Prognostic impact of tumor-infiltrating lymphocytes in high grade serous ovarian cancer: a systematic review and meta-analysis

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Abstract

Background: Tumor-infiltrating lymphocytes (TILs) are involved in the antitumor immune response. The association between prognosis in patients with TILs and high-grade serous ovarian cancer (HGSOC) remains obscure, with some studies reporting conflicting results.

Methods: We conducted an extensive literature search of electronic databases and retrieved prognostic data of each selected subtype of TILs, including CD3+, CD4+, CD8+, CD103+, and PD-1+ TILs. The fixed-effects model was applied to derive the pooled hazard ratio (HR) and 95% confidence interval (CI) of these markers.

Results: The systematic review process yielded 19 eligible studies comprising 6004 patients with HGSOC. We compared TIL-positive and TIL-negative patients, and the pooled HRs from the multivariate analysis revealed that intraepithelial CD8+ TILs were positively correlated with progression-free survival (PFS, HR 0.46, 95% CI 0.25–0.67) and overall survival (OS, HR 0.90, 95% CI 0.86–0.9); stromal CD8+ TILs were positively correlated with OS (HR 0.61, 95% CI 0.36–0.87). Furthermore, the pooled HRs from univariate analysis demonstrated that intraepithelial CD3+, CD4+, CD8+, and CD103+ TILs were positively associated with OS (HR 0.58, 95% CI 0.44–0.72; HR 0.37, 95% CI 0.16–0.59; HR 0.51, 95% CI 0.42–0.60, and HR 0.59, 95% CI 0.44–0.74, respectively); stromal CD4+ and CD8+ TILs were significantly associated with OS (HR 0.63, 95% CI 0.32–0.94 and HR 0.78, 95% CI 0.58–0.97, respectively). However, the pooled HR from the multivariate analysis revealed that PD-1+ TILs were not associated with the OS of patients with HGSOC (HR 0.97, 95% CI 0.90–1.04).

Conclusion: This meta-analysis provided evidence of the association of CD3+, CD4+, CD8+, and CD103+ TILs with the survival benefits (OS and PFS) of patients with HGSOC.

Keywords: high-grade serous ovarian cancer, meta-analysis, prognosis, systematic review, tumor-infiltrating lymphocytes

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Introduction

High-grade serous ovarian cancer (HGSOC) represents the most aggressive gynecologic malignancy, which is frequently diagnosed at an advanced stage and is associated with two-third of gynecological malignancy-related mortality worldwide.[1,2] Despite consistent efforts to augment the efficacy of platinum-based chemotherapy, the overall survival (OS) of women with HGSOC has not substantially improved over the past few decades. Nevertheless, through synthetic lethality, poly-ADP-ribose polymerase inhibitors (PARPis), most active and remarkable therapies approved for the treatment of epithelial ovarian cancer, have changed of ovarian cancer harboring homologous recombination deficiency (HRD) or BRCA mutations.[3,4] Moreover, the recent advancements in molecular genetics have profoundly promoted

an unprecedented understanding of HGSOC in the last few years.[1,5] Notably, attempts have been accelerated to identify biomarkers for predicting treatment response and survival; however, the implications of such predictive parameters remain limited.

Conceivably, the elimination of cancer cells is highly dependent on the immune system of patients. Tumor cells modulate normal immune contexture to orchestrate a supportive but explicitly immunosuppressive tumor microenvironment (TME), which generates neoantigens that attract diverse immune cells.[6,7] Tumor-infiltrating lymphocytes (TILs) are lymphoid cells (T cells) that leave the vasculature and infiltrate the tumor, and localize in the islet of malignant cells (intraepithelial) and reside in the peritumoral space (stromal).[6,8] Moreover, these cells reflect the endogenous antitumor immune response. TILs comprise a heterogeneous population of lymphoid cells, exhibiting diverse antitumor activity and spatial distribution, characterized by the expression of different molecular biomarkers, including CD8, CD3, CD4, CD103, and PD-1.[9,10] CD8+ T cells, known as CD8+ cytotoxic T lymphocytes (CTLs), are activated when their receptors recognize antigens in the context of major histocompatibility complex (MHC) class I molecules and have the capacity to directly kill tumor cells expressing MHC class I molecules. An increasing number of studies have indicated the presence of intraepithelial CD8+ TILs is associated with a prognostically favorable survival benefit in HGSOC patients[11,12] Conversely, Pinto *et al.* reported that stromal CD8+ TILs did not correlate with improved OS and progression-free survival (PFS).[13] Although many markers have been used for evaluating antitumor immune responses of TILs, CD3 represents a reliable marker of TILs. Robust pieces of evidence have shown that high levels of CD3+ TILs were significantly associated with improved OS in HGSOC[14]; however, other studies have reported conflicting results.[15] Moreover, CD4+ TILs orchestrate a diverse range of antitumor immune responses, wherein CD4+ T-helper 1 (Th1) cells secreting cytokines such as IFN-γ and tumor necrosis factor (TNF) may effectively inhibit angiogenesis as well as facilitate the activation and proliferation of CD8+ TILs. Previous studies have confirmed that patients with abundant infiltration of Th1 exhibited improved survival rates by stimulating CD8+ cytotoxic T cells. In contrast, CD4+ T-regulatory cells (Treg) exhibit tumor-promoting activity by limiting the development of autoimmunity and suppressing the function of Th1 cells, which may be inhibited to promote optimal antitumor responses.[16,17] Of note, CD103+ TILs play a crucial role in specific immunity against cancers of epithelial origin by recruiting antigen-specific lymphocytes within epithelial tissues *via* binding through the epithelial cell surface molecule, E-cadherin. An increasing number of studies indicate that TILs often present an impaired capacity to produce an effective antitumor response because of adaptive immune resistance. In this context, Ahmadzadeh *et al.* suggested that programmed cell death-1 TILs (PD-1+ TILs) exhibited an exhausted phenotype and effector dysfunction compared with PD-1– TILs, confirming a crucial role of the PD-1 pathway in suppressing T-cell effector function.[18] Previously, multiple meta-analyses employing extensively differing methodologies have been conducted in ovarian cancer,[10,19] including diverse TILs subtypes. However, they did not provide comprehensive insights, and a conflict exists regarding the association of TILs with the prognosis of patients with HGSOC. Thus, the role of these biomarkers as prognostic indicators of survival in patients with HGSOC remains elusive.

