

## Original Article

# Prognostic value of tumor-associated macrophages in classic Hodgkin's lymphoma: systematic review and meta-analysis

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**Abstract:** This meta-analysis investigated the prognostic value of CD68+/CD163+ tumor-associated macrophages (TAMs) in patients with classic Hodgkin's lymphoma. A search was conducted in the PubMed and EMBASE databases for eligible studies published up to May 2015 containing survival data for patients with classic Hodgkin's lymphoma and CD68+/CD163+ TAMs. Thirteen relevant studies comprising 1951 patients were retrieved and analyzed. CD68+/CD163+ TAMs were associated with shorter overall survival (HR for CD68: 2.63,  $P < 0.001$ ; HR for CD163: 3.1,  $P < 0.001$ ) and shorter progression-free survival (HR for CD68: 1.51,  $P < 0.001$ ; HR for CD163: 1.63,  $P < 0.001$ ). The results indicate that CD68+/CD163+ TAMs are strongly associated with poorer outcomes in patients with classic Hodgkin's lymphoma and constitute a potential therapeutic target.

**Keywords:** Classical Hodgkin lymphoma, tumor associated macrophage, prognosis

## Introduction

Despite advances in the treatment of classic Hodgkin's lymphoma (cHL), roughly 10% of patients with early-stage disease, and 20%-30% of patients in advanced-stage, are incurable under the current therapies [1-3]. Furthermore, a similar proportion of patients are over-treated, resulting in both short-term and long-term therapy-related complications. The International Prognostic Score [4] and other prognostic models [5] for risk stratification of patients with cHL have been used for decades, but their power has weakened with modern treatments. New prognostic markers are required.

During tumor initiation, macrophages transition from an inflammatory to a tumor-associated phenotype, and then have pivotal roles in the development, invasion, and migration of tumor cells [6]. However, the prognostic role of tumor-associated macrophages (TAMs) in cancer has been controversial [7]. In 2010, Steidl et al. [8] reported that high infiltration of TAMs in the microenvironment of cHL, detected by CD68 immunohistochemical (IHC) stain, negatively correlated with prognosis. Supportive and

similar results have been reported since then for CD68 or CD163 [9-14]. Yet, the results of other studies have differed [15, 16], and there has been no large-scale study to confirm or deny this issue to date. Thus, we performed the present systematic review to investigate the prognostic significance of TAMs in patients with cHL.

## Materials and methods

### *Inclusion and exclusion criteria*

The inclusion criteria for this meta-analysis were: cHL patients with new diagnoses; evaluated TAMs using anti-CD 68 antibody or anti-CD 163 antibody, or both via IHC; analyzed a correlation between CD68 or CD163 TAMs and survival or relevant data; and original articles in the English language. Case control studies and studies without survival data were excluded. The primary outcomes of interest were overall survival (OS) and progression-free survival (PFS).

### *Search strategy*

We searched the PubMed and EMBASE databases from commencement until May 2015 to

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**Table 1.** Characteristics of included studies

First author, year	Ref.	Country	Score	Patients, n	Follow-up	Age, y	Stage <sup>a</sup>	Regimens
Agur, 2014	[24]	Israel	24	98	45 m (9-94)	35 (18-85)	36/64 <sup>b</sup>	ABVD/BEACOPP
Azambuja, 2012	[19]	Brazil	29	241	6 y (0.06-11.7)	29 (15-82)	106/135 <sup>b</sup>	ABVD
Choe, 2014	[21]	South Korea	27	121	7 y (0.3-15.7)	38.2 (10-80)	66/49 <sup>b</sup>	ABVD
Jakovic, 2012	[25]	Serbia	28	85	88 m	35 (16-68)	85 <sup>b</sup>	ABVD
Kamper, 2011	[11]	Denmark	21	262	7 y (0.2-18.6)	37 (6-86)	172/90 <sup>c</sup>	ABVD
Kayal, 2014	[15]	India	29	100	68.8 m	21 (8-62)	36/64 <sup>b</sup>	ABVD/EVAP
Klein, 2014	[18]	USA	20	81	NA	NA	23/52 <sup>d</sup>	ABVD
Panico, 2013	[13]	Italy	25	121	42 m (1-112)	33 (12-84)	76/45 <sup>b</sup>	ABVD
Sanchez-Espiridion, 2012 <sup>e</sup>	[16]	Spain	25	266	NA	NA	54/212 <sup>d</sup>	NA
		USA		103	NA	NA	20/83 <sup>d</sup>	NA
Tan, 2012	[10]	USA	36	142	5.5 y	32 (18-79)	31/113 <sup>d</sup>	ABVD/Stanford V
Tzankov, 2010	[20]	Switzerland	25	105	142 m	34 (13-87)	66/39 <sup>c</sup>	ABVD/COPP
Yoon, 2012	[9]	South Korea	30	144	5.4 y (0.7-19)	33.5 (15-77)	48/96 <sup>b</sup>	ABVD/MOPP/BEACOPP
Zaki, 2011	[17]	Japan	22	82	47.5 m (8.8-115)	54 (15-85)	47/32 <sup>c</sup>	ABVD

