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Prognostic Value of Tertiary Lymphoid Structures in Cancer - A Systematic Review and Meta-Analysis

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Abstract

Background: Some studies have found that immune cell spatial structures, also known as tertiary lymphoid structures (TLSs), may have prognostic value in cancer. However, these studies all had insufficient sample sizes and inconsistent conclusions. To overcome these problems and draw conclusions, a systematic review and meta-analysis was conducted to determine the prognostic impact of TLSs in cancer.

Methods: PubMed, Web of Science, and Embase databases were searched to identify eligible articles published before April 2021. Two authors assessed each record, ensuring that the PICOS framework was met, and risk of bias on the study level was assessed using the Cochrane Bias Risk Assessment Tool. We then performed a meta-analysis using Review Manager 5.3 program to obtain a comprehensive estimate of the prognostic role of TLSs.

Results: In total, 28 studies were included in our analysis. We found that in comparison to cancer patients with low TLSs levels, those with high TLSs levels had a better overall survival (OS, hazard ratio [HR]: 0.48, 95% confidence interval [CI]: 0.40-0.57, p<0.00001) and disease-free survival (DFS, HR: 0.52, 95% CI: 0.42-0.63, p<0.00001). Surprisingly, after receiving immune checkpoint inhibitors (ICIs) therapy, cancer patients with high TLSs levels also had a better OS (HR, 0.28; 95% CI, 0.16-0.48; p<0.00001). Furthermore, our subgroup analysis showed that the density of DC-Lamp+ dendritic cells (DCs) and CD20+ B cells in TLSs represented better clinical outcomes.

Conclusion: To the best of our knowledge, this study is the first meta-analysis to show the significant prognostic value of TLSs in cancer, to date. Moreover, TLSs are expected to be a potential marker for predicting anti-tumor immune response, which may help clinicians to select patients with cancer who are sensitive to immunotherapy. In addition to their concentration, TLSs components also had strong prognostic value. In particular, the study may provide a novel therapeutic target for patients with cancer and provide a new theoretical basis for future research on tumor immunotherapy.

1. Introduction

Immune cell spatial structures, also known as tertiary lymphoid structures (TLSs), have been found to play an important role in the adaptive immune system. B-cell-dominated immune cell aggregation, including T cells, DC-Lamp + dendritic cells (DCs), and high endothelial venules (HEVs), contribute to the formation of tumor-associated TLSs(1–3), which only develop in non-lymphoid tissues with persistent defects, such as cancer TLSs develops only in non-lymphoid tissues with persistent defects such as tumors(4–6).

Previous studies have observed that some cancer patients with high T-lymphocyte infiltrations still had poor prognoses(7). A possible logical assumption was that the proliferation and differentiation of T cells required a special structure, which could aggregate other immune components, and was similar to a lymph node. Moreover, this structure was expected to be able to provide sufficient stimulatory signals, inducing anti-tumor response in the tumor microenvironment. Thus, it was reasonable to suppose that the immune cellular structure would play a key role in anti-tumor immunity, despite its number and location.

Recently, researchers have focused on the association of TLSs with the survival prognosis of patients with cancer. Specifically, several studies have shown a better prognosis for patients with TLSs(8–10), whereas others have shown the opposite(11, 12). This inconsistency can be attributed to the insufficient sample sizes and low universality in these studies(13, 14). Therefore, to properly draw a conclusion based on the numerous and varying results, the first systematic review and meta-analysis was conducted to determine the prognostic roles of TLSs in anti-tumor immunity, as well as to provide new clinical curative effect predictors and potential anti-tumor targets.

2. Methods

Our meta-analysis was conducted in accordance with the PRISMA statement and was based on previously published studies (supplementary materials 1). Therefore, ethical approval and patient consent were not required(15).

2.1 Search Strategy

Two independent reviewers conducted a systematic search of PubMed, Web of Science, and Embase databases before April 2021. The following keywords were used for the literature: “tertiary lymphoid structure”, “tertiary lymphoid structures,” “cancer,” “neoplasm,” “tumor.” Details of the literature search strategy are provided in the Appendix.

2.2 Inclusion and Exclusion Criteria

All relevant articles were independently qualified by two investigators. The following inclusion criteria were used :(1) papers written in English; (2) Results: Overall survival (OS, Defined as time from randomization to death)(16), progression-free survival (PFS, defined as time from randomization to first recorded disease progression or death)(17), disease-free survival (DFS, Defined as from the start of treatment to the first recurrence, or to death without any type of recurrence), disease-specific survival (DSS, defined as the period from the start of diagnosis or treatment to the time of death); (3)Study design. The relationship between TLSs levels and prognosis (i.e., OS, PFS, DFS, and DSS) was investigated. (4) Original research that is not restricted in the research design; (5) Studies containing sufficient data to estimate effects (I.For example, HR and corresponding 95% CI (for OS or DFS or PFS or DSS). The following exclusion criteria were considered :(1) comments, reviews, editorials, protocols, case reports, qualitative studies or letters; (2) Insufficient dataDetermine the results; (3) Studies without available data.

2.3 Data Extraction

Two different independent reviewers extracted data from the included studies according to a data extraction form. Disagreements were resolved through consultation or joint discussions with third authors. We extracted the following data for this review: (1) first author and year of publication; (2) cancer type; (3) study type; (4) the detection method of TLSs; (5) Subgroup and details of TLSs(The density of TLSs, or CD20 + B cells in TLSs, or DC-Lamp + dendritic cells (DCs) in TLSs); (6) Sample size; (7) the HR and 95% CI for PFS, DFS, OS and DSS.

2.4 Statistical Analysis

The risk of bias was assessed by the two researchers individually(18). We assessed the quality of each included article. When disagreements arose between two investigators, a third investigator was involved in discussions to resolve these differences.

