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The prognostic role of tumor associated macrophages in squamous cell carcinoma of the head and neck: A systematic review and meta-analysis

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ABSTRACT

Head and neck squamous cell carcinoma (HNSCC) is an immunogenic cancer type, and tumor associated macrophages (TAMs) are a major component of the tumor microenvironment (TME). In this systematic review and meta-analysis, studies assessing tumor infiltration with CD68+, iNOS+, HLA-DR+, CD11b+, CD163+, CD206+, and CD204+TAMs were included, and correlation to survival hazard was studied. A low number of CD68+TAMs correlated to better overall survival (OS) in multivariate analysis (HR 1.36 95 %CI (1.07–1.72) $P = .01$). CD68+TAMs did not correlate to disease free survival (DFS), disease specific survival (DSS), progression free survival (PFS), or recurrence free survival (RFS). A low number of CD163+TAMs correlated to better OS in uni- and multivariate analysis (resp. HR 2.65 95 %CI (1.57–4.46) $P = .01$ and HR 2.42 95 %CI (1.72–3.41) $P < .001$). A low number of CD163+TAMs also correlated to better DFS and PFS, whereas a low number of CD204+TAMs only correlated to PFS. While IHC analysis of pan macrophage marker CD68 and M2-like marker CD163 both show prognostic utility in OS, CD163 is a stronger prognosticator, as indicated by multivariate meta-analysis. CD163+TAMs also correlate to DFS and PFS; outcomes that are more relevant to patients, thus showing promising results for future clinical implementation.

Introduction

Head and neck cancer is the sixth most common cancer worldwide, with approximately 890,000 new cases and 450.000 deaths reported in 2020.[2] More than 90% of head and neck cancers are head and neck squamous cell carcinomas (HNSCC), derived from the mucosal epithelium of the oral cavity, pharynx, or larynx.[3,4] Main risk factors for HNSCC include smoking and tobacco use, as well as Human Papilloma Virus (HPV) specifically for oropharyngeal cancer. For patients diagnosed with early stage HNSCC (stage I or II), the prognosis is relatively favorable, with 5-year overall survival (OS) rates between 70 and 90% after treatment with surgery or radiotherapy. However, around 60% of HNSCC patients are diagnosed with locally advanced stage disease (stage III or IV). The 5-year overall survival rate of locally advanced stage disease is poor (less than 50%) due to an increased risk of recurrence and/or distant metastasis.[5]

Prognostic biomarkers predict the natural course of disease and thus, identify the likelihood of patient survival, irrespective of treatment. They serve several purposes in clinical setting, like predicting the risk of poor outcome in an individual, which could aid in managing patient expectations and guiding treatment decisions.[6] Classical cancer-related prognostic factors such as histological tumor type, tumor site, tumor size, lymph node involvement, distant metastasis, and HPV status are leading in the clinical management process of HNSCC patients.[7] The current standard of care is based on risk stratification according to the tumor-node-metastasis (TNM) classification (eight edition) and HPV status. However, the clinical outcome of patients diagnosed with the same TNM-stage is variable.[8] This underlines a need for further refinement of the TNM staging system to predict the clinical behavior of HNSCC better.

The tumor microenvironment (TME) plays a pivotal role in cancer progression and harbors various immune cells, such as macrophages,

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natural killer cells, and lymphocytes. Although not yet used in clinical practice, extensive studying of the TME in recent years has produced some promising prognostic biomarkers, such as tumor infiltrating lymphocytes (TILs).[8,9] Identifying additional prognostic biomarkers within the TME, with respect to the TNM classification, could refine HNSCC risk stratification, and aid in treatment decision making.

Macrophages are of innate origin and are classically divided into two phenotypes: type 1 macrophages (M1) and type 2 macrophages (M2). Classically activated macrophages, or the M1 macrophages, produce proinflammatory cytokines such as IL-12, IL-23, IFN-Y, TNF-a and show strong antimicrobial resistance through phagocytosis and activation of inducible nitric oxide (iNOS). Alternatively, activated macrophages, or the M2 macrophages, produce anti-inflammatory cytokines such as IL-4, IL-10, and IL-13, and they play a central role in antiparasitic immunity, angiogenesis, tissue remodeling and allergic diseases.[10] It is important to state that the discrimination between M1 and M2 macrophages does not consider that macrophage subtypes exist on a broad spectrum and are polarized by the resident microenvironment. The most frequently used immunohistochemical marker for macrophages is CD68, a pan macrophage marker that does not differentiate between subtypes. M1 macrophages could be detected by staining iNOS (inducible nitric oxide synthase), a toxic cytoplasmic enzyme excreted by M1 macrophages, or by surface markers like CD11b and HLA-DR. M2 macrophages could be detected by CD163, a scavenger receptor for the hemoglobin-haptoglobin complex. They can also be detected by CD204, macrophage scavenger receptor 1, or CD206, a mannose receptor of type C lectin.[11,12]

The TME abundantly accommodates a specific class of macrophages: tumor associated macrophages (TAMs).[12,13] TAMs consist of two major subpopulations, the M1-like macrophages that are potent effector cells in the killing of tumor cells, and the M2-like macrophages which stimulate tumor growth and progression through several mechanisms. TAMs that reside within or near the tumor generally show characteristics of the M2 subtype.[14] Pro-tumorigenic M2-polarized TAMs are strongly involved in angiogenesis, which in turn plays an important role in inducing tumor growth and metastasis.[15] Reasoning from the biological function of TAMs within the TME, we hypothesize that M1-like TAMs are correlated with better survival, whereas M2-like TAMs are correlated with worse survival, and they could therefore function as prognostic biomarkers.[16]

This systematic review and meta-analysis aims to provide insight into the prognostic value of macrophage subsets found in the TME, and to thereby contribute to deliberate decision making in clinical practice.

Methods

Search strategy - An extensive systematic search was conducted on the 27th of June 2022 in two databases: PubMed/Medline and EMBASE. As search terms, synonyms of the term 'head and neck squamous cell carcinoma' and a variety of tumor associated macrophage markers were used. The search also included a filter for prognostic studies. The full search strategy is shown in Supplementary Table 1.

