# Prognostic significance of programmed death ligand-1 immunohistochemical expression in esophageal cancer
## A meta-analysis of the literature

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## Abstract

**Background:** It is thought that expression of programmed death ligand-1 (PD-L1) in esophageal cancer (EC) might compromise patient survival. However, the association between PD-L1 expression and survival of patients with EC remains controversial.

**Methods:** A meta-analysis combining eligible published studies was performed to evaluate the effect of PD-L1 expression in tumor cells detected by immunohistochemistry (IHC) on overall survival (OS) and disease-free survival (DFS) in patients with EC, using pooled hazard ratio (HR) with its 95% confidence interval (CI).

**Results:** The pooled HR for 19 eligible studies (18 publications, n = 3306) suggested that PD-L1 overexpression had an unfavorable impact on OS (HR = 1.42, 95% CI: 1.09–1.86). No significant effect of PD-L1 overexpression on DFS was observed, and the combined HR was 1.08 (95% CI: 0.76–1.53) for 12 eligible studies (11 publications, n = 2260).

**Conclusion:** PD-L1 expression in tumor cells detected by IHC was associated with worse OS in EC. However, the prognostic value of PD-L1 expression in tumor cells on OS in EC still needs further large prospective trials to be clarified.

**Abbreviations:** CI = confidence interval, DFS = disease-free survival, EC = esophageal cancer, ESCC = esophageal squamous cell carcinoma, HR = hazard ratio, IHC = immunohistochemistry, PD-1 = programmed cell death protein-1, PD-L1 = programmed death ligand-1, OS = overall survival.

**Keywords:** biomarker, esophageal cancer, meta-analysis, prognosis, programmed death ligand-1

## 1. Introduction

Despite improvements in multimodality therapy, including surgery combined with chemotherapy and/or radiotherapy, the prognosis for esophageal cancer (EC) is still rather dismal.[1] It is a pressing need for developing new therapy modalities. In the past decade, great interest has been directed toward the cancer immunotherapy.

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It is well known that the development and prognosis of malignant tumors are closely related to host immune functions.[2] Recent advances in cancer immunology have revealed the importance of the programmed cell death protein-1 (PD-1) signaling pathway.[3] PD-1 is a negative costimulatory receptor expressed primarily on activated T cells. The interaction of PD-1 with its specific ligands, programmed cell death ligand 1 or 2 (PD-L1 or PD-L2), plays a pivotal role in antigen-specific T-cell response, mediating PD-1-dependent immune suppression, which facilitates tumor cell to escape from immunosurveillance and promotes tumor progression.[4–6]

Up to now, PD-L1 expression has been observed in a wide variety of malignancies, and several studies suggested that PD-L1 high expression in tumor cells indicates poor prognosis in patients with numerous types of malignancies, including EC.[7–11] However, reports on the influence of PD-L1 expression in patients with EC have been equivocal.[10–13] The aim of this study therefore was to perform a meta-analysis of the influence of PD-L1 expression on overall survival (OS) and disease-free survival (DFS) in patients with EC.

## 2. Materials and methods

### 2.1. Search strategy and selection criteria

This study was approved by the Institutional Review Board of Shanghai Jiao Tong University Affiliated Sixth People's Hospital. PubMed and Web of Science were searched (last search was updated on December 31, 2017), using a search algorithm that was based on a combination of the terms: esophageal OR

esophagus and cancer OR carcinoma, and programmed cell death ligand 1 OR PD-L1 OR B7-H1. All searched studies were retrieved, and their bibliographies were checked for other relevant publications. Reference lists from identified primary studies and review articles were then searched to identify additional eligible studies missed by electronic search strategies.

Two independent reviewers assessed the eligibility of studies by reviewing titles and abstracts identified by the search. Studies were included in the meta-analysis if they met the following criteria: patients included had surgery and their disease was identified as EC by postoperative pathologic check, evaluate the expression of PD-L1 in the primary tumor cells rather than in sera or metastatic tissue or in tissue adjacent to the tumor, nor in the tumor-infiltrating immune cells, the expression of PD-L1 was measured by immunohistochemistry (IHC) of protein only, association of PD-L1 expression with OS and/or DFS, articles published as a full paper in English, studies provided sufficient information to estimate hazard ratio (HR) and 95% confidence interval (CI). When multiple articles pertained to overlapping populations of patients, only the newest, largest, or most informative single article was selected.

### 2.2. Data extraction and quality assessment

Two investigators extracted data from eligible studies independently, discussed discrepancies, and reached consensus for all items. The data collection and assessment of methodologic quality followed the quality of reporting of meta-analysis (QUORUM) and the Cochrane Collaboration guidelines (http://www.cochrane.de). The following data were collected from each study: first author's name, year of publication, country of study, tumor cell pathologic type, number of patients analyzed, clinic stage, treatment received, follow-up time, IHC evaluation method and cut-off value for overexpression, antibody used, antibody dilution, rate of PD-L1 overexpression, and prognostic outcomes of interest (OS and DFS). Duplication of data was avoided by matching the author's name and the name of research centers.