Heterogeneity between studies was measured by the Higgins I^2 statistic and Cochrane's Q test ($P < 0.10$ or $I^2 > 50\%$ is considered to be an indicator of statistically significant heterogeneity). If heterogeneity exists, a random-effects model is used.Otherwise, the fixed effects model is used(19).

We used the software Review Manager 5.3 (Cochrane Collaboration, Oxford, UK) to obtain pooled estimates of the prognostic effect of TLSs. In this meta-analysis, HR and its 95% CI were used as aggregate statistics for OS, PFS, and DFS. P values less than 0.05 were set as statistical significance. Funnel plots were drafted for each meta-analysis to assess potential publication bias.

3. Results

3.1 Search Results

Figure1 illustrated the PRISMA flow chart for studies inclusion and exclusion criteria. Database searches yield 962 records.After the duplicate results were removed, a total of 481 records were used for title and summary review.Then, a comprehensive review of 79 articles was conducted. Then, we eliminated 51 unsuitable documents. Ultimately, 28 studies that met the criteria were included in the meta-analysis.

3.2 Characteristics of the Included Studies

Tables 1 and 2 summarised the basic characteristics and target outcomes extracted from the included studies. In our research, all included studies (n = 28) were full-reported retrospective cohort studies. Studies (n = 21) we included involving prognostic role of TLSs in different tumors in Table 1. Five studies were about melanoma including TGGA chort, Danish chort, Riaz chort, Van Allen chort and Gide chort. These studies evaluated the role of TLSs in prognostic and immunotherapy. Patients received immunotherapy with ICIs (Anti-CTLA4) in Danish chort and van Allen chort, while with ICIs (Anti-PD1) in Van Allen chort and Gide chort(20). The remaining studies also evaluated the survival predictive role of TLSs in patients with sarcomas(21), hepatocellular carcinoma(22, 23), Pancreatic cancer(24, 25), OSCC(26, 27), breast cancer(28–30), LSCC(8), Colorectal Cancer(9, 31), Renal Cell Cancer(10) and gastric cancer(32). The methods used to define TLSs in these studies were mainly HE and immunohistochemistry. In our subgroup, we included 7 studies involving survival predictive role of B cells or DCs in TLSs in different tumors including NSCLC(33–35),Colorectal cancer(36, 37), Gastric cancer(38), high-grade serous ovarian carcinoma(39) (Table 2).

3.3 Methodological Bias of Included Studies

We assessed the risk of bias for all included studies (n = 28). We found that the main source of bias has to do with selection bias. Due to incomplete information (participants were excluded due to missing data), not all available patients were included in the final analysis. The risk of bias was assessed for all included studies, as shown in Fig. 2. Since the articles were prospective clinical trials, the overall risk of bias was relatively low. Figure 3 shows the assessment for each area in the full report study.

3.4 TLSs density and prognostic

3.4.1 TLSs level and OS

Fifteen studies involving 3394 patients were evaluated for the relationship between TLSs and OS. The results showed high TLSs predicted a better OS than low TLS. The pooled HR were 0.48 (95% CI, 0.40–0.57; $p < 0.00001$) for total TLSs level (high vs. low). There was little heterogeneity between these studies ($I^2 = 21\%$) (Fig. 4). From the subgroup analyses on the level of TLSs (high vs. low) with immune checkpoint inhibitors (ICIs), the HR were 0.28 (95% CI, 0.16–0.48; $p < 0.00001$) for total, 0.35 (95% CI, 0.16–0.76; $p = 0.008$) for Anti-CTLA4 and 0.23 (95% CI, 0.11–0.49; $p = 0.0001$)) for Anti-PD1. However, there was no heterogeneity between the subgroup of ICIs (Fig. 5).

3.4.2 TLSs level and DFS

Ten studies, including 2277 patients, assessed the relationship between TLSs and DFS in oncology patients. The results showed high TLSs predicted better DFS, with pooled HR of 0.52 (95% CI, 0.42–0.63; $p < 0.00001$) for TLSs level (high vs. low). There was little heterogeneity between these studies ($I^2 = 21\%$) (Fig. 6).

3.4.3 TLSs level and PFS

Only one included study with 138 patients mentioned the prognostic value of TLSs for PFS in Lung Squamous Cell Carcinoma patients. It showed that high TLSs predicted better PFS, with HR of 0.43 (95% CI 0.26–0.71; $p = 0.001$) for TLSs level (high vs. low) (Fig. 7).

3.4.4 TLSs level and DSS

Two studies including 430 patients were evaluated for the relationship between TLSs and DSS in tumor patients. The results showed high TLSs predicted better DSS, with pooled HR of 0.60 (95% CI, 0.41–0.87; p = 0.007) for TLSs level (high vs. low). There was statistically significant heterogeneity between these studies (I^2 = 73%) (Fig. 8).

3.5 The density of B cells in TLSs and prognostic

Three studies involving 286 patients were evaluated for the association between TLSs and OS, four studies of 346 patients for DFS, and two studies of 196 patients for DSS. The results showed high B cells in TLSs predicted better DSS(HR 0.28; 95%CI 0.13–0.60; p = 0.001), DFS(HR 0.51; 95%CI 0.41–0.65; p < 0.0001), OS(HR 0.57; 95%CI 0.40–0.82; p = 0.002) (Fig. 9a);

3.6 The density of DCs in TLSs and prognostic

Three studies of 583 patients for OS, only one studies of 74 patients for DFS, and two studies of 196 patients for DSS were analysed to investigate the relationship of TLSs with outcomes.The results showed high DCs in TLSs predicted better DSS(HR 0.21; 95%CI 0.10–0.44; p < 0.0001), DFS(HR 0.46; 95%CI 0.26–0.84; p = 0.01) and OS(HR 0.53; 95%CI 0.43–0.65; p < 0.00001) (Fig. 9b);

3.7 Publication bias

We assessed the risk of bias for included studies. Funnel plot analysis did not show significant publication bias affecting THE HR of DFS DSS and OS (Supplement 3).

