Systematic Review

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The Prognostic Value of FOXP3 in Patients With Oral Squamous Cell Carcinoma: A Systematic Review and Meta-Analysis

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Background: Oral squamous cell carcinoma (OSCC) is one of the most prevalent malignancies in the head and neck region, with a high morbidity rate and a poor prognosis. As a result, the search for effective biomarkers to assess OSCC's prognosis has become a research hotspot. Given that the impact of Forkhead box protein P3 (FOXP3) as a significant marker of regulatory T cells (Tregs) on OSCC's prognosis remains a subject of debate, the current study was carried out to elucidate FOXP3 importance in the prognostic assessment of OSCC patients.

Methods: Four major databases, i.e., Web of Science, Embase, Cochrane Library, and PubMed, were searched for relevant articles up to 10 February 2025. The 95% confidence intervals (95% CIs) and hazard ratios (HRs) reported in the selected articles were extracted and combined for analysis. The primary outcome was overall survival (OS), while the secondary outcomes encompassed recurrence-free survival (RFS), disease-specific survival (DSS), and disease-free survival (DFS). These outcomes were pooled and analyzed using Stata 15.

Results: 19 articles were included, comprising a total of 2136 patients. The findings revealed that high FOXP3 did not have a significant association with OS under the random-effects model analysis (HR = 0.96, 95% CI = 0.60–1.54, $p = 0.88$) but had a significant association with DSS under the fixed-effects model analysis (HR = 0.55, 95% CI = 0.44–0.67, $p < 0.001$). The heterogeneity was within an acceptable range ($I^2 = 29.3\%$, $p = 0.175$). High FOXP3 was not significantly correlated with either DFS or RFS.

Conclusions: High expression of FOXP3 is significantly associated with improved DSS in OSCC. However, due to inconsistent relationships with other survival outcomes, its clinical application potential requires further validation.

Systematic Review Registration PROSPERO (CRD420250651264).

Keywords: biomarker; FOXP3; oral squamous cell carcinoma; prognosis; meta-analysis

Introduction

Oral cavity cancer (OCC) is prevalent in the head and neck region, with more than 90% stemming from squamous tissues, which makes it widely known as oral squamous cell carcinoma (OSCC) [1]. According to the 2022 global cancer statistics, OCC ranks 15th in terms of mortality and 16th in terms of incidence. However, the rate of secondary tumor formation for OCC is 3%–7% per year. The incidence is high, with a high rate of recurrence and poor prognosis [2]. Currently, there is no accurate biomarker to predict the prognosis of OSCC, making the study of potential OSCC biomarkers a critical focus.

Forkhead box protein P3 (FOXP3), a key transcription factor regulating the function and development of regulatory T cells (Tregs), ensures immune homeostasis by suppressing effector T cells, dendritic cells (DCs), and other immune cells [3]. In OSCC, the distribution, density, and functional status of FOXP3+ Tregs are closely linked to the immunosuppressive properties of the tumor microenvironment (TME). However, studies on the prognostic value of FOXP3 in OSCC have yielded conflicting results. In some studies, individual marker survival analyses were performed in OSCC patients, and the results showed that higher FOXP3 expression is linked to poorer survival [4–6]. Conversely, other studies demonstrated that low FOXP3 density in the tumor stroma and interstitium is linked to worse overall survival (OS), disease-free survival (DFS), and disease-specific survival (DSS) [7,8]. Therefore, the current study performed a systematic review and meta-analysis of existing studies to assess the prognostic value of FOXP3 in OSCC patients.

Methods

The Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines were strictly followed in the implementation of the current study. PRISMA Checklist is outlined in **Supplementary Material 1**. The protocol was recorded in the International Prospective Register of Systematic Reviews (PROSPERO), under the registration number CRD420250651264.

Retrieval Strategy

Two researchers independently conducted article searches across four major databases: the Cochrane Library, Web of Science, Embase, and PubMed. The search covered a timeframe from database establishment to February 2025. The strategy combined MeSH/Emtree controlled vocabulary terms with free-text keywords, using Boolean operators (AND/OR/NOT) to create nested search strings. An example strategy is: (buccal mucosa tumo*r) OR (Cancer of Mouth) OR (intraoral cancer) OR (intraoral tumo*r) OR (mouth cancer*) OR (mouth neoplasm*) OR (mouth tumo*r) OR (oral cancer*) OR (Oral Neoplasm*) OR (oral tumo*r) AND (forkhead box P3 protein) OR (forkhead box protein P3) OR (FOXP3) OR (IPEX protein) OR (protein FOXP 3) OR (scurfin) OR (SCURFIN protein). Complete search strategies for each database are outlined in **Supplementary Material 2**. Additionally, the references of the included articles were manually searched to identify potentially missed studies.

Eligibility Criteria

In accordance with the PECOS principle, the following criteria were adopted to determine the inclusion of articles: Population: patients with histopathologically confirmed OSCC (irrespective of stage); Exposure: high expression of FOXP3+; Comparison: low expression of FOXP3+ or FOXP3-; Outcome: OS, DSS, DFS, etc.; Study design: cohort studies (prospective or retrospective).

The following criteria were employed to exclude articles: (1) Studies involving animal experiments, reviews, conference abstracts, etc.; (2) Inconsistencies in study diseases, objectives, and types; (3) Lack of 95% confidence interval (CI) and hazard ratio (HR); (4) Non-English articles

Screening Process

Duplicates were removed and the initial screening was performed using EndNote X9 software (Thomson Company, Philadelphia, PA, USA). The study selection was independently conducted by two researchers (Qianyu Liu and Ke Liao). First, the titles and abstracts were screened. Subsequently, the full texts were read to determine their eligibility. For articles whose eligibility was unclear, a consensus was reached through consultation with a third researcher (Yinyu Shang).

Data Extraction and Processing

A standardized data extraction form was developed, and the following data were extracted independently by two researchers (Qianyu Liu and Ke Liao): (a) Basic information (first author, publication year, country); (b) Characteristics of study population (sample size, age distribution, and tumor stage); (c) Prognostic data (HR and 95% CI, with priority given to extracting the results of multivariate analyses); (d) Details of test method; (e) In the meta-analysis, relative risk (RR) was regarded as an approximation of HR if the study findings were assessed as RR. Uniform conversion criteria were used for survival data. When only Kaplan-Meier (KM) curves were provided, Engauge Digitizer 11.1 (Andrew Makhorin, Australia) software was utilized for data extraction. All discrepancies were addressed by group discussion.

