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REVIEW – CLINICAL ONCOLOGY

Prognostic value and clinicopathological roles of phenotypes of tumour-associated macrophages in colorectal cancer

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Abstract

Background The role of tumour-associated macrophages (TAMs) in predicting the prognosis of colorectal cancer (CRC) remains controversial. This is especially so because the prognostic significance and clinicopathological relevance of different subtypes of TAMs in the immune microenvironment of CRC have not yet been established.

Objective To assess the clinicopathological and prognostic value of pan-macrophages, M1-macrophages or M2-macrophages in patients with CRC.

Methods Comprehensive searched on the Medline/PubMed, Web of Science (WoS) and Google Scholar databases was conducted to identify relevant studies published up to April 2019. The association between overall survival (OS), cancer-specific survival (CSS) or disease-free survival (DFS) and TAMs was analysed by meta-analysis.

Results A total of 3749 patients from 17 studies were included. The pooled hazard ratios (HRs) indicated that high-density pan-macrophages improved OS (HR 0.67, $P=0.02$). The pooled HR for M2-macrophages showed that high M2-macrophages infiltration was significantly associated with shorter OS (HR 2.93, $P<0.0001$) and DFS (HR 2.04, $P=0.02$). The pooled odds ratios (ORs) revealed that high-density TAMs was associated with high CD8+ T cell infiltration (OR 2.04, $P=0.007$), no distant metastasis (NDM) (OR 0.38, $P<0.0001$), microsatellite instability-high (MSI-H) (OR 0.38, $P=0.001$), no lymph node metastasis (NLNM) (OR 0.54, $P=0.0002$) and non-mucinous cancer (OR 0.39, $P<0.00001$).

Conclusions Unlike other solid tumours, high-density CD68+ macrophage infiltration can be a good prognostic marker for CRC. However, when macrophages act as targets of combination therapy in CRC treatment, this might be more effective for CRC patients with high CD8+ T cell infiltrate, NDM, MSI-H, NLNM and non-mucinous cancer.

Keywords Colorectal cancer · Prognostic biomarkers · Tumour-associated macrophages · Clinicopathological · Meta-analysis

Introduction

Great progress has been made in screening, prevention and comprehensive treatment including surgery, chemotherapy, radiotherapy and immunotherapy of CRC in recent years. However, the incidence and mortality rate of CRC are still high (Siegel et al. 2019). The clinical practice guidelines

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for the prognosis and treatment of CRC are mainly based on the TNM classification scheme which was created by the American Joint Committee on Cancer. Increased awareness of the complexity of the tumour microenvironment and the importance of tumour immunotherapy, targeting specific immune checkpoints has aroused tremendous excitement due to its long-term efficacy (Le et al. 2015, 2016). Likewise, tumour infiltrating lymphocytes (TILs) are also a major point of concern, This is because the assessment of an immune score based on the density and the location of subsets of tumour-infiltrating CD3+CD8+lymphocytes have the potential to predict the prognosis of patients with CRC (Mlecnik et al. 2018; Pages et al. 2018). However, only some of the patients with the so-called hot tumors or high mutation load or high lymphocytes infiltration may achieve better therapeutic effect (Galon and Bruni 2019). Therefore, it is imperative to identify new biomarkers to predict the prognosis of patients with CRC, and find appropriate treatment targets and treatment population.

Tumour microenvironment (TME) comprises tumour cells and non-malignant cells, such as immune cells, tumour vessels, lymphatic vessels, fibroblasts, adipocytes and vascular endothelial cells (Teng et al. 2016; Yang and Zhang 2017). Tumor immune cells play a distinct role in various stages of tumorigenesis and progression, thereby forming a dynamic immune system (Balkwill et al. 2012). In addition to CD8+T cells in tumour-infiltrating lymphocytes (TILs), which produce direct immune responses and tumour-associated macrophages are also common components in TME (Balkwill et al. 2012; Banerjee et al. 2019). Macrophages are mainly classified into M1-polarized macrophages (M1-macrophages) and M2-polarized macrophages (M2-macrophages) based on their polarization states (Biswas and Mantovani 2010). In the progression of tumours to malignancy, TAMs have unique characteristics one of which is the plasticity of redifferentiation from M1-macrophages to M2-macrophages under the induction of certain factor, and vice versa. This leads to an uncertain relationship between TAMs and cancer cells (Biswas et al. 2006; Pollard 2004; Yahaya et al. 2019). Different subpopulations of macrophages have specialized functions in specific stages, yet they promote growth with an inflammatory mutagenic environment at the initial stage (Gonzalez et al. 2018; Qian and Pollard 2010). During the progression period, the dominant function of M2-macrophages provoke angiogenesis, enhances tumour cell migration and invasion, and inhibit anti-tumour immunity.

In most solid tumours, high-density macrophages infiltration has been associated with significantly poor prognosis (Hasita et al. 2010; Zhang et al. 2011, 2016). However, studies evaluating the significance of prognosis in different subtypes of TAMs infiltration in CRC remain controversial (Cavnar et al. 2017; Kim et al. 2018; Zhou et al. 2010). Therefore, this study performed a comprehensive meta-analysis to evaluate the clinicopathological and prognostic significance of different subpopulation of macrophages in patients with CRC.

Methods

Search strategy

A total of eighteen articles searching from PubMed and Web of Science were included from the inception of each database to 20th April 2019. The search and selection process was as illustrated in Fig. 1. In total, 3749 samples of included articles were obtained searched after performing the search using the keywords ("colorectal cancer"), the determinant ("tumour-associated macrophage", "TAM"), and their synonyms. Non-English language studies and articles retrieved with a sample size of less than 50 were excluded The articles retrieval strategy, as well as predesigned inclusion and exclusion criteria, were based on previous studies (de Ruiter et al. 2017; Zhang et al. 2012). Two authors independently screened the titles and abstracts based on the predetermined inclusion and exclusion criteria. The final selection was made through a full-text reading of the initially incorporated studies. Any discrepancies between the two authors were discussed and consensus reached by involving another author. The reference lists of the retrieved articles and other articles not meeting the inclusion criteria were also screened for other relevant articles.