<sup>a</sup>Ann Arbor stage; <sup>b</sup>Early/advanced; <sup>c</sup>I+II/III+IV; <sup>d</sup>IV/others; <sup>e</sup>Two cohorts included in this article were analyzed separately; NA: Not available.

**Table 2.** The cutoff values of CD68 or/and CD163 in included studies

First author, year	Ref.	Marker	Cutoff points	CD68 Cutoff	High CD68	CD163 Cutoff	High CD163
Agur, 2014	[24]	CD68	Previously reported	S <sup>a</sup>	47%	-	-
Azambuja, 2012	[19]	CD68, CD163	Previously reported	5%	83.4%	5%	57.84%
Choe, 2014	[21]	CD68, CD163	Previously reported	5%	60.33%	33%	63.64%
Jakovic, 2012	[25]	CD68	Previously reported	25%	38.82%	-	-
Kamper, 2011	[11]	CD68, CD163	Highest quartile	7.8%	25.57%	21.1%	24.73%
Kayal, 2014	[15]	CD68	Highest quartile	18.2%	50%	-	-
Klein, 2014	[18]	CD68, CD163	Previously reported	25%	NS	25%	28.4%
Panico, 2013	[13]	CD68	Optimal	30 TAM <sup>c</sup>	38.84%	-	-
Sanchez-Espiridion, 2012 <sup>b</sup>	[16]	CD68, CD163	Median	NS	50%	NS	50%
		CD68, CD163	Median	NS	50%	NS	50%
Tan, 2012	[10]	CD68, CD163	Optimal	12.7%	45.07%	16.8%	43.45%
Tzankov, 2010	[20]	CD68	Optimal	0.82%	40.95%	-	-
Yoon, 2012	[9]	CD68, CD163	Optimal	20%	45.83%	20%	53.15%
Zaki, 2011	[17]	CD68, CD163	Median	60.3 TAM <sup>c</sup>	50%	93.8 TAM <sup>c</sup>	50%

<sup>a</sup>High CD68 expression was defined as  $\geq 3$  of 6 high-power fields showing  $> 25\%$  positive cells, and none of the high-power fields showing  $< 5\%$  positive cells; <sup>b</sup>Two cohorts included in this article were analyzed separately; <sup>c</sup>Mean number of TAMs in high power fields. NS: not stated.

identify eligible articles. The search terms were: 'macrophage' AND "Hodgkin's lymphoma" OR 'Hodgkin's disease' AND "course" OR 'follow-up studies' OR 'prognosis' OR 'predict' OR 'mortality' OR 'epidemiologic studies' OR 'incidence'. All references within the potential literature were manually searched for additional studies.

### Data extraction

Two researchers independently evaluated the potential literature according to the above criteria and extracted data from the eligible studies.

A consensus process was conducted when disagreements were encountered.

### Definition of the methods for cut-off values

There were 3 methods utilized to determine the cut-off values for survival related to CD68+ and CD163+ levels. In the first, the cut-off value was determined as the median number of positive cells, or the quintile of patients with the highest expression, compared with the lower 3 quartile values [11, 17]. The second used the reported cut-off points, that is, the cut-off values of CD68+ and CD163+ cells were set for

5% and 25%, respectively, since these are widely used in the literature [8, 18, 19]. The third method selected the optimal cut-off points, which were considered the cut-off values for CD68+ and CD163+ that best predicted the survival rates [20, 21].

