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Prognostic value of FOXP3$^+$ regulatory T cells in patients with diffuse large B-cell lymphoma: a systematic review and meta-analysis

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ABSTRACT

Objectives We aimed to comprehensively evaluate the relationship between forkhead box P3 (FOXP3$^+$) regulatory T cell (Treg) expression and diffuse large B-cell lymphoma (DLBCL) prognosis and to explore the sources of heterogeneity of the results.

Design Systematic review and meta-analysis.

Data sources We searched the Cochrane Library, PubMed, Embase and Web of Science databases up to 5 December 2021.

Eligibility criteria We included studies that analysed the prognostic significance of FOXP3$^+$ Tregs in DLBCL. We included studies reported in Chinese or English that reported HRs and related 95% CIs for prognosis.

Data extraction and synthesis We extracted data from eligible studies. HRs and 95% CIs were used to assess the prognostic value.

Results Fourteen eligible studies were identified. FOXP3$^+$ Treg expression was not associated with overall survival (OS) (HR=0.72, 95% CI 0.45 to 1.16) or progression-free survival (HR=0.86, 95% CI 0.54 to 1.38). The three approaches used to measure FOXP3$^+$ Treg expression ($p_{interaction}$<0.001) may be the source of the heterogeneity of the results. Subgroup analysis found that a higher expression of FOXP3$^+$ Tregs was associated with better OS in all populations and in Asians when FOXP3$^+$ Treg expression was measured by the number of positive cells (HR=0.36 (95% CI 0.22 to 0.58) in the former, HR=0.33 (95% CI 0.20 to 0.55) in the latter) or the percentage of positive cells (HR=0.49 (95% CI 0.27 to 0.89) in the former, HR=0.38 (95% CI 0.21 to 0.70) in the latter). However, when measured by the score, inverse results were found (HR=1.56, 95% CI 1.01 to 2.42).

Conclusions Approaches to measuring FOXP3$^+$ Treg expression might be the major source of heterogeneity in studies of the prognostic significance of FOXP3$^+$ Tregs in DLBCL. FOXP3$^+$ Treg expression might be used to predict the prognosis of patients with DLBCL when FOXP3$^+$ Treg expression is calculated by the number or the percentage of positive cells, especially in Asian populations.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Our meta-analysis comprehensively evaluated the relationship between forkhead box P3 (FOXP3$^+$) regulatory T cell (Treg) expression and diffuse large B-cell lymphoma (DLBCL) prognosis and explored the sources of heterogeneity of the results.
⇒ The results should be interpreted with caution because the populations were mostly Asian.
⇒ Calculating HRs and the related 95% CIs indirectly may produce some errors.
⇒ The approaches to measuring FOXP3$^+$ Treg expression might be the major source of heterogeneity of the results.
⇒ Subgroup analysis was not performed on histological or molecular DLBCL subtypes due to limitations in the available data.

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is a highly malignant form of non-Hodgkin's lymphoma derived from large mature B cells, with an incidence of 30%–40%.[1] Although the disease can be treated with the R-CHOP (rituximab, cyclophosphamide, hydroxydaunorubicin, vincristine and prednisone) standard therapy, about 30%–40% of patients are not curable[2] and a group of patients have poor prognoses. Early identification of these patients would allow early intervention,[3] which could improve their prognosis. However, there are no current biomarkers to predict this outcome.

The International Prognostic Index (IPI) is widely used to predict the prognosis of patients with DLBCL.[4] In addition, the National Comprehensive Cancer Network-International Prognostic Index and the revised International Prognostic Index are useful for prognostic stratification of patients with DLBCL.[5] However, heterogeneity in the survival rates is seen within the same IPI score groups.[6] Moreover, a recent multicentre study showed that the three scoring systems were not sufficiently accurate in the rituximab treatment era.[7] Lee *et al*[8] pointed out that regulatory T cells (Tregs) expressing the

forkhead box P3 (FOXP3) transcription factor were associated with DLBCL prognosis, independent of the IPI. These cells would be expected to impact DLBCL progression.[9] However, it is unclear whether this biomarker could be used to identify patients with DLBCL with poor prognosis.