Inclusion criteria

In reference to previous inclusion criteria (Fu et al. 2019; Mei et al. 2014), the studies included were those which the prognostic value of pan-TAMs by anti-CD68, M1-TAMs by anti-NOS2 or iNOS+or CD86, M2-TAMs by anti-CD163 or Clev/Stab was investigated in patients with CRC. For TAM response markers, the studies in which TAMs were quantified as no/weak, moderate, strong/robust and massive, as well as none, mild, intermediate, strong were included (Bacman et al. 2007; Forssell et al. 2007). However, the final analysis, the original articles having divided TAMs infiltration into the high-density group and low-density group were included and studies not meeting this criterion were otherwise excluded. The detection method was without special restriction, however, only publications concerning TAMs in the tumour tissue were included, and studies that only investigated lymphocytes in peripheral blood or regional lymph nodes were excluded. The prognostic value had to be investigated by time-to-event survival analysis with either overall survival (OS), cancer-special survival (CSS), relapse/recurrence-free survival (RFS), disease-free

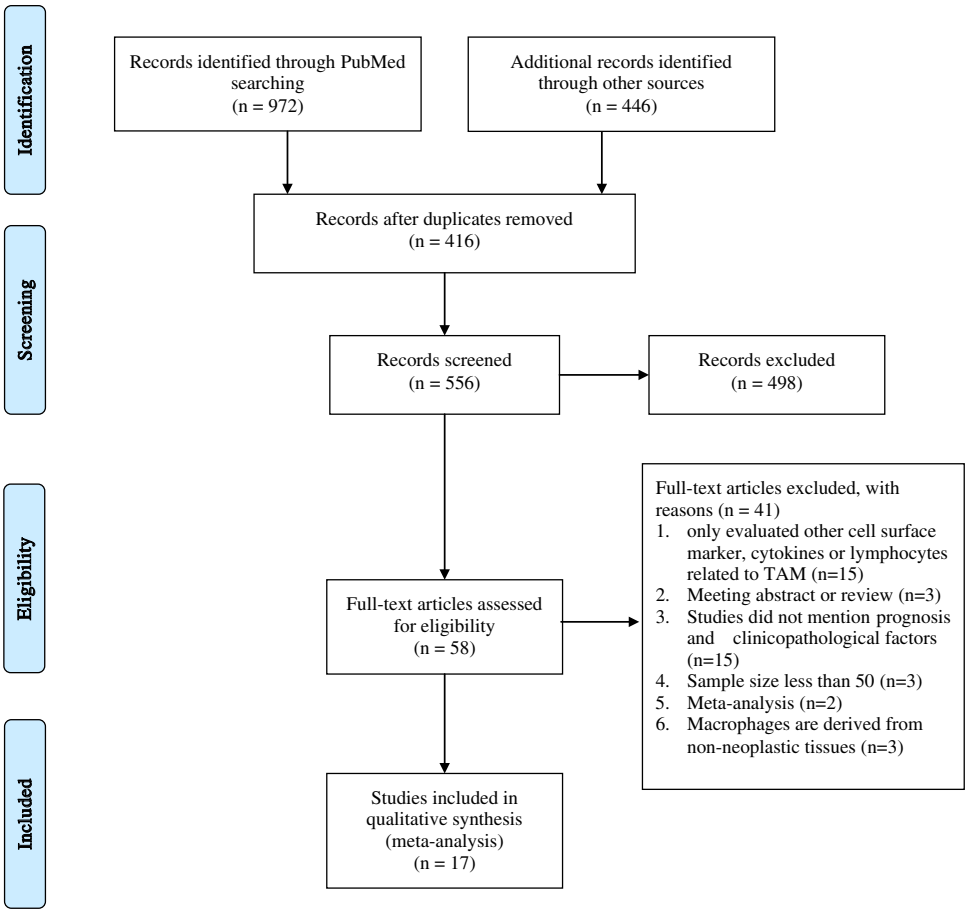

Fig. 1 Flowchart of the study selection process

survival (DFS) or progression-free survival (PFS). Animal studies, case reports, reviews and commentaries were all excluded. In addition, original articles with fewer than 50 cases were excluded.

Data extraction and assessment of study quality

Two authors (ZYM and GXX or WJ) independently selected articles and extracted data from eligible studies. A standardized data abstracted form was developed, and key elements pertaining to the first author, year, country, sample size, stage of disease, detection method, macrophage subset, cut-off value, outcome measure, quality score, clinicopathological parameters, use of multivariate logistic model analysis, adjustment variables, HR estimates (with the corresponding 95%CIs) for the high density over the low density of each TAMs subset and the HR cut-off point were obtained. Disagreements were resolved through a discussion involving a third author (SLF or XMX) who helped the two authors reach a consensus. Whenever the HR involving prognosis was not mentioned in the primary article but the Kaplan–Meier curves were available, the information from the Kaplan–Meier (K–M) curves was extracted and digitized using the Engauge Digitizer software, and the univariate HR was estimated (Tierney et al. 2007). When K–M curves were also not available, or HRs did not match the shown K–M curves, the studies were excluded from this pooled analysis.

The quality of included studies was assessed using an established format that was first applied and used by Mei et al.(2014) and McShane et al.(2005). (Supplementary Table 1) The following seven points were assessed and scored on a scale of 0–8: exclusion and inclusion criteria, prospective or retrospective study, basic characteristics of patients, description of detection method, study outcomes, follow-up time and the number of loss during follow-up.

Statistical analysis

This pooled analysis was conducted to evaluate the association between TAMs infiltrates with OS, DFS, RFS and clinicopathological parameters. Heterogeneity among studies was evaluated using the Chi squared test and I^2. A random-effects model was implemented whenever there was evidence of significant heterogeneity ($I^2 > 50\%$ or P value < 0.1). Conversely, the fixed-effects model was used. Potential publication bias was assessed through a funnel plot. The forest plots were conducted using Review Manager Version 5.3 (RevMan the Cochrane Collaboration; Oxford,

England). All 95% CIs and *P*-values were two-sided, and *P*-values < 0.05 had statistical significance.

Results

Study selection and basic characteristics

After the elimination of 416 duplicates, 556 articles about TAMs in CRC were identified from a primary system literature search in the Medline/PubMed WoS. and Google Scholar. 498 publications were excluded after the screening of titles and abstract, 52 were reviews or commentaries, 6 were unavailable, and 440 were mismatched with our tumor species, determinant and endpoints. The full text of 58 records was read for further assessment. Among which 17 met the inclusion criteria and were included in this study (Algars et al. 2012; Bacman et al. 2007; Cavnar et al. 2017; Edin et al. 2012; Forssell et al. 2007; Funada et al. 2003; Gulubova et al. 2013; Khorana et al. 2003; Kim et al. 2018; Koelzer et al. 2016; Li et al. 2018; Ohnishi et al. 2013; Shabo et al. 2014; Shibutani et al. 2017; Tan et al. 2005; Wei et al. 2019; Zhou et al. 2010). (Figure 1) The basic characteristics of the 16 articles on prognosis are shown in Table 1, while one article only extracted data related to clinicopathological factors. The 16 studies comprising 3749 patients were included in the meta-analysis and they all used IHC assays to evaluate TAMs infiltrating in tumour tissue. The quality scores of all included articles were above 4. All the enrolled studies investigated TAMs by immunohistochemistry in paraffin-embedded tissue. And all articles with independent cut-off value were used to define the criterion for high TAMs infiltrating. 12 studies provided OS data (Cavnar et al. 2017; Funada et al. 2003; Gulubova et al. 2013; Khorana et al. 2003; Kim et al. 2018; Koelzer et al. 2016; Li et al. 2018; Shabo et al. 2014; Shibutani et al. 2017; Tan et al. 2005; Wei et al. 2019; Zhou et al. 2010), 4 studies included DFS/RFS/PFS data (Cavnar et al. 2017; Kim et al. 2018; Shibutani et al. 2017; Wei et al. 2019), 4 studies included CSS data (Algars et al. 2012; Bacman et al. 2007; Edin et al. 2012; Forssell et al. 2007), while 13 studies provided data related to clinicopathological features. In addition, HRs and 95% CIs were extracted directly from all the studies.