4. Discussion

Current immunotherapy aims to reinvigorate immune cells known as killer T cells to fight cancer, but only 20% of patients with cancer have lasting clinical benefit from this treatment(40). In fact, previous studies focusing solely on cell density have shown inconsistent conclusions. Dai et al. found that the function of CD8 + T cells was paradoxical in clear cell renal cell carcinoma in contrast to its antitumor effect in most solid tumors from prior studies(7, 41). Thus, focusing on other immune cells and components in tumor tissues may help in the improvement of these outcomes. In this study, the value of the immune cell spatial structures, namely TLSs, in survival prognosis and immunotherapy outcome of patients with tumors was discussed for the first time.

In this study, we used overall (OS), disease-free (DFS), progression-free (PFS), and disease-specific survival (DSS) as indicators of patient survival prognosis. As previously mentioned, some studies have reported that the presence of TLSs is associated with a good prognosis in multiple solid tumors(8, 28, 29, 42–44), whereas others have shown the opposite results. After synthesizing the small sample sizes and different results of these studies, our study confirmed that cancer patients with high levels of TLSs had a better prognosis than those with low levels (OS, hazard ratio [HR]: 0.48, 95% confidence interval [CI]: 0.40–0.57; DFS, HR: 0.52, 95%CI 0.42–0.63). Additionally, in the current era of tumor immunotherapy(45), we found that higher TLSs also predicted better OS (HR: 0.28, 95%CI: 0.16–0.48). Moreover, our analysis found that that higher B cells and DCs in TLSs predicted better DSS, DFS and OS, suggesting that these components within the TLSs also had strong prognostic values. Although some studies have also focused on the other components of TLSs with positive prognostic values, such as HEVs(42), 12-Chemokine(46), CD3 + T cells(33), and CXCL13(36). data were not enough to conduct a meta-analysis on these components.

Currently, a reasonable logical assumption is that the functional state of immune cells is closely related to their structure in the tumor immune microenvironment(47, 48). Because of the nature of immune cellular growth, development, and proliferation, specific immune system activation requires interactions between immune cells to provide stimulatory signals and cytokines. Furthermore, the aforementioned processes need to be accomplished in specific immune structures known as lymph nodes. However, since the peripheral tissues lack lymph nodes, the immune activation

process mainly occurs in the TLSs(47, 49), which are mainly composed of multiple immune components to provide antigen presentation, co-stimulatory signals, cytokines, and tumor-specific antibodies, followed by an anti-tumor immune response in the tumor microenvironment(50–54). Conversely, in a tumor microenvironment lacking TLSs, the specific immune system T or B cells may not receive enough effective stimulation signals, and as a result, these infiltrating cells in the tumor may be unable to normally exert their specific anti-tumor immune functions(55). Additionally, because these cells can only be activated once during the maturation process, without sufficient stimulatory signals, the T or B cells flooding the tumor microenvironment may develop to become exhausted cells. Therefore, the anti-tumor immune function status cannot be accurately predicted using only the T or B lymphocyte count. More interestingly, the subgroup analysis showed that TLSs were an important predictor of immune checkpoint inhibitor (ICI) efficacy(45). Following immunotherapy bottleneck concerning T cells, TLSs may have the potential to further complement T cell-mediated immunotherapeutic approaches(56). How to combine TLSs with existing immunotherapies to improve their roles in the anti-tumor immune response deserves intensive study. Therefore, the focus on immune cell spatial structures will provide new ideas for anti-tumor immunity, ICI efficacy prediction, and therapeutic target selection.

5. Limitations

Despite these findings, there are certain limitations to our meta-analysis. First, a study suggested that TLSs with different spatial distributions may also have different prognostic significances, wherein TLSs density in the center of the tumor reflected a better prognosis than TLSs density in the invasive margins(32). However, in the studies we included, some defined the location of TLSs as intratumoral or in the tumor periphery, whereas others did not specify the location. Thus, the prognostic significance of spatial distribution could not be explored in this study. Second, although our subgroup analysis results with ICIs were statistically significant, only four studies were included. Thus, more data are needed to support TLSs predictive value in ICIs. Finally, the current studies focused on the TLSs concentration, but the maturity of TLSs was quite different. Therefore, further study of updated information is still required.

6. Conclusions

Previous studies on the number of infiltrating immune cells, especially T and B cells, have shown contradictory results, suggesting that they cannot be independent predictors of survival prognosis and immunotherapy efficacy in patients with cancer. A series of studies focused on the association of TLSs with survival prognosis of patients with cancer had been conducted. However, there was no high-level evidence in this emerging area of research because of the insufficient sample sizes and inconsistent results in those studies. Therefore, this study was the first meta-analysis to show the significant prognostic value of immune cell spatial structures, namely TLSs, in cancer. Moreover, TLSs are expected to be a potential marker for predicting anti-tumor immune response, which may help clinicians to select cancer patients who are sensitive to immunotherapy. In addition to their concentration, the components within the TLSs also had strong prognostic values.

More importantly, the study of TLSs may provide a new therapeutic target for patients with cancer and form a new theoretical basis for future research on tumor immunotherapy.

7. Declarations

Ethical approval and consent participation

Not applicable

Consent to publication

Not applicable

Availability of data and materials

Original data supporting the conclusions of this paper will be provided by the author,No improper reservations.

Competing interests

The authors declare no conflict of interest. Funders have no role in research design; In the collection, analysis, or interpretation of data; Has no role in writing manuscripts or deciding to publish results.

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Authors' contributions

XC and Y-MW had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis; XC and Y-MW contributed equally to this work and are co-first authors; Concept and design: All authors; Acquisition, analysis, or interpretation of data: All authors; Drafting of the manuscript: All authors; Statistical analysis: All authors. All authors contributed to the article and approved the submitted version.