### 2.3. Statistical analysis

Included studies were divided into 2 groups for analysis: those with data regarding OS and those regarding DFS. For the quantitative aggregation of survival results, the impact of PD-L1 overexpression on survival was estimated for each study by the hazard ratio (HR), with its 95% CI, respectively. When HRs and their 95% CIs were described in text or tables, we obtained these values directly. When these statistical variables were not given explicitly in an article, they were calculated from available numerical data using methods described by Parmar et al.[14] When the only available data were in the form of graphical representations, they were calculated from Kaplan–Meier survival curves; the Kaplan–Meier curves were read by 2 persons using Engauge Digitizer 4.1 version independently to reduce inaccuracy in extracted survival rates. By convention, an observed HR of >1 implied a worse survival in the group of PD-L1 overexpression. The impact of PD-L1 on survival was considered to be statistically significant if the 95% CI for the HR did not overlap 1.

The heterogeneity was formally investigated by means of $Q$ test and $I^2$ statistics. If the heterogeneity was existed, we used a random-effects model in place of a fixed-effects model. Evidence of publication bias was evaluated by the funnel plot with Begg

test[15] and Egger linear regression asymmetry test.[16] For all of these analyses, P-values below .05 were considered representative of statistically significant. All the data analyses were performed STATA version 12.0 (Stata Corporation, College Station, TX).

## 3. Results

### 3.1. Search results

The search results have been shown in Figure 1. The primary literature research retrieved 204 records. After screening the title of citations, 51 records were excluded because of duplicated literatures. Next, 89 citations were excluded after screening abstracts of the records due to non-English articles, meeting reports, reviews, not PD-L1 topic, and non-EC. Then we carefully read the full text of the left citations and 46 of those were excluded due to laboratory studies, insufficient OS data, irrelevant study to the current analysis, or repeat study. Finally, there were 18 published studies included in final meta-analysis.

### 3.2. Study characteristics

The characteristics of eligible studies are summarized in Table 1. A total of 18 studies[10–13,17–30] published from 2005 to 2017 met the criteria for this meta-analysis. All of studies were based on the data of retrospective analysis. A total of 3306 patients were subjected to the final analysis (mean: 184 per study; range: 41–536). Surgery was performed for all patients and 611 patients in 6 studies were delivered preoperative chemotherapy and/or radiotherapy. These studies were conducted in 4 countries (China, Germany, Japan, and South Korea), and 16 studies (3080 patients) were performed in Asian population, and 2 studies (226 patients) performed in non-Asian patients. Only squamous cell carcinoma was examined in 15 studies and only adenocarcinoma were analyzed in 1 study. The remaining 2 studies investigated squamous cell carcinoma and adenocarcinoma.

The expression of PD-L1 was measured by IHC in all publications, but the IHC techniques used varied widely among studies, with a wide range of dilutions (from 1:40 to 1:1000). The IHC technique for PD-L1 expression detection was summarized in Table 2. According to the cut-off values for PD-L1 overexpression, as defined by each study's author, 1052 patients (31.8%) in this meta-analysis had PD-L1 overexpression, with a ranged of 14.5% to 63.3%.

In the study of Wakita et al,[21] the impacts of PD-L1 expression on OS and DFS were analyzed in the subgroup of surgery alone and surgery plus adjuvant therapy, respectively. Hence HRs on OS and DFS could be extracted for 19 (18 publications) and 12 (11 publications) of studies, respectively. Of the 19 studies analyzing the impacts of PD-L1 overexpression on OS, 9 directly reported HRs (multivariate analysis), while the other 10 studies provided survival curves. A significant association between PD-L1 overexpression and OS was found in 13 studies, including 11 studies linking PD-L1 expression with worse OS and 2 studies linking PD-L1 expression with better OS. The remaining 6 studies yielded negative results. In the 12 studies analyzing PD-L1 overexpression on DFS, 5 directly reported HRs (multivariate analysis), while the other 7 studies provided survival curves. Four studies suggested PD-L1 overexpression indicated poor prognosis of DFS, and 3 studies resulted in a favorable DFS, and 5 studies resulted in an indeterminate role for PD-L1 overexpression on DFS.
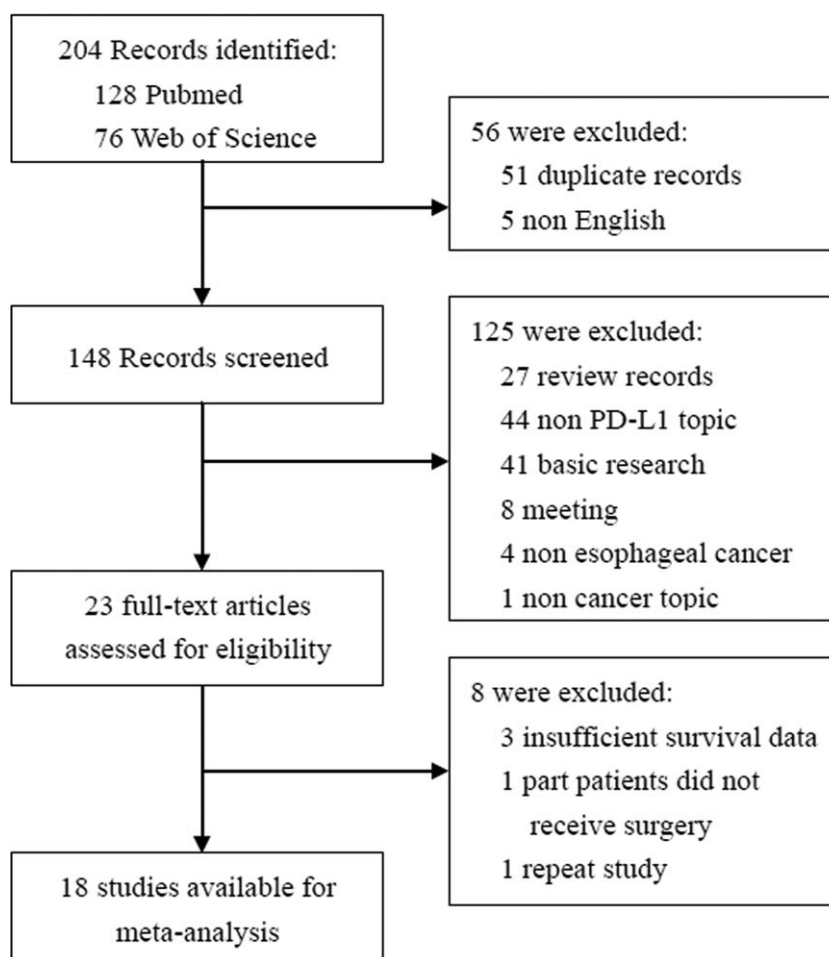
**Figure 1.** Flow chart for eligible studies.