Quality Assessment

Methodological quality assessment was performed via the Newcastle-Ottawa Scale (NOS) [9], which provides a 9-star rating across three domains: outcome measurements (3 points), intragroup comparability (2 points), and subject selection (4 points). The quality of the included studies was rated as follows: 7–9 stars for high quality, 4–6 stars for moderate quality, and 1–3 stars for low quality. The assessment was performed by two independent researchers. Any discrepancies were addressed by referring to the original study protocol and discussing with a third researcher.

Statistical Analysis

Stata 15.0 software (StataCorp LP, College Station, TX, USA) was utilized for statistical analysis. The HRs and the 95% CIs were combined. The heterogeneity was evaluated via the Q-test with an I^2 statistic. A fixed-effects model was adopted if $I^2 \leq 50\%$. When $I^2 > 50\%$, a random-effects model was used, and the source of heterogeneity was investigated. Subgroup analyses were performed on the basis of sample size ($\geq$100 vs. $<$100), study design (prospective and retrospective), and geographic distribution (Asia, Europe, and the USA). The stability of results was verified by excluding studies one by one for a sensitivity analysis. A variety of methods were used to comprehensively evaluate publication bias, including funnel plots, the Egger test ($p < 0.05$ was considered a significant bias), and the trim and fill method. Two-sided tests were performed, and results were considered statistically significant if the p-value was below 0.05.

Results

Screening Results

A systematic search of four databases identified 801 articles (123 from PubMed, 415 from EMBASE, 240 from Web of Science, and 23 from Cochrane Library). Following the removal of duplicates via EndNote software, 585 arti-

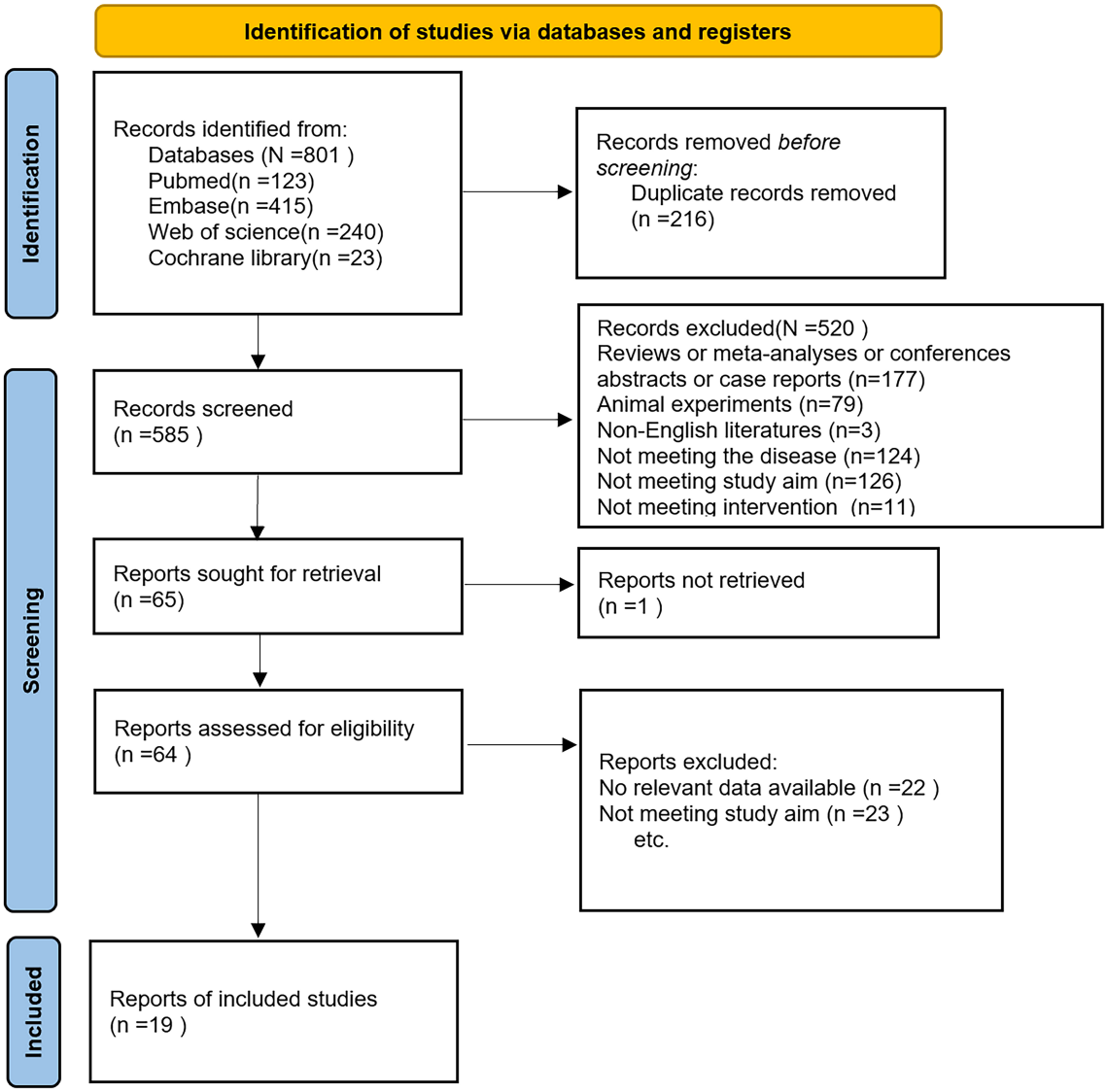

Fig. 1. Selection of studies for inclusion in meta-analysis.

cles were retained. Following a thorough evaluation of the titles and abstracts, 520 ineligible articles were excluded. After a thorough review of the full texts of the 64 remaining articles, 45 studies were removed for ineligible study design (n = 23) and missing data (n = 22). Finally, 19 articles were selected for inclusion. The PRISMA flowchart is presented in Fig. 1.