Table 1 Main characteristic of included studies for meta-analysis

References	Country	No.	Stage	Technique	TAM subset	Cut-off	Outcome	HR (95%CI)	Quality assessment
Algars et al. (2012)	Sweden	159	II–IV	IHC	CD68 Clev/Stab+	≥ 30%	CSS	0.84 (0.5–1.4) 1.41 (0.8–2.5)	5
Bacman et al. (2007)	Germany	310	II–III	IHC	CD68	Intermediate	CSS	1.55 (0.61–3.92)	3
Cavnar et al. (2017)	New York	158	IV	IHC	CD68	Ratio:0.076; 0.0685	OS DFS	0.11 (0.01–2.59) 0.63 (0.43–0.94)	6
Edin et al. (2012)	Sweden	485	I–IV	IHC	NOS2 CD163	Score > 2	CSS	0.67 (0.40–1.12) 0.66 (0.42–1.06)	6
Forssell et al. (2007)	Sweden	446	I–IV	IHC	CD68	Strong or massive	CSS	0.49 (0.29–0.82)	4
Funada et al. (2003)	Japan	98	I–IV	IHC	CD68	Mean	OS	2.93 (0.89–9.69)	3
Gulubova et al. (2013)	Bulgaria	210	I–IV	IHC	CD68	105.20 cell/mm^2	OS	0.69 (0.22–2.16)	5
Khorana et al. (2003)	American	131	II–III	IHC	CD68	2%	OS	0.84 (0.65–1.09)	5
Kim et al. (2018)	Korea	584	I–IV	IHC	CD68 CD163	Median	OS PFS	1.347 (0.967–1.877) 1.447 (1.076–1.947)	3
Koelzer et al. (2016)	Greece	205	I–IV	IHC	CD68	Average	OS	0.53 (0.29–0.95)	5
Li et al. (2018)	China	419	I–IV	IHC	CD68	NA	OS	0.448 (0.273–0.737)	5
Shabo et al. (2014)	Sweden	75	I–IV	IHC	CD163	Median	OS	1.6 (0.7–4.0)	4
Shibutani et al. (2017)	Japan	168	II–III	IHC	CD163	Median	OS RFS	4.123 (1.464–11.61) 3.692 (1.743–7.822)	4
Tan et al. (2005)	China	60	I–IV	IHC	CD68	25%	OS	0.44 (0.24–0.79)	4
Wei et al. (2019)	China	81	I–III	IHC	CD68 CD163	Median	OS RFS OS RFS	1.571 (0.743–3.322) 1.653 (0.091–3.033) 4.149 (1.761–9.776) 2.144 (1.016–4.523)	5
Zhou et al. (2010)	China	160	IIIB–IV	IHC	CD68	20%	OS	0.433 (0.194–0.966)	6

Association between TAMs infiltrating and prognostic parameters

Many studies have indicated that TAMs are an adverse prognostic factor for patients with solid tumours but in CRC, previous reports have indicated an inconsistent correlation between different TAMs intensities or subtypes and prognostic value. The prognostic value of pan-TAMs present was assessed in 13 studies that were eligible for inclusion in the pooled analysis, among which 10 studies analysed the prognostic roles of pan-TAMs which was marked by CD68+ for OS. As shown in Fig. 2a, the pooled results showed significantly improved prognosis for high-density TAMs present (HR: 0.67 [0.47–0.95]) for OS. Although no significant difference was calculated for CSS, the pooled meta-analysis of 3 articles indicated an advantage trend for high-density pan-TAMs (HR: 0.72 [0.51–1.02]), (Fig. 2b). However, for DFS, the synthetic analysis of high-density pan-TAMs showed a trend of adverse prognosis in 3 studies (HR: 1.15 [0.62–2.15]), (Fig. 2c).

None of the studies mentioned the prognostic roles of M1 for OS and DFS. For the prognostic roles of M1 for CSS, only one study from which data could be extracted. Therefore, it was impossible to evaluate the correlation between M1 and CSS directly by pooled analysis. However, from a single study, high-density infiltration of M1 was associated with a favourable trend for CSS (HR: 0.67 [0.40–1.12]) (Fig. 2c).

The results showed obvious predictive roles of M2 for OS or DFS, among which only M2 present was involved in 3 articles and indicated a unfavorable prognostic role for OS (HR: 2.93 [1.73–4.95]) (Fig. 2a). M2 was assessed in 3 studies involving DFS (HR: 2.04 [1.09–3.80]) (Fig. 2b). For CSS, 2 studies were included in this meta-analysis and did not show statistical differences of M2 (HR: 0.89 [0.62–1.28]) (Fig. 2c).

The results showed obvious predictive roles of M2 for OS or DFS, among which only M2 present was involved in 3 of the articles and indicated an unfavourable prognostic role for OS (HR: 2.93 [1.73–4.95]) (Fig. 2a). M2 was assessed in 3 studies involving DFS (HR: 2.04 [1.09–3.80]) (Fig. 2b) For CSS, only 2 studies were included in this meta-analysis and did not show statistical differences of M2 (HR: 0.89 [0.62–1.28]) (Fig. 2c) (Table 2).

Association between TAMs and clinicopathological characteristics

CD8+ TIL

The association between TAMs and CD8+ T cell was evaluated in 3 studies, comprising 1087 patients. 269 (57.60%) of 467 patients with high-density CD8+ T cell infiltrates and 203 (41.51%) of 489 patients with low-density CD8+ T cell infiltrates had high-density infiltration of TAMs. The pooled OR indicated that there was an obvious association between TAMs and CD8+ T cell infiltrates (OR: 1.91[1.48–2.47]) (Fig. 3a).

Lymph node metastasis (LNM)

The data of 5 articles with 614 patients were extracted, of which 282 were LNM positive, 105 (37.23%) had high-density TAMs infiltrates. Of the 327 LNM negative, 166 (50.76%) had high-density TAMs infiltrates. Low-density TAMs infiltrates were significantly associated with LNM based on pooled analysis (OR: 0.58 [0.42–0.81]) (Fig. 3b).

MMR status

In total meta-analysis, the results indicated that high-density TAM was significantly associated with microsatellite instability (MSI). However, in the subgroup analysis, pan-macrophages showed an obvious association with MSI in only two studies (OR: 3.46 [1.35–8.85] (Fig. 3c). The pooled analysis of M1-macrophages and M2-macrophages showed that there was no significant association between high macrophage infiltrates and MMR status.

Pathological type

Only four studies provided data with regard to the association between macrophage infiltration and pathological type of CRC (Fig. 3d), with three assessing pan-macrophage, two M1 and two M2. Regardless of the overall analysis or subgroup analysis, these results revealed that an association between a high-density macrophage infiltration and non-mucinous cancer, of pathological type, was related to a good prognosis, compared to mucinous cancer (total pooled OR: 0.39 [0.28–0.53]).