### 3.3. Impacts of PD-L1 expression on OS and DFS

The impact of PD-L1 expression in tumor cells on OS was shown in Figure 2. Overall, the pooled HR for all 19 eligible studies (18 publications, n = 3306 patients) evaluating PD-L1 overexpression on OS was 1.42 (95% CI: 1.09–1.86, $Z = 2.58$, $P = .01$), suggesting that PD-L1 overexpression detected by IHC was an indicator of poor prognosis for EC (Fig. 2A). For obvious heterogeneity was observed ($Q = 94.67$, $I^2 = 81.0\%$, $P < .001$), random effect model was used to analyze the effect size. It is necessary to analyze the value of PD-L1 subgrouped by HR provided way. In Figure 2B, the combined HR for 9 studies evaluating PD-L1 overexpression on OS by multivariate analysis was 1.52 (95% CI: 1.06–2.19, $Z = 2.25$, $P = .024$). However, the pooled HR of 10 studies provided by survival curve was 1.34 (95% CI: 0.89–2.02, $Z = 1.40$, $P = .162$). Then, stratified analysis according to countries, the pooled HRs of Asian country studies and non-Asian country studies were 1.43 (95% CI: 1.10–1.88, $Z = 2.63$, $P = .008$) and 1.40 (95% CI: 0.24–8.0, $Z = 0.38$, $P = .706$), respectively, indicating PD-L1 is an indicator of poor prognosis of OS in Asian patients but not in non-Asian patients (Fig. 2C). At last, we limited the analysis to the studies dealing mostly (>90%) with squamous cell carcinoma, the combined HR was 1.36 (95% CI: 1.04–1.78, $Z = 2.26$, $P = .024$), suggesting that PD-L1 overexpression was significantly correlated with worse OS in esophageal squamous cell carcinoma (ESCC) (Fig. 2D).

The impact of PD-L1 overexpression in tumor cells on DFS was shown in Figure 3. However, no statistically significant effect of PD-L1 overexpression on DFS was observed, and the combined HR for 12 eligible studies (11 publications, n = 2260 patients) involving DFS was 1.08 (95% CI: 0.76–1.53, $Z = 0.41$, $P = .683$) in patients with EC (Fig. 3A). When stratified analysis according to countries, PD-L1 overexpression had no significant impact on DFS in Asian patients (HR = 1.08, 95% CI: 0.78–1.51, $Z = 0.47$, $P = .640$) nor in non-Asian patients (HR = 1.06, 95% CI: 0.14–8.12, $Z = 0.05$, $P = .957$) (Fig. 3B). The combined HR was 0.99 (95% CI: 0.70–1.39, $Z = 0.08$, $P = .934$) in studies dealing mostly (>90%) with squamous cell carcinomas, suggesting that PD-L1 overexpression had no significant impact on DFS in ESCC (Fig. 3C).

### 3.4. Publication bias

Begg funnel plot[15] and Egger test[16] were performed to evaluate the publication bias of the eligible studies (Fig. 4). About 19 and 12 studies investigating PD-L1 overexpression on OS and DFS yielded an Egger test score of $P = .08$ and .598 (Fig. 4A and C), respectively, indicating the absence of publication bias in the studies. About 18 and 11 studies investigating PD-L1 overexpression on OS and DFS in ESCC yielded an Egger test score of $P = .144$ and .856, respectively (Fig. 4B and D). Similar results were found for the subgroup analysis of PD-L1 overexpression

