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Review

Prognostic significance of CD68$^+$ and CD163$^+$ tumor associated macrophages in head and neck squamous cell carcinoma: A systematic review and meta-analysis

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ABSTRACT

Objective: Tumor associated macrophages (TAMs) are among the most abundant cells of the tumor microenvironment. Several studies have been performed to investigate whether TAM markers, namely CD68 and CD163, could serve as prognostic factors in patients with squamous cell carcinoma of the head and neck (SCCHN). The aim of this systematic review and meta-*analysis* was to synthetize the available evidence of the literature about the role of CD68+ and CD163+ TAMs as prognostic factors in SCCHN.

Materials and methods: This systematic review was performed according to the guidelines reported in the Cochrane Handbook and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. Meta-analysis of overall survival, disease-free survival and progression-free survival was performed using the inverse of variance test. A random- or a fixed- effect model was used on the basis of the presence of heterogeneity. Risk of bias assessment and subgroup analysis were also performed.

Results: High stromal expression of CD163+ TAMs correlated with both poor overall survival (HR, 2.26; 95% CI: [1.47, 3.47]; P < 0.001) and progression-free survival (HR, 2.29; 95% CI: [1.11, 4.71]; P = 0.03). Conversely, abundance of CD68+ TAMs was not associated with overall survival (HR, 1.25; 95% CI: [0.86, 1.80]; P = 0.24) and disease-free survival (HR, 2.06; 95% CI: [0.84, 5.05]; P = 0.11).

Conclusions: Findings from this study revealed that whilst IHC analysis of the generic macrophage marker CD68+ has no prognostic utility in patients with SCCHN, the M2-like marker CD163+ predicts poor prognosis. Our data suggest that assessment of CD163+ TAMs in SCCHN has potential for future clinical use. Further well-standardized studies should be performed to confirm these results.

Introduction

Squamous cell carcinoma of the head and neck (SCCHN) is a common neoplasm in humans [1]. SCCHN involves the epithelium of different anatomical sites, such as the oral cavity, nasal cavity, oropharynx, hypopharynx and larynx [2,3]. Treatment of SCCHN relies on different combinations of surgery, radiation and chemotherapy, and the therapy of choice is currently informed by TNM staging and site involved [4]. Despite the improvement of these treatment modalities in the last few years, the overall 5-year survival rate for SCCHN has not decreased and is still about 50% [5]. Targeted therapy against specific molecules and immunotherapy have recently emerged as promising approaches to cancer treatment. Whilst these novel therapies have demonstrated some clinical efficacy, survival rate of these patients has only slightly increased [6].

Most tumors develop tolerance mechanisms against both traditional and target therapy approaches. Tumor resistance to anticancer drugs is a well-known limitation of oncological treatment. It is believed that both tumor and stromal cells are responsible for this phenomenon, which is the result of complex mechanisms taking place in the microenvironment surrounding the tumor cells [7,8]. Foremost in the tumor microenvironment are tumor associated macrophages (TAMs), the most bountiful non-cancerous cell types in the tumor nest and stroma [9]. Evidence indicates that macrophages may be either tumoricidal or

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adopt a pro-tumoral phenotype in vivo. In particular, M1-like are usually activated in response to antigen presentation and inflammatory response [10], whereas M2-like can be involved in cancer growth, angiogenesis, metastasis and therapy resistance [11,12].

Several studies have evaluated the prognostic role of TAMs in different types of cancer, with dubious results. High density of TAMs was linked to a worst prognosis in gastric, thyroid and breast cancer, whereas controversial results were found in bladder cancer [13]. It also emerged that the differential polarization (i.e. M1 or M2 phenotype) of TAMs was linked to differential prognosis [14,15]. Specifically, CD68+ refers to both M1 and M2 activated TAMs, meanwhile CD163+ is a M2-related antigen [16]. The aim of this study was to investigate the link between the expression of CD68+/CD163+ TAMs with both prognostic and clinical outcomes in patients with SCCHN through a meta-analysis of the current literature.

Materials and methods

Protocol and eligibility criteria

This systematic review was performed according to the guidelines reported in the Cochrane Handbook [17] and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [18]. Only studies fulfilling the following inclusion criteria were included in the qualitative and quantitative analysis: (i) prospective and retrospective cohort studies analyzing the expression of CD68+ and/or CD163+ TAM in clinical sections from resective surgery of SCCHN (ii) a minimum number of 20 patients included in each study; (iii) semi-quantitative evaluation performed via immunohistochemistry (IHC); (iv) studies who evaluated the correlation of TAM with patients prognosis calculating at least one of the following parameters: Overall Survival (OS), Disease Free Survival (DFS) and Progression Free Survival (PFS). Studies were included only if either hazard ratio (HR) with its 95% Confidence Interval (CI) or the Kaplan Meier graph were directly reported. In this latter case, the HR of survival analysis and its 95% CI were estimated applying the method by Tierney et al. [19]. Non-human studies, case series with less than 20 patients and case reports were excluded from this systematic review. In addition, only English article were included while no restrictions were applied about the year of publication.