Some studies showed that higher FOXP3$^+$ Treg expression was associated with improved prognosis in patients with DLBCL,[8 10 11] whereas others reported opposite results[12] or no association at all.[13] Therefore, this issue is still controversial. Only one systematic review and meta-analysis has evaluated the association between FOXP3$^+$ Tregs and the prognosis of patients with DLBCL in a subgroup analysis.[14] The DLBCL subset in the meta-analysis included seven studies. Overall survival (OS) was the only outcome in the subgroup analysis. However, progression-free survival (PFS) and event-free survival (EFS) were vital prognostic indicators and new evidence has been published.[12 15] Moreover, the previous meta-analysis mentioned that the laboratory testing methods and reagents might cause heterogeneity. Therefore, we conducted this systematic review and meta-analysis to comprehensively evaluate the relationship between FOXP3$^+$ Treg expression and the prognosis of patients with DLBCL and to explore the potential source of heterogeneity by analysing DLBCL subgroups.

METHODS

This meta-analysis was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.[16] The study protocol was not registered.

Search strategy

We searched the Cochrane Library, PubMed, Embase and Web of Science databases (up to 5 December 2021) for relevant studies analysing the prognostic significance of FOXP3$^+$ Treg expression in patients with DLBCL. The following key terms were used: ("T-Lymphocytes, Regulatory", "Regulatory T Lymphocyte" or "FOXP3 protein, human", "Transcription Factor FOXP3", "FOXP3") and ("Lymphoma, Large B-Cell, Diffuse", "Lymphoma, Large Lymphoid, Diffuse", "Diffuse Large B Cell Lymphoma", "DLBCL"). Detailed search strategy is shown in online supplemental table S1.

Selection criteria

Two authors (YB and LZ) independently evaluated all eligible studies. Any discrepancies were resolved by consensus. The selected studies had to meet the following criteria: (1) studies that analysed the prognostic significance of FOXP3$^+$ Tregs in patients with DLBCL; (2) patients with DLBCL were diagnosed by histopathological analysis; (3) FOXP3$^+$ Tregs were evaluated by immunohistochemistry; (4) the HRs and related 95% CIs could be obtained either directly or indirectly; and (5) studies were written in Chinese or English. The exclusion criteria included (1) studies that involved cytological tests or were animal trials, reviews, conference abstracts or case reports; and (2) studies that included duplicate data.

Data extraction

Two independent investigators (YB and LZ) extracted the data from eligible studies and reached a consensus in case of discrepancies. The following information was extracted from all the included studies: basic information (authors, country, year of publication and study design), characteristics of the patients (number of patients, age, gender, DLBCL subgroup, DLBCL stage, IPI score and follow-up), approaches to measuring FOXP3$^+$ Treg expression (one involved calculating the percentage of positive cells using a tissue microarray (TMA) technique (%); one involved calculating the number of positive cells via TMA (cells/mm^2); and one calculated the overall scores (a score of ≥3 or 4 indicated high or positive expression) based on the staining intensity of the cells multiplied by the percentage of positive cells or only the staining intensity (score)), and the association between FOXP3$^+$ Treg expression and prognosis (cut-off points, HR (95% CI) for OS, PFS, EFS and disease-specific survival (DSS), as well as the adjusted factors in the model).

When the studies did not include the desired data, we calculated the HR and the related 95% CI from the Kaplan-Meier survival curves by employing Engauge Digitizer V.9.8 (http://sourceforge.net/projects/digitizer/) and following the method defined by Tierney *et al*.[17] We also used the survival rates to compute the HR and 95% CI for studies without Kaplan-Meier survival curves according to the method defined by Tierney *et al*.[17]

Quality assessment

The Newcastle-Ottawa Scale (NOS) criteria were used to assess the quality of each eligible study.[18] The NOS score evaluated the following three aspects: (1) selection, (2) comparability and (3) outcomes. The total score ranged from 0 to 9 for each of the studies. A study was considered of high quality when the score was ≥6.[19]

Statistical analysis

The HRs and related 95% CIs were used to analyse the influence of FOXP3$^+$ Treg expression levels on OS and PFS in patients with DLBCL. Study heterogeneity was assessed by the χ^2 statistical test and I^2. Strong statistical heterogeneity was defined as I^2 >50%.[20] The random-effects model and the fixed-effects model will give identical pooled-effect sizes when there is no heterogeneity among the eligible studies. However, the former can be applied to combine some heterogeneity. Therefore, we employed the random-effects model to evaluate the pooled-effect size (https://training.cochrane.org/handbook/current/chapter-10#section-10-10-4). We used forest plot to present this meta-analysis.