Baseline Information

Nineteen studies were included. Of these, three studies were from China, five were from Japan, and the remaining 11 were from other countries. A total of 2136 patients were involved, and the age range was 49 to 68 years. A summary of the basic characteristics of the included studies is provided in Table 1 (Ref. [4–8,10–23]). The detailed detection methods of FOXP3 is shown in Table 2 (Ref. [4–8,10–23]).

Methodological Quality Assessment

The observational studies were evaluated for quality using the NOS. All nineteen studies had a score of $\geq$7, indicating a high quality. The analysis did not include any low-quality studies (score $<$5). The specific scores are shown in Table 3 (Ref. [4–8,10–23]).

Meta-Analysis Results

Primary Outcome: OS

Fourteen studies with a total of 1619 patients reported OS. The combined results of the random-effects model demonstrated that high FOXP3 had no significant association with OS (HR = 0.96, 95% CI = 0.60–1.54, $p = 0.88$). Furthermore, high heterogeneity ($I^2 = 81.2\%$, $p < 0.001$) was observed, as illustrated in Fig. 2A. Subgroup analyses were performed to address potential sources of heterogene-

Table 1. Baseline characteristics of included studies.

Author	Year	Region	Design	Sample size	Age	Percentage of males	Source	Follow-up time	Outcome	Stage	Percentage of advanced	Preoperative chemoradiotherapy	Primary treatment
Takahashi [10]	2019	Japan	Retrospective cohort study	77	68.13 ± 12.27	64.94%	NR	NR	OS	I–IV	35.06%	NR	Surgery
Boxberg [7]	2019	Germany	Retrospective cohort study	66	63.44 ± 8.94	61.80%	Epithelium, stroma	Median follow-up time: 50 M (8–97 M)	OS, DFS, DSS	I–IV	63.60%	No preoperative neoadjuvant therapy	Surgery
Zhang [11]	2019	China	Retrospective cohort study	45	60.63 ± 9.99	53.34%	Epithelium, stroma	NR	OS	I–IV	64.40%	No preoperative neoadjuvant therapy	Surgery
Ramalingam [4]	2024	India	Retrospective cohort study	260	54.97 ± 10.82	74.43%	NR	NR	OS	I–IV	37.31%	NR	Unspecified
Song [5]	2016	China	Retrospective cohort study	273	59.69 ± 10.76	68.86%	NR	NR	RFS, 5-year OS	I–II, III–IV	47.98%	NR	Unspecified
Kindt [12]	2017	Belgium	Retrospective cohort study	110	58.47 ± 10.07	73.64%	Epithelium, stroma	NR	RFS, OS	*In situ*, I–II, III–IV	NR	NR	Surgery
Ikeuchi [13]	2023	Japan	Retrospective cohort study	69	66.36 ± 12.05	63.77%	Epithelium, stroma	Median follow-up time: 45 M (2–156 M)	DSS	I–IV	IV 73.9%	Concurrent chemoradiotherapy in partial patients	Chemoradiotherapy + surgery
Peña-Cardelles [14]	2022	Spain	Retrospective cohort study	65	65	62%	Epithelium, stroma	71.23 ± 38.67; median follow-up time (OS): 73 M	DSS, DFS, OS	I–IV	66.67%	No preoperative neoadjuvant therapy	Unspecified
De Meulenaere [15]	2017	Belgium	Retrospective cohort study	78	65	17%	Epithelium, stroma	NR	OS, DFS	I–IV	86%	Concurrent chemoradiotherapy (n = 27) or radiotherapy alone (n = 12)	Surgery in most patients
Lequerica-Fernández [8]	2021	Spain	Retrospective cohort study	125	58.69 ± 14.34	65.60%	Epithelium, stroma	6–230 M, median follow-up time: 61 M	DSS	I–IV	58%	No preoperative neoadjuvant therapy	Surgery
Hori [6]	2021	Japan	Retrospective cohort study	62	60.69 ± 12.04	69%	Epithelium, stroma	73.59 ± 41.51	DFS, 5-year RC	I–II	0%	No preoperative neoadjuvant therapy	Surgery
Wongpattaraworakul [16]	2024	The United States	Retrospective cohort study	231	61.99 ± 14.0284	58.90%	Tumor cells and immune cells in the tumor microenvironment	Early stage: Mean follow-up time: 133 M, advanced stage: Mean follow-up time: 141 M	OS	NR	60.4% (Clinical stage: T3–4)	NR	Surgery
Chen [17]	2018	China	Retrospective cohort study	93	49.23 ± 10.11	82.80%	Tumor cells and immune cells in TME	Median follow-up time: 31.4 M (0.2–99.8 M)	RFS	I–VI	44.1% (Clinical stage: T3–4)	No preoperative neoadjuvant therapy	Surgery
Bron [18]	2013	Switzerland	Prospective cohort study	35			Epithelium, stroma	47.59 ± 19.26	OS	I–IV	66%	NR	Surgery
Koike [19]	2020	Japan	Retrospective cohort study	137	67.54 ± 11.50	55.50%	Epithelium, stroma	79 M (range: 4–164 M)	OS, DSS, RFS, MFS	I–IV	24.80%	No preoperative neoadjuvant therapy	Surgery
Piersiala [20]	2024	Germany	Prospective cohort study	49	62.32 ± 14.31	38.80%	Epithelial, lymph node metastasis	NR	DFS, 3 OS	I–IV	36.7% (Clinical stage: T3–4)	No preoperative neoadjuvant therapy	Surgery

Table 1. Continued.

Author	Year	Region	Design	Sample size	Age	Percentage of males	Source	Follow-up time	Outcome	Stage	Percentage of advanced	Preoperative chemoradiotherapy	Primary treatment
Ljokjel [21]	2022	Norway	Retrospective cohort study	168	62.1 ± 11.0	76.19%	Epithelium, stroma	NR	DSS	NR	54.93% (Clinical stage: T3–4)	Yes	Radiotherapy (preoperative) + surgery
Hayashi [22]	2022	Japan	Retrospective cohort study	106	62.03 ± 14.49	56%	Epithelium	NR	OS	I–IV	64%	NR	Surgery + chemoradiotherapy in most patients
Watanabe [23]	2010	Japan	Retrospective cohort study	87	64.38 ± 13.07	59.77%	NR	NR	OS	I–IV	37%	No preoperative neoadjuvant therapy	Surgery

The percentage of advanced patients refers to the ratio of the number of patients in stage iii–iv or T3–4 to the total number of patients. Abbreviations: OS, overall survival; RFS, recurrence-free survival; DFS, disease-free survival; DSS, disease-specific survival; MFS, metastasis-free survival; NR, non-reported.