Information source and search strategy

A direct research was performed independently by two authors (GT and VCA) on the following online databases: PUBMED, SCOPUS and Web of Science. MESH terms and free text words were used to search for eligible studies; two Boolean operators (AND, OR) were used to combine the selected key words. The following protocol was used: ((((macrophage OR TAM OR "tumor-associated macrophage" OR CD68 OR CD163))) AND ((head neck OR HNSCC OR SCCHN OR "oral cancer" OR "oral squamous cell carcinoma" OR OSCC OR tongue))) AND ((survival OR prognosis OR biomarker)).

Study selection, data collection and data items

The process of study selection was divided in two phases. In the first phase, authors screened for articles by reading only title and abstract of the studies. The full-text of publications meeting the initial inclusion criteria were analyzed in the second round. At the end of the second phase, the two reviewers (GT and VCA) provided independently a final judgement (include, exclude or uncertain) of inclusion for the selected articles and notified such recommendations to a third author (LLM). This author calculated a value of k-statistic to ascertain the level of reviewers' agreement. In cases of disagreement the same author took a final decision of the inclusion after discussion with the first two reviewers in a joint meeting. At the end of the selection process, papers fulfilling all inclusion criteria were included in the systematic review and meta-analysis.

Data extraction was performed independently by two authors (IA and MT) using an *ad hoc* extraction sheet; subsequently, data were double checked in a joint session with a third author (GT). The following parameters were extracted from each included study: name of the first author, year of publication, nation where the study was performed, head and neck tumor sub localization, TAM localization, TAM biomarker used (CD68+, CD163+ or both), cut-off values, gender, staging, tumor size, rate of lymph node metastasis, HRs and 95% CI for: overall survival (OS), disease-free survival (DFS) and progression-free survival (PFS).

Risk of bias assessment

Risk of bias of the included studies was performed using parameters derived from the Reporting Recommendations for Tumour Marker Prognostic Studies (REMARK) [20,21]. As previously reported, the scale consists of six parameters evaluating: (a) samples, (b) clinical data of the cohort, (c) immunohistochemistry, (d) prognosis, (e) statistics and (f) classical prognostic factors. On the basis of the REMARK guidelines each factor was considered as: adequate, inadequate or not evaluable. Furthermore, an analysis of the risk of bias across studies was performed using Q and I^2 tests. A threshold of P-value lower than 0.05 was considered significant for the presence of heterogeneity in the Q test. The Higgins index was also assessed and classified as follows: low heterogeneity (< 30%), medium heterogeneity (30–60%) and high heterogeneity (> 60%).

Summary measures and planned methods for analyses

The pooled analyses were performed using the software Review Manager version 5.2.8 (Cochrane Collaboration, Copenhagen, Denmark; 2014). For survival analysis of CD68+and CD163+ TAM, the natural logarithmic of the HR and its standard error (SE) were calculated and entered into the software. For the analysis of the influence of CD68+ and CD163+ TAM on clinicopathological features, the risk ratio (RR) and its confidence interval (CI) were calculated in Reviewer Manager. Subsequently, relative risk reduction (RRR) was calculated by authors using the formula: $RRR = 100\% \times (1 - RR)$. The overall effects were calculated using the inverse of variance test setting a P-value lower than 0.05 as threshold of statistical significance, in addition the results of the meta-analysis were summarized in forest plots.

Results

Study selection process and study features

A total of 942 records were screened by title and abstract (first phase). Among these, 54 articles met the initial inclusion criteria and were read full-text. At the end of this second phase, 17 articles [22–38] were considered eligible for inclusion in this systematic review and meta-analysis. Details of the selection process are provided in Fig. 1, while reasons for exclusion of articles that had undergone the full-text reading is reported in Supplemental Material 1. The value of k-statistic was 0.84 which indicates an excellent level of agreement between reviewers. A total of 1528 patients were analyzed in the 17 articles and were all included in our meta-analysis. Geographically, 14 of the included studies were performed in Asia, one in North America, one in South America, and one in Europe (Table 2). CD68+ TAMs were assessed in 12 studies [22,23,26,27,29–37], while CD163+ TAMs were analyzed in 8 studies [24–27,34,36–38]; notably, 4 studies [24,26,34,37] evaluated both TAM subpopulations. Seven studies [23,27,29,30,32,33,35] analyzed CD68+ TAMs in the tumor core, six studies [22,27,29,32,34,37] analyzed TAMs in the stroma, while in 4