#### Quality assessment

The quality assessment was performed in accordance with the guidance provided by the Centre for Reviews and Dissemination Systematic Reviews 2009, for undertaking reviews in healthcare with modification [22]. Briefly, all studies were evaluated based on 6 main categories with a total possible score of 40: representativeness of patients; follow-up time; definition and measurement of outcome; measurement of IHC markers; analysis; intervention standardization or randomization.

#### Statistical analysis

Study-specific estimates of the hazard ratio (HR) and related confidence interval (CI) were extracted if reported. The logarithm of the HR, and standard errors for the logarithm of the HR, were calculated. The total number of events, the number of patients at risk in each group, and the log-rank statistic or its *P* value was used to derive an approximate estimate of the HR for the rest. The Q test was employed for heterogeneity evaluation and the  $I^2$  statistic was calculated for quantitative analysis. The fixed effects model was selected for meta-analysis if no statistical heterogeneity existed among the studies ( $P \geq 0.10$ ,  $I^2 \leq 50\%$ ); otherwise the random effects model was chosen ( $P < 0.10$ ,  $I^2 > 50\%$ ). Potential publication bias was examined by Begg's funnel plot and Egger's test. Influence analysis was used for sensitivity analyses. All statistical analyses were performed with Stata 12.0 software.

#### Results

In PubMed and EMBASE, 263 and 260 articles, respectively, were initially identified. Thirty-one articles were retrieved based on screening the title and abstract. After further review, 13 studies comprising 1951 patients were found eligible for the meta-analysis (Table 1). The sample sizes of the included studies ranged from 81 to 369, with a median follow-up of 42 months.

Eight studies included both CD68 and CD163 as TAM markers, and the rest used only CD68. The levels of CD68 and CD163 revealed via IHC

were analyzed by computer in 5 studies, and visual scoring by manual in the remaining studies. To determine cut-off values, 4 studies used optimal cut-off points, 4 selected median percentile or highest quartile values, and 5 chose the reported cut-off points (Table 2). The results of survival analyses by individual study are shown in Table 3.

Nine studies reported the OS for the CD68 TAM marker with HR, or data from which the HR could be calculated (Figure 1). HRs ranged from 1.4 to 3.5. For the entire population, the overall HR of an increased number of CD68+ TAMs infiltration for OS was 2.63 (95% CI: 2.0-3.45;  $P < 0.001$ ; Figure 1A). For the subpopulation of patients with TAMs measured by manual visual scoring, the HR was 2.72 (95% CI: 1.9-3.89;  $P < 0.001$ ). For the subpopulation of patients with TAMs measured by computer, the HR for OS was 2.5 (95% CI: 1.64-3.82;  $P < 0.001$ ). For studies in which the cut-off points were the median percentile or highest quartile values, the overall HR for OS was 2.25 (95% CI: 1.44-3.51;  $P < 0.001$ ). For studies that chose the reported cut-off points, the overall HR for OS was 2.65 (95% CI: 1.52-4.6;  $P = 0.001$ ). For studies that used the optimal cut-off points, the overall HR was 3.04 (95% CI: 1.96-4.73;  $P < 0.001$ ). No heterogeneity was observed.

HRs for PFS ranged from 0.62 to 2.34 (Figure 1B). For the entire population, the overall HR of high CD68 TAMs for PFS was 1.51 (95% CI: 1.25-1.83;  $P < 0.001$ ). For the subpopulation of patients with TAMs measured by manual visual scoring, the HR for PFS was 1.51 (95% CI: 1.02-2.25;  $P = 0.04$ ). For the subpopulation of patients with TAMs measured by computer, the HR for PFS was 1.49 (95% CI: 1.17-1.89;  $P = 0.001$ ). For studies in which the cut-off points were the median percentile or highest quartile values, the overall HR for PFS was 1.35 (95% CI: 1.03-1.78;  $P = 0.031$ ). For studies that chose the reported cut-off points, the overall HR for PFS was 1.43 (95% CI: 0.98-2.09;  $P = 0.06$ ). For studies that used the optimal cut-off points, the overall HR for PFS was 1.91 (95% CI: 1.34-2.72;  $P < 0.001$ ). No heterogeneity was found.