Table 2. The detailed detection methods of FOXP3.

Author	Year	High and Low FOXP3 Expression Criteria	FOXP3 Detection Methods
Takahashi [10]	2019	A semi-quantitative scoring system was applied: Score 0: no infiltration; Score 1: $FOXP3^+$ cells present around <30% of tumor cells; Score 2: 30–60%; Score 3: >60% High expression was defined as Score 3 (>60% infiltration), while all other scores were classified as low expression.	IHC
Boxberg [7]	2019	Intraepithelial $FOXP3^+$ TILs (FOXP3i): quantified as the number of $FOXP3^+$ cells per 100 tumor cells. Stromal $FOXP3^+$ TILs (FOXP3s): assessed as the percentage of $FOXP3^+$ cells within the tumor stromal area. Samples were stratified into low, intermediate, and high groups based on the 33rd and 66th percentiles, and subsequently consolidated into low vs. high categories for survival analysis.	IHC
Zhang [11]	2019	$FOXP3^+$ cells were manually counted in five high-power fields (×400, 0.0625 mm^2), and cases were classified into high and low groups using the median value as the cutoff.	IHC
Ramalingam [4]	2024	Expression intensity was evaluated using the H-score system: (1) Staining intensity was graded as 1^+ (weak), 2^+ (moderate), or 3^+ (strong). (2) The optimal cut-off value was determined using a ROC curve, and an H-score ≥37.5 for FOXP3 was defined as high expression, whereas lower values were classified as low expression.	IHC
Song [5]	2016	Tumor-cell expression of FOXP3 was evaluated by immunohistochemistry (IHC) using the IRS (Immunoreactive Score) system: Staining intensity (1–3) × percentage of positive cells (1–3). Cases were categorized into three groups: low (score 1), intermediate (score 2), and high (score 3). For survival analyses, the high-expression group (score 3) was used as the reference, while the low and intermediate groups were combined as the comparison group.	IHC
Kindt [12]	2017	$FOXP3^+$ cells were manually counted in five high-power fields (×400), and the median value (114 cells) was used as the cutoff.	IHC

Table 2. Continued.

Author	Year	High and Low FOXP3 Expression Criteria	FOXP3 Detection Methods
Ikeuchi [13]	2023	The optimal cut-off value was determined based on the ROC curve. The proportion of FOXP3$^+$ cells among every 250 stromal cells was manually quantified. A proportion ≥10% was defined as "positive (high)", whereas <10% was classified as "negative (low)".	IHC
Peña-Cardelles [14]	2022	The percentage of positive expression was categorized into four groups: 1–5%, 5–10%, 10–20%, and 20–100%.	IHC
De Meulenaere [15]	2017	A semi-quantitative scoring system was applied: 1$^+$ (absent/scattered), 2$^+$ (moderate), and 3$^+$ (abundant). The median score of 2$^+$ was used as the cutoff, classifying cases into low (1$^+$) versus high (2$^+$ or 3$^+$) expression. Scoring was performed separately in the stromal and intraepithelial compartments, and only stromal FOXP3 expression was included in the survival analyses.	IHC
Lequerica-Fernández [8]	2021	Positive cells were quantified separately in tumor nests and stroma as the number of positive cells per mm^2. The median value was used as the cutoff. Tissue microarrays (TMAs) were analyzed and independently evaluated by multiple blinded observers.	IHC
Hori [6]	2021	Cell density was quantified using a computer-assisted image analysis system (Patholoscope), and the mean value of the two regions with the highest density was used. High density was defined as a FOXP3-positive cell count ≥the median value (153 intratumoral; 251 stromal), and low density as <the median.	IHC
Wongpattaraworakul [16]	2024	High expression (+) was defined as an immunoscore of 2 or 3, and low expression (–) as a score of 0 or 1. The scoring system (0–3) was based on the density of positive inflammatory cells: 0: absent or minimal positive cells; 1: single cells or clusters of 2–4 cells; 2: clusters of more than 2–4 cells; 3: band-like or continuous cellular aggregates.	IHC
Chen [17]	2018	Under a 20× objective, the percentage of positive cells was determined, and the median value served as the cutoff. High expression was defined as a FOXP3-positive cell percentage ≥the median (70%), and low expression as <the median.	IHC
Bron [18]	2013	Using the median as the cutoff: High expression: cell count ≥median; Low expression: cell count <median	IHC
Koike [19]	2020	Cell counts per high-power field were obtained in four tumor regions, using region-specific median values as cutoffs: Tumor center (TCe), epithelium: median = 2; Invasive front (IF), epithelium: median = 2; Tumor center (TCe), stroma: median = 16; Invasive front (IF), stroma: median = 28.	IHC
Piersiala [20]	2024	The median percentage of FOXP3$^+$CD4$^+$ cells was used as the threshold: High expression: percentage ≥median; Low expression: percentage <median.	flow cytometry
Ljokjel [21]	2022	Based on quartile distribution, the high-expression group included cases within the top 25% of cell counts. Five adjacent fields (630× magnification) were assessed, and the mean value was used.	IHC
Hayashi [22]	2022	An area containing >50 positive cells per 0.09 mm^2 was defined as "high expression", whereas <50 positive cells was considered "low expression". Counts were performed manually in the region with the highest positive-cell density.	IHC
Watanabe [23]	2010	Fifteen tumor nests and fifteen stromal regions were evaluated, and the mean value was calculated. Cases were classified into high- or low-expression groups according to the median value.	IHC

Abbreviations: IHC, immunohistochemical method; FOXP3, forkhead box protein P3; ROC, receiver operating characteristics.

Table 3. NOS assessment.