In- and exclusion criteria - The selection of studies was conducted by title and abstract screening, followed by full-text reading of the selected articles. The title/abstract screening and full-text reading were conducted by researchers SKB and MvdK, and discrepancies were resolved by discussion. Studies were eligible for inclusion if they assessed the prognostic value of CD68+, iNOS+, HLA-DR+, CD11b+, CD163+, CD206+ and/or CD204 + macrophages in patients with HNSCC by a time-to-event analysis, described as overall survival (OS), disease free survival (DFS), disease specific survival (DSS), progression free survival (PFS), recurrence free survival (RFS) or locoregional control (LRC). See Supplementary Table 2 for the definition of the survival terms. Nasopharyngeal carcinomas were excluded due to distinct pathogenesis. Macrophages had to be evaluated by immunohistochemistry (IHC) and/or immunofluorescence (IF) techniques. Only original articles published in English were included. Animal studies, case reports, reviews, meta-analyses, conference abstracts, or repetitive studies were excluded.

Data extraction - From the studies selected by full-text screening, the following data were extracted: author's last name, year of publication, biomarkers, sample size, tumor subsite, HPV status, treatment modalities, staining and scoring methods, confounders, hazard ratios (HRs) for outcome, confidence intervals, and p-values. Between studies, different definitions regarding tumor compartment were used. Most studies assessed macrophages in the intra-tumoral compartment, meaning the tumor epithelium (TE) or tumor stroma (TS) within the tumor mass. Two studies[17,18] assessed macrophages in the intra-tumoral compartment (IT), which included both TE and TS, and in the peritumoral compartment (PT), which only included stroma on the outskirts of the whole tumor. In this study, HRs for survival were separately extracted for tumor epithelium (TE) and tumor stroma (TS) in the intra-tumoral compartment. These data were entered in a standardized form creating a synopsis of all relevant articles.

Outcome - This study focused on macrophage markers and the correlation with survival (OS, DFS, DSS, PFS, RFS, and LRC) using meta-analysis. The Cochrane handbook advises pooling univariate and multivariate HRs for survival separately because of their different statistical interpretation. Studies were excluded from the meta-analysis if HRs were missing. Data from studies that used IHC were pooled separately from studies that used IF to limit potential heterogeneity.

Critical appraisal - The Quality in Prognosis studies (QUIPS) was used to assess the risk of bias, as described by Hayden et al (2006).[19] This tool comprises six items: study participation, attrition, prognostic factor measurement, outcome measurement, confounding and statistical analysis, and reporting. For each of these items, the risk of bias was scored as low, moderate, or high by two independent researchers (SKB, MvdK). Discrepancies between the researchers were resolved by discussion, after which, the official QUIPS score was determined.

Quality of evidence - The quality of the evidence summarized in this study was rated by GRADE (Grading of Recommendations, Assessement, Development and Evaluations).[20]

Statistical analysis

Concordance between the QUIPS of the reviewers was measured by calculating a Weighted Kappa. In the meta-analysis, HRs for survival were defined by low macrophages versus high macrophages. If the study mentioned HRs as high macrophages versus low macrophages, the reciprocal was used. Statistical heterogeneity was assessed using the I^2 statistics. The meta-analysis was performed in Review Manager version 5.3 [54] using the inverse of variance test with a random effect analysis. Additionally, publication bias was assessed by funnel plots and Egger's test. The funnel plots were created of meta-analysis with at least 10 included studies. Egger's test of $p < 0.05$ indicated the presence of publication bias. Funnel plots and Egger's test were conducted in Stata version 17 [53].

Results

Study selection - The initial search yielded 2009 articles after removing duplicates (Figure 1). After title/abstract screening, 80 articles were eligible for full-text screening, of which 25 articles[17,17,20–42] met the inclusion criteria. Table 1 gives an overview of study characteristics of the studies included in the meta-analysis.

Critical appraisal - The 25 studies included in the meta-analysis were critically appraised using the QUIPS-criteria by the two independent researchers.[19] Concordance between the researchers was considered fair (κ 0.04 CI 0.03–0.78). Four studies[23,28,31,41] scored moderately in the study participation domain due to non-consecutive cohorts, insufficient reporting of treatment method, or inclusion/exclusion criteria. The domain for study attrition was disregarded