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Tables

Table 1 Characteristics of the included studies.

Author (year)	Cancer type	Study type	TLS detection method	Patients	Harzard Ratio (95%CI)			
					PFS	DFS	OS	DSS
Florent Petitprez (2020)(21)	sarcomas (STSs)	retrospective	HE	202			0.418 (0.1911-0.9146)	
Hui Li (2020)(22)	early-stage HCC	retrospective	HE	159		0.534 (0.334-0.855)	0.742 (0.472-1.167)	
Wu Hu Zhang (2020)(24)	(G1/G2) NF-PanNETs	retrospective	HE	182		0.3 (0.14–0.69)	0.3 (0.12–0.74)	
Qunxing Li (2020)(26)	OSCC	retrospective	IHC	168		3.296 (1.279-8.490)	3.784 (1.498-9.562)	
Rita Cabrita TGGA data (2020)(20)	melanoma	retrospective	IHC	349			1.81 (1.20-2.74)	
Rita Cabrita Danish data (2020)(20)	melanoma	retrospective	IHC	37			2.36 (0.76-7.29)	
Van Allen (2015)(57)	melanoma	retrospective	IHC	40			3.50 (1.17-10.51)	
Gide (2019)(58)	melanoma	retrospective	IHC	69			4.01 (1.47-10.99)	
Riaz (2017)(59)	melanoma	retrospective	IHC	40			4.72 (1.53-14.56)	
Qiaowei Lin (2020)(60)	GIST	retrospective	HE	118		0.412 (0.192–0.887)	0.180 (0.061–0.534)	
Michae Sofopoulos (2019)(29)	breast cancer	retrospective	HE	112		0.2190 (0.0692-0.6933)	0.2245 (0.0557-0.9050)	
Karīna Siliņa	LSCC	retrospective	HE	138	0.4			

(2018)(8)					(0.26-0.71)			
XIA LIU (2017)(28)	Breast Cancer	retrospective	HE IHC	245		0.222 (0.075-0.654)		
Giuseppe Di Caro (2014)(9)	Early-Stage Colorectal Cancer	retrospective	HE	351		0.72 (0.46-1,14)		
Nicolas A. Giraldo (2015)(10)	Renal Cell Cancer	retrospective	IHC	135		0.78 (0.3-1.9)	0.72 (0.2-2.3)	
Wenting He (2020)(32)	gastric cancer (GC)	retrospective	HE	914			0.411 (0.229–0.740)	
Julien Calderaro (2018)(23)	hepatocellular carcinoma	retrospective	HE	273		0.46 (0.27-0.80)		
Anna M Wirsing (2014)(27)	OSCC	retrospective	HE	80	-	-	-	2.409 (0.556-10.448)
N Hiraoka (2015)(25)	Pancreatic cancer	retrospective	HE	534		1.611 (1.092–2.375)	1.637 (1.115–2.403)	
Juha P. Vayrynen (2013)(31)	Colorectal cancer	retrospective	HE	350				0.54 (0.37–0.80)
Miseon Lee (2018)(30)	breast cancer	retrospective	HE	335			0.49 (0.320–0.748)	

Notes: TLS: Tertiary lymphoid structure; early-stage HCC: 0-A Hepatocellular carcinoma; (G1/G2) NF-PanNETs: Grade 1 or Grade 2 Non-functional pancreatic neuroendocrine tumors; OSCC: Oral squamous cell carcinoma; GIST: Gastrointestinal stromal tumors; LSCC: Lung squamous cell carcinoma; CLR: colorectal cancer associated lymphoid reaction or Crohn's like lymphoid reaction; HR: hazard ratio; ICI: Immune checkpoint inhibitor; OS: overall survival; PFS: progression-free survival; DFS: disease-free survival; DSS: disease-specific survival; lo: low, hi:high;

Table 2 Characteristics of the included studies.

Author (year)	Cancer type	Study type	TLS detection method	patients	Subgroup and details	Harzard Ratio (95%CI)		
						DSS	DFS	OS
Claire Germain (2014)(34)	NSCLC	retrospective	IHC	122	DCs	11 (2.3–54)		
				122	CD20+B cells	11 (1.4–9)		
Jeremy Goc (2013)(35)	NSCLC	retrospective	IHC	362	DCs			0.53 (0.40-0.7)
Dieu-Nosjean (2008)(33)	NSCLC	retrospective	IHC	74	CD20+B cells	3.62 (0.96-13.68)	1.95 (0.61-6.17)	2.18 (0.90-5.32)
				74	DCs	3.34 (1.64-8.68)	2.11 (1.19-3.89)	1.88 (1.21-2.96)
Gabriela Bindea (2013)(36)	Colorectal cancer	retrospective	gene expression	125	CD20+B cells		2.76 (1.0–7.6)	
Audrey Hennequin (2015)(38)	Gastric cancer	retrospective	IHC	82	CD20+B cells		0.4 (0.2-1)	
Anastasia Meshcheryakova (2014)(37)	Colorectal cancer	retrospective	IHC	65	CD20+B cells		0.534 (0.411-0.693)	0.640 (0.431-0.950)
Iva Truxova (2018)(39)	HGSC	retrospective	IHC	147	CD20+B cells			0.09 (0.01–0.72)
				147	DCs			0.52 (0.34–0.81)

Notes: TLS: Tertiary lymphoid structure; NSCLC: non-small-cell lung carcinoma; HGSC: high-grade serous ovarian carcinoma; DCs: DC-Lamp+ dendritic cells; HR: hazard ratio; OS: overall survival; DFS: disease-free survival; DSS: disease-specific survival;