Sensitivity analysis was performed to estimate the stability of the pooled HR values. Subgroup analysis was based on the region of the patients, approaches to measuring FOXP3$^+$ Treg expression, statistical methods

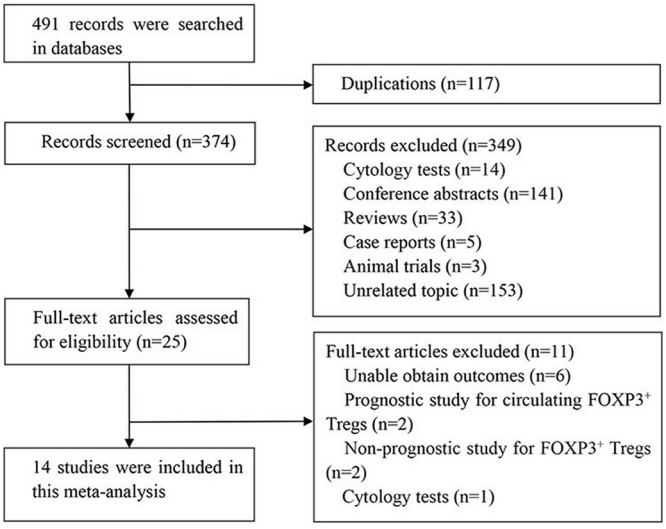

Figure 1 Selection process for the included studies. FOXP3+, forkhead box P3; Tregs, regulatory T cells.

and how the data were obtained. Egger's test and funnel plot were employed to evaluate potential publication bias.[21] Statistical analyses and graphs were generated using Stata V.16.0. P<0.05 was considered statistically significant.

Patient and public involvement

Neither patients nor the public were involved in the design, conduct, reporting or dissemination plans of our research.

RESULTS

Study selection and characteristics

The study selection process is shown in figure 1. We reviewed a total of 491 studies downloaded from the four previously mentioned databases. Nam *et al* published two similar studies in 2014[22] and 2018,[11] so only the latter study was included. Ultimately, 14 studies including 1501 patients were included in our meta-analysis. The key characteristics of the 14 studies[8 10–12 15 23–31] are presented in table 1.

Among the 14 studies, 13[8 10–12 15 23–27 29–31] included the HR with 95% CI for OS, 5 studies [10 11 15 24 30] reported the HR and 95% CI for PFS, 1 study[10] provided the HR and 95% CI for EFS, and 1 study[28] reported the HR and 95% CI for DSS. The HRs and the related 95% CIs in four articles[10 15 25 26] were based on multivariable analysis models.

All studies were performed in Asian and Western populations. The mean age of the patients was between 54.0 and 69.0 years and most patients were male. The DLBCL histological subtypes included were primary DLBCL (531), not otherwise specified DLBCL (156), Epstein-Barr virus-associated DLBCL (17) and anaplastic DLBCL (30). Moreover, 387 patients had germinal centre B-cell-like (GCB) DLBCL, while 592 cases were non-GCB. The number of patients classified as stage I–II (475) and stage III–IV (506) was similar. The IPI score was 0–2 in 596 cases, while 398 patients had IPI scores of 3–4 or 3–5. The mean follow-up time was from 16.0 to 56.6 months, while the longest follow-up time was 178.0 months. One study[12] reported that 40 patients were lost to follow-up.

Nine studies[8 10 15 23 25–27 30 31] were of high quality based on the NOS score of ≥6. In total, we found that five studies[11 12 24 28 29] achieved a score of 5, four[8 27 30 31] achieved a score of 6, four[10 15 23 25] achieved a score of 7, and one[26] achieved a score of 8.

FOXP3+ Treg expression and OS

Thirteen studies[8 10–12 15 23–27 29–31] including a total of 1231 patients with DLBCL assessed the relationship between FOXP3+ Treg expression and OS. FOXP3+ Treg high-expression compared with FOXP3+ Treg low-expression did not improve OS (HR=0.72, 95% CI 0.45 to 1.16, p=0.176; I^2=82.87%) (figure 2).