Distant metastasis

Four studies, involving 523 patients, assessed the association between high-density macrophages and distant metastasis in a fixed-effects model. 49 (36.03%) of 136 patients with DM and 171 (44.76%) of 382 patients with no DM had a high density of macrophage infiltration. The pooled analysis revealed a significant association between no DM and high-density macrophages (OR: 0.43 [0.27–0.67]) (Fig. 3e).

TNM stage

Just as the pooled analysis of T-stages, four studied assessed pan-macrophage, one M1-macrophage, four M2-macrophage. Further, six studies revealed that there was no

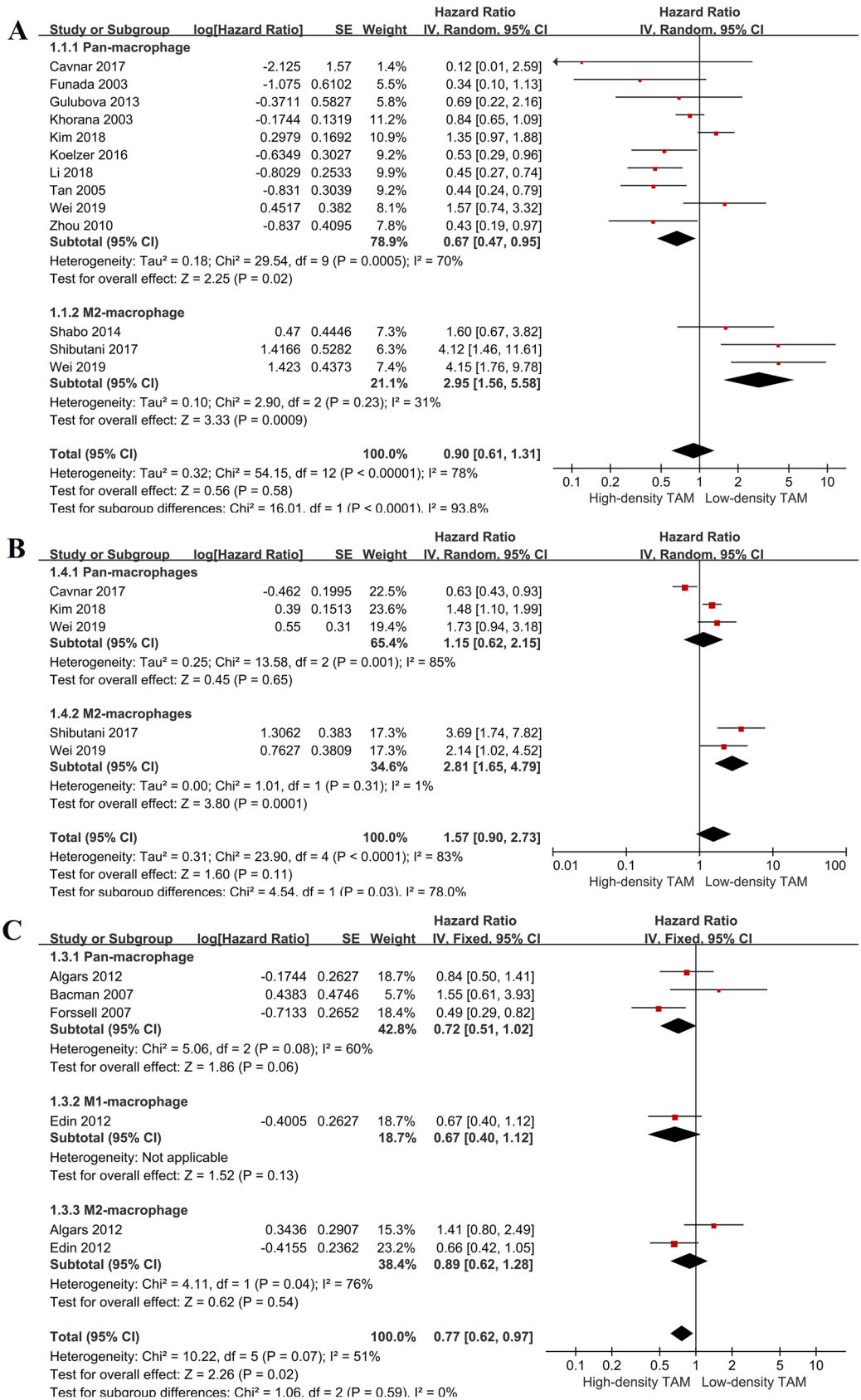

Fig. 2 Forest plots showing studies evaluating the association between different subtype of TAMs infiltration and prognostic parameters in CRC patients (**a** OS, **b** DFS, **c** CSS)

obvious association between high-density macrophage and TNM stage (stage I–II vs stage III–IV) in the meta-analysis or subgroup analysis with a random-effects model (total pooled OR: 0.87[0.53–1.42]) (Fig. 3f).

T classification

This analysis evaluated the association between high-density macrophage and T stage in three articles, with three assessed pan-macrophage, only one M1, two M2. The pooled HR indicated that there was no significant association between high macrophage infiltration and T stage, whether it involved pan-macrophages or M1 or M2 (total pooled OR: 1.31[0.68–2.53]) (Fig. 3g).

Publication bias and heterogeneity

The funnel plots revealed that low publication bias influencing the HRs for OS was observed in the thirteen studies (Fig. 4). For OS, significant heterogeneity was observed for pan-macrophage ($I^2 = 72\%$, $P = 0.0002$), whereas moderate heterogeneity was observed for M2-macrophage ($I^2 = 31\%$,