Figures

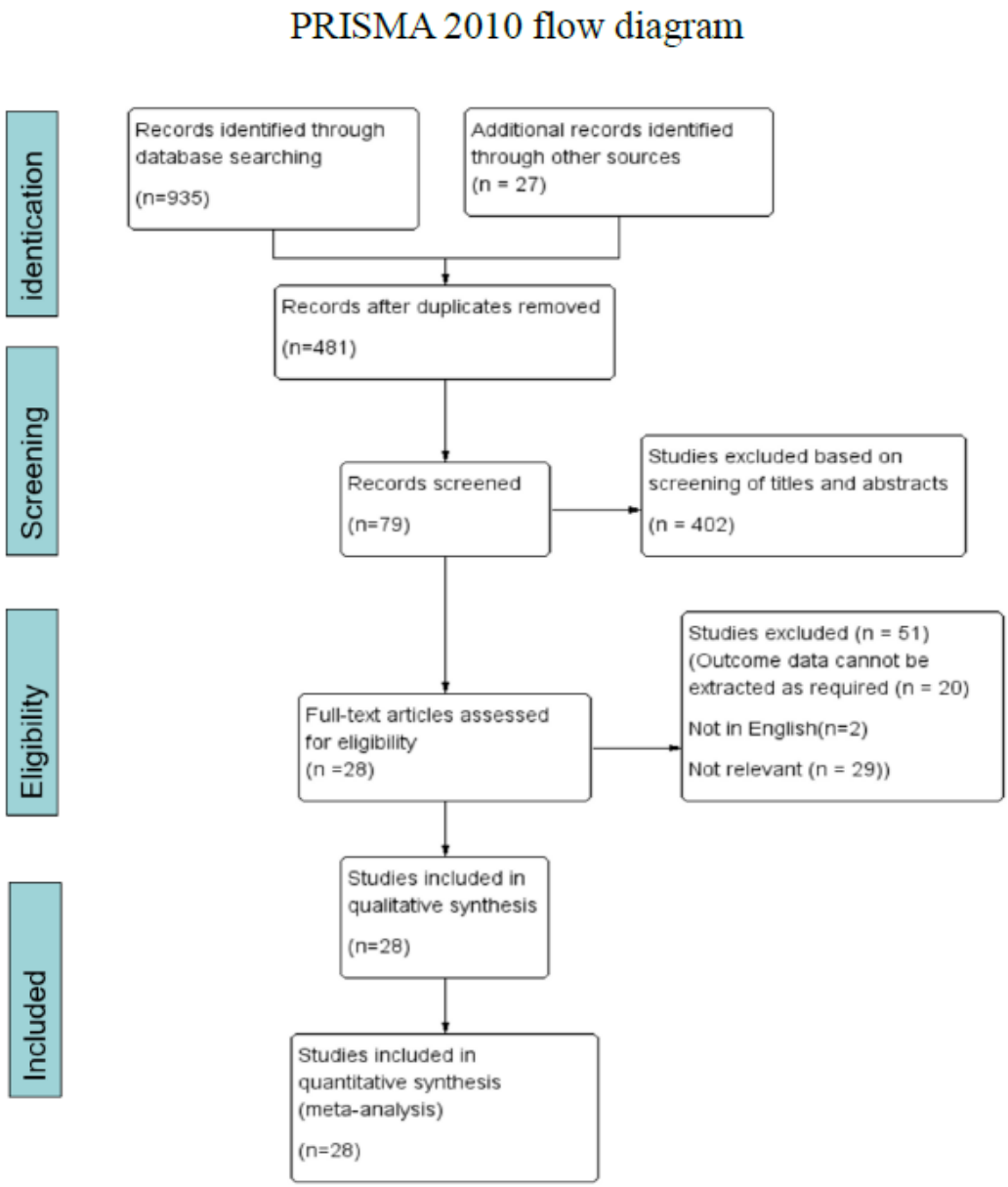


Figure 1

PRISMA 2010 flow diagram

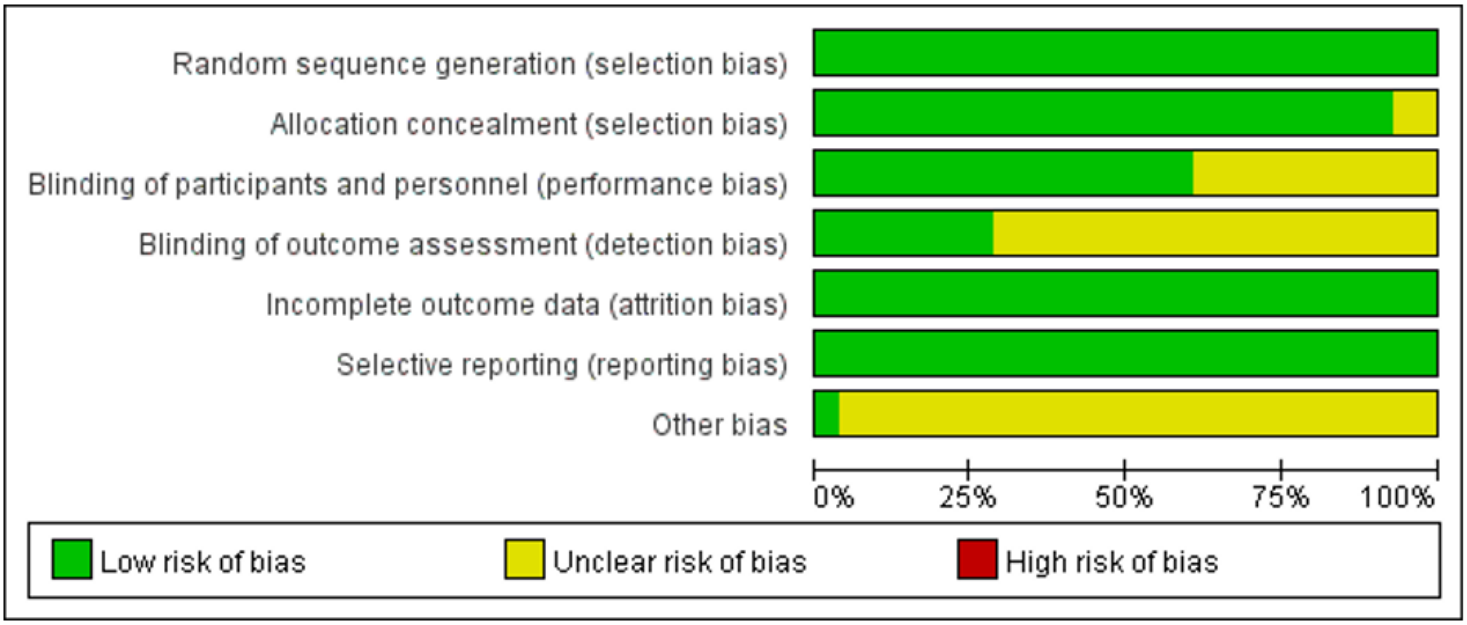


Figure 2

Risk of bias graph.

Figure 3

Risk of bias summary.