**Table 1**

**Main characteristics and results of the eligible studies.**

| First author | Year | Country | Stage | N pts | Pathology | PD-L1 overexpression (%) | Treatment | Median follow-up | Outcome | Multivariate/ univariate | Result |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Zhang[12] | 2017 | China | II–III | 344 | ESCC | 50 (14.5) | 197 Surgery alone 147 Surgery+RT | NR | OS | Survival curve | NS |
| | | | | | | | | | DFS | Survival curve | NS |
| Tsutsumi[17] | 2017 | Japan | NR | 90 | ESCC | 57 (63.3) | Surgery; Postop treatment: NR | NR | OS | Multivariate | NS |
| | | | | | | | | | DFS | Multivariate | S (worse) |
| Momose[18] | 2017 | Japan | I–IV | 251 | 245 ESCC 6 EAC | 39 (15.5) | CRT/CT+Surgery | 53.7 mo | OS | Survival curve | S (worse) |
| Hatogai[19] | 2017 | Japan | I–IV | 286 | ESCC | 67 (23.4) | 249 Surgery alone 37 Surgery+CT | 5.5 y | OS | Multivariate | S (better) |
| | | | | | | | | | DFS | Multivariate | S (better) |
| Jesinghaus[20] | 2017 | Germany | I–IV | 125 | ESCC | 38 (30.4) | Surgery; Postop treatment: NR | 65.1 mo | OS | Survival curve | S (better) |
| | | | | | | | | | DFS | Survival curve | S (better) |
| Wakita[21] | 2017 | Japan | IB–IIIC | 72 | ESCC | 15 (20.8) | Surgery alone | NR | OS | Survival curve | NS |
| | | | | | | | | | DFS | Survival curve | S (better) |
| Wakita[21] | 2017 | Japan | IB–IIIC | 105 | ESCC | 34 (32.4) | Surgery+CT | NR | OS | Survival curve | NS |
| | | | | | | | | | DFS | Survival curve | NS |
| Yagi[22] | 2017 | Japan | I–III | 305 | 279 ESCC 15 EAC | 53 (17.4) | 109 CRT/CT+Surgery 196 Surgery+ CRT/CT | 3.7 y | OS | Multivariate | S (worse) |
| | | | | | | | | | DFS | Multivariate | S (worse) |
| Chen[13] | 2016 | China | I–IV | 536 | ESCC | 222 (41.4) | Surgery Postop treatment: NR | 32.7 mo | OS | Multivariate | NS |
| | | | | | | | | | DFS | Multivariate | NS |
| Chen[23] | 2016 | China | NR | 162 | ESCC | 74 (45.7) | 115 Surgery alone 47 CRT+Surgery | 39.7 mo | OS | Survival curve | S (worse) |
| Ito[24] | 2016 | Japan | NR | 90 | ESCC | 17 (18.9) | Surgery Postop treatment: NR | NR | OS | Survival curve | S (worse) |
| | | | | | | | | | DFS | Survival curve | NS |
| Kim[25] | 2016 | South Korea | I–IV | 200 | ESCC | 67 (33.5) | 20 CRT/CT+Surgery 180 Surgery±CT/RT | NR | OS | Survival curve | NS |
| Leng[26] | 2016 | China | I–IV | 106 | ESCC | 57 (53.8) | Surgery; Postop treatment: NR | 55 mo | OS | Survival curve | S (worse) |
| Lim[27] | 2016 | South Korea | I–III | 73 | ESCC | 41 (56.2) | 64 CRT+Surgery 9 CT+Surgery | NR | OS | Multivariate | S (worse) |
| | | | | | | | | | DFS | Multivariate | NS |
| Tanaka[28] | 2016 | Japan | I–IV | 180 | ESCC | 53 (29.4) | 69 Surgery 111 CT+Surgery | NR | OS | Survival curve | S (worse) |
| Zhu[29] | 2016 | China | II | 133 | ESCC | 56 (42.1) | Surgery alone | 42.6 mo | OS | Multivariate | S (worse) |
| | | | | | | | | | DFS | Multivariate | S (worse) |
| Chen[11] | 2014 | China | I–IV | 106 | ESCC | 57 (53.8) | Surgery alone | NR | OS | Multivariate | S (worse) |
| Loos[30] | 2011 | Germany | I–IV | 101 | EAC | 37 (36.6) | Surgery Postop treatment: NR | 75 mo | OS | Multivariate | S (worse) |
| | | | | | | | | | DFS | Multivariate | S (worse) |
| Ohigashi[10] | 2005 | Japan | NR | 41 | ESCC | 18 (43.9) | Surgery Postop treatment: NR | 25 mo | OS | Survival curve | S (worse) |

CT = chemotherapy, CRT = chemoradiotherapy, DFS = disease-free survival, EAC = esophageal adenocarcinoma, ESCC = esophageal squamous cell carcinoma, N pts = number of patients, NR = not reported, NS = non significant, OS = overall survival, Postop = postoperative, RT = radiotherapy, S = significant.

on OS in multivariate analysis ($P = .115$), Asian ($P = .127$), respectively. These results suggested that there were no publication biases in these subgroup analyses.

## 4. Discussion

Recently, PD-1/PD-L1 pathway has attracted much attention as an immune-based treatment of several types of malignancies. Some clinical trials using PD-L1-targeting antibodies, such as avelumab and durvalumab, were performed in gastro-ECs.[31] Understanding the mechanisms of action of anti-PD-L1 therapy requires correct evaluation of the impact of PD-L1 expression on survival of patients.