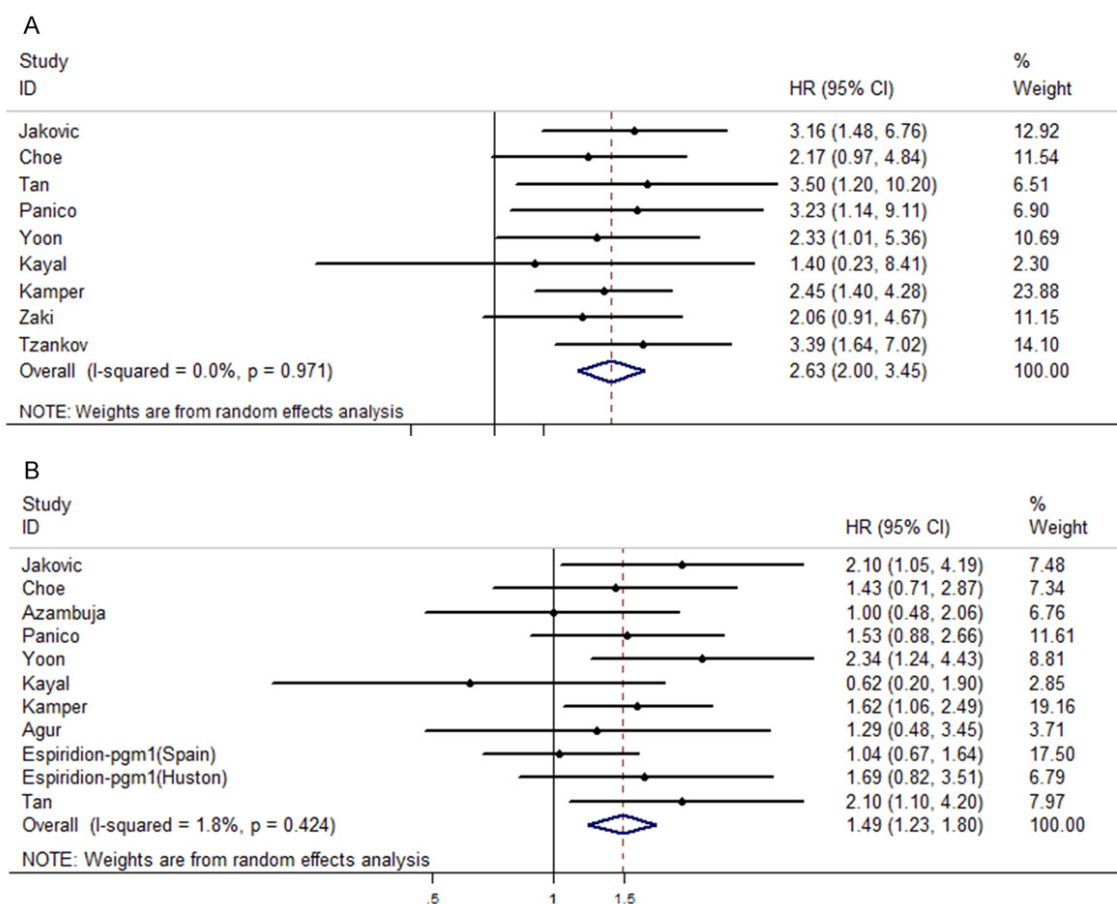
The data for OS for CD163 were extracted from 5 studies with HRs ranging from 1.82 to 14.58 (Figure 2A); PFS data were extracted from 5 studies with HRs ranging from 1.15 to 2.5 (Figure 2B). For the entire population, the over-

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**Table 3.** Results of survival analyzed by individual study