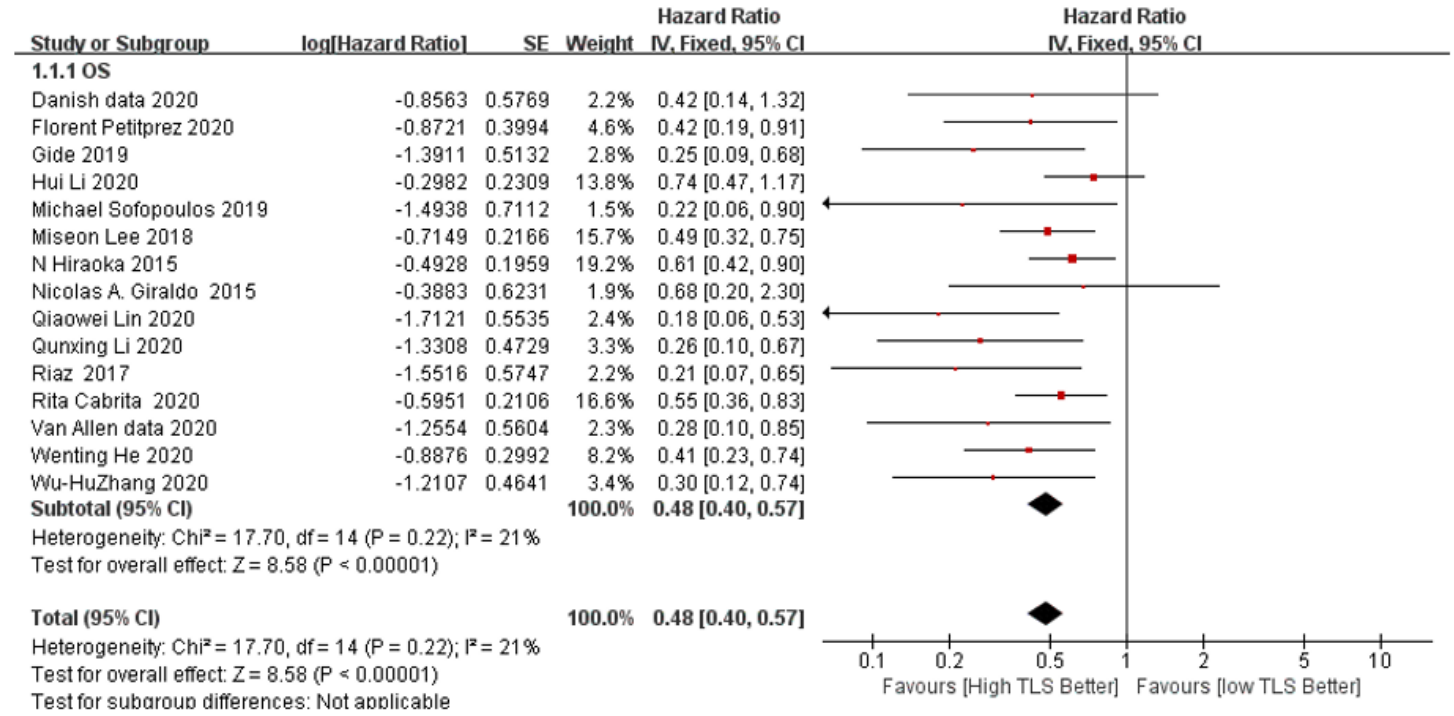


Figure 4

Forest plots of the random-effects meta-analysis for the efficacy of tertiary lymphoid structures (TLSs) for overall survival (OS).