Subgroup analysis by patient region showed that FOXP3+ Treg high-expression had no prognostic OS value in Asian populations (HR=0.67, 95% CI 0.40 to 1.13; I^2=87.03%) or Western populations (HR=1.04, 95% CI 0.39 to 2.72; I^2=0.00%) (figure 3). Based on the approaches used to define FOXP3+ Treg high-expression and low-expression, patients with FOXP3+ Treg high-expression had poor OS when the FOXP3+ Treg expression was calculated by the score (HR=1.56, 95% CI 1.01 to 2.42; I^2=79.45%) and had better OS when the FOXP3+ Treg expression was calculated by the percentage of positive cells (HR=0.49, 95% CI 0.27 to 0.89; I^2=7.48%) or by the number of positive cells (HR=0.36, 95% CI 0.22 to 0.58; I^2=0.00%) (figure 4). In Asian populations, the results of the subgroup analysis based on the three FOXP3+ Treg methods of measurement were similar to the all-population results (in the group with FOXP3+ Treg expression calculated by the score (HR=1.56, 95% CI 1.01 to 2.42; I^2=79.45%), in the group with FOXP3+ Treg expression calculated by the percentage of positive cells (HR=0.38, 95% CI 0.21 to 0.70; I^2=0.00%) and in the group with FOXP3+ Treg expression calculated by the number of positive cells (HR=0.33, 95% CI 0.20 to 0.55; I^2=0.00%; figure 5). FOXP3+ Treg high-expression was not associated with OS in the multivariable analysis (HR=0.99, 95% CI 0.35 to 2.82; I^2=73.03%) or the univariable analysis (HR=0.62, 95% CI 0.35 to 1.11; I^2=84.67%) (figure 6). In addition, FOXP3+ Treg high-expression was not related to OS in directly obtained data (HR=0.76, 95% CI 0.39 to 1.47; I^2=85.60%) or indirectly obtained data (HR=0.65, 95% CI 0.33 to 1.25; I^2=65.94%) (figure 7).

FOXP3+ Treg expression and PFS

We analysed five studies[10 11 15 24 30] including 471 cases to probe the influence of FOXP3+ Treg expression on PFS. FOXP3+ Treg high-expression had no PFS prognostic value (HR=0.86, 95% CI 0.54 to 1.38, p=0.542; I^2=0.00%) (figure 8). The pooled HRs based on subgroup analysis are shown in table 2. There were no statistically significant differences in the four subgroups.

Table 1 Characteristics of the included studies

Authors	Year	Country	Study design	Patients (n)	Age, mean (range) (years)	Gender (male) (%)	DLBCL histological subtype (n)	DLBCL molecular subtype		DLBCL stage		IPI score (n)	Follow-up, median (range) (months)	Cut-off points (cells/mm^2, %, score)	Adjusted factors (OS/PFS/EFS/DSS)	NOS score
								GCB (n)	Non-GCB (n)	I–II (n)	III–IV (n)					
Lee *et al*[8]	2008	Korea	Retrospective	96	58.0 (20–83)	64.6	Primary DLBCL (96)	25	42	48	48	0–1 (40), 2 (18), 3 (23), 4–5 (15)	16.0 (1.0–132.0)	2.3%	–	6
Tzankov *et al*[28]	2008	Switzerland	Retrospective	270	63.0	54.4	Primary DLBCL (270)	81	98	78	83	0–2 (97), 3–5 (55)	–	4.4 or 6.1 cells/mm^2	–	5
Xu *et al*[27]	2013	China	Retrospective	92	62.0 (23–84)	62.0	–	–	–	42	50	0–2 (66), 3–4 (26)	0.0–80.0	3 (score)	–	6
Ahearne *et al*[26]	2014	UK	Prospective	70	67.0 (30–88)	58.6	–	–	–	44	26	0–1 (46), 2–4 (24)	–	–	–	8
Gomez-Gelvez *et al*[10]	2016	USA	Retrospective	74	59.1 (21–91)	54.1	DLBCL-NOS (74)	–	–	–	–	–	49.2 (7.2–144.0)	17.0%	Stage, LDH, type of DLBCL (ABC vs GCB), CD68, MVD	7
Wu *et al*[25]	2016	China	Retrospective	112	61.0 (22–81)	59.8	–	–	–	52	60	0–2 (76), 3–4 (36)	56.6 (4.0–70.0)	4 (score)	Gender, age, stage, pretreatment LDH, extranodal sites, IPI score, B7-H4 expression	7
Lee *et al*[24]	2017	Korea	Retrospective	100	61.0 (15–86)	63.0	–	30	70	55	45	0–1 (47), 2 (14), 3 (22), 4–5 (17)	54.0 (0.0–84.0)	4 (score)	–	5
Nakayama *et al*[23]	2017	Japan	Retrospective	82	68.3	58.5	DLBCL-NOS (82)	25	57	34	48	0–1 (23), 2 (22), 3 (31), 4–5 (6)	0.0–133.3	40.0 cells/mm^2	–	7
Nam *et al*[11]	2018	Korea	Retrospective	114	58.7 (10–82)	62.3	Primary DLBCL of the central nervous system (114)	14	78	–	–	0–2 (64), 3–5 (50)	31.4 (0.2–178.0)	24.0 cells/mm^2	–	5
Zhao *et al*[12]	2020	China	Retrospective	208	60.0 (34–78)	59.6	–	110	95	–	–	–	0.0–91.0	3 (score)	–	5
Chang *et al*[29]	2021	China	Retrospective	70	59.5 (30–86)	50.0	DLBCL EBV-associated (7)	26	28	41	29	0–2 (37), 3–5 (27)	0.5–87.0	16.0 cells/mm^2	–	5
Autio *et al*[30]	2021	Finland	Cohort	51	54.0 (22–64)	67.0	Primary DLBCL (no primary mediastinal B-cell lymphoma) (51)	24	17	5	46	0–2 (10), 3–5 (41)	61	–	–	6
Carreras *et al*[15]	2022	Japan	Retrospective	132	69.0 (14–97)	60.6	DLBCL EBV-associated (10)	47	82	72	50	0–2 (73), 3–5 (33)	–	4.5%	CD68, CD16, MITF, CD163, PTX3, IL-10, IPI, cell-of-origin (Hans classifier), EBER and high-grade B-cell lymphoma genotype	7
Xu *et al*[31]	2021	China	Cohort	30	61.5 (26–89)	66.7	A-DLBCL (30)	5	25	4	21	0–2 (9), 3–5 (16)	–	–	–	6