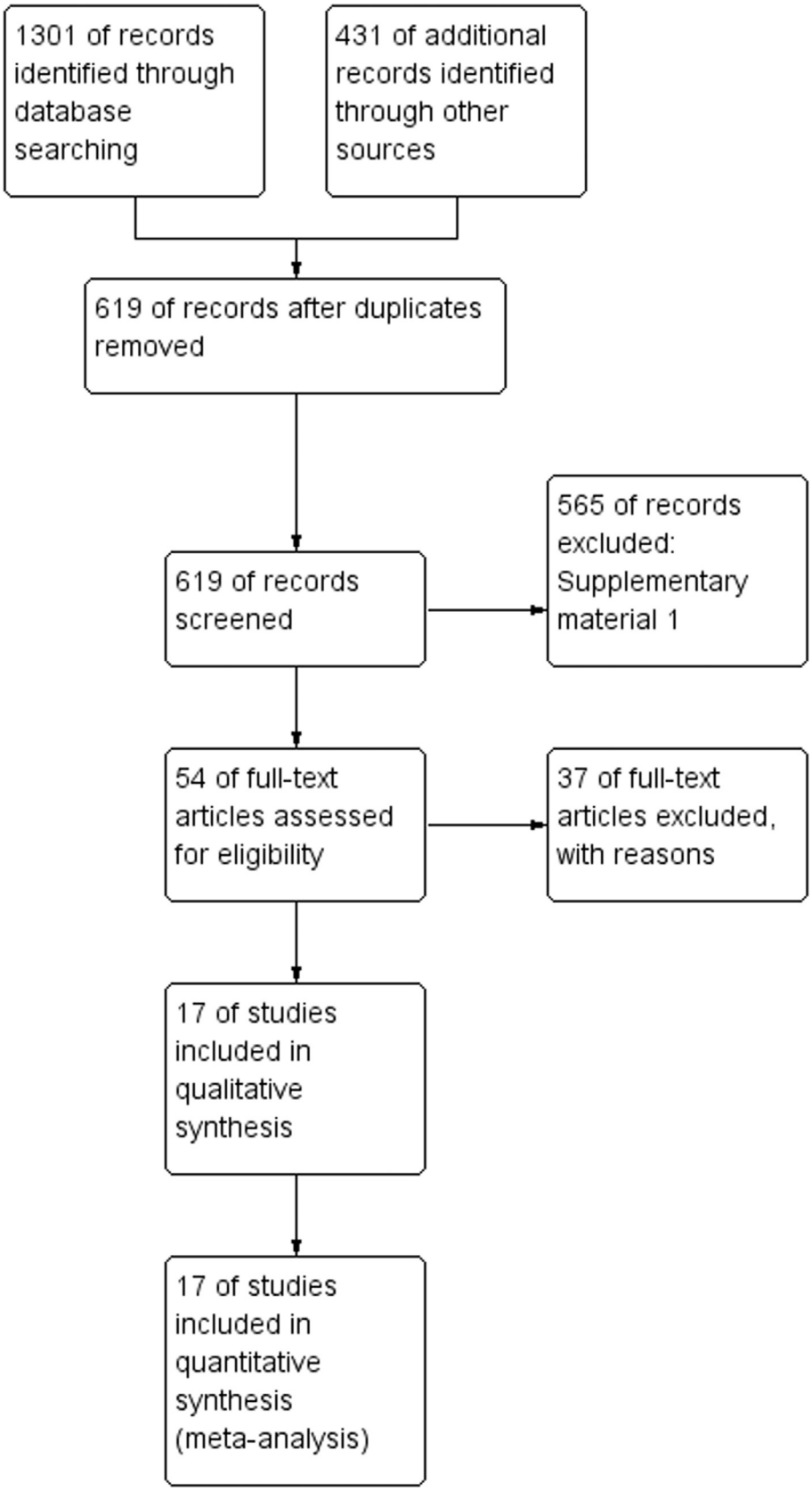

Fig. 1. PRISMA flowchart of the selection process.

Table 1
Evaluation criteria used to assess the quality of studies included in the meta-analysis according to the REMARK guidelines – Included Studies for which: A: Adequate; I: Inadequate; N/A: no description.

Author/year	Country	Samples	Clinical data	Immunohistochemistry	Prognostication	Statistics	Classical prognostic factors
Costa/2012	Brazil	A	A	A	A	I	I
Fang/2017	China	A	A	A	A	I	A
Fujii/2011	Japan	A	A	A	A	A	A
Fujita/2014	Japan	A	A	A	I	I	A
He/2014	China	A	A	A	A	I	A
Hu/2016	China	A	A	A	A	I	A
Kubota/2017	Japan	A	A	A	A	I	A
Lin/2011	Japan	A	A	A	A	I	A
Liu/2007	China	A	A	A	A	I	I
Lu/2009	China	A	A	A	A	I	A
Ni/2015	China	A	A	A	A	A	A
Russell/2014	USA	A	A	A	I	I	I
Sakakura/2016	Japan	A	A	A	A	I	I
Seminerio/2018	Belgium	A	A	A	A	I	A
Sun/2017	China	A	A	A	A	I	I
Takahashi/2016	Japan	A	A	A	A	A	A
Wang/2014	China	A	A	A	A	A	A

studies [25,31,35,36] the area of IHC analysis was not specified. Quantification of CD163+ TAMs was carried out in tumor stroma (five studies [24,25,27,34,37]), tumor core (two studies [27,36]), whole sample [38], whereas in one study [26] the spot selected was unclear. OS, DFS and PFS were used jointly or separately as prognostic endpoints in the included studies (Table 3).

Risk of bias within studies

Four studies fully complied with the REMARKS guidelines, while the remaining thirteen showed weakness in some of the parameters. Absence of risk of bias was detected for the following parameters: samples, clinical data, and immunohistochemistry; deficiencies were present for the other parameters analyzed. More details about the assessment of risk of bias within studies are reported in Table 1.