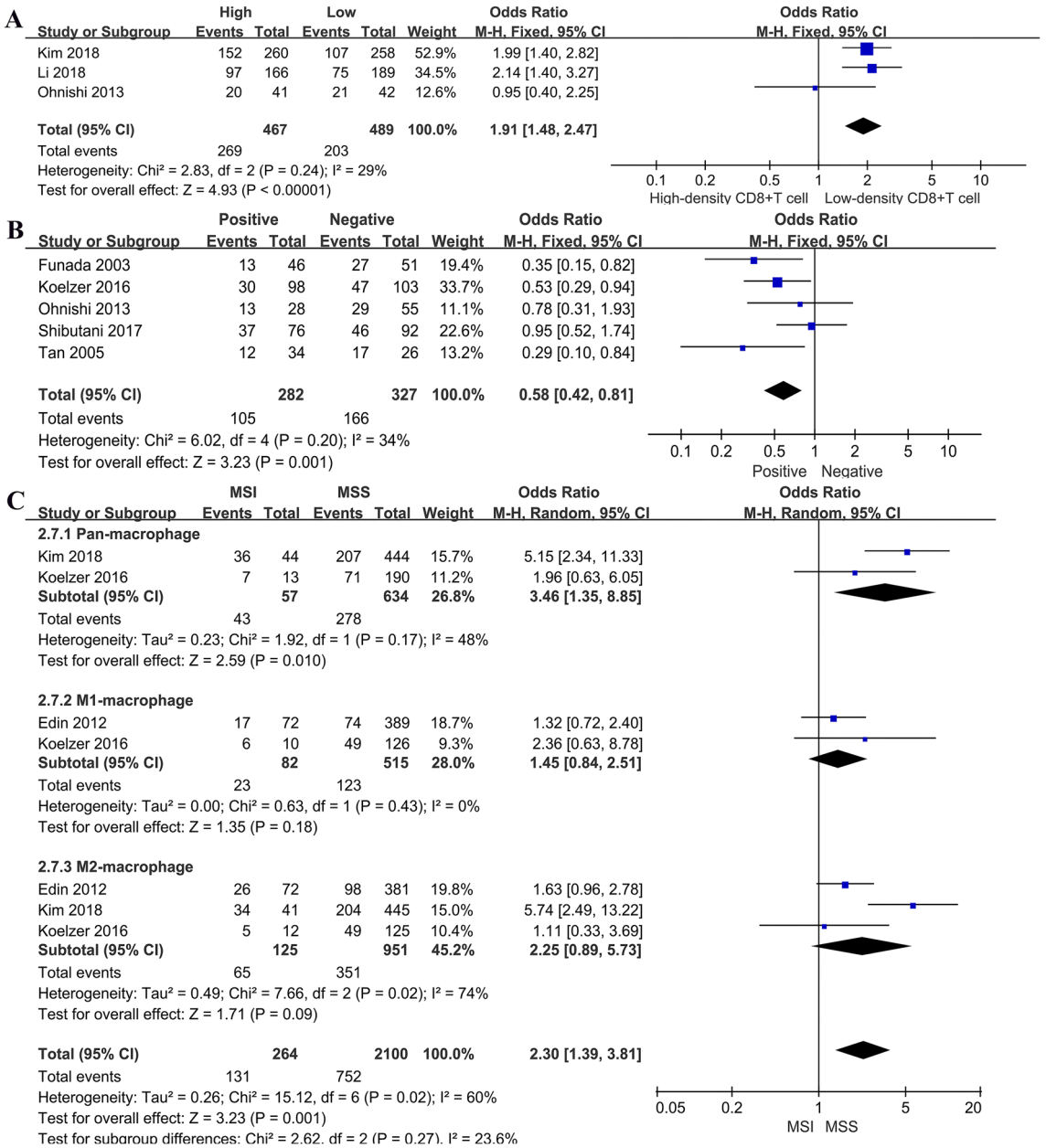

Fig. 3 Forest plots showing the association of TAMs infiltration with clinicopathological factors in CRC patients (**a** CD8+T cell, **b** lymph node metastasis, **c** MMR status, **d** pathological type, **e** distant metastasis, **f** TNM stage, **g** T stage)

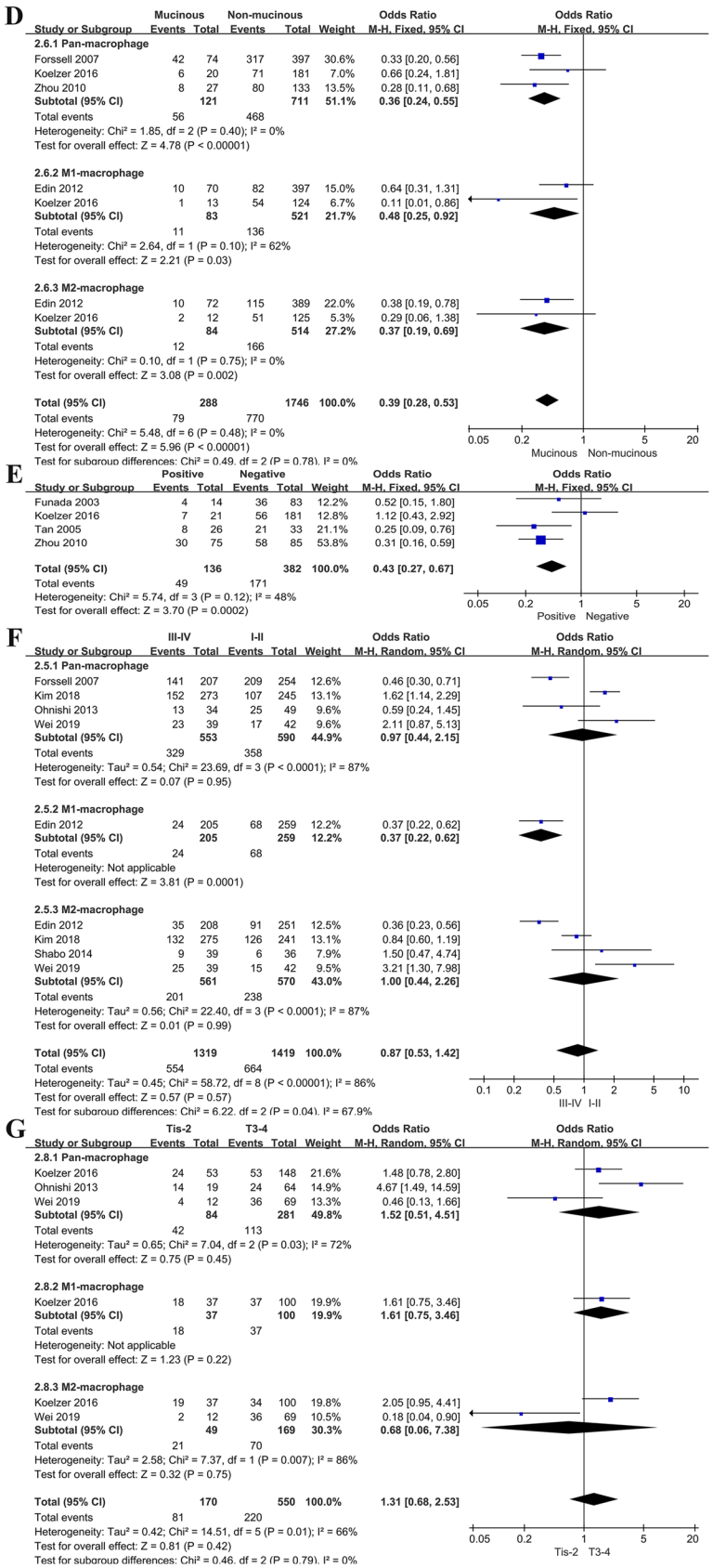

Fig. 3 (continued)

Table 2 The pooled analysis of OS, DFS (RFS), CSS and clinicopathological factors in different subgroup