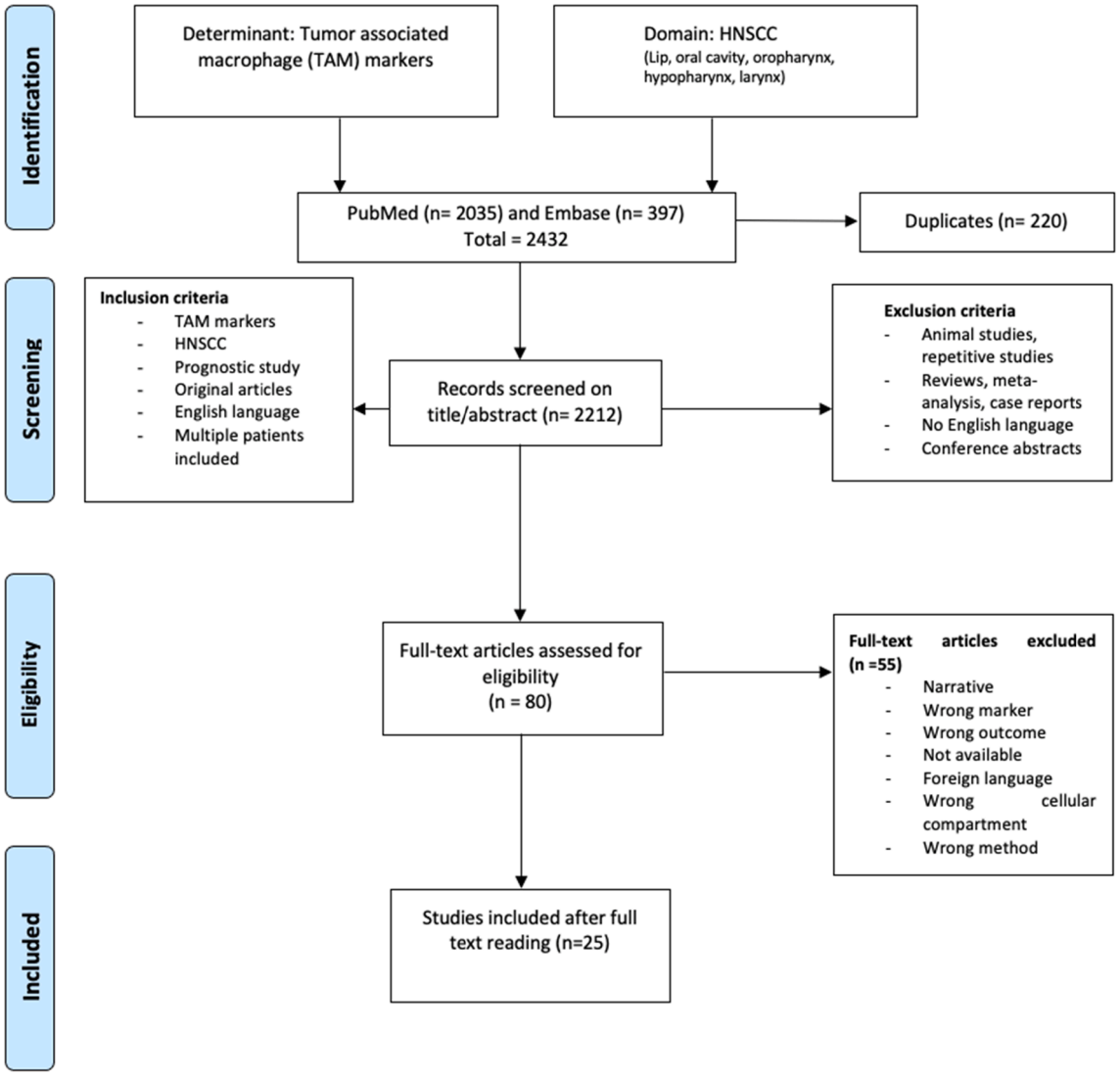


Fig. 1. Of the 80 articles subjected to full-text reading, 25 articles were eligible for inclusion.

because only one study[21] provided information about patients' loss to follow-up. In the prognostic factor domain, many studies used the median value as a cut-off value. Since no consensus is reached about a reasonable cut-off value, the use of medians was scored as low risk. Two studies[22,35] scored moderately in this domain because they provided insufficient information about the histological techniques used to assess the prognostic factor. One study[39] scored moderately in the outcome measurement domain because no follow-up time was mentioned. In the statistical analysis and reporting domain, it was observed that several studies did not conduct multivariate analysis, and this was scored accordingly.[22,32] Data reported in the study by Lee et al. (2015) [35] could not be reproduced in our meta-analysis; this domain was scored as high risk of bias. Therefore, this study was excluded from the meta-analysis. The full quality assessment is summarized in Table 2.

Classical marker CD68 as a prognostic biomarker for survival – A total of nine studies reported on CD68+TAMs and their correlation with OS in a univariate manner by the IHC technique. [17,21,25,27,27,30,33,33,37] The pooled meta-analysis showed no correlation between CD68+TAMs and OS (HR 1.27 95 %CI (0.90–1.79) $P = .17$, Figure 2a). Seven of the studies included in the univariate meta-analysis also reported multivariate HRs. Three studies only reported multivariate HRs.[38,43,44] Pooled meta-analysis of the final ten studies investigating the relationship between CD68+TAMs and OS showed that a low number of CD68+TAMs correlated to a better OS (HR 1.36 95 %CI (1.07–1.72) $P = .01$, Figure 2b).

In the univariate and multivariate pooled analysis, a total of four studies assessed CD68 in both tumor epithelium and tumor stroma. [21,25,37,43] Kikuchi et al. (2020) reported a trend that low CD68+TAMs correlated to better OS in the tumor epithelium. However, this was not the case in tumor stroma. [21] Lin et al. (2011) also supported the latter finding.[43] Ni et al. (2015) reported that low CD68+TAMs were associated with a better OS in the tumor stroma, but tumoral CD68 expression did not have a prognostic impact.[37] Of the studies that did not specify the scoring compartment, Sun et al. (2018), Seminerio et al. (2018), and Wang et al. (2014) also reported a positive correlation between low CD68+TAMs and OS.[27,27,40] The remaining studies found no correlation between CD68+TAMs and OS. In contrast with the previous studies, Ou et al. (2019) report a trend following a high number of CD68+TAMs in tumor epithelium correlating with better OS.[25] When stratifying studies in subgroup based on tumor compartment, no difference was found between TAMs found in tumoral or surrounded stromal tissue (Figure 2c). However, these results need to be interpreted with caution, as high statistical heterogeneity is present.

Three studies[22,29,32] employed IF techniques to study the relationship between CD68+TAMs and OS and reported no significant correlation in univariate analysis (Supplementary Figure 1).

Table 1
Study characteristics of the studies included in the meta-analysis.