CD68+ TAM and prognosis in SCCHN

The meta-analysis assessing the association between CD68+ TAM and OS showed no statistically significant differences (HR, 1.25; 95% CI: [0.86, 1.80]; P = 0.24). This meta-analysis was performed at random effect model because of the presence of heterogeneity (I^2 = 63%); it is to note that in this analysis we included studies who evaluated twice the expression of CD68+ TAM in more areas (i.e stroma and tumor area) of samples from the same cohort of patients. The results were similar after excluding these duplicates (HR, 1.47; 95% CI: [0.76, 2.82]; P = 0.25) (Figure 2). Next, we evaluated whether the expression of CD68+ TAM in different sample locations (tumor *vs* stroma) held differential prognostic significance for SCCHN patients. The subgroup analysis revealed no association between tumor (HR, 0.97; 95% CI: [0.58, 1.62]; P = 0.89) or stromal (HR, 1.39; 95% CI: [0.77, 2.49]; P = 0.27) expression of CD68+ TAM and OS (Figure 3). Subgroup analysis was also performed for studies that reported univariate (HR, 1.60; 95% CI: [0.87, 2.92]; P = 0.13) or multivariate (HR, 1.00; 95% CI: [0.96, 0.05]; P = 0.86) survival analysis, revealing the absence of statistically significant differences. Meta-analysis for DFS was performed and again showed no statically significant associations (HR, 2.06; 95% CI: [0.84, 5.05]; P = 0.11). (See Tables 4 and 5).

Table 2
Main characteristics of included studies. N/A: not reported.

Study	*Country*	*N° of patients*	*Staging edition*	*Detection Method*	*Cut-off*
Costa/2012	Brazil	45	N/A	IHC	Median-N/A
Fang/2017	China	78	UICC	IHC	Mean-N/A
Fujii/2011	Japan	108	UICC	IHC	2/HPF for CD68 1.6/HPF CD163
Fujita/2014	Japan	50	WHO	IHC	Median-N/A
He/2014	China	43	UICC	IHC	Mean 17.59 ± 1.91 for CD68Mean 18.33 ± 1.29 for CD163
Hu/2016	China	127	UICC	IHC	6/HPF in the tumor 8/HPF in the stroma
Kubota/2017	Japan	46	IUAC	IHC	Mean-N/A
Lin/2011	Japan	84	2002 TNM	IHC	Mean-N/A
Liu/2007	China	112	AJCC	IHC	Median-163/HPF
Lu/2009	China	92	UICC	IHC	196/HPF
Ni/2015	China	91	WHO	IHC	75% percentile
Russell/2014	USA	32	N/A	IHC	Median-N/A
Sakakura/2016	Japan	74	AJCC/UICC	IHC	199/HPF for CD68 89/HPF for CD163
Seminerio/2018	Belgium	110	N/A	IHC	32/HPF in the tumor 67/HPF in the stroma
Sun/2017	China	65	UICC	IHC	Mean-N/A
Takahashi/2016	Japan	73	UICC	IHC	Median and interquartile range 204 ± 200 for CD68 64 ± 55 for CD163
Wang/2014	China	298	UICC	IHC	iOD: 50

Table 3
Synthesis of data extracted from the included studies related to outcomes pooled in the meta-analysis. N/A: not reported.

Study	*Cluster of Differentiation*	*Type of analysis*	*Follow-up*	*Overall Survival*		*Disease Free Survival Progression Free Survival*	
				HR	*95% C.I.*	*HR*	*95%CI*
Costa/2012	CD68 in the stroma near the invasion front	U	N/A	3,05	Estimated		
Fang/2017	CD68 in tumor	U and M	48 months (median)	0,733	0,411–1,308 (Univariate)		
				1,533	0,825–2,848 (Multivariate)		
Fujii/2011	CD163 tumor invasive front	M	N/A	2,636	1,021–6,803		
Fujita/2014	CD163 in the stroma invasive front	U and M	N/A	4,54	Estimated	3,13	Estimated
He/2014	CD68 in unclear spot	U	21,6 ± 1,2 months (mean)	2,71	Estimated		
	CD163 in unclear spot			3,08	Estimated		
Hu/2016		U	41,2 months (mean)		−0,01–1,390		
	CD68 in stroma		39 months (median)	0,69			
	CD68 in tumor			3,08	2,236–3,914		
	CD163 in stroma						
	CD163 in tumor			0,78	0,085–1,473		
				2,83	1,991–3,669		
Lin/2011	CD68 in tumor	M	N/A	1,006	1,001–1,011 (Multivariate)	4,204	(0,906–19,497) (Multivariate)
	CD68 in peritumor			1,409	0,551–3,603 (Multivariate)	5,455	1,168–25,47 (Multivariate)
Liu/2007	CD68 in tumor	U	N/A	2,28	Estimated		
Lu/2009	CD68 in unclear spots	U		2,95	Estimated	2,35	Estimated
Ni/2015	CD68 in stroma	U and M	N/A	1,947	1,512–10,379 (Univariate)		
				1,55	0,122–19,515 (Multivariate)		
	CD68 in tumor			0,904	0,18–4,552 (Univariate)		
Russell/2014	CD68 in tumor	U	N/A	0,41	0,14–1,17		
Sakakura/2016	CD68 in adjacent to cancer cell field	U and M		1,85	Estimated	2,78	Estimated (PFS)
	CD163 in adjacent to cancer cell field			3,81	Estimated		
Seminerio/2018	CD68 in unclear spot	U and M	N/A	2,353	1,222–4,531 (Univariate)	3,638	1,755–7,539 (Univariate)
				2,833	1,218–6,589 (Multivariate)	4,21	1,672–10,602 (Multivariate)
Sun/2017	CD163 in tumor	M	N/A	2.58	1.95–4.18 (Multivariate)		
Takahashi/2016	CD68 in the stroma invasive front	U and M	N/A	2,332	0,859–6,332 (Univariate)	4,307	1,259–14,742
						2,382	(Univariate PFS)
	CD163 in the stroma invasive front			2,335	0,993–5,49 (Univariate)		0,633–8,961 (Multivariate PFS)
				1,114	0,345–3,597 (Multivariate)	2,304	
						1,322	0,957–5,543 (Univariate PFS)