Studies	Outcomes	TAM subtypes	Heterogeneity	HR/OR (95%CI)
Cavnar et al. (2017), Funada et al. (2003), Gulubova et al. (2013), Khorana et al. (2003), Kim et al. (2018), Koelzer et al. (2016, Li et al. (2018), Tan et al. (2005), Wei et al. (2019), Zhou et al. (2010)	OS	Pan-macrophage	Random	0.67 (0.47–0.95)
Shabo et al. (2014), Shibutani et al. (2017), Wei et al. (2019)		M2-macrophage	Random	2.95 (1.56–5.58)
		Pooled analysis	Random	0.90 (0.61–1.31)
Cavnar et al. (2017), Kim et al. (2018), Wei et al. (2019) Shibutani et al. (2017), Wei et al. (2019)	DFS	Pan-macrophage	Random	1.15 (0.62–2.15)
		M2-macrophage	Random	2.81 (1.65–4.79)
		Pooled analysis	Random	1.57 (0.90–2.73)
Algars et al. (2012), Bacman et al. (2007), Forssell et al. (2007),	CSS	Pan-macrophage	Fixed	0.72 (0.51–1.02)
		M1-macrophage	Fixed	0.67 (0.40–1.12)
Edin et al. (2012)		M2-macrophage	Fixed	0.89 (0.62–1.28)
Algars et al. (2012), Edin et al. (2012)		Pooled analysis	Fixed	0.77 (0.62–0.97)
Kim et al. (2018), Li et al. (2018), Ohnishi et al. (2013)	High-density CD8+ T cell	Pan-macrophage	Fixed	1.91 (1.48–2.47)
Funada et al. (2003), Koelzer et al. (2016), Ohnishi et al. (2013), Shibutani et al. (2017), Tan et al. (2005)	LNM	Pan-macrophage	Fixed	0.58 (0.42–0.81)
Kim et al. (2018), Koelzer et al. (2016)	MMR status	Pan-macrophage	Fixed	3.46 (1.35–8.85)
Koelzer et al. (2016)		M1-macrophage	Random	1.45 (0.84–2.51)
Edin et al. (2012), Kim et al. (2018), Koelzer et al. (2016)		M2-macrophage	Random	2.25 (0.89–5.73)
		Pooled analysis	Random	2.30 (1.39–3.81)
Forssell et al. (2007), Koelzer et al. (2016), Zhou et al. (2010)	Pathological type	Pan-macrophage	Fixed	0.36 (0.24–0.55)
Edin et al. (2012), Koelzer et al. (2016)		M1-macrophage	Fixed	0.48 (0.25–0.92)
Edin et al. (2012), Koelzer et al. (2016)		M2-macrophage	Fixed	0.37 (0.19–0.69)
		Pooled analysis	Fixed	0.39 (0.28–0.53)
Funada et al. (2003), Koelzer et al. (2016), Tan et al. (2005), Zhou et al. (2010)	Distant metastasis	Pan-macrophage	Fixed	0.43 (0.27–0.67)
Forssell et al. (2007), Kim et al. (2018), Ohnishi et al. (2013), Wei et al. (2019)	TNM stage	Pan-macrophage	Random	0.97 (0.44–2.15)
		M1-macrophage	Random	0.37 (0.22–0.62)
Edin et al. (2012)		M2-macrophage	Random	1.00 (0.44–2.26)
Edin et al. (2012), Kim et al. (2018), Shabo et al. (2014), Wei et al. (2019)		Pooled analysis	Random	0.87 (0.53–1.42)
Koelzer et al. (2016), Ohnishi et al. (2013), Tan et al. (2005), Wei et al. (2019)	T stage	Pan-macrophage	Fixed	1.62 (0.68–2.53)

$P = 0.23$). Significant heterogeneity existed in both the pan-macrophage and M2-macrophage panels (pan-macrophage, DFS: $I^2 = 85\%$, $P = 0.001$; CSS: $I^2 = 60\%$, $P = 0.08$, respectively) (M2-macrophage, DFS: $I^2 = 70\%$, $P = 0.03$; CSS: $I^2 = 76\%$, $P = 0.04$, respectively). For the analysis of clinicopathological factors, except for the TNM stage and T stage group, the heterogeneity of other groups was low.

Discussion

This meta-analysis compared the prognostic value of high and low-density TAMs infiltration in patients with CRC based on patient prognostic data from selected studies. The pooled results indicated that high pan-TAMs infiltration marked by CD68+ could be a relatively pronounced predictive marker, with a better prognosis than low infiltrating density. This is in terms of OS and clinicopathological factors including CD8+ T cell infiltration, no LNM, no DM, and dMMR-MSI-H. The sensitivity analysis revealed the stability of the HR estimates. There was low publication bias in the clinicopathological factors panels.

During the screening process, 4 studies on prognosis were excluded as some of those that had limited sample size (Nagorsen et al. 2007; Xiong et al. 2018). In addition, TAMs of two studies not derived from tumor tissue, but from peripheral blood or lymphoid tissue (Grossman et al. 2018; Ohnishi et al. 2013) and one without high or low-density grouping was also excluded (Chaput et al. 2013). 17 retrospective studies were selected based on rigorous exclusion criteria, and this led to more accurate results in this meta-analysis. Sensitivity analysis was performed by removing the articles one by one and this revealed that among the OS data involving pan-macrophages in 10 of the included studies, when one study was excluded, the heterogeneity could have been reduced from 70 to 52% (Kim et al. 2018). Similarly, in the pooled analysis of M2-macrophages involving OS, excluding the study of Shabo et al. the heterogeneity could

have been reduced to 0. Two studies with adverse prognostic trend demonstrated that high CD68+ macrophage infiltration was associated with decreased OS (Kim et al. 2018; Wei et al. 2019). The relationship between CD68+ macrophages and the adverse prognosis was not limited to two articles in this meta-analysis. Previous studies evaluating high-density TAMs were significantly associated with poorer OS in breast cancer, gastric cancer, ovarian cancer, oral cancer and thyroid cancer (Alves et al. 2018; Yin et al. 2017; Yuan et al. 2017; Zhang et al. 2012). However, in a previous multinomial study on CRC, inconsistent prognostic values involving TAMs have confirmed the complex function of TAMs in the initiation and growth of CRC. Just as the expression of CD4+, CD25+, Foxp3+, Treg cells and PD-1 in CRC show the incongruent function and prognostic value compared to other solid tumours (Li et al. 2019; Mei et al. 2014; Salama et al. 2009; Vlad et al. 2015).