We assumed that different subtypes of TILs might have different prognostic roles in HGSOC and that the spatial distribution of TIL subtypes might be of specific prognostic relevance. Therefore, we conducted a comprehensive systematic review and meta-analysis of relevant publications to evaluate the prognostic efficacy of the different TIL subtypes, and also assessed the effect of their anatomical location. We hypothesized that the prognostic effects of TILs might be identical in some subtypes; however, the magnitude of the effect might differ, considering the subtype and spatial distribution.

Materials and methods

This systematic review and meta-analysis were performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (http://www.prisma-statement.org/).[20]

Data sources and search strategy

We conducted an extensive literature search of electronic databases, including PubMed/Medline,

Web of Science, Excerpta Medica Database (Embase), and the Cochrane Library database up to and including 31 March 2020. Proceedings from the annual scientific meetings, including the American Society of Clinical Oncology (ASCO) and European Society of Medical Oncology (ESMO), were also searched to identify unpublished data on the association between TILs and HGSOC. The search strategy was based on published articles,[10,19] and the determinant domains were as follows: ("ovarian cancer") AND ("tumor-infiltrating lymphocytes" OR TILs OR "T lymphocytes" OR "T cells" OR "Tregs"). Besides, the references of the selected articles and reviews were also retrieved manually to obtain all potentially eligible records that were not identified through a database search. In the initial search, we did not apply any advanced restrictions to the date or study design or publication language in our search strategy.

Study selection

All retrieved articles were independently scrutinized for eligibility by two reviewers (Hao and Yu). Studies were eligible if they met the following selection criteria: (1) patients enrolled were histopathologically diagnosed with HGSOC; (2) prognostic efficacy of TILs were evaluated in patients with HGSOC; (3) time-to-event outcomes was included with survival outcome measures, such as PFS, OS, or disease-specific survival (DSS) with relative hazard ratios (HRs) and 95% confidence intervals (CIs) or survival curves. We excluded studies that were not original articles (such as letters, reviews, meta-analyses, case reports, animal trials, *in vitro* studies, and comments), duplicate articles, data on the relationship between TILs and HGSOC that could not be acquired and articles with incomplete data. When multiple publications reported on the same patient cohort or overlapping population, only the most recent or complete publication was selected for the meta-analysis. When the study details were not sufficient to determine eligibility, corresponding authors were contacted for further information. Any disagreements in the determination of the study's eligibility between the reviewers were resolved by consensus through discussion with the third reviewer (Xue).

Data items and collection

For each eligible publication, the data extraction was performed independently by the two investigators (HAO and YU), using a predefined table. Any disagreements between investigators were resolved through panel discussions. The following data were extracted: the first author's last name, year of publication, country, sample size, tumor stage, detection method, cut-off value, biomarker(s), site of infiltration, and survival endpoints of univariate and/or multivariate analysis defined by the HRs with 95% CIs. When survival data were not mentioned in the article, the data were extracted indirectly from the Kaplan–Meier curves using Engauge Digitizer software (http://digitizer.sourceforge.net/).[21,22] However, when calculated HRs did not match the existing curves, studies were excluded. For time-to-event data, HRs were used to evaluate the risk of progression or death of patients with high-level TILs *versus* low-level TILs. In studies that reported HRs for low-level TILs *versus* high-level TILs, the reciprocals of HRs and 95% CIs were recorded. Of note, this meta-analysis extracted and analyzed survival data based on biomarkers and site of infiltration, and classified the locations as intraepithelial, stromal, and pan-tumor.

Statistical analyses

Pooled analyses of HRs and 95% CIs were used to evaluate the prognostic efficacy of TILs in this meta-analysis. The primary outcomes of interest were PFS and OS/DSS of TIL-positive HGSOC patients compared with TIL-negative HGSOC patients. In this meta-analysis, we estimated the pooled HRs according to the degree of variation (I^2) attributable to heterogeneity.[23] Heterogeneity analysis was assessed by I^2 statistic with a significant threshold of p-value < 0.05. A value of I^2 of 0–25% represented insignificant heterogeneity, $>25\%-\leqslant 50\%$ represented low heterogeneity, $>50\%$ considered high heterogeneity. The fixed-effects model was applied when $p > 0.05$ and $I^2 < 50\%$; otherwise, the random-effects model was selected.[24] Quantitative measurement of inconsistency across studies was eventually demonstrated through visual inspection of forest plots. When heterogeneity was observed, a sensitivity analysis was performed to test the stability of the original results. The publication bias was assessed by visual inspection of funnel plots, and quantitative evaluation by Beggr's regression and Egger's linear regression method.[25,26] The non-parametric trim-and-fill procedure was applied to assess the possible effect of publication bias. All statistical analyses were performed using STATA version 12.0 (Stata Corporation, College Station,