Study	Selection				Comparability	Outcome			Quality scores
	Representativeness of exposed cohort (*)	Selection of nonexposed cohort (*)	Ascertainment of exposure (*)	Demonstration that outcome of interest was not present at the start of study (*)	Comparability of cohorts on the basis of the design or analysis (**)	Assessment of outcome (*)	Follow-up was long enough for outcomes to occur (*)	Adequacy of follow-up of cohorts (*)	
Takahashi 2019 [10]	*	*	*	*	*	*	*	*	8
Boxberg 2019 [7]	*	*	*	*	**	*	*	*	9
Zhang 2019 [11]	*	*	*	*	*	*	*	*	8
Ramalingam 2024 [4]	*	*	*	*	*	*	*	*	8
Song 2016 [5]	*	*	*	*	**	*	*	*	9
Kindt 2017 [12]	*	*	*	*	**	*	*	-	8
Ikeuchi 2023 [13]	*	*	*	*	**	*	*	*	9
Peña-Cardelles 2022 [14]	*	*	*	*	*	*	*	*	8
Lequerica-Fernández 2021 [8]	*	*	*	*	*	*	*	*	8
De Meulenare 2017 [15]	*	*	*	*	**	*	*	*	9
Hori 2021 [6]	*	*	*	*	*	*	*	*	8
Wongpattaraworakul 2024 [16]	*	*	*	*	**	*	*	*	9
Chen 2018 [17]	*	*	*	*	**	*	*	*	9
Bron 2013 [18]	*	*	*	*	*	*	*	*	8
Koike 2020 [19]	*	*	*	*	*	*	*	*	8
Piersiala 2024 [20]	*	*	*	*	**	*	*	*	9
Ljokjel 2022 [21]	*	*	*	*	**	*	*	*	9
Hayashi 2022 [22]	*	*	*	*	*	*	*	*	8
Watanabe 2010 [23]	*	*	*	*	*	*	*	*	8

Regarding the quality assessment criteria for cohort studies using the Newcastle-Ottawa Scale (NOS), a score of "*" indicates achievement of the criterion, while a score of "-" indicates non-achievement.

ity on the basis of sample size (Fig. 2B), region (Fig. 2C), and study design (Fig. 2D). The sample size-based grouping resulted in two categories: 0–100 and >100. The HR was 0.83 (95% CI = 0.45–1.53, $p = 0.55$) and 1.09 (95% CI = 0.57–2.10, $p = 0.793$) in the 0–100 group and the >100 group, respectively. The grouping based on respective geographical origins, resulted in three categories: Asia, Europe, and the US. The HR was 1.27 (95% CI = 0.70–2.30, $p = 0.426$), 0.61 (95% CI = 0.31–1.21, $p = 0.156$), and 1 (95% CI = 0.60–1.67, $p = 1$) in the Asia group, the Europe group, and the US group, respectively. Finally, the study design-based group yielded two categories: prospective and retrospective. The HR was 0.93 (95% CI = 0.12–7.33, $p = 0.943$) and 0.97 (95% CI = 0.59–1.59, $p = 0.913$) in the prospective group and the retrospective group, respectively. None of the above analyses identified possible sources of heterogeneity.

Secondary Outcomes: DSS, DFS, RFS

Twelve studies reported DSS, DFS, and RFS, of which six provided DSS, five provided DFS, and four provided RFS. These studies explored the correlation between high and low FOXP3 expression and DSS, DFS, and RFS, respectively. The fixed-effects model analysis revealed a significant association between high FOXP3 and DSS (HR = 0.55, 95% CI = 0.44–0.67, $p < 0.001$) with acceptable heterogeneity ($I^2 = 29.3\%$, $p = 0.175$), as illustrated in Fig. 3A. High FOXP3 did not significantly correlate with either DFS or RFS. For DFS, the overall effect size was HR = 1.05 (95% CI = 0.39–2.84, $p = 0.917$), with considerable heterogeneity ($I^2 = 80.5\%$, $p < 0.001$), as illustrated in Fig. 3B. For RFS, the effect size was HR = 0.99 (95% CI = 0.55–1.79, $p = 0.969$), also with considerable heterogeneity ($I^2 = 76.9\%$, $p < 0.001$), as illustrated in Fig. 3C.

Meta-Regression Analysis

The relationship between FOXP3 expression levels and OS, DFS, and RFS exhibited significant heterogeneity. To investigate its sources, regression analysis was conducted, as shown in Table 4. In the meta-regression for OS, the following factors were evaluated: proportion of advanced-stage cases, age, sex ratio, sample size, and publication year. Results indicate that patient age is a significant moderator of the relationship between FOXP3 expression and OS (coefficient = –0.15, $p = 0.031$), suggesting that in elderly populations, high FOXP3 expression may not indicate poor prognosis and may even exhibit a protective trend. None of other variables (gender ratio, proportion of advanced cases, sample size, publication year) showed significant moderating effects (all $p > 0.05$). In the meta-regression for RFS, the model nearly fully explained inter-study heterogeneity. We identified advanced disease proportion, sample size, publication year, and patient age as sources of heterogeneity, while sex ratio was not a source of heterogeneity. In contrast, no sources of heterogeneity were identified in the meta-analysis for DFS.

Sensitivity Analysis and Publication Bias

Sensitivity Analysis

A sensitivity analysis was performed by sequentially excluding individual studies. The HRs and 95% CIs for OS (Fig. 4A), DSS (Fig. 4B), DFS (Fig. 4C), and RFS (Fig. 4D) did not change significantly, indicating stable results.

Publication Bias

The Egger test and funnel plots were employed to assess the potential publication bias of OS. The funnel plot indicated no publication bias in OS (Fig. 5A). However, the Egger test for OS yielded a p-value of 0.017, indicating the presence of publication bias. To determine whether the bias affected the results, a trim and fill method was adopted. The method demonstrated that the significance of the effect value remained unchanged, and the result was robust, with a minimal impact on the meta-analysis results. Funnel plots and an Egger test were used to assess publication bias in DSS, DFS, and RFS. The funnel plots demonstrated no publication bias in the DSS, DFS, and RFS (Fig. 5B–D). The p values of the Egger test for DSS, DFS, and RFS were 0.161, 0.44, and 0.340, respectively.