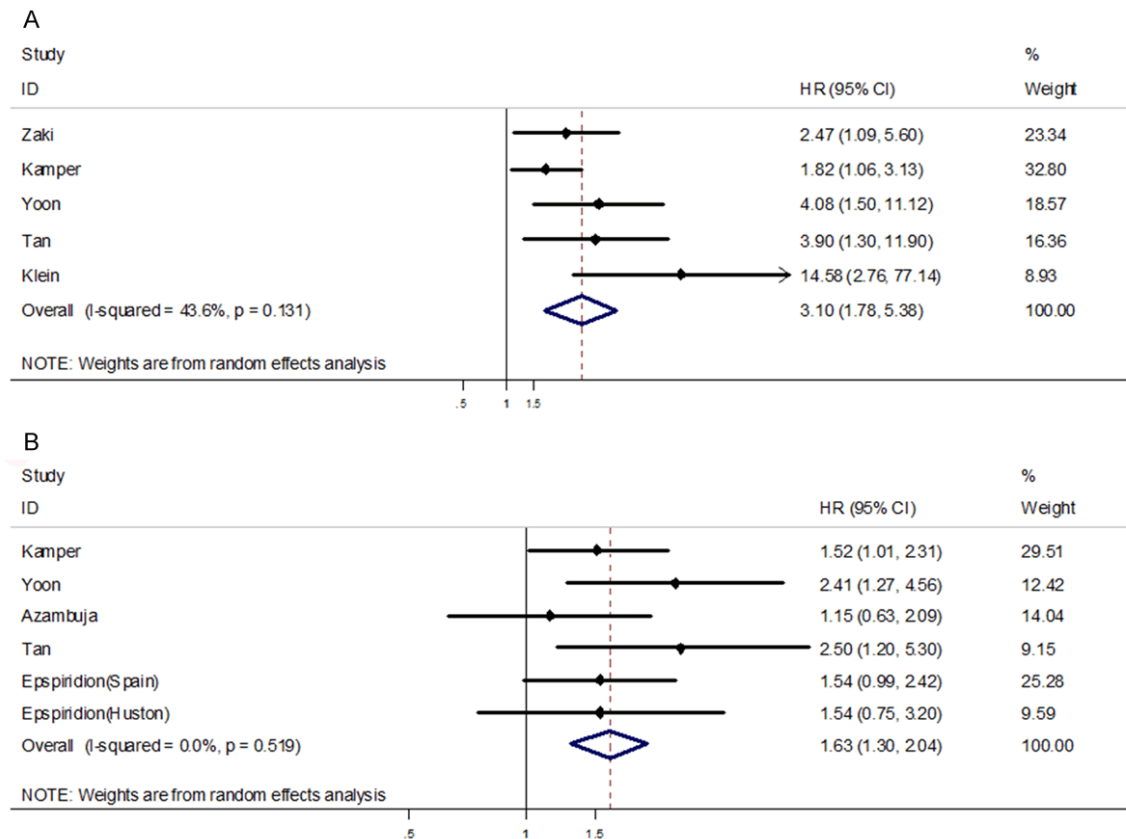
First author, year	Ref.	CD68 HR (95% CI)		CD163 HR (95% CI)	
		OS	PFS	OS	PFS
Agur, 2014	[24]	-	1.29 (0.48, 3.45)	-	-
Azambuja, 2012	[19]	-	1.0 (0.48, 2.06)	-	1.15 (0.63, 2.09)
Choe, 2014	[21]	2.17 (0.79, 4.84)	1.43 (0.71, 2.87)	-	-
Jakovic, 2012	[25]	3.16 (1.48, 6.76)	2.1 (1.05, 4.19)	-	-
Kamper, 2011	[11]	2.45 (1.4, 4.28)	1.62 (1.06, 2.49)	1.82 (1.06, 3.13)	1.52 (1.02, 2.31)
Kayal, 2014	[15]	1.4 (0.23, 8.41)	0.62 (0.2, 1.9)	-	-
Klein, 2014	[18]	-	-	14.58 (2.76, 77.14)	-
Panico, 2013	[13]	3.23 (1.14, 9.11)	1.53 (0.88, 2.66)	-	-
Sanchez-Espiridion, 2012*	[16]	-	1.25 (0.8, 1.96)	-	1.54 (0.99, 2.42)
		-	1.37 (0.66, 2.83)	-	1.54 (0.75, 3.2)
Tan, 2012	[10]	3.5 (1.2, 10.2)	2.1 (1.1, 4.2)	3.9 (1.3, 11.9)	2.5 (1.2, 5.3)
Tzankov, 2010	[20]	3.39 (1.64, 7.02)	-	-	-
Yoon, 2012	[9]	2.33 (1.01, 5.36)	2.34 (1.24, 4.43)	4.08 (1.5, 11.2)	2.41 (1.27, 4.56)
Zaki, 2011	[17]	2.06 (0.91, 4.67)	-	2.47 (1.09, 5.6)	-

\*Two cohorts included in this article were analyzed separately.



