B) for the meta-analysis of studies evaluated TILs in oropharyngeal cancer.

[27,28] (De Meulenaere et al., 2017, De Meulenaere et al., 2017, and De Keukeleire et al., 2021) that included overlapping cases. Therefore, they were not included together in one meta-analysis. This makes a total of 5 cohorts (n = 950 patients) that were included in our meta-analysis of disease-free survival, 6 cohorts (n = 1153 patients) for overall survival, and 3 cohorts (n = 547 patients) for disease-specific survival as presented in Figs. 3, 4 and 5.

There were two studies (De Meulenaere et al., 2018 [33] and Brcic et al. [34]) that assessed TILs in HE- stained slides without reporting the prognostic value, and therefore they were not included in our meta-analysis. Of note, these two studies reported a good agreement between the score of TILs in biopsies and in resection samples [33,34].

A high interobserver agreement was widely reported in all relevant studies including assessment of TILs in a binary system (Kappa = 0.87) or in a ternary system (Kappa = 0.72), as in the study by Ruangritchankul et al. [25]. Furthermore, perfect agreement (Kappa = 1.0) was also reported [29]. Interestingly, TILs in the stromal area were clinically more relevant than TILs in the intra-tumoral area when both areas were analyzed.

Our meta-analyses (Figs. 3, 4, and 5) showed that a high infiltration of TILs in HE-stained slides was associated with improved disease-free survival (HR 0.39, 95% CI 0.24–0.65, $P = 0.0003$), overall survival (HR 0.38, 95% CI 0.29–0.50, $P < 0.0001$) and improved disease-specific survival (HR 0.32, 95% CI 0.19–0.53, $P < 0.0001$). As presented in Table 2, all studies included in our meta-analyses were associated with a low risk of bias. Further, no bias was expected based on the funnel plots (Figs. 3B, 4B, and 5B) as the individual studies were distributed equally on both sides of the meta-analysis estimates and they remain mostly within (13 out of 14 estimates) or just outside the 95% region (1 out of 14 estimates). There was no heterogeneity ($I^2 = 0\%$, Fig. 4A) in meta-analysis of overall survival. However, some heterogeneity was observed in the meta-analyses of disease-free survival ($I^2 = 46\%$, Fig. 3A) and disease-specific survival ($I^2 = 22\%$, Fig. 5A).

4. Discussion

The immune response is a critical player during cancer progression [35]. TILs constitute a major part in the adaptive immune response, and the clinical significance of TILs has been widely studied in recent decades [17]. In many tumor types, a high infiltration of TILs in HE-stained specimens was an indicator of immune activation and has been recognized as an independent marker for improved survival [36–38]. Here for the first time, we report a meta-analysis on the prognostic significance of TILs evaluated in HE-stained sections of OPSCCs.

Our findings indicate that overall assessment of TILs scored in HE stained sections of OPSCC can be used to predict tumor behavior (Figs. 3, 4 and 5). Of note, in recent studies using the most recent AJCC 8 staging system, TILs score was an independent prognostic factor for OPSCC [13, 29]. Furthermore, when cases at an early stage were analyzed separately, TILs were reported as an important risk classifier in multivariate analysis, [29] and similarly in studies including only early-stage OPSCC [31]. In addition, TILs score was a useful prognostic indicator in both HPV-positive and HPV-negative tumors [11], which indicates that overall score of TILs can be considered universally in risk assessment of OPSCC. Of note, all the published studies included in our current meta-analysis focused specifically on OPSCC (Table 1) and their findings support the implementation of overall assessment of TILs in daily practice. On the other hand, analysis of the subtypes of TILs using

Table 2
Quality assessment of the articles included in meta-analyses of TILs in OPSCC using Quality In Prognosis Studies (QUIPS) tool.