Discussion

The current study systematically assessed the prognostic significance of FOXP3 in OSCC by including 19 studies involving 2136 patients with malignancies. The results showed that high FOXP3 expression had no significant correlation with OS, but was significantly associated with DSS, with higher FOXP3 expression corresponding to longer DSS. These findings suggest that FOXP3 expression levels are associated with OSCC prognosis and may serve as a potential biomarker.

Sun *et al.* [24], in their study, suggested that higher levels of FOXP3 TILs in HER2-positive and triple-negative breast cancers were linked to better pathological complete response (pCR) and OS. Similarly, Idos *et al.* [25] posited that in colorectal cancer, higher overall infiltration of FOXP3 tumor-infiltrating inflammatory cells was linked to improved OS and DFS. However, these claims were contradicted by another study [26], which showed that a higher density of FOXP3 T cells in gastric cancer was not associated with poorer outcomes (HR = 1.382, 95% CI = 0.944–2.025). In pancreatic cancer, higher FOXP3 expression has been linked to poorer OS (HR = 1.48, 95% CI = 1.20–1.83) [27]. Similarly, gallbladder cancer patients with higher FoxP3+ Tregs have been shown to have diminished OS (HR = 1.55, 95% CI = 1.11–2.00; $p < 0.001$) [28]. Furthermore, a subgroup analysis demonstrated an association between higher FOXP3 expression and unfavorable outcomes in gallbladder cancer, although this association was not statistically significant in bile duct cancer. Another study [29] suggested that patients with non-small-cell lung cancer who presented with TIL infiltration had significantly

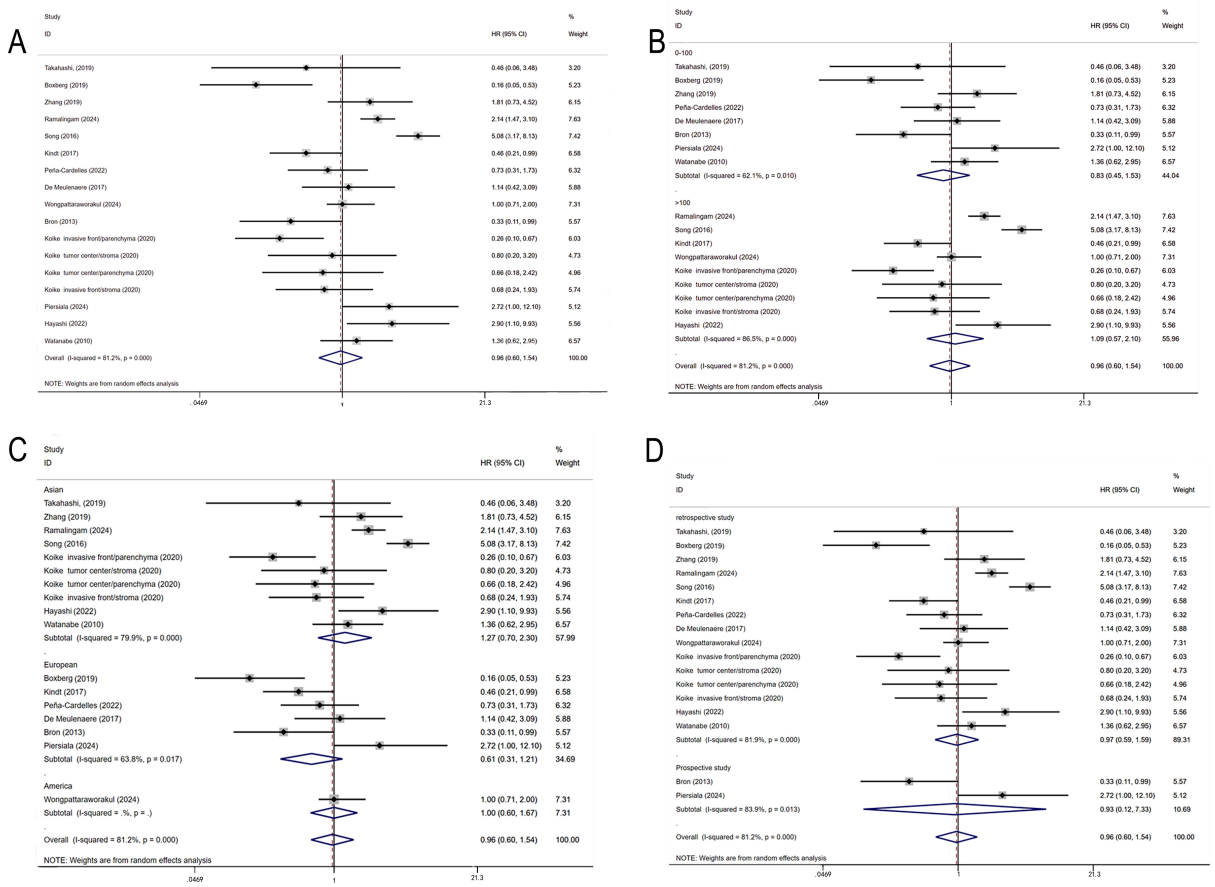

Fig. 2. The prognostic effect of FOXP3 on OS (A); the prognostic effect of FOXP3 on OS according to sample size (B); the prognostic effect of FOXP3 on OS according to region (C); the prognostic effect of FOXP3 on OS according to study design (D). HR, hazard ratio; CI, confidence interval; FOXP3, forkhead box protein P3; OS, overall survival.

better OS (HR = 0.67, 95% CI = 0.55–0.81). Nevertheless, FOXP3+ was linked to poorer OS (HR = 1.35, 95% CI = 0.87–2.11). These findings indicate that FOXP3 exerts a multifaceted influence on tumors. Its paradoxical effects are potentially associated with its immunosuppressive function, anti-inflammatory protective effects, and cellular subpopulation heterogeneity [3].