**Figure 1.** Forrest plots of HRs and 95% CI: (A) CD68+ TAM overall survival, (B) CD68+ TAM progression-free survival. The weight for the fixed-effect model in the meta-analysis was displayed at the right. HR higher than unity indicates that the presence of CD68+ TAM is associated with worse prognosis, but if the CI crosses this line this result is not statistically significant.

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**Figure 2.** Forrest plots of HRs and 95% CI: (A) CD163+ TAM overall survival, (B) CD163+ TAM progression-free survival. The weight for the fixed-effect model in the meta-analysis was displayed at the right. HR higher than unity indicates that the presence of CD163+ TAM is associated with worse prognosis, but if the CI crosses this line this result is not statistically significant.

all HR was 3.1 for OS (95% CI: 1.78-5.38;  $P < 0.001$ ) and 1.63 for PFS (95% CI: 1.3-2.04;  $P < 0.001$ ). The heterogeneity test was 43.6% for OS and zero for PFS, indicating no significant heterogeneity.

### Evaluation of publication bias and sensitivity analyses

There was no publication bias detected in the overall meta-analysis of OS ( $P = 0.435$ ) or PFS ( $P = 0.770$ ) based on CD68+ TAM status, and PFS based on CD163+ TAM status ( $P = 0.394$ ; **Figure 3**). Publication bias was found for the analysis of OS based on CD163+ TAM status ( $P = 0.003$ ). The sensitivity analysis showed stable results in all four sub-groups (**Figure 4**).