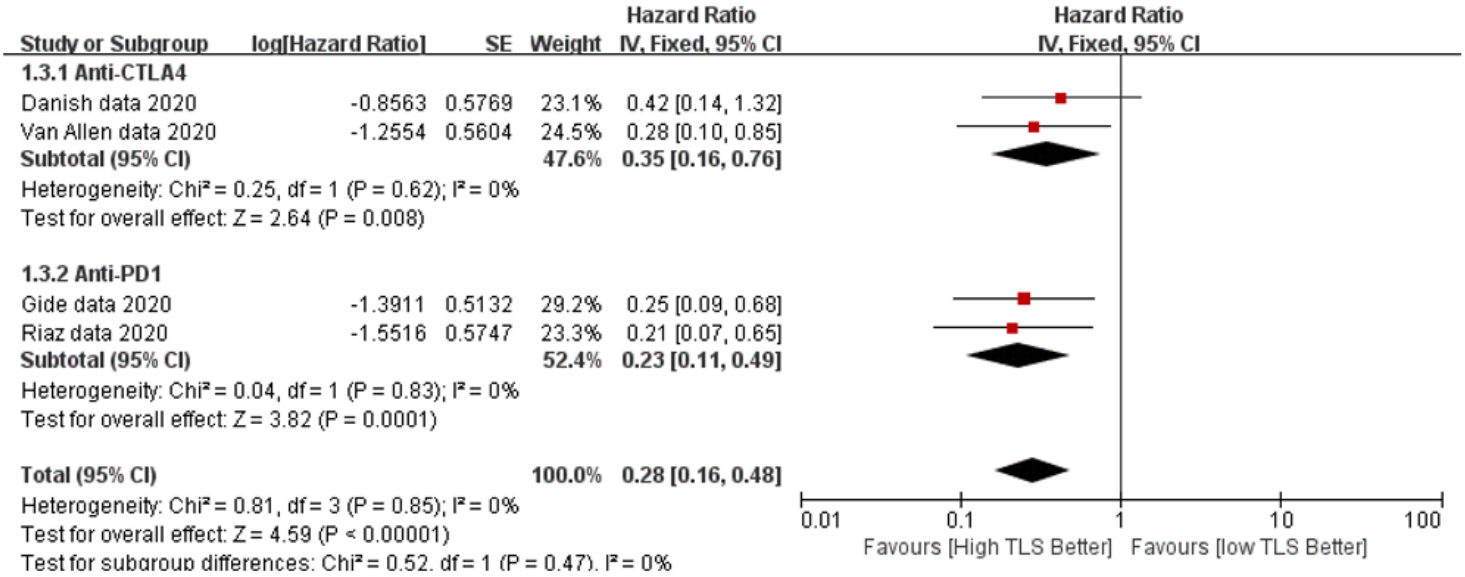


Figure 5

Forest plots of the random-effects meta-analysis for the efficacy of tertiary lymphoid structures (TLSs) for overall survival (OS) in immune checkpoint inhibitors (ICIs).

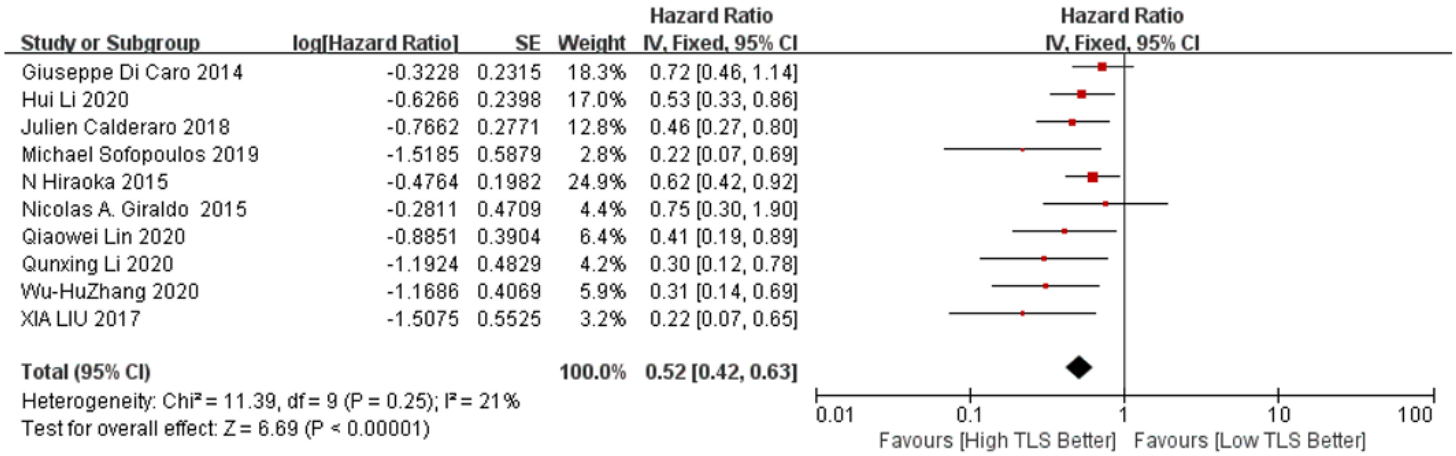


Figure 6

Forest plots of the random-effects meta-analysis for the efficacy of tertiary lymphoid structures (TLSs) for disease-free survival (DFS).

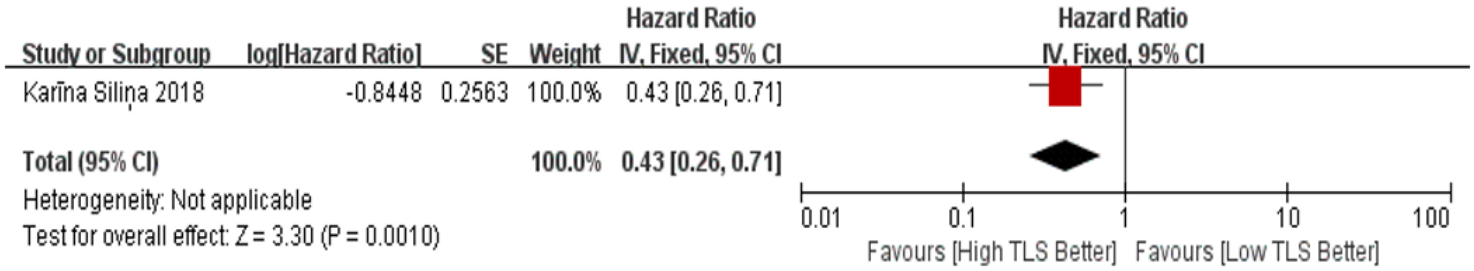


Figure 7

Forest plots of the random-effects meta-analysis for the efficacy of tertiary lymphoid structures (TLSs) for disease-free survival (PFS).