Study	Sample size	Subsite	HPV +/-	Stage	Treatment	Biomarkers	Material	Scoring compartment	Technique
Hori 2021	62	T	–	I-IVb	S, S + RR, S + CRT	CD163, Pan-CK, CD4, CD8, FOXP3, CD45RO	FFPE	IT/PT	IHC
Kikuchi 2020	103	OC	–	All	S, S + RT, S + CRT	CD68, PD-1, PD-L1, CD3, CD8, CD4	FFPE	TE, TS	IHC
Tsakiroglou 2020	72	OP	Both	All	RT, CRT	CD68, CD8, PD1, PD-L1	FFPE	NS	IF
Haque 2019	44	OC	–	All	?	CD3, CD20, CD162, CD204, CD206, EGF	FFPE	NS	IHC
Kwon 2019	54	OC	–	All	S, S + RT, S + CRT	CD68, Stabilin-1	FFPE	NS	IHC
Ou 2019	95	OC, OP, L, HP	Both	III, IV	CRT, BRT	CD68, CD163, HLA 1	FFPE	TE, TS	IHC
Zhou 2019	71	L	–	All	S	CD68, CD163, CD3, CD8, CD4	FFPE	IT/PT	IHC
Ryu 2018	396	OC, OP, HP, L, NP, T	–	All	S + CT, RT	CD163, P16, CD3, CD8, C8/144B, FOXP3 ICOS/CD278 , LAG-3 , TIM-3 , CTLA-4, PD-L1, PD-1, c-MetNUT, Trk, TrkB panTrk, cyclin D1	FFPE	TE	IHC
Seminerio 2018	110	OC, OP, HP, L, NP	Both	All	S	CD68	FFPE	NS	IHC
Sun 2018	72	OC	–	I-IVb	CT, RT	CD68, CD31	FFPE/FT	NS	IHC
Cioni 2018	142	OP	Both	All	RT, CRT	CD4, CD8, CD68, FoxP3, CD163, panCK	FFPE/FT	TE, TS	IF
Fang 2017	78	OP	–	All	S	CD57, CD68, CD8, CD4, T-bet	FFPE	TS	IHC
Kubota 2017	46	OC	–	All	?	CD163, CD204, CD25, IL10, CD69	FFPE/FT	TE	IHC
Oguejiofor 2017	124	OP	Both	All	RT, S + RT, CRT, S + CRT	CD68, CD8, PDL1	FFPE	NS	IF
Takahashi 2017	73	T	–	All	S	CD68, CD163, a-SMA, Ki67, p53	FFPE	TS	IHC
Nguyen 2016	278	OC, OP, HP, L	Both	All	S, CRT, RT, P	CD68, CD104, CD8, CD4, CD1a	TMA	TE	IHC
Lee 2015	79	TS	Both	All	CRT	CD68, CD8, CD4	FFPE	TE, TS	IHC
Matsuoka 2015	60	OC	–	II,III, IV	CRT	CD163, a-SMA	FFPE	TS	IHC
Ni 2015	91	OC	–	All	S	CD68	FFPE	TE, TS	IHC
Balermpas 2014	106	OC, OP, HP, L	Both	I-IVb	CRT	CD68, CD163, CD11b, p16, CD31	FFPE	NS	IHC
Fuijta 2014	50	OC	–	All	S	CD163, IL8, FOXp3	FFPE	TE	IHC
Wang 2014	298	OC	–	All	S	CD163, IL-10, IFN-y	FFPE	NS	IHC
Russell 2013	35	OC, OP, HP, L, SN	Both	All	S	CD68, CD3, CD8, FOXp3, CD20, CD16,HLA-DR, HLA-A, HLA-G	FFPE	TE	IHC
Fujii 2012	108	OC	–	All	S	CD68, CD163, a-SMA	FFPE	TE	IHC
Lin 2011	84	L	–	All	S	CD68	FFPE	TE, TS	IHC

Oral Cavity (OC), Oropharynx (OP), Hypopharynx (HP), Larynx (L), Lip (Lip) Tongue (T), Sinonasal (SN), Nasopharynx (NP), Tonsil (TS), Surgery (S), Radiotherapy (RT), Chemotherapy (CT), Chemoradiotherapy (CRT), Immunotherapy (IT), Bio Radiotherapy (BRT), Formalin Fixed, Paraffin Embedded material (FFPE), Fresh tissue (FT), Tissue Microarray (TMA), Tumor epithelium (TE), Tumor stroma (TS), Intra-tumoral (IT), Peritumoral (PT), Immunohistochemistry (IHC), Immunofluorescence (IF), Not specified (NS).

One study, Lin et al. (2011) [43] reported that a low number of CD68+TAMs correlate to a better DFS, especially for stromal TAMs (HR 5.4 95 %CI (1.17–25.47) $P = .02$). Three studies [24,34,41] reported on the correlation between CD68 + TAMs and DSS, and not one significant correlation was found in univariate analysis (Supplementary Figure 2). Another three [25,33,38] studies reported on the correlation between CD68+TAMs and PFS. In both univariate and multivariate analysis, Ou et al. (2019) reported that high CD68+TAMs correlated to a better PFS, whereas the two other studies did not report significant findings. The pooled meta-analysis conducted for multivariate analysis revealed no significant correlation (HR 1.04 95 %CI (0.45–2.40) $P = .93$) (Supplementary Figure 3). Contrary to Ou et al. (2019), Takahashi et al. (2017) did report a significant univariate correlation, in which low CD68+TAMs correlate with better PFS; however, this finding was not maintained in multivariate analysis (resp. HR 4.3 95 %CI (1.26–1.47) $P = .02$ and HR 2.38 95 %CI (0.63–8.90) $P = .20$). [33] Pooled analysis between three studies [24,27,34] that reported on RFS revealed no correlation between CD68+TAMs and RFS (HR 1.62 95 %CI (0.70–3.75) $P = .25$) (Supplementary figure 4).

M2-like markers CD163, CD204, and CD206 as prognostic biomarkers for survival – The pooled analysis for studies reporting univariate HRs found that low CD163+TAMs correlate to better OS (HR 2.65 95 %CI (1.57–4.46) $P = .01$) (Figure 3a). The pooled analysis for studies that reported multivariate HRs also found that low CD163+TAMs correlate to better OS (HR 2.42 95 %CI (1.72–3.41) $P < .001$) (Figure 3b). Three studies reported on the correlation between CD163+TAMs and DFS in a multivariate manner. [18,36,39] The pooled analysis showed that low CD163+TAMs correlated to a better DFS (HR 2.51 95 %CI (1.55–4.09) $P < .001$) (Figure 4). The pooled result of two studies [23,31] revealed no significant correlation between CD163+TAMs and DSS (Supplementary Figure 5). The pooled result for both univariate and multivariate studies showed that low CD163+TAMs correlate to a better PFS (resp. HR 1.86 95 %CI (1.22–2.84) $P = .00$ and HR 1.50 95 %CI (1.02–2.22) $P = .04$) (Figure 5). Hori et al. (2021) also reported on LRC and found that low CD163+TAMs in tumor epithelium correlates to a better LRC (Multivariate HR 5.06 95 %CI (1.12–22.88) $P = .04$). [18]

Haque et al. (2019) and Kubota et al. (2017) reported on the correlation between CD204+TAMs and DSS. [23,31] The pooled result revealed no significance between CD204+TAMs and DSS (Supplementary Figure 6). These two studies also reported on the correlation between CD204+TAMs and PFS. The pooled result revealed that low CD204+TAMs correlate to a better PFS (HR 1.96 95 %CI (1.15–3.35) $P = .02$) (Supplementary Figure 7).

Haque et al. (2019) [23] reported on CD206+TAMs and their correlation with DSS and PFS. For both endpoints, low CD206+TAMs

Table 2
Quality assessment of the studies included in the meta-analysis.