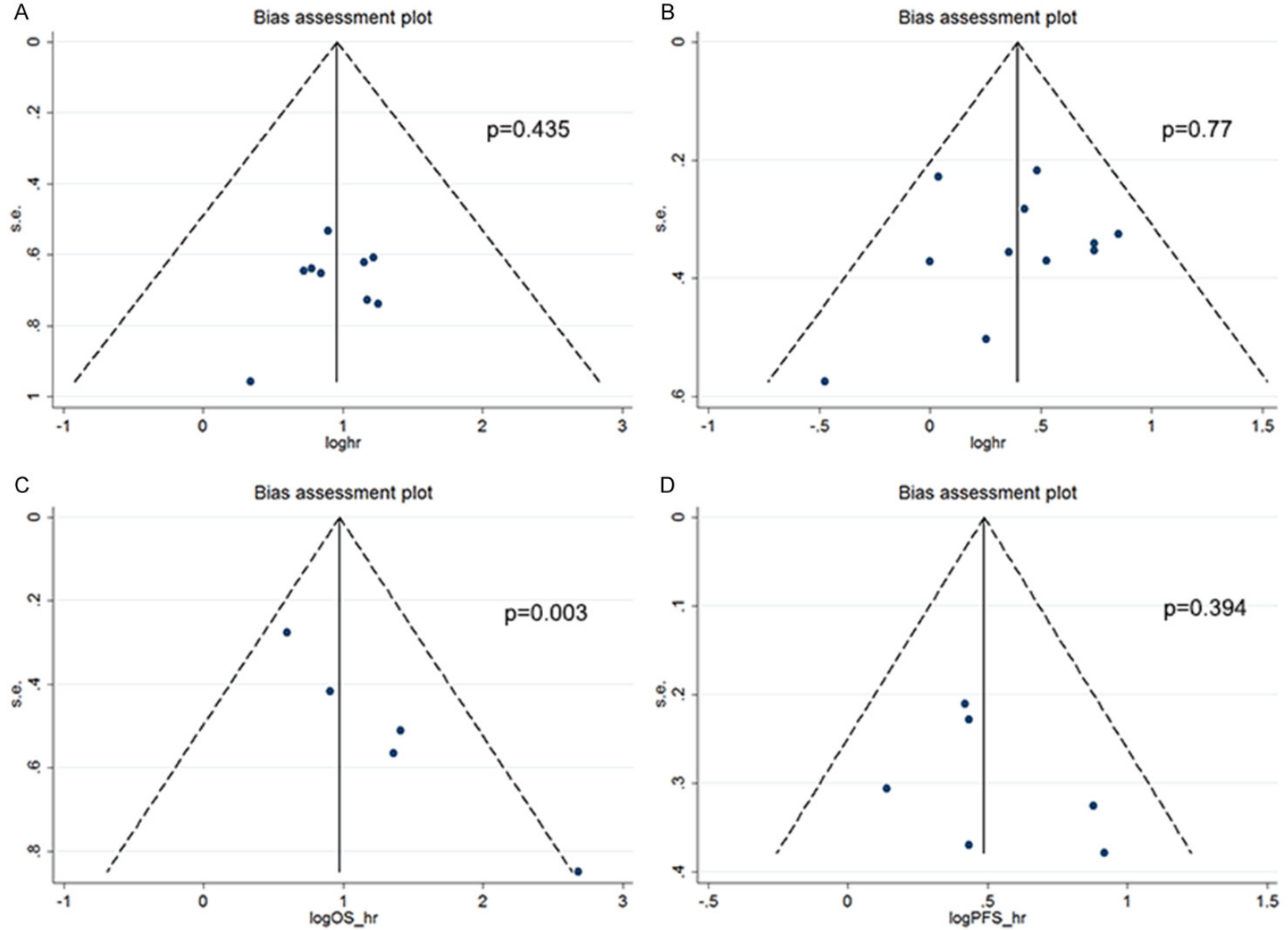
### Discussion

In the present meta-analysis, we found that in cHL patients both CD68+ TAMs and CD163+ TAMs were significantly associated with shorter

OS ( $P < 0.001$ ) and PFS ( $P < 0.001$ ). TAMs have been reported to predict poor prognosis in many human malignancies [6]. However, the prognostic value of TAM in cHL is controversial. To answer this question, we performed a meta-analysis of 13 relevant original articles. All publications had similar designs and investigated the correlations of the TAMs CD68+, CD163+, or both, with either OS or PFS. The studies of Stedil et al. [8] and Greaves et al. [14] were excluded either for a case control or tri-categorical design. Therefore, our results are representative and resolve the inconsistencies observed among different study groups.

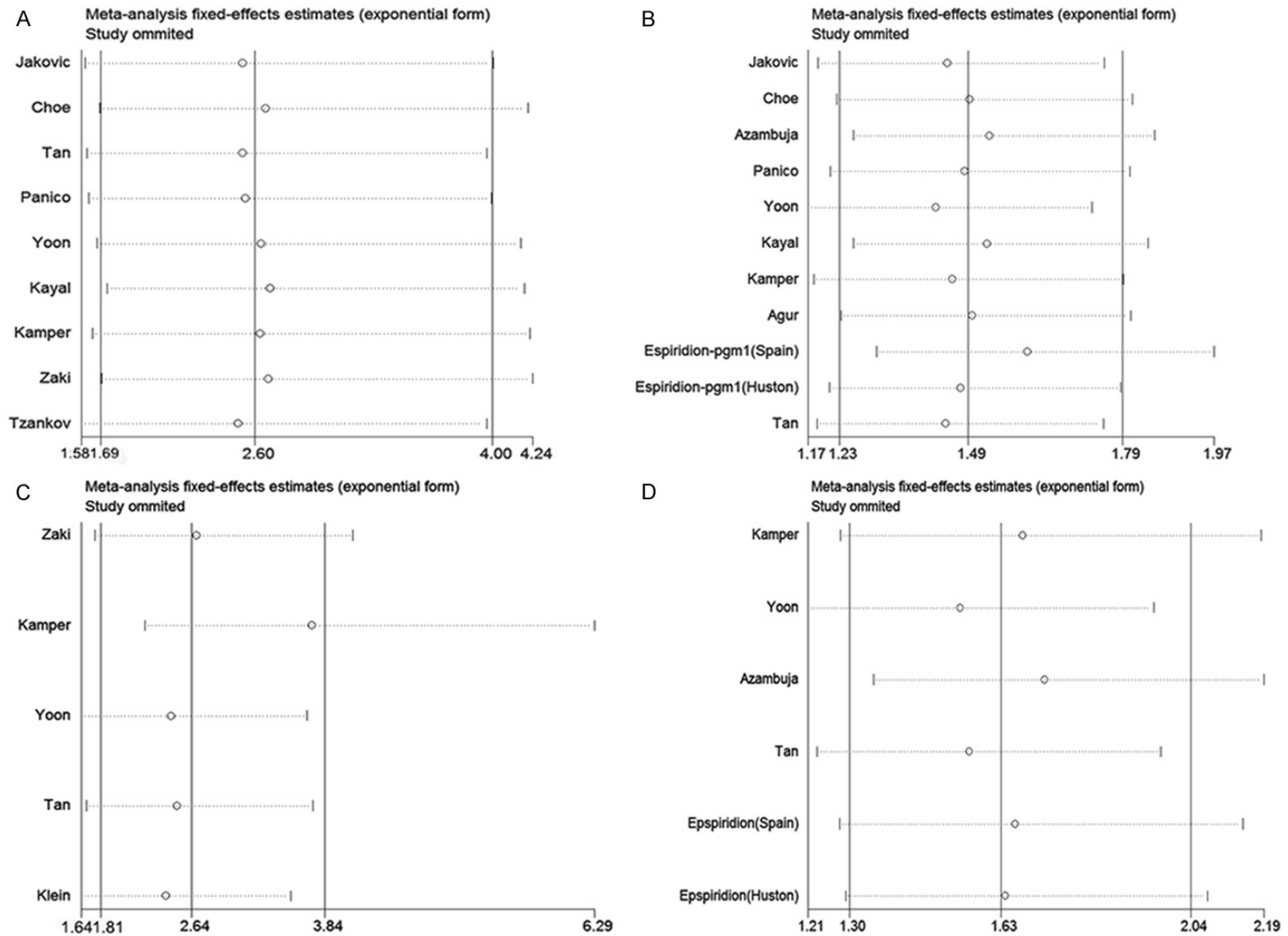
Using a gene-expression profile, Stedil et al. [8] demonstrated that the overexpression of a TAM-associated gene signature in cHL significantly correlated with worse outcomes. The same group also found that an increased number of CD68+ TAM infiltration detected by IHC