Our present analysis combining 18 published studies which included 3306 patients with EC yielded summary statistics indicating that PD-L1 overexpression has an unfavorable impact on OS, with the pooled HR of 1.42 (95% CI: 1.09–1.86), but not on DFS (HR = 1.08, 95% CI: 0.76–1.53). Conversely, 2 studies included in our analysis showed PD-L1 expression to be a factor predicting favorable OS in EC.[19,20] Similar association have also been found in different tumor types.[32,33] Several possible reasons for these discordant results have been speculated. One reason is likely that heterogeneous baseline characteristics exist in different studies. Moreover, primary antibody used, the definitions of positive staining applied and the cut-off values adopted were also different. This also explained the heterogeneity

## Table 2

**Immunohistochemical technique used in studies.**

| First author | Antibody for IHC | Dilution | Counting method and cut-off for overexpression of PD-L1 | PD-L1 location |
|---|---|---|---|---|
| Zhang[12] | Primary antibody (clone SP142; Spring Bioscience, Pleasanton, CA) | NR | The proportion of PD-L1 positive cells was estimated as the percentage of total tumor cells: 0, 0–1%; 1, 1–5%; 2, 5–10%; 3, >10%. Cut-off value of ≥5% of tumor cells with staining | Membrane cytoplasm |
| Tsutsumi[17] | Rabbit PAb (Lifespan Bioscience, Seattle, WA) | 1:200 | Cut-off value of ≥5% of tumor cells staining | Membrane cytoplasm |
| Momose[18] | Rabbit MAb (clone SP142; Spring Bioscience) | 1:100 | Cut-off value of ≥5% of the cells was stained | Membrane |
| Hatogai[19] | Rabbit MAb (E1L3N, Cell Signaling Technology, Cambridge, UK) | 1:400 | Cut-off value of ≥1% of tumor cells with membrane at least weak staining | Membrane |
| Jesinghaus[20] | PD-L1 primary antibody (VENTANA, clone: SP-263) | 1:100 | The intensity: "no staining" (0), "weak staining" (1+), "intermediate staining" (2+), and "strong staining" (3+). Patients were stratified into 3 subgroups: lower (below 33 percentile), intermediate (33–66 percentile) and upper third (exceeding 66 percentile). Cut-off value of: cases within the upper third | Membrane |
| Wakita[21] | Rabbit MAb (13684; Cell Signaling Technology, Danvers, MA) | 1:200 | Samples were deemed positive when the stained area was 10% or more of the whole cancer area and patients were partitioned by this value into PD-L1-positive and PD-L1-negative groups | Membrane cytoplasm |
| Yagi[22] | Rabbit MAb (clone E1L3N; Cell Signaling Technology, Danvers, MA) | 1:200 | Cut-off value of ≥25% of tumor cells displayed at least moderate staining or strong expression in any portion of tumor cells | Membrane cytoplasm |
| Chen[13] | Rabbit MAb (clone SAB2900365; Sigma-Aldrich, Saint Louis, MO) | 1:400 | Cut-off value of ≥5% of the tumor cells displayed at least moderate staining | Membrane cytoplasm |
| Chen[23] | PD-L1-neutralizing antibody purchased from Biolegend (San Diego, CA), | NR | Calculated by multiplying intensity (0=no staining, 1=weak staining, 2=moderate staining, and 3=strong staining) by the percentage of positively stained cells (0=less than 10% of cells stained, 1=11–50% of cells stained, 2=51–80% of cells stained, and 3=more than 81% of cells stained). Cut-off value of ≥ score 2 | membrane cytoplasm |
| Ito[24] | Rabbit PAb (cat no: LS-B480; Lifespan Biosciences) | NR | Staining intensity: 0, no staining; 1, weak; 2, moderate; 3, strong. Area of stained cells: 0, 0%; 1, 1–10%; 2, 11–30%; 3, 31–66%; 4, 67–80%; and 5, ≥80%. Scores=added of area and intensity. Cut-off value of ≥score 4 | Membrane cytoplasm |
| Kim[25] | Rabbit MAb (E1L3N) XP (Cell Signaling Technology, Danvers, MA) | NR | Scored as 0 (no or any staining less than 10% of cells), 1+ (weak), 2+ (moderate), or 3+ (strong staining in more than 10% of tumor cells); cut-off value of ≥score 1 | Membrane cytoplasm |
| Leng[26] | Rabbit PAb (ab58810; Abcam, Cambridge, MA) | 1:40 | Staining intensity: 0, no staining; 1, faint yellow; 2, clay bank; 3, sepia. Area of stained cells: 1, <10%; 2, 10–50%; 3, >50%. Scores=multiplication of the area and intensity. Cut-off value of >score 3 | Membrane cytoplasm |
| Lim[27] | MAb (5H1, Mayo Foundation for Medical Education and Research) | NR | Intensity: 0, no appreciable staining; 1+, <10% of tumor cell staining; 2+, moderately to intensely positive tumor cell staining in a single group; 3+, intensely positive tumor cell staining matching or exceeding control material, in more than a single group or small groups of cells. H-scores were obtained by multiplying the grades of extent and intensity of staining; cut-off value of ≥score 20 | Membrane cytoplasm |
| Tanaka[28] | Mouse MAb (clone 27A2; MBL, Woburn, MA) | NR | Staining intensity: 0, no staining; 1, weak; 2, moderate; 3, strong; Area of stained cells: 0, 0%; 1, 1–30%; 2, 30–60%; 3, 60–100%. Scores=multiplication of the area and intensity. Cut-off value of ≥score 4 | Cytoplasm |
| Zhu[29] | Antibody (clone SP142; Zhongshan Golden Bridge Biotechnology Company, Beijing, China) | NR | Distinct membranous or cytoplasmic staining was observed in tumor cells | Membrane cytoplasm |
| Chen[11] | Rabbit MAb (NBP1-03220; Novus Biologicals, Littleton, CO) | 1:200 | At least weak staining in the tumor cells | Membrane cytoplasm |
| Loos[30] | Primary antibody B7-H1 (Abcam, Cambridge, UK) | NR | Quantification was made as follows: 33% of the cancer cells or less, 1; more than 33% to 66% of the cancer cells, 2; and more than 66% of the cancer cells, 3. Intensity of staining was stated as absent or weak, 1; moderate, 2; and strong, 3. Each section had a final grade that derived from the multiplication of the area and intensity scores. Cut-off value of ≥score 4 | Membrane cytoplasm |
| Ohigashi[10] | PD-L1 MAb (MIH1, mouse immunoglobulin G1) | NR | Cut-off value of >10% PD-L-positive tumor cells staining | Membrane cytoplasm |