(*continued on next page*)

Table 3 (*continued*)

Study	Cluster of Differentiation	Type of analysis	Follow-up	Overall Survival		Disease Free Survival Progression Free Survival	
				HR	95% C.I.	HR	95%CI
							0,524–3,338 (Multivariate PFS)
Wang/2014	CD163 in the whole sample	U and M	61,5 months (median)	4,411 3,561	2,578–7,547 (Univariate) 1,733–7,32 (Multivariate)		

CD163+ TAM and prognosis in HNSCC

This meta-analysis was performed at fixed effect model because of the low rate of heterogeneity (I^2 = 30%). Pooled results for the association between CD163+ TAM and OS revealed that higher expression of CD163+ TAM correlates with worse survival in SCCHN patients (HR, 2.26; 95% CI: [1.47, 3.47]; P < 0.001). In this analysis, one study that evaluated the expression of CD163+ TAM twice (more areas of samples from the same patients) was included. These same results were confirmed after excluding this study (HR, 2.77; 95% CI: [1.70, 4.53]; P < 0.001) (Figure 4). Subgroup analysis revealed that these results were dependent upon the type of survival analysis performed. Specifically, the association between CD163+ TAM and OS was significant for studies that performed univariate (HR, 2.52; 95% CI: [1.60, 3.97]; P < 0.001) but not multivariate (HR, 0.99; 95% CI: [0.28, 3.50]; P = 0.99) survival analysis. In addition, when localization of TAM expression was considered, subgroup analysis revealed that the association was significant for stromal (HR, 2.46; 95% CI: [1.43, 4.24]; P < 0.001) but not for intratumoral (HR, 1.93; 95% CI: [0.81, 4.62]; P = 0.14) expression (Fig. 5). Meta-analysis for DFS was not performed because of the absence of available data. Finally, pooled results for PFS revealed a significant association between higher level of CD163+ TAM expression and a worse PFS (HR, 2.29; 95% CI: [1.11, 4.71]; P = 0.03).

Association between CD68+ and CD163+ TAMs and clinicopathological features

The association between clinicopathological characteristics and expression of CD68+ and CD163+ TAMs was also evaluated. In particular, a high expression of CD68+ (OR, 0.65; 95% CI: [0.45, 0.95]; P = 0.03) and CD163+ (OR, 0.63; 95% CI: [0.41, 0.96]; P = 0.03) TAMs was more frequent in females than in males. Subgroup analysis confirmed these results for the stromal (OR, 0.50; 95% CI: [0.30, 0.84]; P = 0.009) but not the intratumoral (OR, 0.88; 95% CI: [0.81, 1.51]; P = 0.63) expression. In addition, no association was found between CD68+ TAMs expression and lymph node metastasis (OR, 0.98; 95% CI: [0.56, 1.72]; P = 0.94), tumor stage (OR, 1.30; 95% CI: [0.74, 2.27]; P = 0.36) and grade (OR, 0.86; 95% CI: [0.57, 1.27]; P = 0.44). Similar results were obtained for the association of CD163+ TAM with lymph node metastasis (OR, 1.48; 95% CI: [0.96, 2.26]; P = 0.07) and tumor stage (OR, 1.50; 95% CI: [0.98, 2.28]; P = 0.06).

Discussion

In the present meta-analysis, we demonstrate that the abundance of CD163+ TAM, but not of CD68+ TAM, correlates with poor survival in HNSCC patients. Furthermore, the predictive power of this association correlates with CD163 expression in the tumor stroma, but not in the tumor core.

These results support the role of the microenvironment in cancer progression and strongly suggest that distinct subgroups of TAM are pro-tumorigenic.

The findings obtained after years of cancer research has revealed the important role of the tumor microenvironment in cancer initiation, progression and response to therapies [39]. TAMs are usually abundant in the tumor microenvironment and can play different role on the basis of their polarization. It is known that TAMs can be distinguished in M1 and M2 subtypes [40]. M1 TAMs usually act against cancer cells showing proinflammatory and antitumoral proprieties, while M2 TAMs play an immunosuppressive and protumoral role favoring cancer progression [41]. CD68 and CD163 are the biomarkers more frequently