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**Figure 3.** Bias assessment plots for studies included in meta-analyses: (A) CD68+ TAM overall survival, (B) CD68+ TAM progression-free survival, (C) CD163+ TAM overall survival, and (D) CD163+ TAM progression-free survival. *p* value came from Egger's test.

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**Figure 4.** Influence analysis for studies included in meta-analyses: (A) CD68+ TAM overall survival, (B) CD68+ TAM progression-free survival, (C) CD163+ TAM overall survival, and (D) CD163+ TAM progression-free survival.

was associated with a poor PFS. However, their inclusion of cases of treatment failure may have caused bias [8]. Our present results are consistent with 2 studies with smaller cohorts of cHL patients [11, 20], and a cohort study in a clinical trial setting using both CD68 and CD163 markers detected by IHC [10]. In the clinical trial which included 287 patients with cHL [10], the authors concluded that an increased number of CD68+ and CD163+ infiltration were significantly associated with inferior clinical outcomes and both CD68 and CD163 were significantly independent predictors of PFS and OS. Two studies questioned the prognostic value of CD68+ TAMs, and proposed that CD163+ was the better TAM marker [17, 18]. Another two studies reported no link between TAM and survival, with either CD68+ or CD163+ TAMs [15, 16, 23].

The poor interobserver reliability of CD68 interpretation has been identified as a potential pitfall, especially when using manual visual scoring methods [18]. Using computerized analysis to produce better objectivity in scoring may overcome this drawback [11]. However, after stratifying according to IHC quantitating method, our present subgroup analysis found that the overall HRs for high CD68+ TAMs, CD163+ TAMs, or both, for either OS or PFS, were similar, whether measured by manual visual scoring or by computer. Both of them have statistical significance. The interobserver reliability for CD163 interpretation is acceptable [23], but considering the publication bias, the result regarding the association of CD163+ TAMs with prognosis in this meta-analysis should be interpreted with caution.

The optimal method for assessing TAMs of high CD68+, CD163+, or both is currently unclear. There were 3 methods used to determine the cut-off points in the 13 studies included in this meta-analysis. In the sub-analysis, we found that the overall HRs were statistically significant in all of the 3 sub-groups. The overall HR was the lowest for those studies in which the median percentile or highest quartile values as cut-off points were selected, and the highest for those studies in which the optimal cut-off points were chosen.

In conclusion, the present study supports the notion that TAMs CD68+, CD163+, or both can predict poorer prognosis in cHL, and proposes

a new biomarker for risk stratification and novel therapeutic targets. For example, to treat cHL patients with high TAM involvement, clinical trials could be developed that test agents that shift macrophages from tumor-associated to antitumor phenotypes.

### Disclosure of conflict of interest

None.

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