Specifically, the mechanism of action of FOXP3, a pivotal transcription factor that regulates Tregs development and function, is inextricably linked to Tregs biology. Tregs possess both immunosuppressive and anti-inflammatory protective functions, which may favorably affect the organism. The collective effect of these processes depends on the primary function of either process in tumor development [28,30,31]. FOXP3 is essential for Tregs in terms of immunosuppressive function. It does so by directly inhibiting the expression of the *IL-2* gene and enhancing *CTLA4* gene transcription [28]. An association between increased levels of CD8 T cells and increased levels of FOXP3 Tregs has also been suggested [32]. This indicates that the favorable prognostic impact of FOXP3 Tregs might result from immunosuppressive negative feedback. In another study [33], they posited that due to the different roles of the Treg subpopulations, there might be conflicting impacts on tumor invasion and on the response of oral tumors to treatment. For OSCC patients, both the potential anti-inflammatory effects of Tregs and the role of other anti-infective medications (e.g., antifungal and antibacterial) in the immune response should be taken into account [34].

This study, despite showing substantial heterogeneity (I^2 = 81.2%, $p < 0.001$), found no significant association between higher FOXP3 expression and overall survival (OS). Although subgroup analyses by country, study design, and sample size failed to clearly identify all the potential sources of heterogeneity, further regression analysis provided clues to explain this variability: The mean age of patients may have served as a significant moderating factor (coef = –0.15, $p = 0.031$), suggesting that among older populations, those with higher FOXP3 expression may demonstrate improved overall survival. This potentially reflects the complexity of Treg function interacting with host immune status within the context of immune senescence [35]. However, the mean patient age explained only 26% of the heterogeneity. Other clinical variables, such as gender ra-

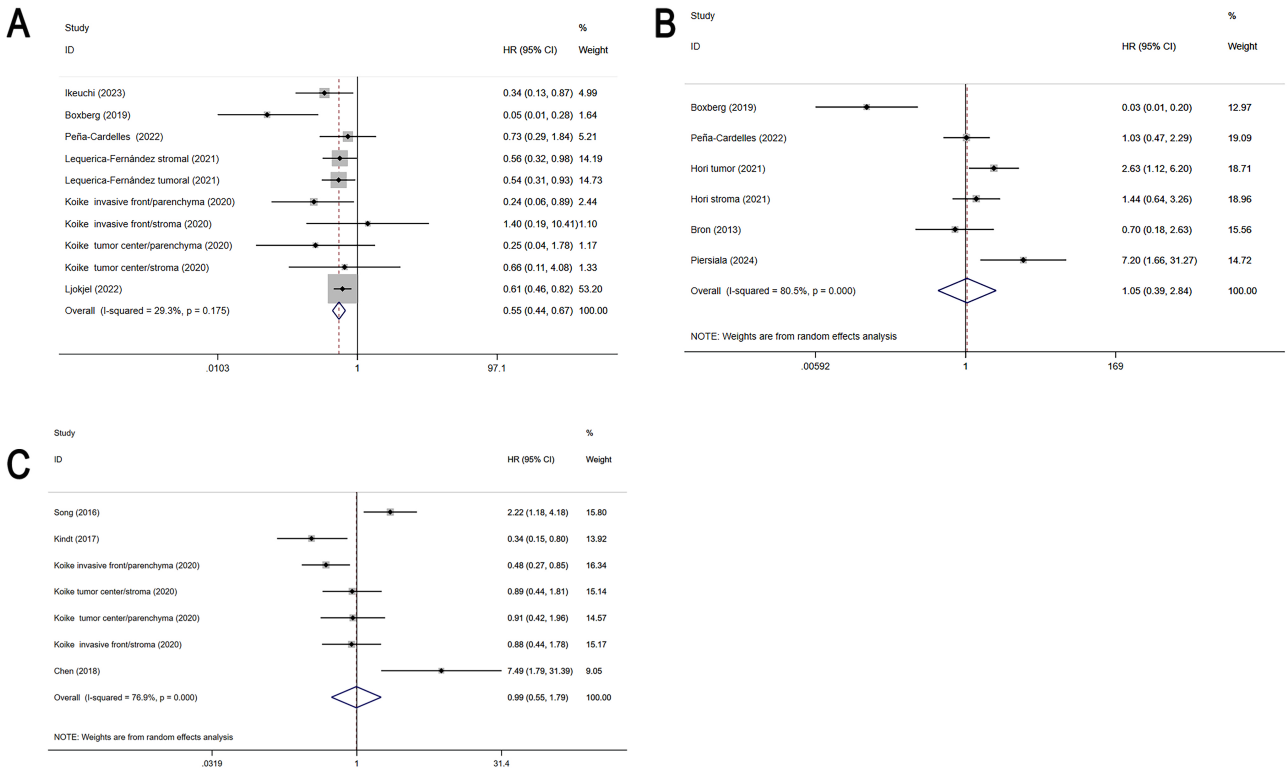

Fig. 3. The prognostic effect of FOXP3 on DSS (A); the prognostic effect of FOXP3 on DFS (B); the prognostic effect of FOXP3 on RFS (C). HR, hazard ratio; CI, confidence interval; DSS, disease-specific survival; DFS, disease-free survival; RFS, recurrence-free survival.

Table 4. Meta-regression analysis.

	Meta-regression coefficient	*p*	95% CI
OS	Adj R^2 = 26.17%, I^2_res = 76.32%		
Total	0.005	0.154	–0.002 to 0.011
Year	–0.015	0.817	–0.154 to 0.124
Age	–0.152	0.031	–0.288 to –0.0162
Percentage of males	–2.093	0.263	–5.955 to 1.769
Percentage of advanced	0.658	0.634	–2.238 to 3.555
RFS	Adj R^2 = 96.86%, I^2_res = 0%		
Total	0.190	0.033	0.003 to 0.035
Year	0.904	0.049	0.009 to 1.798
Age	–0.272	0.033	–0.503 to –0.041
Percentage of males	4.475	0.252	–4.406 to 13.357
Percentage of advanced	5.832	0.043	0.312 to 11.353
DFS	Adj R^2 = 83.16%, I^2_res = 57.47%		
Total	–0.117	0.126	–0.316 to 0.081
Year	0.376	0.133	–0.283 to 1.036
Age	–0.523	0.481	–3.143 to 2.096
Percentage of males	–7.501	0.505	–47.526 to 32.524
Percentage of advanced	–2.061	0.283	–8.166 to 4.042

The age represents the median age; the percentage of advanced patients refers to the ratio of the number of patients in stage iii–iv or T3–4 to the total number of patients.
Abbreviations: OS, overall survival; RFS, recurrence-free survival; DFS, disease-free survival.