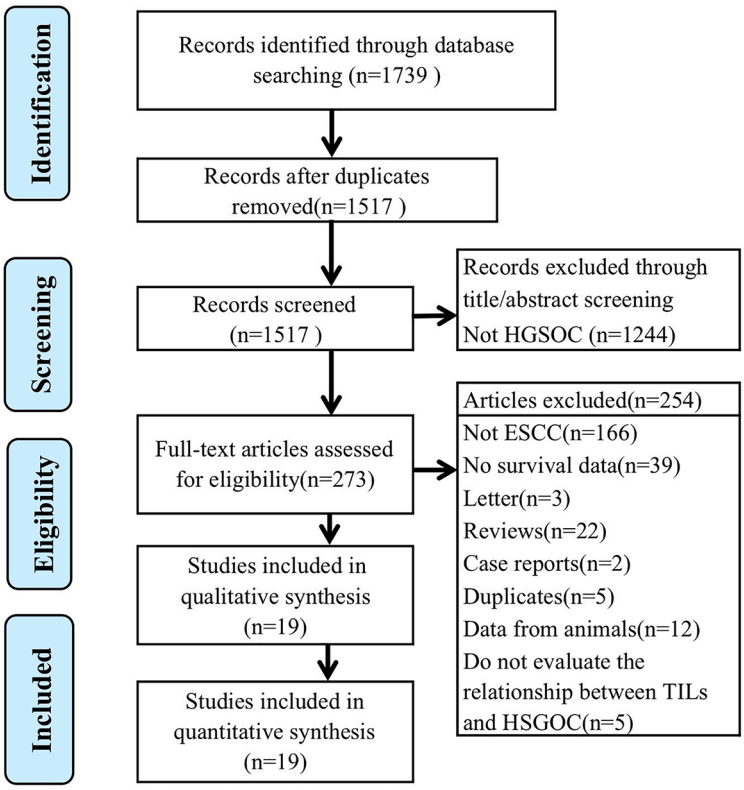

Figure 1. Schematic diagram of the selection procedure for studies included in this meta-analysis.
ESCC, esophageal squamous-cell carcinoma; HGSOC, high-grade serous ovarian cancer; TILs, tumor-infiltrating lymphocytes.

TX, USA) software. All statistical tests were two-sided, and a p-value of < 0.05 was considered statistically significant.

Results

Study characteristics

A total of 1739 potentially relevant records were initially retrieved from different databases. After the full-text screening, we identified 19 articles that met our eligibility criteria, comprising 6004 participants. All included studies were published between 2008 and 2019 in English peer-reviewed journals with a median sample size of 150 (interquartile range, 82–3196). Among all the studies, 16 studies were conducted in Europe and America,[11,12,15,27–39] 2 in Asia,[14,40] and 1 in South America.[13] Four studies only included patients with stage III–IV HGSOC, 5 studies included stage I–III patients, two studies included stage II–IV patients, and eight studies included all combined stage I–IV HGSOC patients. Most of the included studies used formalin-fixed paraffin-embedded (FFPE) HGSOC tissue specimens. All studies used immunohistochemistry (IHC) as a method for detecting hotspot markers presented on TILs, with diverse scoring systems and cut-off values. For case ascertainment, almost all studies had a retrospective design and barely provided data on the follow up of HGSOC patients who were lost during follow up. Figure 1 illustrated the schematic diagram representing the detailed search and the PRISMA study selection strategy. Of the total eligible studies, 14 studies investigated the association between intraepithelial TILs and survival outcome, whereas three studies reported pan-tumor TILs, and five studies reported stromal TILs. Almost all of the included studies evaluated HRs and 95% CIs from univariate and/or multivariate analysis; however, a few of them were calculated using survival curves. Quality In Prognosis Studies (QUIPS) was used to appraise the risk of bias in the included 19 studies.[41] Low, moderate, and high risks of bias were noted in seven, four, and eight included studies, respectively, with scores ranging from 1 to 6. Importantly, we did not exclude studies based on a QUIPS score for overall quality. A summary of the characteristics and quality assessment of the included studies was presented in Table 1.

Table 1. Main characteristics of included studies.

Study	Country	Cases	Stage	Method	Cut-off value	Subtype	Location	Outcome	UA	MA	Total risk of bias
Callahan *et al.*[27]	US	184	IIIB–IV	IHC	75th percentile	CD8+	ST	OS	NR	Yes	Low
Darb-Esfahani *et al.*[28]	Germany	215	I–IV	IHC	density for each marker	CD3+, PD-1+	IT	PFS, OS	NR	Yes	High
Lo *et al.*[15]	Canada	90	III–IV	IHC	median density	CD3+, CD8+, PD-1+	IT	OS	NR	Yes	Low
Leonard *et al.*[29]	US	354	I–IV	IHC+IF	median density	CD3+, CD4+, CD8+	GT	PFS, OS	NR	Yes	Low
Stumpf *et al.*[12]	Germany	100	III	IHC	5 cells/HPF	CD8+	IT	OS	NR	Yes	Low
Wang *et al.*[14]	China	107	I–IV	IHC	score = 2 or 3	CD3+, CD4+, CD8+	IT, ST	OS	Report	Yes	High
Wouters *et al.*[30]	Netherlands	87	II–IV	IHC	highest tertile	CD8+	IT	DSS	NR	Yes	Moderate
Bösmüller *et al.*[31]	Germany	150	II–IV	IHC	density for each marker	CD3+, CD103+	IT	OS	Report, SC	No	High
Clarke *et al.*[32]	Canada	155	I–III	IHC	1 cell/HPF	CD8+	IT	DSS	SC	No	Moderate
deLeeuw *et al.*[33]	Canada	187	I–III	IHC	1 cell/HPF	CD8+	IT	PFS	Report	No	Moderate
Komdeur *et al.*[34]	Netherlands	82	IIb	IHC+IF	intermediate	CD8+, CD103+	GT, ST	DSS	SC	No	High
Strickland *et al.*[35]	US	245	I–IV	IHC	13 cells/HPF	CD3+	GT	DFS, OS	SC	No	High
Webb *et al.*[36]	Canada	198	I–III	IHC	5 cells/0.6 mm^2	CD103+	IT	DSS	SC	Yes	Low
Goode *et al.*[11]	US	3196	I–IV	IHC	20 cells/HPF	CD8+	ST	OS	SC	Yes	Low
Pinto *et al.*[13]	Chile	128	I–IV	IHC	10 cells/HPF	CD4+, CD8+	IT, ST	PFS, OS	Report	Yes	Low
Stanske *et al.*[37]	Germany	113	I–IV	IHC	25th percentile	CD3+, CD4+, CD8+	IT	PFS	SC	No	High
Webb *et al.*[38]	Canada	195	I–III	IHC	5 cells/HPF	CD3+, CD8+	IT	DSS	Report	No	Moderate
Huang *et al.*[40]	China	84	I–IV	IHC	density for each marker	CD3+, CD4+, CD8+	IT	PFS	SC	Yes	High
Adams *et al.*[39]	US	134	III–IV	IHC	10 cells/HPF	CD8+	IT	OS	SC	No	High