–, no data; ABC, activated B-cell-like; A-DLBCL, anaplastic diffuse large B-cell lymphoma; DLBCL, diffuse large B-cell lymphoma; DLBCL EBV, DLBCL Epstein-Barr virus-associated; DLBCL-NOS, DLBCL not otherwise specified; DSS, disease-specific survival; EBER, Epstein-Barr virus-encoded small RNA; EFS, event-free survival; GCB, germinal centre B-cell-like; IL-10, interleukin 10; IPI, International Prognostic Index; LDH, lactate dehydrogenase; MITF, microphthalmia transcription factor; MVD, microvasculature density; NOS, Newcastle-Ottawa Scale; OS, overall survival; PFS, progression-free survival; PTX3, pentraxin 3.

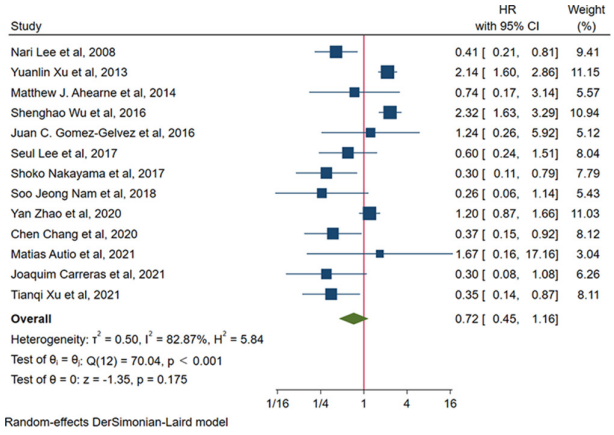

Figure 2 Forest plot of overall survival. Random-effects DerSimonian-laird model.

FOXP3+ Treg expression and EFS or DSS

One study[10] reported that the HR for EFS in patients with DLBCL was 2.93 (95% CI 0.87 to 9.84, p=0.082). Another study[28] found that the HR for DSS was 2.27 (95% CI 0.93 to 5.55) in patients without GCB DLBCL, but that it was 0.39 (95% CI 0.14 to 1.08) in patients with GCB DLBCL.

Publication bias and sensitivity analysis

Egger's test (p=0.006) and the funnel plot (online supplemental figure S1) indicated publication bias for the pooled HRs used in this study for OS. Egger's test (p=0.051) showed that there was no publication bias for the pooled HRs for PFS.

Sensitivity analysis evidenced that the pooled values were sturdy when deleting any one of the selected studies on OS (online supplemental figure S2). Sensitivity analysis confirmed that the pooled analysis was not impacted by any single study on PFS (online supplemental figure S3).

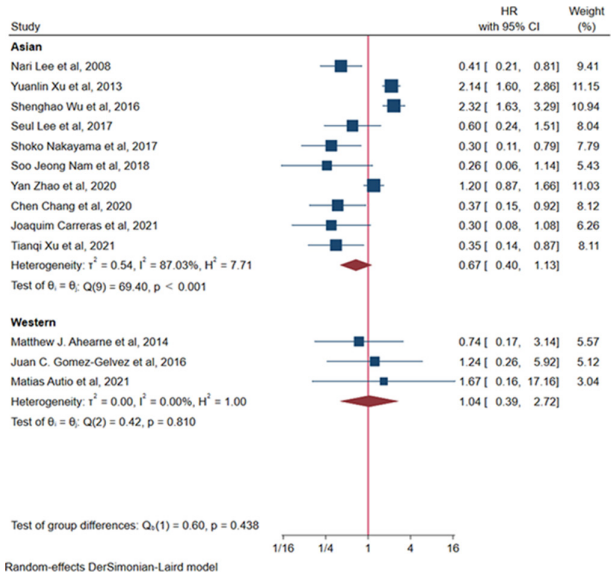

Figure 3 Subgroup analysis of overall survival by region. Random-effects DerSimonian-laird model.