Study	Study participation	Study attrition	Prognostic factor meassurment	Outcome meassurement	Study confounding	Statistical analysis and reporting	Total bias score
Hori 2021	○	●	○	○	○	○	Low
Kikuchi 2020	○	◐	○	○	○	○	Low
Tsakiroglou 2020	○	●	◐	○	○	◐	Moderate
Haque 2019	◐	●	○	○	○	○	Low
Kwon 2019	○	●	○	○	○	○	Low
Ou 2019	○	●	○	○	○	○	Low
Zhou 2019	○	●	○	○	○	○	Low
Ryu 2018	○	●	○	○	○	○	Low
Seminerio 2018	○	●	○	○	○	○	Low
Sun 2018	◐	●	○	○	○	○	Low
Cioni 2017	○	●	○	○	○	○	Low
Fang 2017	○	●	○	○	○	○	Low
Kubota 2017	◐	●	○	○	○	○	Low
Oguejiofor 2017	○	●	○	○	○	◐	Low
Takahashi 2017	○	●	○	○	○	○	Low
Nguyen 2016	○	●	○	○	○	○	Low
Lee 2015	○	●	◐	○	○	●	Moderate
Matsuoka 2015	○	●	○	○	○	○	Low
Ni 2015	○	●	○	○	○	○	Low
Balermpas 2014	○	●	○	○	○	○	Low
Fuijta 2014	○	●	○	◐	○	○	Low
Wang 2014	○	●	○	○	○	○	Low
Russell 2013	◐	●	○	○	○	○	Low
Fujii 2012	○	●	○	○	○	○	Low
Lin 2011	○	●	○	○	○	○	Low

○ = low risk of bias, ◐ = moderate risk of bias, ● = high risk of bias.

correlated with better survival (HR 3.29 95 %CI (1.1–14.1) $P = .03$ and HR 3.28 95 %CI (1.1–14.1) $P = .03$).

M1-like markers iNOS, HLA-DR, and CD11b as prognostic biomarkers for survival – No studies reported on the correlation between iNOS, HLA-DR, or CD11b expressed by TAMs and OS, DFS, DSS, PFS, RFS or LRC.

Quality of Evidence – The strongest certainty in the quality of evidence was present in PFS as outcome, based on the GRADE approach. OS, DSS and RFS had a lower GRADE score on inconsistency, due to high statistical heterogeneity in the pooled meta-analysis. DFS had a lower GRADE score on imprecision, due to the relatively smaller sample size (Supplementary Table 3). Publication bias is an important component of the GRADE approach. To address this, funnel plots were created of the correlation of CD68 with OS, because these meta-analyses were the only ones with at least 10 studies included. The funnel plots showed a slight assymetric distribution, which could indicate potential publication bias for OS as outcome (Supplementary Figure 8A and 8B). However, Egger's test displayed a $p < .19$ for the univariate meta-analysis and a $p < .52$ for the multivariate meta-analysis. Our pooled result of the correlation between CD68 and OS was thus not affected by publication bias.

Discussion

This systematic review and meta-analysis demonstrate that a low number of CD68+TAMs found in the TME of HNSCC correlate with better OS. Furthermore, a low number of CD163+TAMs correlate to better OS, DFS, and PFS. A low number of CD204+TAMs correlate to better PFS.

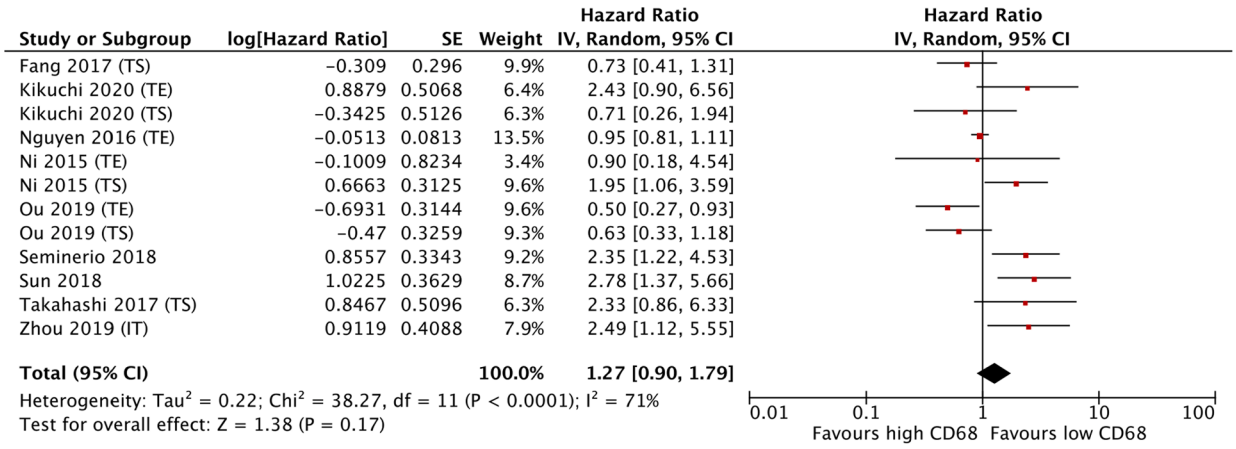
HNSCC is thought to be an immunogenic cancer type, and TAMs are the most abundant type of immune cell present in the TME. [44] In the early stages of neoplastic development, TAMs within the TME predominantly express M1-like features, probably attempting to eliminate tumor cells. As the tumor progresses, tumor cells can excrete soluble biomolecules which activate and polarize TAMs to work for the tumor's benefit, skewing them in the M2-like direction. [15,45] Tumor evasion mechanisms are characteristic traits of cancer, and TAMs have various strategies to achieve this, like the upregulation of checkpoint inhibitors like Programmed cell Death Ligand 1 (PD-L1) to deactivate cytotoxic lymphocytes and the secretion of immunosuppressive cytokines that affect other immune cells. TAMs can also promote tumor metastasis by secreting soluble factors that damage the endothelial basement membrane of blood vessels, like matrix metalloproteases (MMPs). TAMs are drawn to hypoxic tumor regions, where they often upregulate hypoxia inducible factor (HIF)-1a and HIF-2a. This hypoxic environment aids TAMs in angiogenesis by inducing the secretion of angiogenic molecules, like vascular endothelial growth factor (VEGF). [15] Due to the duality in TAM function and subsequent controversial evidence on TAMs in HNSCC, its prognostic role has yet to be determined. This systematic review and meta-analysis fulfill an unmet need by including a broad spectrum of TAM markers (CD68, CD163, CD204, CD206, iNOS, HLA-DR and CD11b) in the quest for clarification of the prognostic role of TAMs within the TME in HNSCC.