DSS, disease-specific survival; HPF, high power field; IHC, immunohistochemistry; IT, intraepithelial; MA, multivariate analysis; NR, not reported; OS, overall survival; PFS, progression-free survival; PT, pan-tumor; SC, survival curve; ST, stromal; UA, univariate analysis; US, United States.

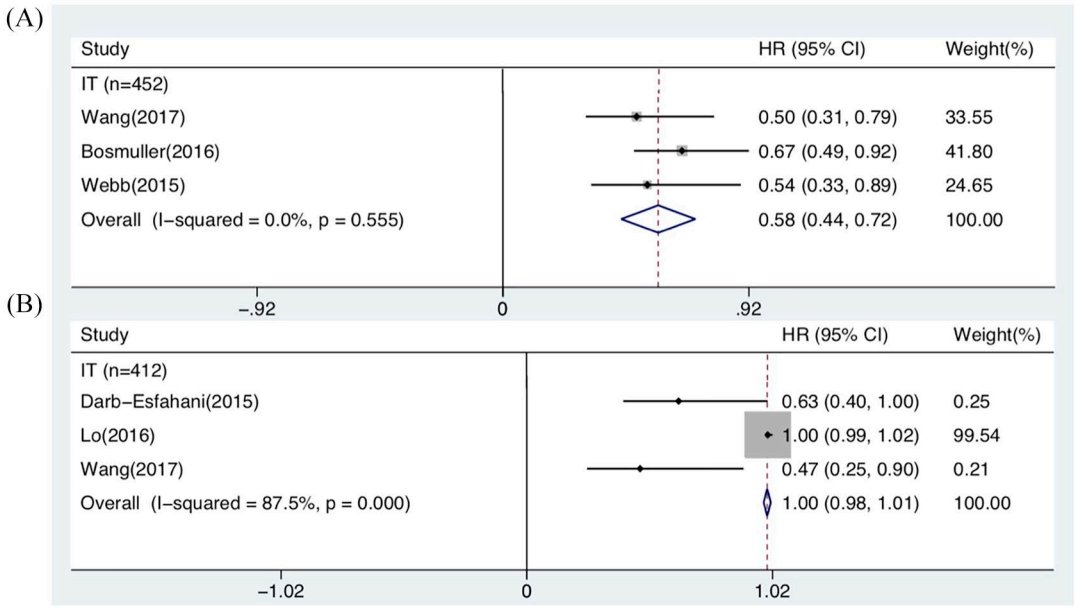

Figure 2. Pooled HRs of intraepithelial CD3+ TILs from univariate (A) and multivariate (B) analysis for OS.
CI, confidence interval; HR, hazard ratio; IT, intraepithelial; OS, overall survival; TILs, tumor-infiltrating lymphocytes.

Prognostic value of CD3+ TILs

The prognostic value of CD3+ TILs was assessed in nine studies. We pooled survival data only for patients with intraepithelial infiltration due to lack of data. The pooled results from univariate analysis indicated that PFS (HR 0.68, 95% CI 0.55–0.81) and OS (HR 0.58, 95% CI 0.44–0.72) was positively associated with CD3+ TILs, with no clear evidence of heterogeneity (PFS $I^2=0.0\%$, $p=0.885$; OS $I^2=0.0\%$, $p=0.555$) (Figure S1, Figure 2). Pooled HR from the multivariate analysis revealed no trend towards a favorable OS (HR 1.00, 95% CI 0.98–1.01), and there was considerable heterogeneity among studies (OS $I^2=87.5\%$, $p=0.000$) (Figure 2). When the random-effects model was applied, the observed impact of TILs on OS (HR 0.73, 95% CI, 0.36–1.09) did not change.

Prognostic value of CD4+ TILs

Five studies presented data on the prognostic value of CD4+ TILs in patients with HGSOC. All survival data were derived from univariate analysis. The pooled HRs indicated that high levels of intraepithelial CD4+ TILs were associated with improved PFS (HR 0.74, 95% CI 0.61–0.87), with some evidence of heterogeneity between the results of studies (PFS $I^2=84.8\%$, $p=0.001$), and the results altered significantly when the random-effects model was applied (HR 0.73, 95% CI 0.39–1.06) (Figure S2). Notably, patients with intraepithelial CD4+ TILs were significantly associated with an improved OS (HR 0.37, 95% CI 0.16–0.59). Although there was some evidence of heterogeneity between the results of studies ($I^2=59.7\%$, $p=0.115$), the survival benefits (HR 0.42, 95% CI 0.05–0.79) remained unaltered when the random-effects model was applied. In patients with stromal CD4+ TILs, pooled HRs indicated that high-levels of CD4+ TILs were associated with favorable OS (HR 0.63, 95% CI 0.32–0.94) with no significant evidence of between-study heterogeneity ($I^2=0.0\%$, $p=0.804$) (Figure 3).