Macrophages mainly originate from the peripheral hematopoietic organ serving as immune cells that not only provide crucial innate immune defense and function as tissue homeostasis and repair but also are involved indirectly in T cell-mediated adaptive immune response and participate in the formation and progression of tumours (Lavin et al. 2015; Noy and Pollard 2014). Studies have reported that macrophages recruited from peripheral blood monocytes may take up the majority of TAMs whose functions differ between tumour types and between macrophage phenotypes (Hasita et al. 2010; Li et al. 2018; Sorensen et al. 2018; Yang and Zhang 2017). Distinct activated polarization factor profiles and cytokines or chemokines production profiles exist in different subpopulations of TAMs (Biswas and Mantovani 2010; Gordon and Taylor 2005; Schmieder et al. 2012; Yahaya et al. 2019). M1-macrophage phenotype was activated with microbial moieties (lipopolysaccharide, LPS) and T helper type 1 (T_H1) cytokine (interferon-γ, IFN-γ; Tumour necrosis factor-α, TNF-α; granulocyte–macrophage colony-stimulating factor, GM-CSF)) by classical pathway (Gordon and Taylor 2005; Joshi et al. 2014; Murray et al. 2014), and M2-macrophage phenotype was polarized with T helper type 2(T_H2) cytokine (IL-4, IL-13, glucocorticoid, CSF-1) by alternative pathway (Fleetwood et al. 2009; Martinez et al. 2008; Schmieder et al. 2012). M1-macrophages have high expression of major histocompatibility complex class II (MHC-II) with efficient antigen presentation capability and a strong ability to secrete proinflammatory cytokines and immunostimulatory cytokines, such as interleukin (IL)-12, IL-23, CXCL9, CXCL10. Consequently, this makes them possess the function of killing bacteria and viruses in cells and promotes the polarization and recruitment of T_H1 cells, thereby enhancing a type 1 response (Biswas and Mantovani 2010; Hagemann et al. 2008). M2-macrophages express a large number of anti-inflammatory cytokines (IL-10), immunomediators (transforming growing factor-β (TGF-β), prostaglandin, indoleamine and dioxygenase IDO), growth factors (VEGF, EGF), chemokines (CCL2, CCL17, CCL22) and matrix metallopeptidases (MMPs) (Bailey et al. 2007; Biswas et al. 2006; Biswas and Mantovani 2010; Giraudo et al. 2004; Noy and Pollard 2014; Qian and Pollard 2010; Ruffell et al. 2012). This makes them participate in the processes of maintaining homeostasis,

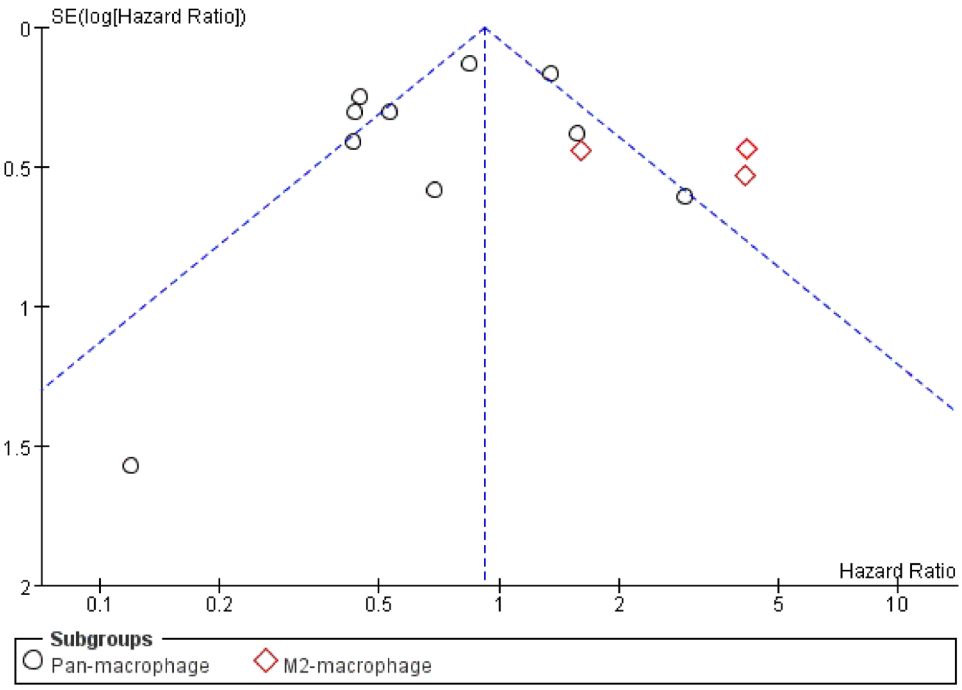

Fig. 4 Funnel plots of the relationship between the size of the effect in individual studies and the precision of the study estimate (log (HR), horizontal axis; SE (log (HR)), vertical axis) for pan-macrophages (CD68+) for OS

such as anti-inflammatory, tissue remodelling, wound healing, angiogenesis and the processes of tumorigenesis and progression including tumor cells aggregation, tumour cells proliferation and immune escape, thereby recruiting Treg cells, T_H2 cells and amplifying of polarized T_H2 responses (Mantovani et al. 2004), the origin and polarization of TAMs are shown in Fig. 5. Previous studies reported that M2-TAMs were the most common type of TME in malignant tumours, which furnishes an immunosuppressive microenvironment for tumour progression (Hao et al. 2012; Lewis and Pollard 2006). These mechanisms explain the results of our meta-analysis and diverse solid tumors of other studies that M2-TAMs are associated with poor prognosis, while M1-TAMs show the opposite results (Hasita et al. 2010; Kawachi et al. 2018; Kim et al. 2018; Sugimura et al. 2015; Yuan et al. 2017).

TAM is a momentous pathway linking inflammation and cancer (Mantovani et al. 2009). Monocytes with some regulator and chemotactic factors in circulation are induced by corresponding ligands (CCL2+, CCL18+, CCL20+, CXCL12, Colony-stimulating factor (CSF) 1) produced by cancer cells and endothelial cells to primary or metastatic sites where they differentiate into M2 phenotype, hence inhibiting immune responses or promotes metastasis (Franklin et al. 2014; Nandi et al. 2016; Srivastava et al. 2014; Yang and Zhang 2017). M1 phenotype could be transformed into M2 during the progression of tumours, and this has been confirmed in animal models. In the early stage of tumours, the majority of TAMs with high expression of MHC-II have been found, while in advanced tumours, the majority are TAMs with low expression of MHC-II and high expression of typical M2 markers (Wang et al. 2011). Despite the fact that the mechanism of macrophage subtype transformation has not been fully understood in the progress of human cancer progression, the acidification of tumour microenvironment (PH changes) and oxygen partial pressure predict poor prognosis or metastasis and these are partly related to the polarization of macrophage subtypes in the course of tumor growth (Bohn et al. 2018; Colegio et al. 2014; Vaupel 2008). Previous studies have confirmed that TME of most solid tumours was acidified, in this "acidic constitution" tumour microenvironment, acidification of tumours is sensed by macrophage G protein-coupled receptor (GPCR), which leads to high expression of ICE (inducible cyclic AMP (cAMP) early repressor), which is a transcription inhibitor of macrophages, and this causes