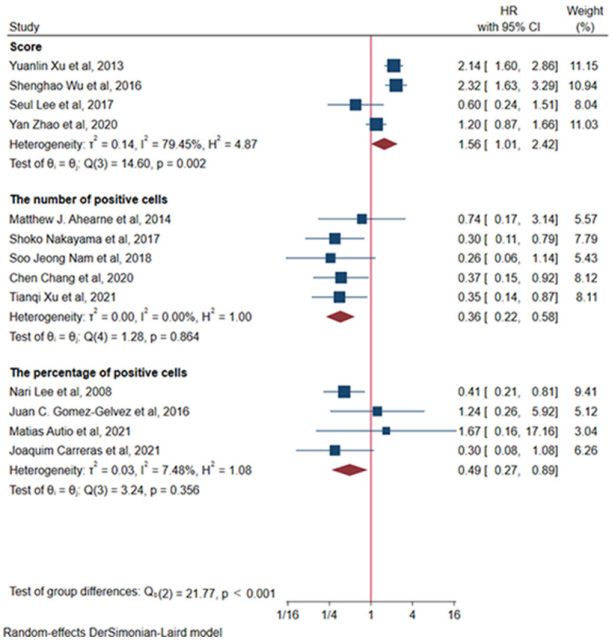

Figure 4 Subgroup analysis of overall survival by approaches to measuring cut-off points. Random-effects DerSimonian-laird model.

DISCUSSION

Our meta-analysis showed that FOXP3+ Treg expression was not associated with OS and PFS in patients with DLBCL regardless of the region of the patients, statistical methods or how the data were obtained. FOXP3+ Treg high-expression in patients with DLBCL indicated better OS when the FOXP3+ Treg expression was calculated by the number or percentage of positive cells, but poor OS when the FOXP3+ Treg expression was calculated by the score.

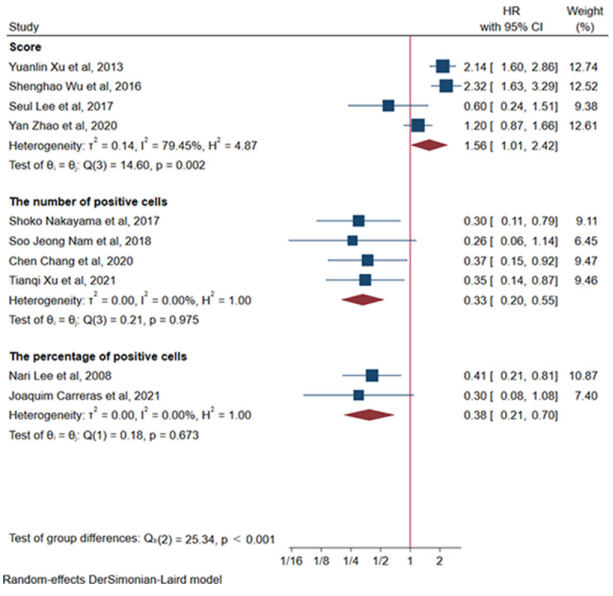

Figure 5 Subgroup analysis of overall survival by approaches to measuring cut-off points in Asian populations. Random-effects DerSimonian-laird model.

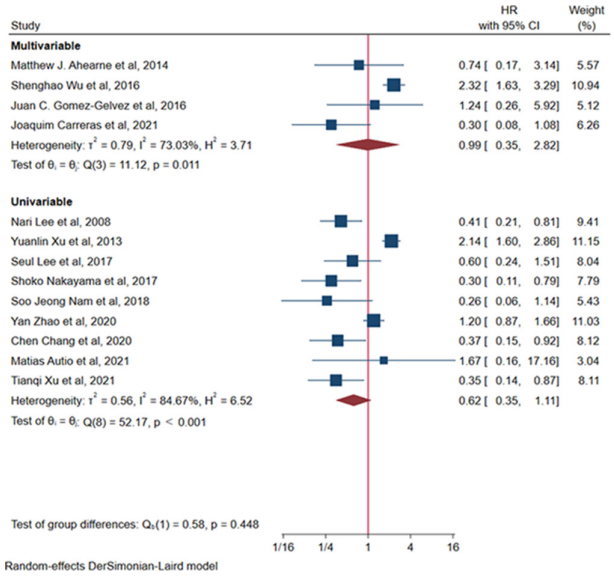

Figure 6 Subgroup analysis of overall survival by statistical methods. Random-effects DerSimonian-laird model.