Prognostic value of CD8+ TILs

A total of 14 articles investigated the prognostic value of CD8+ TILs. Pooled HRs indicated that patients with high levels of intraepithelial CD8+ TILs exhibited a favorable outcome for PFS (HR from univariate analysis 0.54, 95% CI 0.41–0.67; HR from multivariate analysis 0.46, 95% CI 0.25–0.67); the relationship was consistent across studies (univariate analysis $I^2=0.0\%$, $p=0.570$; multivariate analysis $I^2=0.0\%$, $p=0.384$) (Figure S3A and S3B). In patients with high-level intraepithelial CD8+ TILs, pooled HRs indicated that CD8+ TILs were correlated positively with OS (HR from univariate analysis 0.51, 95% CI 0.42–0.60; HR from multivariate analysis 0.90, 95%CI

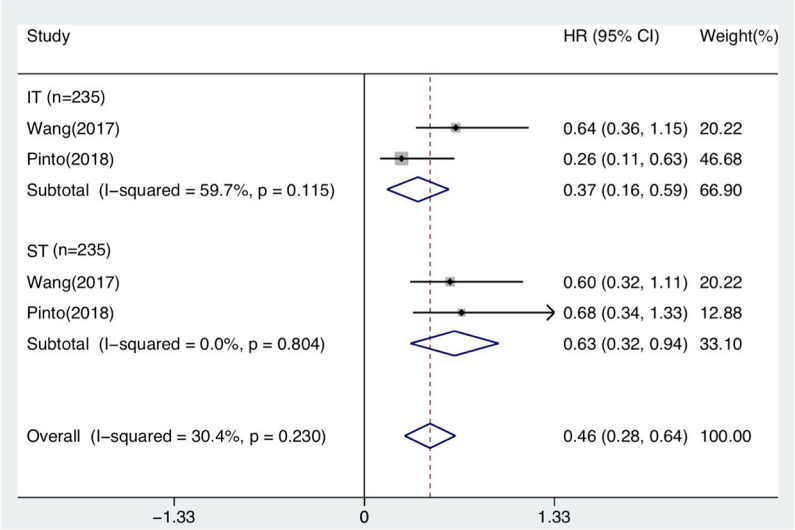

Figure 3. Pooled HRs of intraepithelial and stromal CD4+ TILs from univariate analysis for OS.
CI, confidence interval; HR, hazard ratio; IT, intraepithelial; OS, overall survival; ST, stromal; TILs, tumor-infiltrating lymphocytes.

0.86–0.93); however, there was considerable evidence of variation between the studies for OS from multivariate analysis ($I^2 = 95.7\%$, $p = 0.000$). The result did not change significantly when the random-effects model was applied (HR 0.68, 95% CI 0.39–0.98). Similarly, in patients with high-level stromal CD8+ TILs, pooled results revealed that CD8+ TILs were positively associated with OS/DSS (HR from univariate analysis 0.78, 95% CI 0.58–0.97; HR from multivariate analysis 0.61, 95% CI 0.36–0.87) with no evidence of between-study heterogeneity (univariate analysis $I^2 = 3.0\%$, $p = 0.357$; multivariate analysis $I^2 = 40.2\%$, $p = 0.196$) (Figure 4).

Prognostic value of CD103+ TILs

Only two studies that evaluated the impact of CD103+ TILs on survival were included in this meta-analysis. The studies investigating CD103+ TILs only assessed the relationship between intraepithelial infiltration level and OS. Pooled data from the univariate analysis revealed that high levels of intraepithelial CD103+ TILs improved OS (HR 0.59, 95% CI 0.44–0.74); however, there was evidence of between-study heterogeneity ($I^2 = 55.5\%$; $p = 0.134$), which was reflected when the random-effects model was applied (HR 0.59, 95% CI 0.37–0.82) (Figure 5A).

Prognostic value of PD-1+ TILs

Only two studies evaluated the impact of intraepithelial PD-1+ TILs on survival. Therefore, scant data was available to determine the effect of PD-1+ TILs on OS in HGSOC patients. The pooled HR from the multivariate analysis was 0.97 (95% CI 0.90–1.04), indicating that there was no correlation between PD-1+ TILs and OS, and evidence of significant between-study variation for OS existed ($I^2 = 88.4\%$, $p = 0.003$). However, no significant benefit of intraepithelial PD-1+ TILs on OS was observed when the random-effects model was applied (HR 0.81, 95% CI 0.40–1.22) (Figure 5B).

Sensitivity analyses and publication bias

Sensitivity analyses were performed to assess the stability of the results when heterogeneity was observed. No individual study altered the pooled data qualitatively according to the leave-one-out trial. Moreover, the funnel plots exhibited a symmetrical distribution, indicating the absence of publication bias. In addition, the results of Begg's test and Egger's test showed no significant publication biases that could have significantly influenced the results of this meta-analysis.