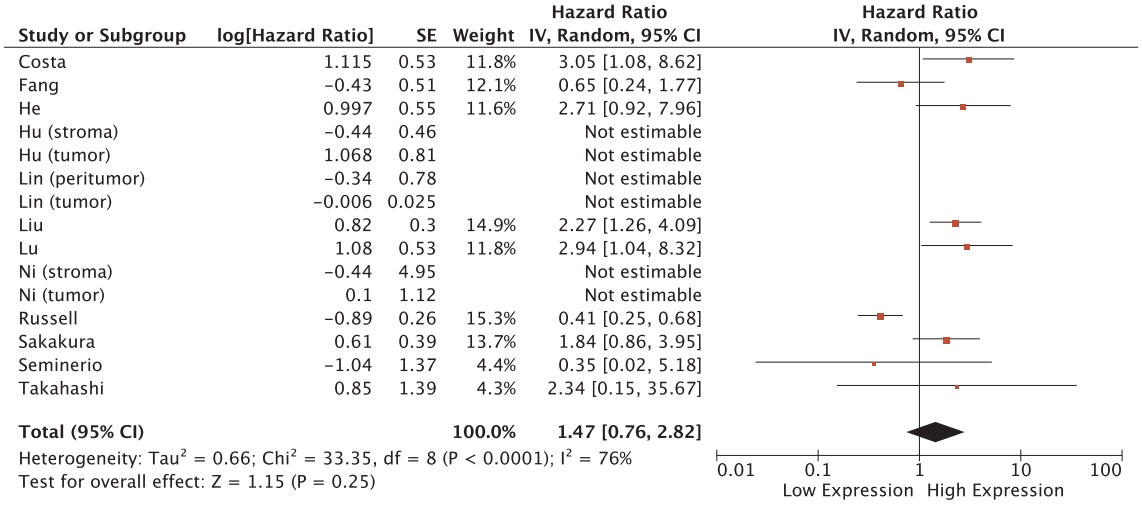

Fig. 2. Meta-analysis at random-effect model for the correlation between CD68+ TAMs expression and OS in SCCHN patients, omitting studies who evaluated twice the expression of CD68+ TAMs in more areas of samples from the same cohort.

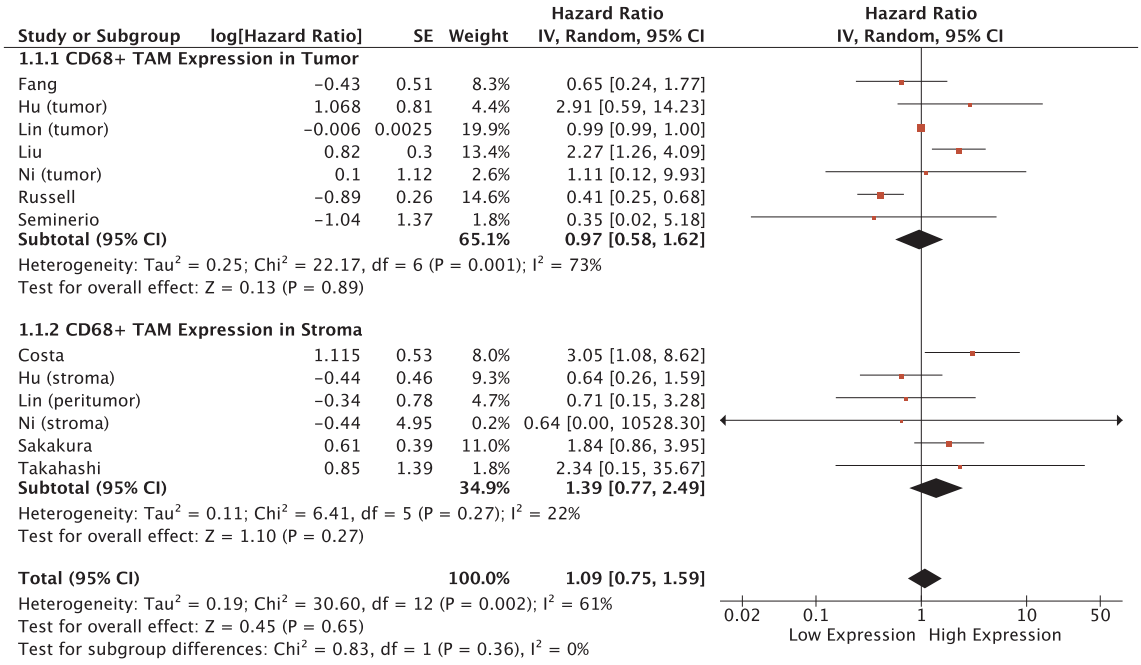

Fig. 3. Subgroup analysis on the basis of the intratumoral or stromal localization of CD68+ TAMs in SCCHN samples.

used to detect TAMs in tumor specimens. In particular, CD68 identifies the whole population of TAMs regardless of their polarization, while CD163 is mainly used for the detection of polarized M2 TAMs [42]. CD163 is a member of the scavenger receptor cysteine-rich family (SRCR) and is exclusively expressed on cells of the monocyte lineage [43]. This molecule has gained considerable attention in cancer research as it is expressed in tumor-promoting, M2-like macrophages. Whether CD163 plays a role in cancer progression in vivo, however, has not been elucidated to date.

Several studies have been published that assessed the prognostic significance of CD68+ and CD163+ TAMs in SCCHN, with inconsistent results. For this reason, we undertook a systematic review with meta-analysis of the literature in order to provide stronger evidence on this topic.