Tregs are one type of tumour-infiltrating lymphocytes with the ability to inhibit the host's antitumour response by suppressing $CD8^+$ cytotoxic T cells, which play a key role in the tumour immune microenvironment.[32–34] A previous meta-analysis showed that $FOXP3^+$ Treg expression was associated with longer OS in patients with DLBCL.[14] However, owing to inconsistencies in the included studies, this conclusion warranted verification. The present study showed that there may be no association between $FOXP3^+$ Treg expression and the prognosis of patients with DLBCL. These differences might be explained by the multiple mechanisms underlying the effects of Tregs in tumours, the classification of $FOXP3^+$ Tregs and the different approaches to measuring $FOXP3^+$ Treg expression.

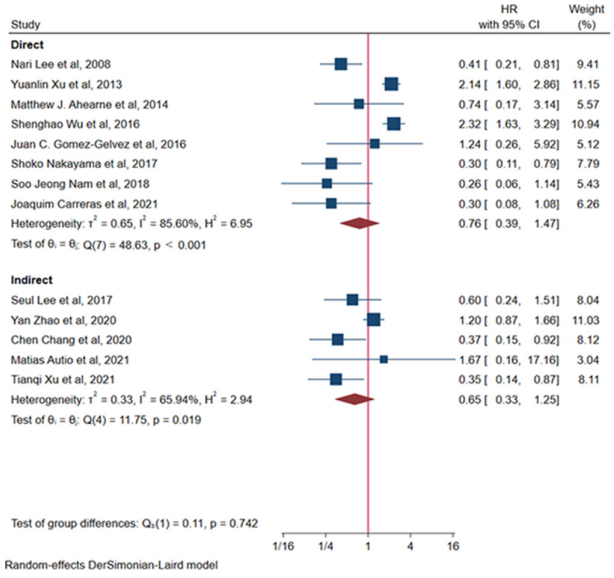

Figure 7 Subgroup analysis of overall survival by data sources. Random-effects DerSimonian-laird model.

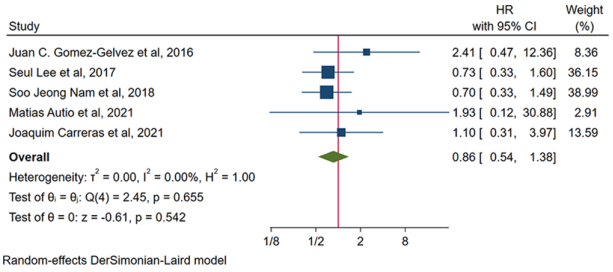

Figure 8 Forest plot of progression-free survival. Random-effects DerSimonian-laird model.

Tregs can have two effects in various diseases: pathological or protective.[35] The pathological role involves the suppression of immunity, whereas the protective role involves maintaining balanced immunity.[35] From this point of view, it is reasonable to conclude that $FOXP3^+$ Treg expression may not correlate with the prognosis of patients with DLBCL.

Studies have shown that $FOXP3^+$ Tregs could be classified into three distinct subpopulations based on their function and phenotype: resting or naive Tregs, activated or effector Tregs (eTregs), and non-Tregs.[36] Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is expressed by activated T cells and eTregs and contributes to their suppressive function.[37 38] Furthermore, research has shown that patients with colorectal cancer with high eTreg infiltration may have better prognosis than those with high non-Treg infiltration.[37] Thus, tumour-specific $FOXP3^+$ Tregs have a significant impact on the prognosis of patients with tumours by enhancing or suppressing tumour immunity. However, another study showed that single-positive $FOXP3^+$ Tregs were linked to a better prognosis in patients with DLBCL, whereas double-positive (CTLA-4 and FOXP3) Tregs were associated with poor prognosis in patients with DLBCL.[23] Hence, the function of $FOXP3^+$ Tregs in DLBCL may be influenced by other factors, including whether they are double-positive for CTLA-4 and FOXP3. Furthermore, the phenotype and levels of $FOXP3^+$ Treg infiltration in tumour tissues varied in the different stages of disease progression.[39] Therefore, it is particularly crucial to define the stage of the disease and the $FOXP3^+$ Treg phenotype in the studies of patients with DLBCL, instead of just the number of $FOXP3^+$ Tregs.