Discussion

In this systematic review and meta-analysis, we verified the association of high levels of major subsets of TILs with a favorable survival outcome in HGSOC patients. Previous studies have suggested that the presence of TILs can increase the likelihood of improved survival in patients with

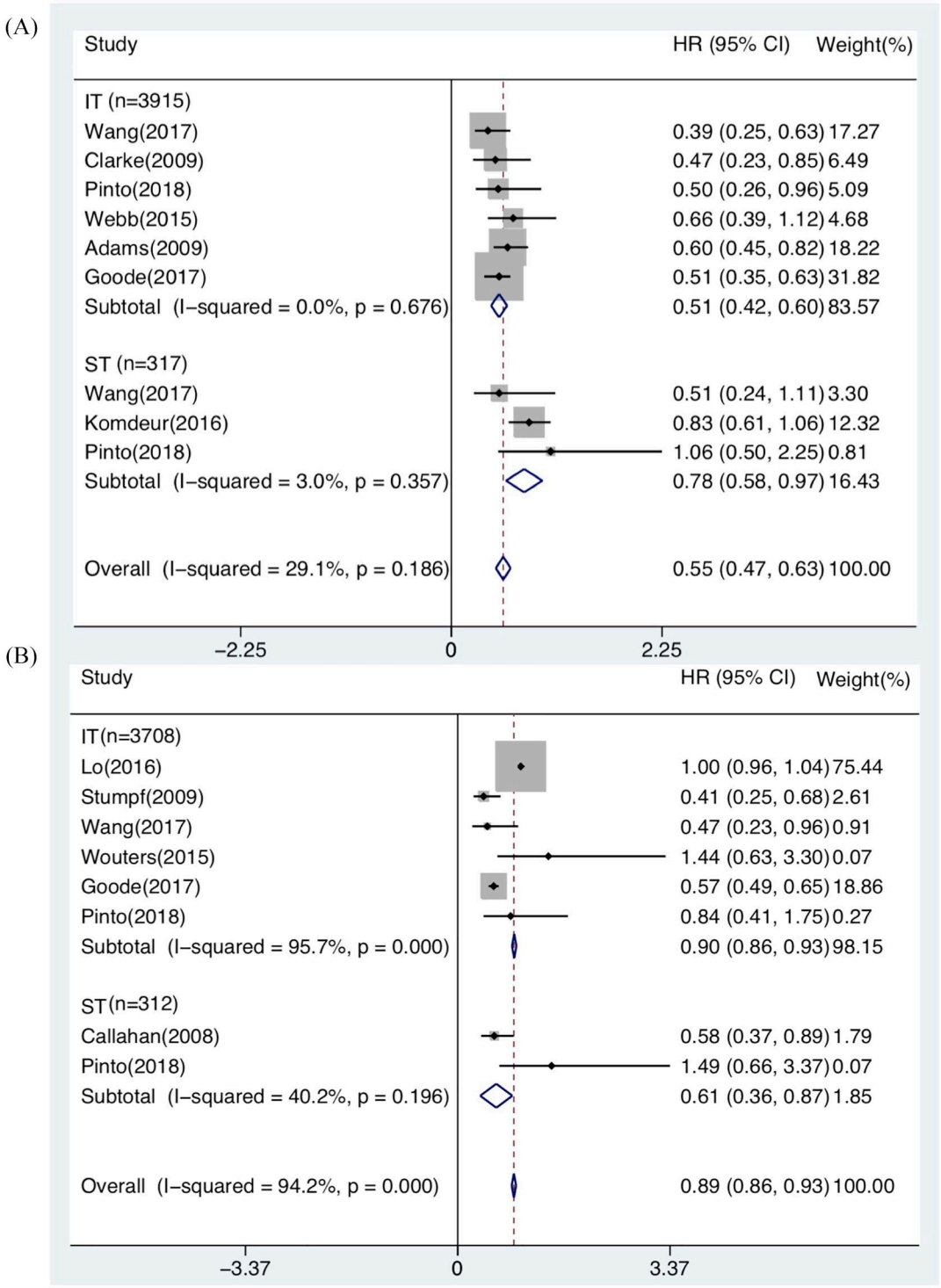

Figure 4. Pooled HRs of intraepithelial and stromal CD8+ TILs from univariate (A) and multivariate (B) analysis for OS/DSS.

CI, confidence interval; DSS, disease-specific survival; HR, hazard ratio; IT, intraepithelial; OS, overall survival; ST, stromal; TILs, tumor-infiltrating lymphocytes.

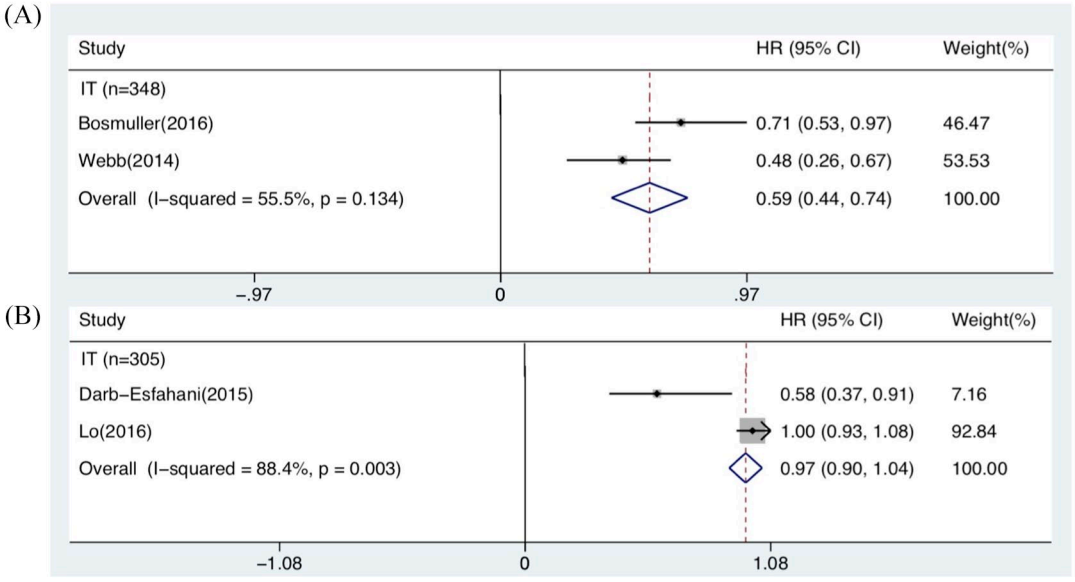

Figure 5. Pooled HRs of intraepithelial CD103+ TILs from univariate analysis (A) and PD-1+ TILs from multivariate analysis (B) for OS.
CI, confidence interval; HR, hazard ratio; IT, intraepithelial; OS, overall survival; PD-1, programmed cell death 1; TILs, tumor-infiltrating lymphocytes.

ovarian cancer.[10,19] However, the prognostic significance of TILs in HGSOC remains elusive. Therefore, we collected available evidence from all relevant studies to individually assess the prognostic effect of TILs in patients with HGSOC.