After a thorough selection process, 16 studies were included in this review and, among these, CD68+ TAMs were analyzed in 12 studies and CD168 + in 8 studies. Risk of bias assessment revealed that most studies present limits concerning: reporting, statistics and prognostic factors (Table 1). However, all the studies included in the synthesis were adequate in more than half of the parameter analyzed, hence the risk of bias of these studies can be considered medium overall. Results of the meta-analysis for CD68+ TAMs revealed the absence of a significant correlation with either OS or DFS. Such findings were also confirmed by a subgroup analysis performed on the basis of CD68+ TAMs localization (tumor or stroma) and type of statistical analysis (univariate or multivariate). Conversely, a high expression of CD163+ TAMs correlated with worst OS of SCCHN patients. Interestingly, only stromal expression of CD163+ resulted to be statistically significant (HR, 2.46; 95% CI: [1.43, 4.24]; $P < 0.001$), whereas no significance was detected for intratumoral expression (HR, 1.93; 95% CI: [0.81, 4.62]; $P = 0.14$). However, these results could be influenced by the type of statistical analysis because significance was present only for

Table 4
CD68 expression related to the rate of: Lymph-node metastasis (LNM), Gender, Tumor Size, Grading and Staging in SCCHN patients. N/A: not reported; Unclear: data were reported but they were not clear.

CD68 Expression									
Study	Subsite location	High with LNM	Low with LNM	High in male	Low in male	High in female	Low in female	High expression	Low expression
Fang J/in tumor	Oral	13	17	28	30	9	11	37	41
Fujii N/in tumor invasive front	Oral	22	15	27	40	24	17	51	57
Hu Y/in stroma	Oral	39	16	44	30	40	13	84	43
Hu Y/in tumor	Oral	33	22	40	34	35	18	75	52
Lin Jia-Y/in tumor	Supraglottic larynx	10	24	40	37	2	5	42	42
Lin Jia-Y/in peritumor	Supraglottic larynx	18	16	38	39	4	3	42	42
Study	Subsite location	High with pT3-pT4	Low with pT3-pT4	High in G2-G3 grade	Low in G2-G3 grade	High in Stage III-IV	Low in Stage III-IV	High expression	Low expression
Fang J/in tumor	Oral	7	13	8	13	17	25	37	41
Fujii N/in tumor invasive front	Oral	26	21	Unclear	Unclear	35	27	51	57
Hu Y/in stroma	Oral	23	15	32	19	51	22	84	43
Hu Y/in tumor	Oral	23	15	33	18	45	28	75	52
Lin Jia-Y/in tumor	Supraglottic larynx	30	24	32	37	N/A	N/A	42	42
Lin Jia-Y/in peritumor	Supraglottic larynx	30	24	34	35	N/A	N/A	42	42

Table 5
CD163 expression related to the rate of: Lymph-node metastasis (LNM), Gender, Tumor Size, Grading and Staging in SCCHN patients. N/A: not reported; Unclear: data were reported but they were not clear.

CD163 Expression									
Study	Subsite location	High with LNM	Low with LNM	High in male	Low in male	High in female	Low in female	High expression	Low expression
Fujii N/in tumor invasive front	Oral	21	16	28	39	23	18	51	57
Hu Y/in stroma	Oral	32	23	42	32	31	22	73	54
Hu Y/in tumor	Oral	36	19	38	36	37	16	73	54
Study	Subsite location	High with pT3-pT4	Low with pT3-pT4	High in G2-G3 grade	Low in G2-G3 grade	High in Stage III-IV	Low in Stage III-IV	High expression	Low expression
Fujii N/in tumor invasive front	Oral	22	25	Unclear	Unclear	31	31	51	57
Hu Y/in stroma	Oral	21	17	32	19	44	29	73	54
Hu Y/in tumor	Oral	24	14	34	17	47	26	73	54

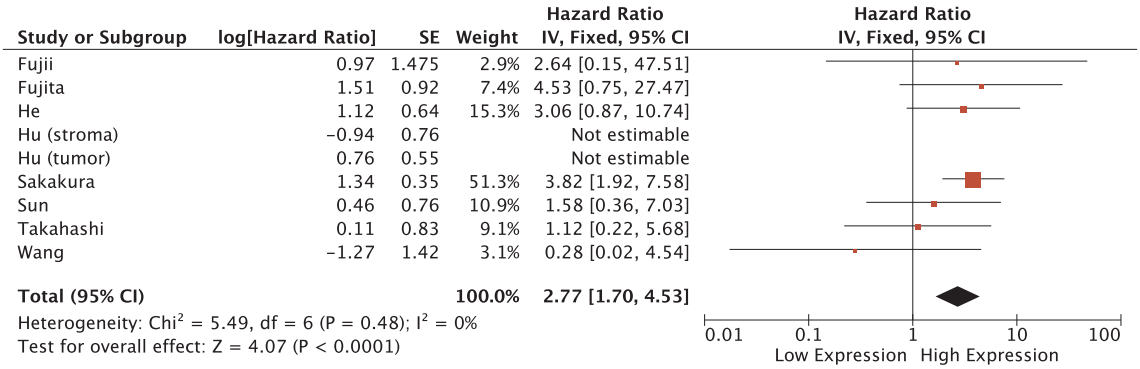

Fig. 4. Meta-analysis at fixed-effect model for the correlation between CD163+ TAMs expression and OS in SCCHN patients, omitting studies who evaluated twice the expression of CD163+ TAMs in more areas of samples from the same cohort.