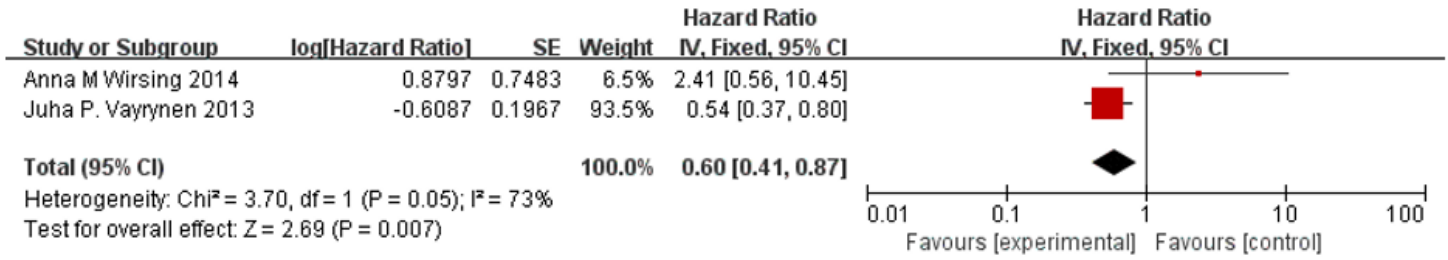
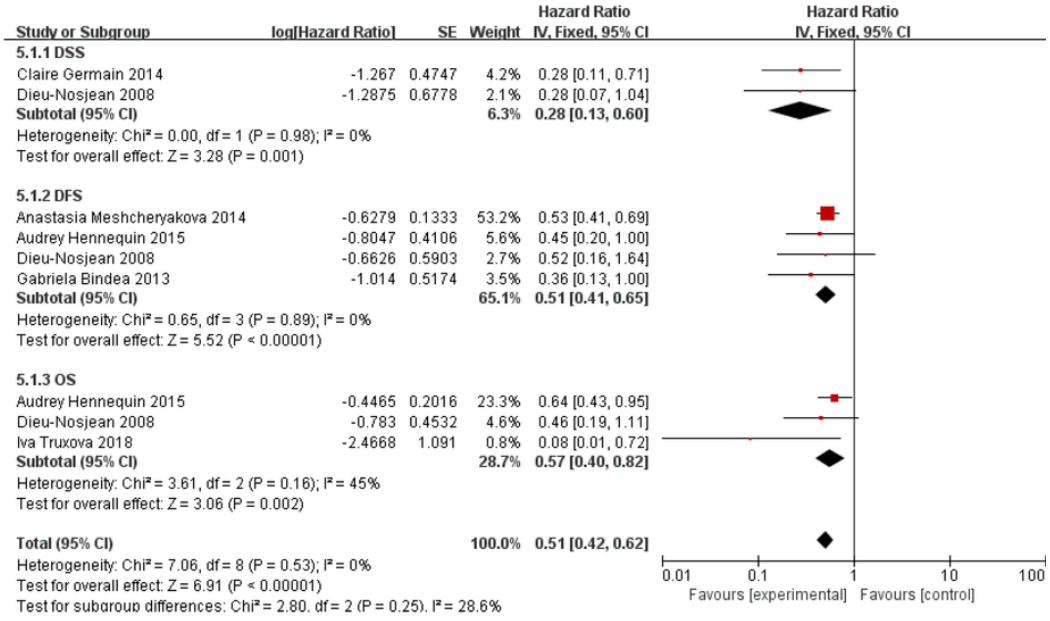
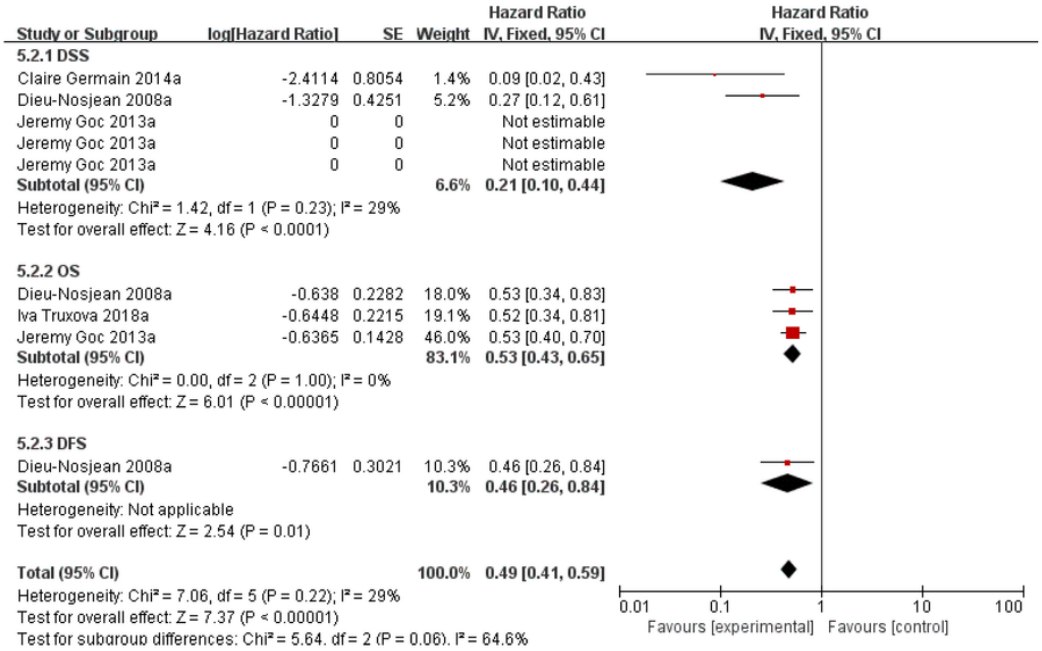


Figure 8

Forest plots of the random-effects meta-analysis for the efficacy of tertiary lymphoid structures (TLSs) for disease-specific survival (DSS).



a



b

Figure 9

Forest plots of the random-effects meta-analysis for the efficacy of B cells (a) and DCs(b) in tertiary lymphoid structures (TLSs) for OS、PFS、DFS and DSS.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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