Included studies	Bias Domains					
	Study participation	Study attrition	Prognostic factor measurement	Outcome measurement	Study confounding	Statistical analysis and reporting
Ward et al. 2014 (United Kingdom)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
De Meulenaere et al. 2017 (Belgium)	Low risk	Low risk	Low risk	Low risk	Low risk	High risk
Ruangritchankul et al. 2019 (United Kingdom)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Faraji et al. 2020 (USA)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Gurin et al. 2020 (Czech Republic)	Low risk	Low risk	Low risk	Low risk	Moderate risk	High risk
Kreinbrink et al. 2021 (USA)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
De Keukeleire et al. 2021 (Belgium)	Low risk	Low risk	Low risk	Low risk	Low risk	High risk
Corredor et al. 2022 (USA)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Shaban et al. 2022 (United Kingdom)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Almangush et al. 2022 (Finland)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk

immunohistochemistry has usually been performed only in cohorts of head and neck squamous cell carcinomas (HNSCC) from heterogenous locations. For example, the meta-analysis on the prognostic significance of CD3, CD4, CD8, and FoxP3 of de Ruiter et al. [39] reported that the accumulated findings were based mainly on cohorts from various subsites of HNSCC. Results regarding the subtypes (e.g. CD4, CD8, FoxP3) of TILs in HNSCC [40] have often been contradictory. Therefore, a definitive conclusion on subtype analysis of TILs in OPSCC has not yet been reached. Furthermore, the costs of immunohistochemical stainings and the problems of their standardization can be avoided by assessing TILs in HE-stained sections.

Different methods have been used for the overall assessment of TILs, including the standardized method introduced by the International TILs Working Group (2014) [15,16]. Remarkably, the efficacy of the latter method has been documented and it was recognised as a reliable, reproducible and practical method in breast cancer where it was firstly applied [16], as well as in other solid tumors including esophageal cancer [41], colorectal cancer [42] and OPSCC [13,14]. It is important to mention that TILs in OPSCC and other tumors have been usually evaluated in full section slides which are more representative than biopsies [24]. However, a recent study on breast cancer [43] showed good performance in scoring of TILs in core biopsies. In the management protocols for OPSCC, primary surgery is recommended only for a minority of patients, and thus evaluation of TILs is rather restricted to biopsy samples. Of note, a good match between biopsies and resection samples with regard to TILs score has been reported recently in OPSCC [33,34].

Assessment of prognostic biomarkers using digital analysis has been studied in head and neck cancers. Studies that used automated image analysis (machine learning and/or deep learning) for TILs in HE-stained sections of OPSCC [26,31] showed promising prognostic values that were even superior to manual assessment [26]. This can be useful for precise and standardized scoring of TILs and for its incorporation into clinical daily practice. Furthermore, semiautomated assessment of TILs (pathologist guiding automated evaluation by determining the area assessed) can be more effective for the evaluation of TILs. Indeed, further research is necessary to validate recent findings [26,31] on this type of automated analysis.

Multiple prognostic factors (i.e. predictive model) for clinical decision making has been emphasized in recent literature [44]. One important finding (Table 1) is that TILs can identify HPV-positive cases of OPSCC with a favorable prognosis. In Ward et al. study [24], survival was better in HPV-positive OPSCC cases which have a high infiltration of TILs, while HPV-positive cases with low TILs had a prognosis similar to that of HPV-negative cases. Therefore, combination of TILs with other factors (e.g. HPV status) can be useful during treatment planning. Such a combination of prognostic markers is useful also for the prediction of response to immunotherapy [45]. To date, p16/HPV status and PD-L1 expression are the only validated biomarkers that have been incorporated in daily clinical practice, but TILs may prove to be a valuable addition to the current armamentarium of prognosticators and/or predictive biomarkers.

In conclusion, our meta-analysis reported an improved survival of patients with tumors that have a high infiltration of TILs. This group of tumors may represent a distinct clinical entity requiring a specific treatment strategy different from tumors with low TILs but at the same TNM stage. The findings of our meta-analysis combined with the standardized assessment method proposed by IIBWG for TILs in HE-stained sections form an important step toward implementation of TILs in routine daily practice for better risk stratification and treatment planning in OPSSC.

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CRediT authorship contribution statement

Alhadi Almangush: Conceptualization, Study design, Data production, Analysis and interpretation, Manuscript writing, Writing – review & editing. **Rasheed Omobolaji Alabi:** Conceptualization, Study design, Data production, Analysis and interpretation, Manuscript writing. **Stijn De Keukeleire:** Manuscript writing, Writing - review & editing. **Makitie Antti A. Mäkitie:** Conceptualization, Study design, Manuscript writing, Writing - review & editing. **Matti Pirinen:** Conceptualization, Study design, Data production, Analysis and interpretation, Manuscript writing, Writing - review & editing. **Ilmo Leivo:** Conceptualization, Study design, Manuscript writing, Writing – review & editing. All authors have reviewed the manuscript and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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