Remarkable results of this study are that the univariate meta-analysis did not show a correlation between CD68+TAMs and OS. In contrast, the multivariate meta-analysis showed that a low number of CD68+TAMs correlate to better OS than a high number of CD68+TAMs. This could be explained by greater statistical power in the multivariate analysis, due to a greater sample size. In many studies, markers are only included in the multivariate analysis if they show a certain level of significance in the univariate analysis, which could result in greater statistical power, due to narrower confidence intervals. One pattern can be recognized in the univariate- and multivariate meta-analysis; most individual studies mention that low CD68+TAMs correlated to better survival. Other individual studies mention a trend in that low CD68+TAMs correlates to worse survival, or they mention no correlation at all.

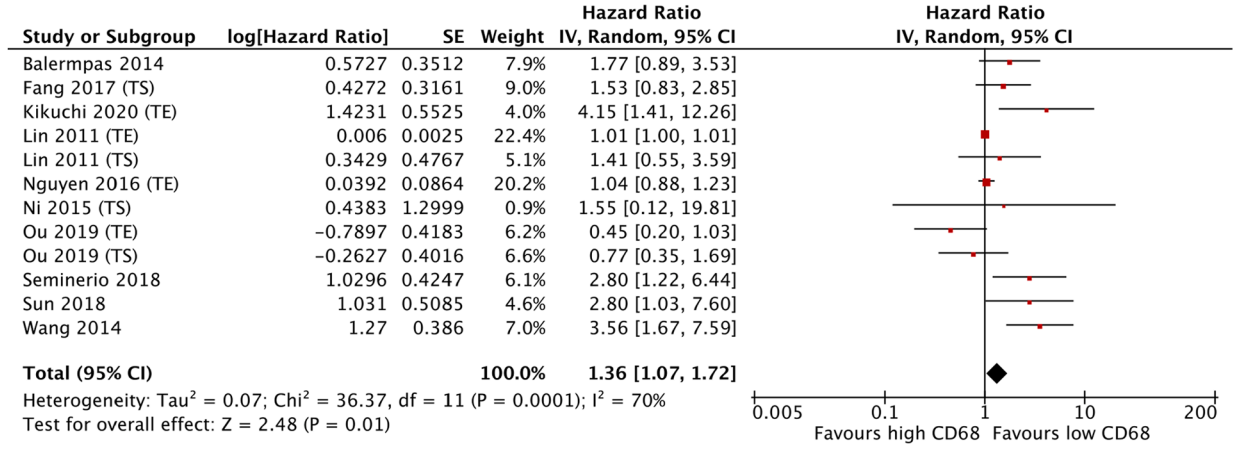
These inconsistencies between individual studies could have different explanations.

One partial explanation could be the specificity of CD68. CD68 is a glycosylated type 1 transmembrane glycoprotein located in lysosomes, intracellularly. It is not specific for cell lineage, but for lysosomal activity. Although it is highly expressed on the monocyte/macrophage

a. Univariate Forest plot CD68+ TAMs and OS



b. Multivariate Forest plot CD68+ TAMs and OS



c. Subgroup analysis of the correlation between CD68+ TAMs and OS in tumor epithelium and tumor stroma

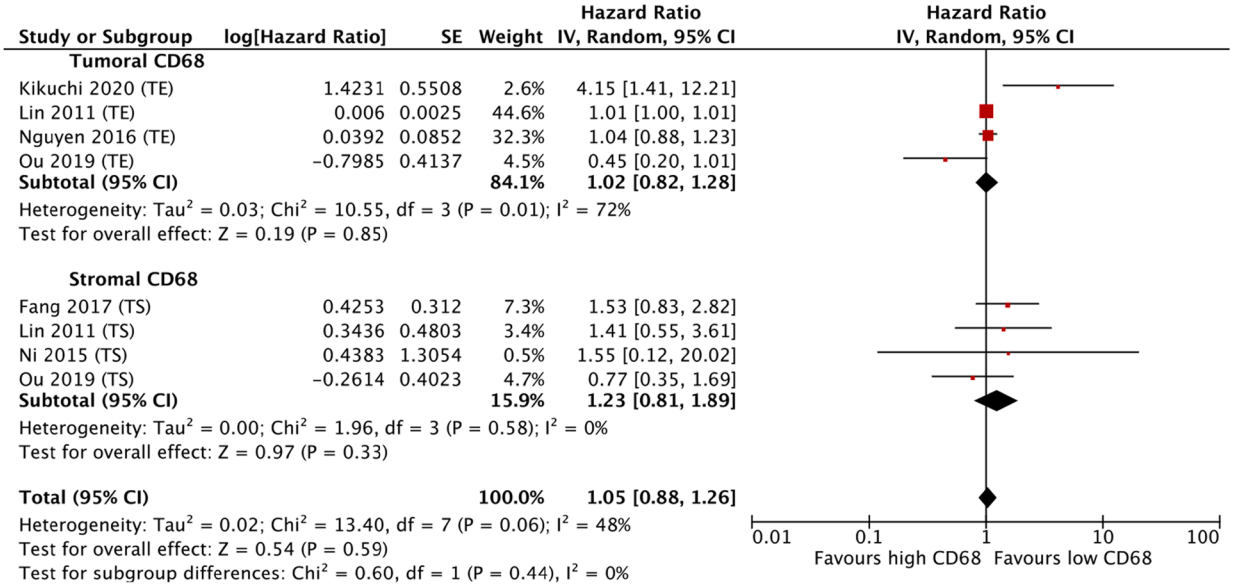
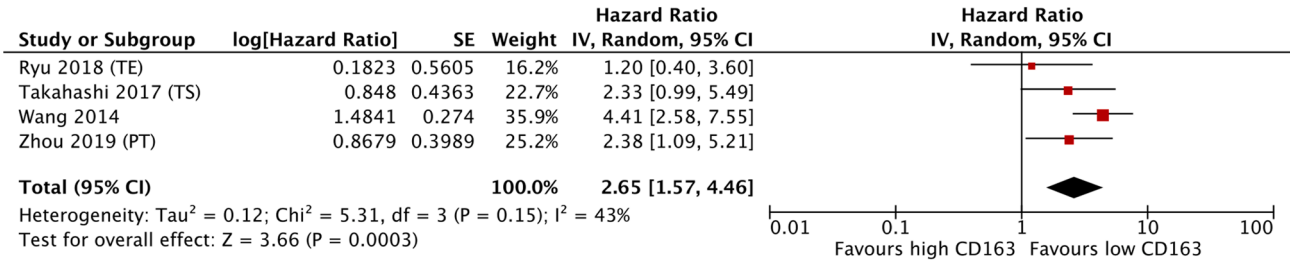