TILs elicit a potent antitumor immune response that is mediated in part by direct cell-to-cell contacts and provides a favorable survival advantage to patients with HGSOC.[6] Furthermore, TILs infiltrating in stroma or intraepithelial may have different prognostic value. In this context, we attempted to pool the HRs of stromal and intraepithelial TILs independently. Our study confirmed that intraepithelial CD3+, CD4+, CD8+, and CD103+ TILs in tissue specimens are reliable biomarkers for OS and PFS of patients with HGSOC. Among these subtypes, overexpression of CD8+ TILs presented a more consistent and robust association with improved survival. It is worth mentioning that emerging immunotherapies are being developed based on the implication of the adoptive transfer of marker-specific TILs to produce an antitumor immune response.[42] Thus, it is plausible for pathologists and clinicians to adopt the status of CD8+ TILs for predicting survival and guiding clinical management. Although our study demonstrated no significant association between survival benefit and PD-1 expression in TILs, the relationship between PD-1+ TILs and survival still warrants further investigation due to extensively increasing implications of immunotherapy. In addition, stromal CD4+ and CD8+ TILs are also significantly associated with the survival of HGSOC patients. The findings of this meta-analysis remain valid despite the existence of heterogeneity across studies concerning patient characteristics, study design, and scoring methodology.

Tumor-reactive T lymphocytes, defined by the presence of CD3+ TILs, have been investigated in ovarian cancer. In 2003, Zhang *et al.* confirmed that the presence of intratumoral CD3+ TILs correlated with improved PFS and OS among women with ovarian carcinoma.[9] However, the study did not independently analyze the association between intratumoral CD3+ TILs and clinical outcomes in HGSOC, which does not meet the first inclusion criterion of our study. The study also found that increased expression of vascular endothelial growth factor (VEGF) was associated with the absence of intratumoral CD3+ TILs, the VEGF may, therefore, affect the behavior of ovarian cancer, not only by promoting angiogenesis but also by reducing the number of tumor-infiltrating T cells.[9] This may possibly explain the results of our study that intratumoral

CD3+ TILs were only associated with PFS but not with OS. In our study, the stratified analysis of CD3+ TILs based on infiltration sites was limited because the number of studies was insufficient to provide a firm conclusion on stromal CD3+ TILs. Taken together, further studies are warranted to validate the implication of the detection of intratumoral and stromal CD3+ TILs in the treatment assessment of patients with HGSOC. TILs expressing pan T cell marker, CD3, are used to assess the overall quantity of infiltrating T lymphocytes, and interplay with different subsets such as CD4+ TILs. This meta-analysis suggested that HGSOC patients with high levels of intraepithelial or stromal CD4+ TILs presented improved survival. Consistently, melanoma patients with increased levels of CD4+ TILs exhibited longer OS than TIL-negative patients.[10] Indeed, the CD4+ T-cell family exhibits versatile roles in modulating antitumor immune responses due to various functional diversities ranging from effector to regulatory subsets.[43] As for functional subsets, CD4+ T-helper 1 and T-helper 2 cells can either directly eliminate tumor cells through cytolytic mechanisms or indirectly enhance antitumor responses and function as antigen-presenting cells for CD8+ TILs. Besides, T-regs may function to suppress immune responses.[43,44] In cancer patients, the characterization of CD4+ TILs has been mostly investigated, whereas the functional subtypes such as T-helper 1, T-helper 2 and T-regs remain scantly analyzed. Thus, to decipher CD4+ TILs biology in relation to tumor development and progression in HGSOC patients, further studies and testing on functional subtypes are warranted in future investigations.

Furthermore, previous studies have demonstrated that deficient CD4+ T-helper reduces the response of CD8+ T cells, and optimizing CD4+ T-helper may improve outcomes in patients receiving cancer immunotherapy.[44] Therefore, the balance between CD4+ and CD8+ TILs can also influence the antitumor competence of TILs. A suitable CD8+/CD4+ TILs ratio may be associated with a better prognosis of patients with HGSOC. For instance, a higher CD8+ TILs/T-reg cells ratio, which indicates that the beneficial effect of CD8+ T cells outweighs the immunosuppressive effect of the T-regs, was presented as a superior indicator for survival outcome than CD8+ or CD4+ TILs alone. Therefore, the balance of CD8+ and CD4+ TILs remains critical for the prognosis. However, an inadequate number of studies that evaluated these ratios were available in this meta-analysis. Thus, further investigations are warranted to comprehensively understand the significance of these ratios as a prognostic marker.