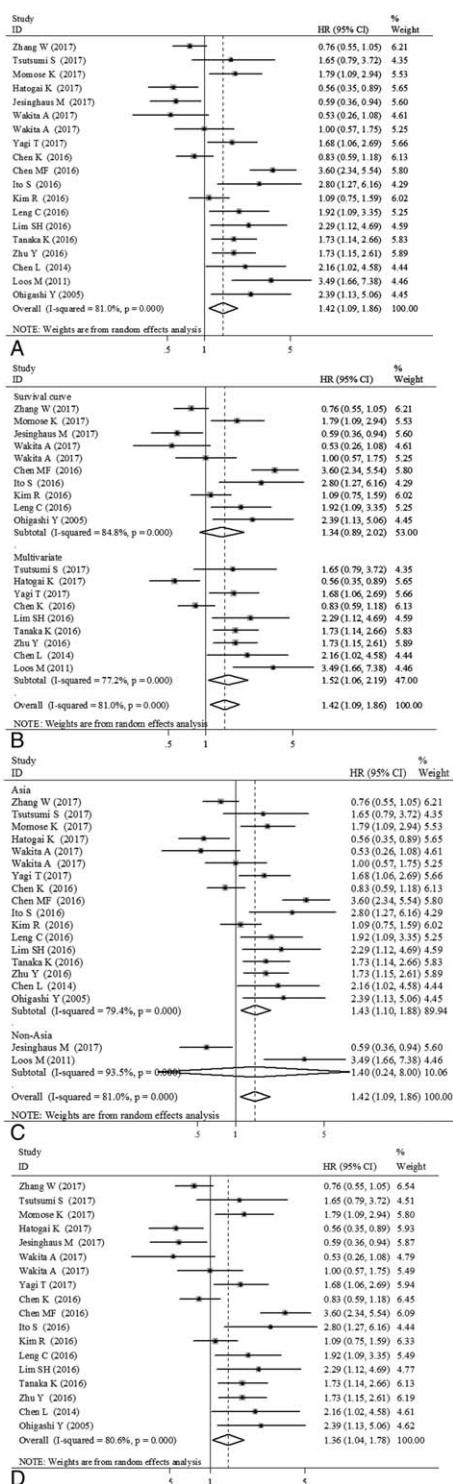IHC = immunohistochemistry, NR=not reported, PD-L1 = programmed death ligand-1.

Figure 2. Forest plot showing the association between programmed death ligand-1 (PD-L1) expression status with overall survival (OS). (A) All 19 eligible studies (18 publications) for OS. (B) Subgrouped by hazard ratio (HR) provided way (multivariate analysis/survival curve). (C) Subgrouped by country (Asia/non-Asia). (D) 18 eligible studies (17 publications) for OS in esophageal squamous cell carcinoma (ESCC).
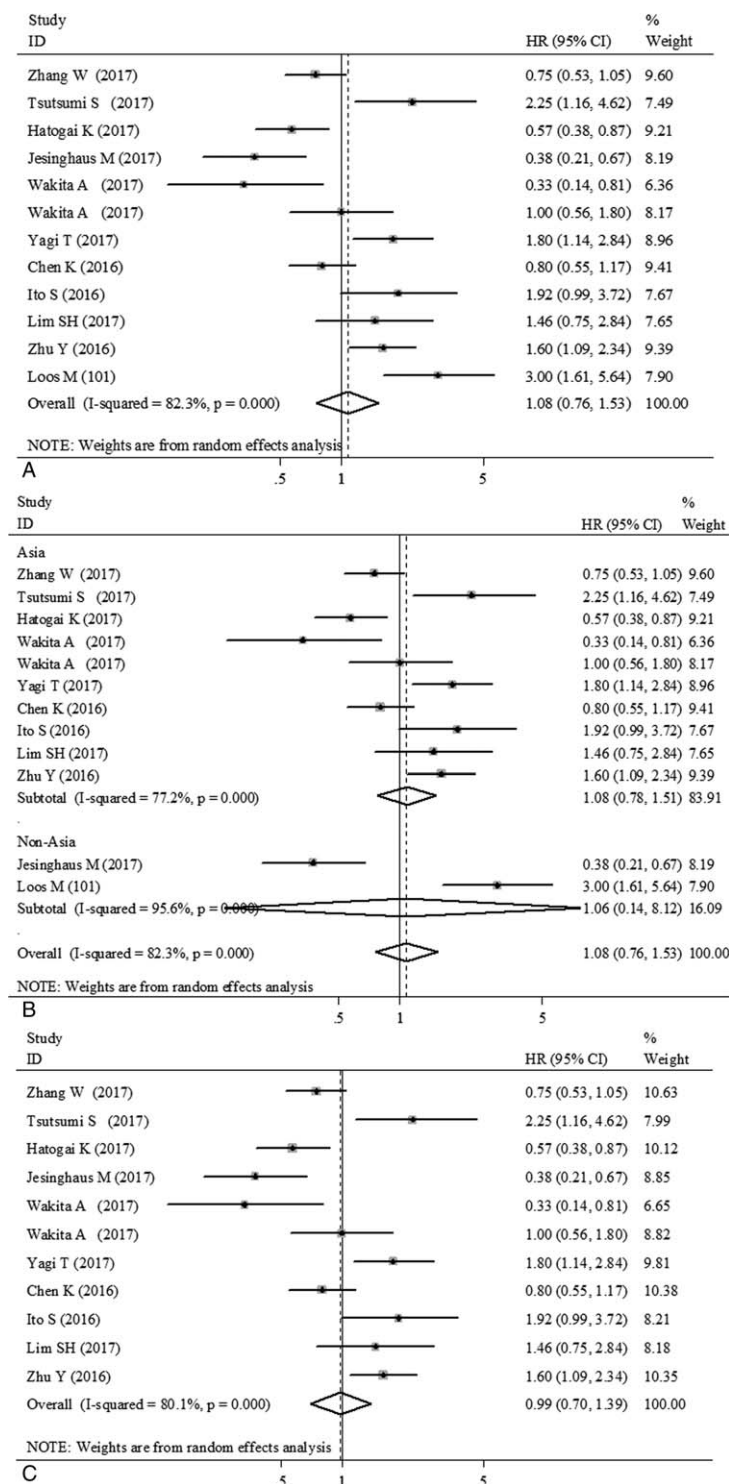