Fig. 2. These forest plots assessed the correlation between CD68+TAMs stained by IHC techniques and OS: 2a. The pooled univariate analysis revealed no correlation between CD68+TAMs and OS. 2b. The pooled multivariate analysis revealed that a low number of CD68+TAMs correlated to a better OS. 2c. Subgroup analysis revealed that there was no significant difference on the number of TAMs in tumoral or stromal tissue.

a. Univariate Forest plot CD163+ TAMs and OS



b. Multivariate Forest plot CD163+ TAMs and OS

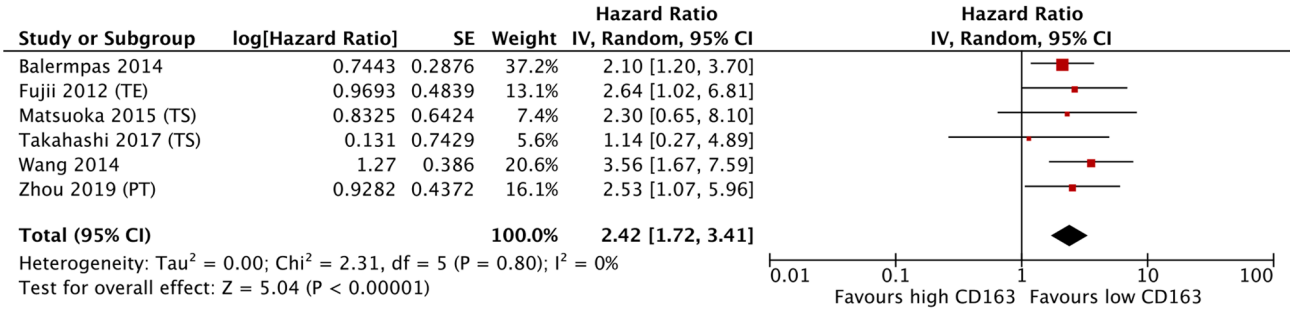


Fig. 3. These forest plots assessed the correlation between CD163+TAMs stained by IHC techniques and OS: 3a. The pooled univariate analysis revealed that a low number of CD163+TAMs correlates to a better OS. 3b. The pooled multivariate analysis also revealed that a low number of CD163+TAMs correlates to a better OS.

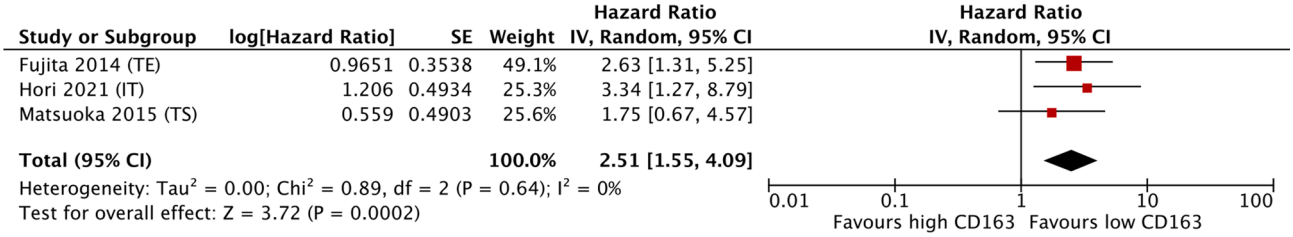


Fig. 4. The pooled multivariate analysis revealed that a low number of CD163+TAMs correlates to a better DFS.

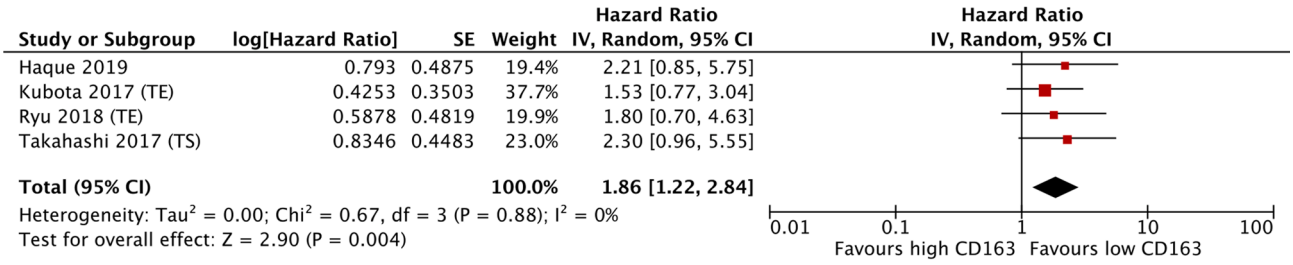
lineage, it is also incidentally expressed on fibroblasts, endothelial cells, dendritic cells, B cells, T cells, basophils, neutrophils, and osteoclasts. [11] Therefore, results on pan-macrophage marker CD68 should be interpreted with discretion.

Another explanation could be the different TAM detection and quantification techniques among studies. [12] Most studies used the 'hotspot' method, but the amount of 'hotspots' differs between studies. Some studies quantified TAMs manually, meaning that at least two pathologists manually counted the macrophages. However, other studies used automatic quantification methods. There is no consensus on a cut-off value of TAMs, resulting in some studies using median values, others using the mean, which could aid in differences. These limitations indicate the need for standardization of TAM detection and quantification, for example, by reaching consensus on the number of 'hotspots' and which cut-off value to use.