CD8+ TILs are the pivotal effector for targeting tumor cells and are the most frequently assessed subtype, as they detect intracellular antigens that are presented by MHC class I molecules expressed by all tumor cell types.[11,12] In accordance with our results, numerous preclinical studies have identified a positive association between survival benefits and high-level CD8+ TILs in melanoma[10] and esophageal cancer.[45] Notably, Goode *et al.*[11] conducted the largest prospective study on intraepithelial CD8+ TILs in 3196 patients with HGSOC. They demonstrated a steady dose-dependent increase in survival with increasing levels of intraepithelial CD8+ TILs. However, other studies failed to report any prognostic significance of intraepithelial CD8+ TILs.[13] This discrepancy may be explained by adoptive immune-resistance induced by the upregulation of the PD-1/PD-L1 signaling pathway. Moreover, preclinical studies revealed an inverse correlation between the expression of PD-1 and the densities of CD8+ TILs on cancer cells, suggesting that PD-1 inhibits the recruitment of TILs.[18] Furthermore, immunosuppressive cytokines released by tumor cells, deficient presentation of tumor antigen by dendritic cells, and reduced production of co-stimulating cytokines by helper CD4+ T-cells could also impair the antitumor function of CD8+ TILs.[18,44]

α_E(CD103)β_7 integrin binds to an E-cadherin domain that is frequently expressed on tumor cells.[31,36] Increasing evidence suggested that CD103+ TILs might be trapped within the tumor islets through adhesive interactions with E-cadherin in specific immunity against cancers of epithelial origin.[36] Possibly because intraepithelial TILs are in direct contact with tumor cells, the interaction of E-cadherin with CD103 may afford improved prognostic advantage. Therefore, we included CD103 in our panel of biomarkers. In the present meta-analysis, we confirmed the prognostic significance of CD103+ TILs. In addition, CD103+ TILs have been shown to be critical for the recognition and killing of cervical and breast cancer cells.[46,47] Most CD103 molecules are preferentially expressed by intraepithelial CD8+ TILs rather than the associated stromal counterparts in ovarian cancer.[36]

Importantly, the $CD8^+$ TILs that exhibit a highly activated and cytolytic phenotype are strongly associated with survival. Furthermore, previous studies have reported that CD8+CD103+ TILs exert regulatory functions *via* secretion of IL-10 or by contact-mediated suppressive mechanisms. The study also revealed that CD103 defines a subset of TILs that appear to mediate protective antitumor immunity in HGSOC.[36] Noticeably, CD103 could also be expressed on CD4 T-regulatory cells and on a substantial proportion of CD4/CD8 double-positive TILs[48]; however, further investigations are warranted to clarify the immunological role of CD103+ TILs in the tumor microenvironment.

TILs become exhausted with repeated antigen stimulation, and *T cell* exhaustion has a critical role in immune dysfunction in cancer.[6,7] PD-1, an immunoinhibitory receptor, is highly expressed on activated TILs, helps to negatively regulate TILs activation. Earlier studies indicated that PD-1+ TILs displayed functional exhaustion, referred to as an impaired effector function to proliferate and produce cytokines, providing a plausible explanation for tumor progression despite the presence of TILs in the tumor stroma.[8,18] Our findings on PD-1+ TILs are mainly in agreement with the study by Lo and colleagues,[15] who also found that overexpression of PD-1+ TILs was not associated with the survival of patients with HGSOC. Similarly, Ahmadzadeh *et al.* also reported that levels of PD-1 expression in melanoma cells were inversely correlated with the function of CD8+ TILs,[18] indicating a possible role of PD-1 in suppressing immune surveillance. In addition to PD-1+ TILs, this meta-analysis could not pool the HRs of PD-L1+ TILs due to insufficient data. Collectively, PD-1+ and PD-L1+ TILs may be promising biomarkers for identifying patients who may benefit from immune-checkpoint inhibitors. However, further studies are required for a more definitive conclusion concerning the prognostic impact of PD-1+ TILs in HGSOC.

While the results of the present meta-analysis are credible, this meta-analysis has certain limitations that must be acknowledged, which are inherent to its study design and characteristics of the included articles. First, heterogeneity among primary studies represented the major limitation of this meta-analysis. The prognostic value of biomarkers analyzed is most likely to differ among different subtypes and infiltrating locations. However, the small number of included studies did not allow stratification for these circumstances. Thus, more homogeneous patient cohorts may strengthen the prognostic values of these biomarkers and provide better insight into the differences between patient subgroups. Besides, the prognostic value of biomarkers is also dependent on the therapy administered; however, only a few studies have evaluated treatment modality in their analysis. Furthermore, for the application of prognostic T-cell markers in routine clinical practice, future prognostic studies using large homogeneous patient cohorts with regard to subtype, infiltrating location, and treatment modalities are highly desirable. Second, the validated scoring system and cut-off differed among the included studies. Thus, to include TILs in immunotherapy, we propose to make the scoring system more consistent and easy to interpret and to standardize the cut-off, to facilitate the direct comparisons across studies. However, a consensus was not reached to provide a universally applicable cut-off in this study. Third, this meta-analysis was based on literature-based abstracted data, and thus pooled analyses were not based on individual patient data. Fourth, CD4 and CD8 are not exclusively representative of T helper cells and cytotoxic T-cells. They are also expressed on macrophages and dendritic cells. Conceivably, advanced techniques that are competent to identify subsets of T cells more specifically might provide additional robust biomarkers for HGSOC. All these factors limited the evidence quality of this meta-analysis; thus, further large-scale, well-designed prospective studies are warranted to validate these findings.

Conclusion

This meta-analysis confirmed that high levels of CD3+, CD4+, CD8+, and CD103+ TILs are positively associated with survival (OS and PFS) of patients with HGSOC. Thus, the assessment of TILs subtypes and degree of infiltration may help predict precise prognosis and guide the optimal management of patients with HGSOC. For the incorporation of TILs into routine clinical practice, robust and well-designed prospective studies with homogeneous patient cohorts are warranted.

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Authorship

Jiatao Hao and Hui Yu contributed equally to this work. RFA and YX are the guarantor of the study and take all responsibility for the content of the manuscript. JTH and HY were involved in the concept and design of the study and in the acquisition and interpretation of the study data. JTH drafted the manuscript. YX revised the manuscript for important content. JTH, HY, and THZ were involved in extracting and analyzing the data. YX has approved the final version for submission and publication. All authors involved have read and approved the final manuscript.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Supplemental material

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