Figure 3. Forest plot showing the association between programmed death ligand-1 (PD-L1) expression status with disease-free survival (DFS). (A) All 12 eligible studies (11 publications) for DFS. (B) Subgrouped by country (Asia/non-Asia). (C) 11 eligible studies (10 publications) for DFS in esophageal squamous cell carcinoma (ESCC).

problem in our meta-analysis. Also, in certain circumstance, high PD-L1 expression might promote immune responses through interaction of PD-L1 with unknown receptors, resulting in T-cell proliferation and secretion of certain cytokines, which in turn activate strong antitumor effects.

Then, we performed analysis in subgroup of different country. The PD-L1 is a poor prognostic factor for OS in EC patients in Eastern Asian countries (China, Japan, and South Korea) (HR = 1.43, 95% CI: 1.10–1.88), but not in non-Asian patients (HR = 1.40, 95% CI: 0.24–8.0), which raises a question whether the
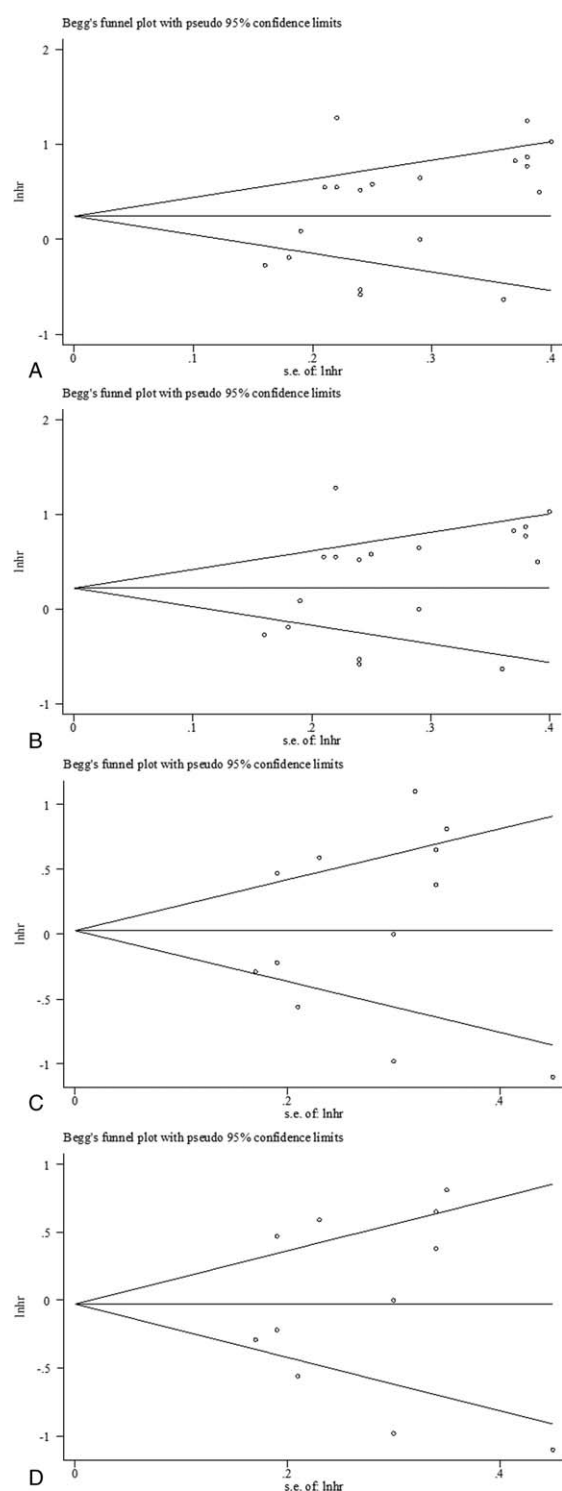
**Figure 4.** Begg funnel plot for publication bias test. Plots are arranged as follows: (A) all 19 eligible studies (18 publications) for overall survival (OS); (B) 18 studies (17 publications) for OS in esophageal squamous cell carcinoma (ESCC); (C) all 12 eligible studies (11 publications) for disease-free survival (DFS); (D) 11 studies (10 publications) for DFS in ESCC.