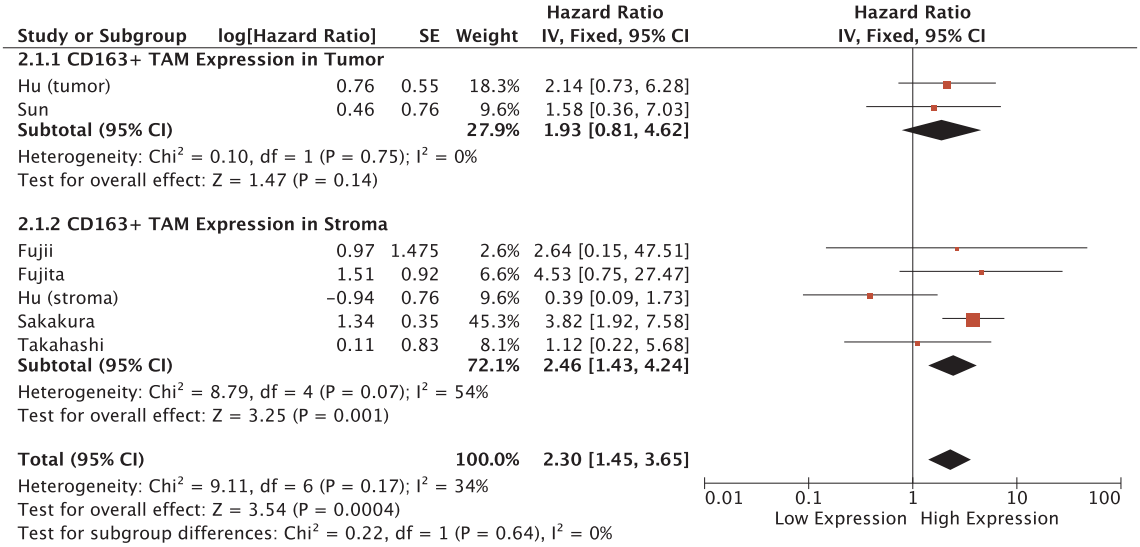

Fig. 5. Subgroup analysis on the basis of the intratumoral or stromal localization of CD163+ TAMs in SCCHN samples.

studies that performed univariate survival analysis, but not for those reporting multivariate analysis. These findings support the role of M2 polarized macrophages as prognostic factors in SCCHN, although more well-standardized studies are needed to confirm these results. In addition, our results revealed a correlation of CD163+ TAMs with PFS, however only two studies were included in this meta-analysis. Hence, the power of this evidence is too low to be taken into consideration for clinical purposes. With regards to the association with clinicopathological features, this meta-analysis showed that neither CD68+ nor CD163+ TAMs correlated with the rate of lymph node metastasis or with tumor stage and grade. Interestingly, a high expression of both CD68+ and CD163+ TAMs was significantly more frequent in females than in males. This is consistent with previous studies investigating other immune biomarkers such as programmed cell death ligand-1 (PD-L1) [44,45].

In this meta-analysis, we demonstrated that stromal CD163+ TAMs

correlate with poor prognosis in SCCHN. These results have salient clinical and translational implications. Clinically, stromal expression of CD163+ TAMs, but not of pan-macrophage marker CD68, could be used to inform the selection of appropriate treatment and follow up of SCCHN patients. Translationally, the phenotype of M2-like macrophages, and specifically CD163, constitutes a potential target for immunotherapies or for targeted anticancer treatments. Several approaches to block TAMs have been investigated to date for SCCHN, including TAMs inactivation by means of the colony stimulating factor 1 (CSF-1)/CSF-1 receptor (CSF-1R) inhibitors or strategies to reprogram TAMs from M2 protumoral phenotype toward M1 antitumoral phenotype [41]. All these studies, however, have restricted their analyses to CD68+ cells. Evidence now exists that CD163, rather than CD68, can be successfully targeted in cancer treatment: for example, knocking-down CD163 in cancer cells inhibits tumor growth in vivo [46] and a CD163-targeted STAT3-inhibitory drug consisting of corosolic acid (CA) packaged within long-circulating liposomes (LCLs) induces M1-like reprogramming [47]. Therefore, it will be of paramount importance to plan future research in light of the key role of CD163+ macrophages in cancer biology.

This study presents some limitations. First, we included studies who evaluated tumors regardless of the head and neck subsite involved without taking into account the rate of HPV infection. Secondly, some studies did not perform multivariate survival analysis or did not report HR and its 95% CI. Multivariate analysis allows for adjustment for patient-related factors, which could potentially affect the survival time of the patients. In addition, cut-off values in the studies included in the meta-analysis differ, thus adding another confounding variable. On the other hand, the number of studies included in the meta-analysis of OS is adequate leading to a good statistical power of the results.

Conclusion

Results of this study demonstrated that the stromal expression of CD163+ TAMs correlated with a worse prognosis of SCCHN patients. Conversely, the immunohistochemical analysis of CD68+ TAMs did not show prognostic utility in patients with SCCHN. Further well-standardized prospective randomized studies should be performed to confirm these results.

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Conflict of Interest Statement

All the authors declare the absence of a conflict of interest related to this study.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.oraloncology.2019.04.019.

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