The studies included in this meta-analysis used three approaches to assessing $FOXP3^+$ Treg expression. One estimated the percentage of positive cells, another the number of positive cells, and another the overall score based on the staining intensity of the cells multiplied by the percentage of positive cells. We performed a subgroup analysis based on the approaches to measuring high-expression versus low-expression. Not unexpectedly, the three methods yielded quite different results. In the percentage method, the percentage could be heterogeneous depending on how it is calculated, such as the percentage of Tregs within all cells of the tumour, within the T cells or by digital image quantification estimation. However, the heterogeneity was relatively low (I^2=7.48%)

Table 2 Subgroup analysis of progression-free survival

Subgroup	Studies (n)	Patients (n)	HR (95% CI)	Heterogeneity		Interaction p value
				I^2 (%)	P value	
Region of patients						0.152
Western	2	125	2.28 (0.56 to 9.31)	0.00	0.892	
Asian	3	346	0.76 (0.46 to 1.26)	0.00	0.829	
Approaches to measuring the FOXP3+ Treg expression						0.393
Score	1	100	0.73 (0.33 to 1.60)	–	–	
Number of positive cells	1	114	0.70 (0.33 to 1.49)	–	–	
Percentage of positive cells	3	257	1.53 (0.59 to 3.95)	0.00	0.7	
Statistical method						0.234
Multivariable	2	206	1.48 (0.54 to 4.07)	0.00	0.459	
Univariable	3	265	0.74 (0.43 to 1.27)	0.00	0.786	
Obtained data						0.752
Direct	3	320	0.92 (0.50 to 1.68)	0.00	0.385	
Indirect	2	151	0.78 (0.37 to 1.67)	0.00	0.508	

–, no data; FOXP3+ Treg, regulatory T cells expressing forkhead box P3.

because those included studies measured the percentage of Tregs via TMA. Moreover, it looks like the most reliable data were obtained when the FOXP3+ Treg expression was measured by the number of positive cells. I^2 (0.00%) was very low and Tregs correlated with good patient prognosis, which makes biological or pathological sense.

A previous study reported that 2.3% of the FOXP3+ Tregs per 10 high-power fields were approximately equal to 163.3 cells/mm^2.[8] Moreover, the scoring method relied on the percentage of positive cells. This supports that the three approaches to measuring FOXP3+ Treg expression significantly differed from each other. Thus, the cut-offs used by the three methods could result in distinctly different survival estimates. Therefore, the results of our study were influenced by the different methodologies. It is necessary to unify the methodologies used to evaluate FOXP3+ Treg expression in future studies, considering that it is associated with the prognosis of patients with DLBCL. Undoubtedly, the more accurate the determination of the expression of FOXP3+ Tregs, the more reliable the prognosis of patients with DLBCL. Alternatively, determining an exact FOXP3+ Treg expression cut-off for predicting the prognosis of patients with DLBCL can be done if the original study provides the method so the three approaches can be correlated.

Although our meta-analysis provides some valuable insight, we acknowledge some limitations. First, the number of patients may not have been sufficiently large and the populations were mostly Asian. Therefore, our conclusions may not apply to other populations. Second, several histological or molecular DLBCL subtypes are well established and the impact of the stage is also known. However, it was not possible to perform subgroup analysis on these parameters because the original data were not reported comprehensively. Third, calculating HRs and the related 95% CIs indirectly may produce some errors. However, we considered this effect and performed a subgroup analysis based on the method used to obtain the data. The subgroup analysis showed that this variable did not influence the pooled results. Finally, the included studies had publication bias and the results need to be cautiously interpreted.

CONCLUSIONS

Our study revealed that FOXP3+ Treg expression was not associated with OS and PFS in patients with DLBCL and that FOXP3+ Treg expression may not be used to predict the prognosis of patients with DLBCL. However, the approaches to measuring FOXP3+ Treg expression caused qualitative interactions and might be the major source of heterogeneity. FOXP3+ Treg expression may be used to predict the prognosis of patients with DLBCL when FOXP3+ Treg expression is calculated by the number or percentage of positive cells, especially in Asian populations. More studies with a larger number of patients and standardised methods are required to confirm our conclusions.

Contributors All authors contributed to the study conception and design and take full responsibility for the integrity of the data and the accuracy of the data analysis. YB extracted and analysed the patient data and was the major contributor in the preparation of the manuscript. TH analysed part of the patient data. LZ performed the literature search and extracted the data. QL was responsible for statistical analysis. JY and ZZ made substantial contribution to the conception of the study. The first draft of the manuscript was written by KY and MZ and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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