validity of results in present meta-analysis would also be applicable to non-Asian patients. In our meta-analysis, only 2 studies included non-Asian patients,[20,30] which occupied 6.8% (n = 226), and negative result may due to the small sample. Also, variability in measurements, experimental procedures, and criteria for PD-L1 expression among countries may contribute

to different results. In the study of Jesinghaus et al,[20] only membranous staining patterns were scored as positive and the intensity of PD-L1 staining was scored using a 4-tiered grading system. While in some studies from Asian countries,[10,13,17] membranous and cytoplasm staining patterns were scored as positive, and the cut-off point of high expression of PD-L1 was defined as ≥5% or 10% of the cells were stained.

In subgroup analysis of patients with ESCC, we also observed an adverse influence of PD-L1 overexpression on OS (HR = 1.36, 95% CI: 1.04–1.78). One previous meta-analysis[34] found a trend that overexpression of PD-L1 might be associated with poor survival in patients with ESCC, but the difference was not statistically significant (HR = 1.65; 95% CI 0.95–2.85; P = .07). However, that analysis only combined 7 studies and several studies showing poor survival in tumors overexpressing PD-L1 was excluded, which might have affected the results. Unfortunately, studies involving the role of PD-L1 in esophageal adenocarcinoma (EAC) are limited, so we are unable to draw similar conclusions in the subgroup of patients with EAC for the time being.

Some studies also investigated the prognostic value of PD-L1 expression detected by IHC in tumor-infiltrating immune cells of EC. According to the search strategy in our study, we found 3 studies investigated the prognostic value of PD-L1 expression detected by IHC in tumor-infiltrating immune cells of EC.[12,18,20] In the study of Zhang et al,[12] tissue specimens from 344 patients with ESCC were obtained for IHC analysis of PD-L1 expression on tumor-infiltrating immune cells. The results demonstrated that PD-L1 expression on tumor-infiltrating immune cells is an independent prognostic factor in patients with ESCC and patients with positive immune cell PD-L1 expression had improved survival. However, the results from the study of Momose et al[18] demonstrated that high expression of PD-L1 on the immune cells was associated with unfavorable prognosis in patients with EC. Jesinghaus et al[20] also investigated the influence of immune cell PD-L1 expression on survival in ESCC. In this study, a prognostic value for PD-L1 expression on immune cells was not found for OS or DFS. The literature about the effect of immune cells PD-L1 expression on survival in EC is limited, so we did not perform a quantitative aggregation of survival results to analyzing the association between the immune cells PD-L1 expression and survival in EC.

Association of PD-L1 expression with unfavorable OS provides a rationale for antitumor use in the treatment of EC, especially in ESCC, but the association of PD-L1 expression with traditional prognostic factors such as clinical TNM stage or differentiation is still needed to investigate. Moreover, the prognostic role of PD-L1 in EC should be examined in the context with other molecular biomarkers. Some studies enrolled in this meta-analysis had already addressed the association of PD-L1 with other biomarkers, such as MLH1,[18] HLA Class I,[24] and FOXP3+.[29]

As we performed a meta-analysis, we had to deal with heterogeneity problems. Heterogeneity is a potential problem that may affects the interpretation of the results of all meta-analyses. In the present meta-analysis, obvious heterogeneity was observed between studies. Although only studies performing IHC staining were enrolled in our meta-analysis, some variations in methodologic factors may contribute to heterogeneity between studies, such as different primary antibodies and wide range of dilutions (from 1:40 to 1:1000) were used for immunodetection of PD-L1 across the studies. Some studies even did not clarify the antibody used in detail. Also, different quantitative evaluation

systems for the IHC findings and cut-off value from arbitrary choices by investigators conduced to a wide range of protein overexpression. As there is no standard cut-off point for the expression of PD-L1 in the primary tumor cells in EC at present, the cut-off values are different among included studies, which may have interference in judging the prognostic value of PD-L1 expression. In this study, the rate of PD-L1 overexpression was ranged from 14.5% to 63.3%, which may also have contributed to heterogeneity.

Although we did not detect significant publication bias in this meta-analysis, some kind of potential bias still exists between studies and cannot be completely eliminated. We have restricted our analysis to published studies written in English, and a majority of studies that met eligibility criteria were excluded based on language criteria, which may have led to an overestimation of effect sizes. Another potential source of bias is related to the method for extrapolating HR. Some HRs were extrapolated from survival curves, which unavoidably developed a decrease of reliability. Therefore, the results must be interpreted with caution.

In conclusion, the results of our meta-analysis revealed that PD-L1 overexpression was significantly associated with poor OS in EC. Increased expression of PD-L1 might be a predicative factor of poor prognosis and provide a rationale for inhibiting PD-L1 in EC. In the future, higher quality studies and superior patient selection are expected.

## Author contributions

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