A third contributor to the discrepancies could be explained by differences in the tumor compartment in which the TAMs were assessed. [12] For a long time, it was believed that a high abundance of TAMs led to poor survival in various types of solid tumors. However, in the last decade, accumulating evidence arose on the complexity of TAMs residing within the TME. In lung cancer, a high number of M2-like TAMs in the tumor stroma were associated with a poor OS. Interestingly, abundant M1-like TAMs in the tumor epithelium was associated with a favorable OS. [46,47] On the contrary, in breast cancer, a high number of TAMs correlate to worse survival, especially for TAMs found in the tumor stroma. [48] For this matter, a subgroup analysis was conducted in this study to investigate a potential difference between the prognostic value of CD68+TAMs in tumor stroma and tumor epithelium. No difference between TAMs in the tumor or stroma was found. These results, however, need to be interpreted with caution because of high statistical heterogeneity. It is also notable that a clear definition of intra-tumoral and peritumoral compartment within HNSCC seems to be lacking, as studies used different definitions, which complicates the interpretation of the results regarding tumor compartment.

Furthermore, in this study, a low number of CD163+TAMs correlated with better OS in both univariate and multivariate analysis. In univariate analysis, Troiano et al. (2019) found that a low number of CD163+TAMs correlated to better OS. [49] Our study strengthens the results of the former study by the additional finding that a low number of CD163+TAMs correlated to better OS in multivariate analysis. CD163+TAMs also correlated to better DFS and PFS. CD163 is exclusively expressed on cells of the monocyte lineage (monocytes, macrophages, dendritic cells) and could therefore function as a good prognostic biomarker and target for therapy. [50] Based on these results, it is concluded that CD163 is potentially a stronger prognosticator for survival than CD68. Also, CD204 could potentially function as a prognostic biomarker based on the results of this meta-analysis. However, only two studies assessed the prognostic role of this marker, and they reported HRs with wide confidence intervals, which warrants careful interpretation.

a. Univariate Forest plot CD163+ TAMs and PFS



b. Multivariate Forest plot CD163+ TAMs and PFS

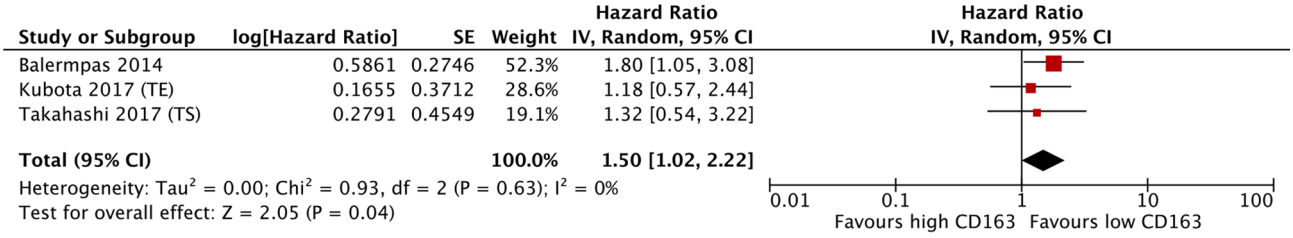


Fig. 5. These forest plots assessed the correlation between CD163+TAMs stained by IHC techniques and PFS: 5a. Even though separate studies did not report a significant correlation, the pooled univariate analysis revealed that a low number of CD163+TAMs correlates to a better PFS, due to increased statistical power. 5b. The pooled multivariate analysis revealed that a low number of CD163+TAMs correlates to a better PFS.

Lastly, in this study, the prognostic role of M1 marker iNOS expressed on TAMs in HNSCC was aimed to be identified. However, no studies on this topic were found. Even though iNOS is a widely known M1-marker, iNOS immunohistochemistry bears challenges, like unreliable results when using paraffine-embedded blocks, due to mRNA degradation.[51] iNOS can also be expressed on tumor cells and endothelial cells, which could blur the actual effect of M1-like TAMs on survival. No studies were found on some other markers for M1-macrophages, including CD11b and HLA-DR. Agarbati et al. (2021) investigated the prognostic role of CD11c M1-like TAMs in tongue squamous cell carcinoma and reported a better DFS in a subgroup of patients with histologic grade 3 differentiation.[52] However, CD11c is a nonspecific marker, mostly expressed on dendritic cells, limiting its specificity. This study was not included in our study because HRs were not reported. Specifically, for M1-like TAMs, a robust marker for IHC seems to be lacking.

The strength of this study lies in the large number of studies included, resulting in the possibility of pooling both univariate and multivariate results. Our study also calculated the interobserver variability between the raters of the QUIPS criteria and employed the GRADE approach to rate the quality of the body of evidence. In meta-analysis conducted on the correlation of CD68+TAMs and OS, Egger's test revealed no publication bias was present, however high statistical heterogeneity was observed, which is most likely the result of clinical heterogeneity. Therefore, the first general limitation of this study is heterogeneity in tumor subsite, among and within studies included in this review. The studies showed heterogeneity in treatment modalities with different mechanisms of action, so the prognostic role of TAMs could also differ. This analysis included both HPV + and HPV- HNSCC, even though we know that HPV+HNSCC is a different entity with better survival outcomes. Furthermore, Seminerio et al. (2018) report a higher number of CD68+TAMs in HPV+/p16+tumors, indicating that HPV is an important confounder.[27] In multivariate analysis, correction of patient related factors is included, allowing a more accurate interpretation of results. A limitation of pooling the multivariate results together in this meta-analysis is that the studies did not all correct for the same variables. Lastly, several studies would have been eligible for inclusion if they provided hazard ratios.

In conclusion, this study showed that in multivariate meta-analysis, CD163 is a potentially better prognosticator for OS than CD68. CD163+TAMs also correlated with DFS and PFS, outcomes that are more relevant in clinical practice. The quality of evidence regarding PFS as outcome was high, indicating strong confidence in the effect estimate. For M1-like TAMs, limited studies have been conducted, which could be attributed to the lack of a robust IHC marker.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The authors confirm that the data supporting the findings of this systematic review and meta-analysis are available within the article and its supplementary materials.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.oraloncology.2022.106227.

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