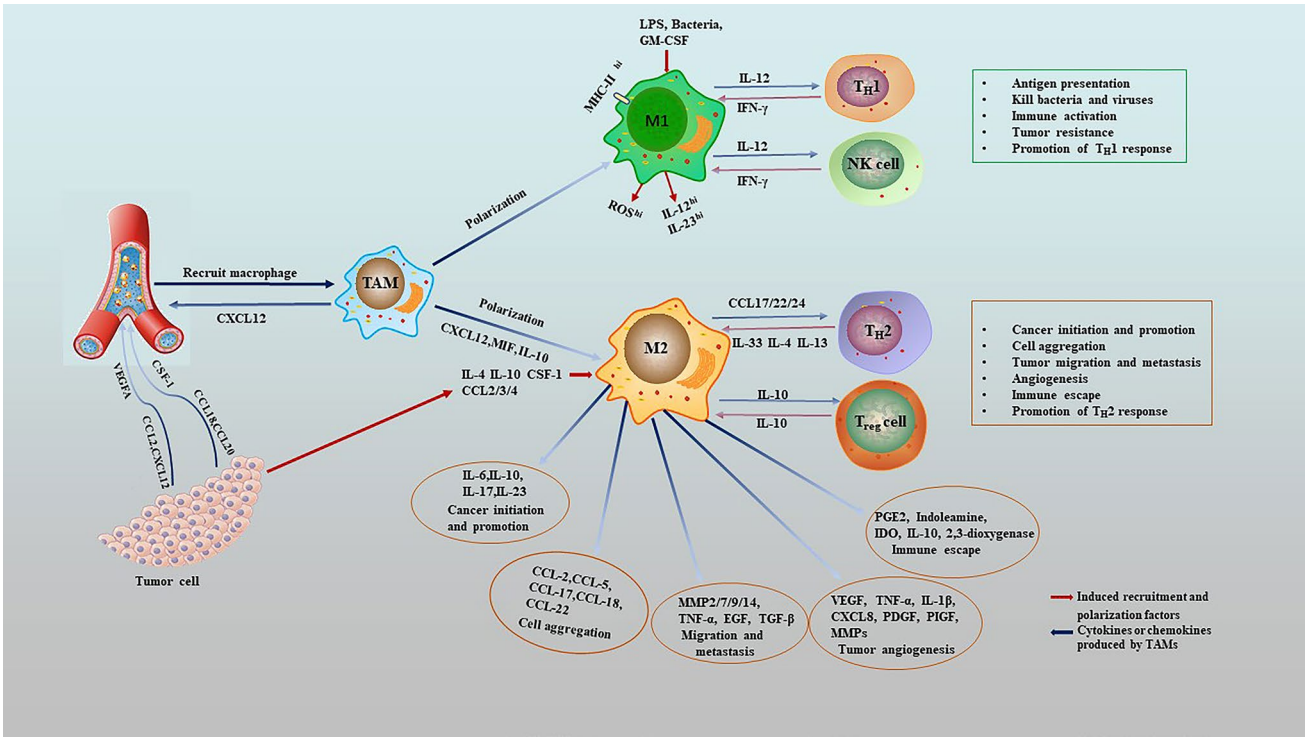

Fig. 5 The origin and polarization of TAMs. Peripheral blood monocytes are recruited locally and differentiate into macrophages in response to various chemokines and growth factors produced by tumour cells in the tumour microenvironment (CCL2, CCL12, CCL18, CCL20, VEGFA and CXCL12) and secreted by TAMs (CXCL12). Tumour cells (CCL2/3/4, CSF1, IL-4, IL-10), Treg cells (IL-10), T_H2 cells (IL-4/13/33) or TAMs (MIF, IL-10, CXCL12) produce various factors that induce the polarization of TAMs to a non-inflammatory phenotype (M2). Other factors (LPS, GM-CSF, IFN-γ) induce the polarization of TAMs to inflammatory phenotype (M1). The secretion of various factors by M1 and M2-TAM mediates the process of tumorigenesis and development

polarization of non-inflammatory macrophages which lead to immune escape. However, colon cancer does not seem to have this acidic constitution as compared to melanoma and lung cancer, which are strongly acidified in TME (Bohn et al. 2018). This explains these study findings that a high level of pan-macrophages is associated with increased OS (HR 0.67 [0.47–0.95]). This was similar to the results in the previous meta-analysis with small sample sizes, on the effect of TAMs on the prognosis of patients with CRC (Zhang et al. 2012). This may be that the polarization of non-inflammatory macrophages (M2-macrophages) in total macrophages of intestinal tumours without acidic microenvironment were weaker than those of other solid tumours, which leads to different prognostic phenomena. However, for DFS and CSS, no definite predictive value was obtained. We found that only three articles from these two subgroups were less likely to show prognostic trends.

The previous study by Franklin and colleagues indicated that the depletion of TAMs originating from CCR2+ inflammatory monocytes restored tumour-infiltrating cytotoxic CD8+ T cells response by reducing the number of immunosuppressive PD1+, GzmB−, CD8+ T cells and thus inhibiting the growth of tumours. However, in intestinal tumours, the symbiotic environment of intestinal bacteria largely shapes gut-related immune system (Zigmond et al. 2012). Particularity the intestinal microenvironment has different effects on certain immune cell subpopulation from other tumours, such as the number and function of FOXP3+ T cell and the abundance of T helper cells (Atarashi et al. 2011; Ivanov et al. 2009). Did this particular microenvironment affect TAMs differently from other tumours? Bohn et al. explained that compared with melanoma having higher glycolytic activity, colon cancer that has lower glycolytic activity had a lower ability to sense acid signals through TAMs to reduce the activity of cytotoxic T cells. The pooled analysis of clinicopathological factors in this meta-analysis confirmed the above findings that in colon cancer, high-density TAMs infiltration is related to high-density CD8+ T cell infiltration, and associated with pathological factors predicting good prognoses, such as no lymph node metastasis, no distant metastasis, non-mucinous cancer, and MSI.

This study provided moderate evidence which evaluated the association of TAMs infiltration with prognostic outcomes and clinicopathological factors. However, this meta-analysis was associated with some limitations. First, a relatively small sample of DFS and CSS was used to evaluate the prognostic value of TAMs, therefore, no definite conclusion was obtained. Second, no restriction was placed on the staining methods and evaluation methods, however, different experimental methods and evaluation methods may have different TAMs, and this led to high heterogeneity in a pooled analysis of prognosis, especially for DFS and CSS. Therefore, unified experimental and evaluation methods are needed in the future. Third, although the heterogeneity of pooled analysis of clinicopathological factors was relatively small, a small number of studies meeting the inclusion criteria were analysed in subgroup and pooled analyses. Despite these limitations, the results of this analysis to improvements in the outcomes of targeting TAMs therapy through stratifying patients or combining clinicopathological parameters to predict the prognosis of patients with CRC.

Conclusion

This meta-analysis corroborated with previous studies that pan-macrophages (CD68+) are favourably associated with overall survival. M2-macrophages (CD163+) were associated with poorer OS and DFS/RFS. Further, high-density TAMs were found to be related to NLNM, NDM, MSI, non-MUC and high-density CD8+ T cell infiltration. These results can be used to develop new therapeutic regimen targeting TAMs. They can also serve as a guide to the treatment and prognosis by detecting phenotype and status of TAMs in patients with CRC. However, further studies are required to develop strategies for accurate application of TAM markers as predictive biomarkers in clinical practice, in combination with other biomarkers including TILs, and genetic phenotype.

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Compliance with ethical standards

Conflict of interest No potential conflicts of interest were disclosed.

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