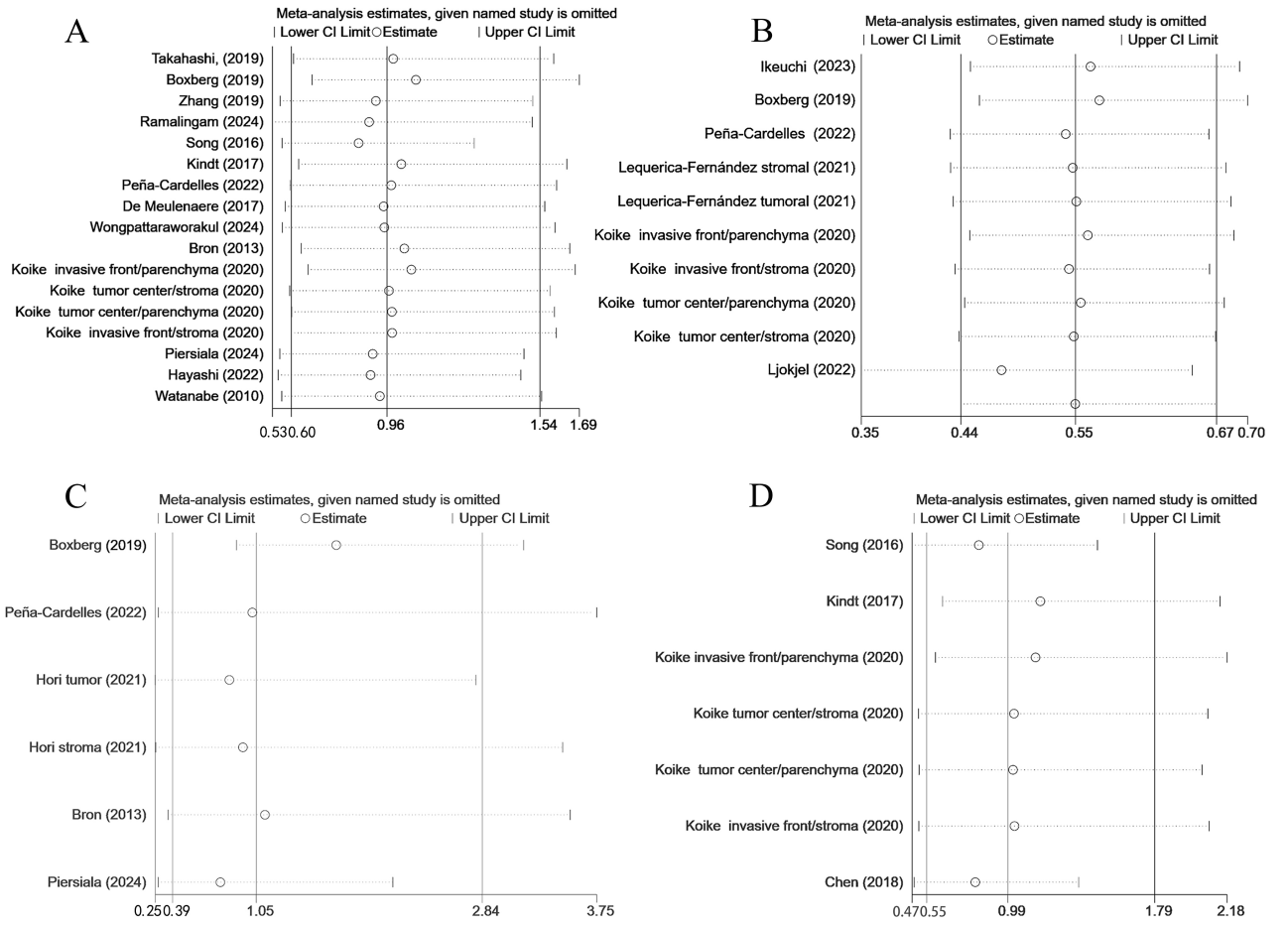

Fig. 4. Sensitivity analyses on OS (A), DSS (B), DFS (C), and RFS (D). OS, overall survival; DSS, disease-specific survival; DFS, disease-free survival; RFS, recurrence-free survival.

tio and proportion of advanced-stage cases, did not demonstrate significant explanatory power.

Moreover, recurrence-free survival (RFS) and disease-free survival (DFS) similarly exhibited high heterogeneity. The meta-regression model for RFS accounted for nearly all of the observed between-study heterogeneity (Adj R^2 = 96.86%). Analysis revealed that patient mean age, study sample size, publication year, and proportion of advanced-stage cases were potential sources of RFS heterogeneity. In contrast, no clear source of DFS heterogeneity was identified through regression analysis, suggesting the possible presence of unmeasured confounding factors.

Concurrently, this study indicates that higher FOXP3 expression may correlate with longer disease-specific survival (DSS). The lack of association between FOXP3 and overall survival (OS) while showing a link to DSS underscores the critical importance of endpoint selection in clinical research. As an endpoint event, DSS excludes non-tumor-related competing mortality risks (such as cardiovascular events or accidents), thereby providing a purer reflection of tumor aggressiveness and treatment response. Chronic inflammation serves as a key driver in the development and progression of OSCC. The potent anti-inflammatory function of FOXP3+ Tregs may directly reduce cancer-related mortality by suppressing excessive, pro-tumorigenic tumor-associated inflammation. These findings demonstrate an association between FOXP3 expression and OSCC prognosis, positioning FOXP3 as a potential prognostic biomarker.

This study, however, is subject to several limitations. The lack of significant correlation between higher FOXP3 expression and overall survival (OS), disease-free survival (DFS), or recurrence-free survival (RFS) may have stemmed from several factors: First, the populations in the included studies were of different races, and the significant differences in genetic backgrounds may have affected the Treg functional phenotype. Second, in terms of microenvironmental dynamics, FOXP3+ cells are predominantly anti-inflammatory and protective in early-stage tumors, whereas they shift to immunosuppressive dominance in advanced-stage tumors. Third, the OS is vulnerable to non-tumor mortality factors (e.g., cardiovascular

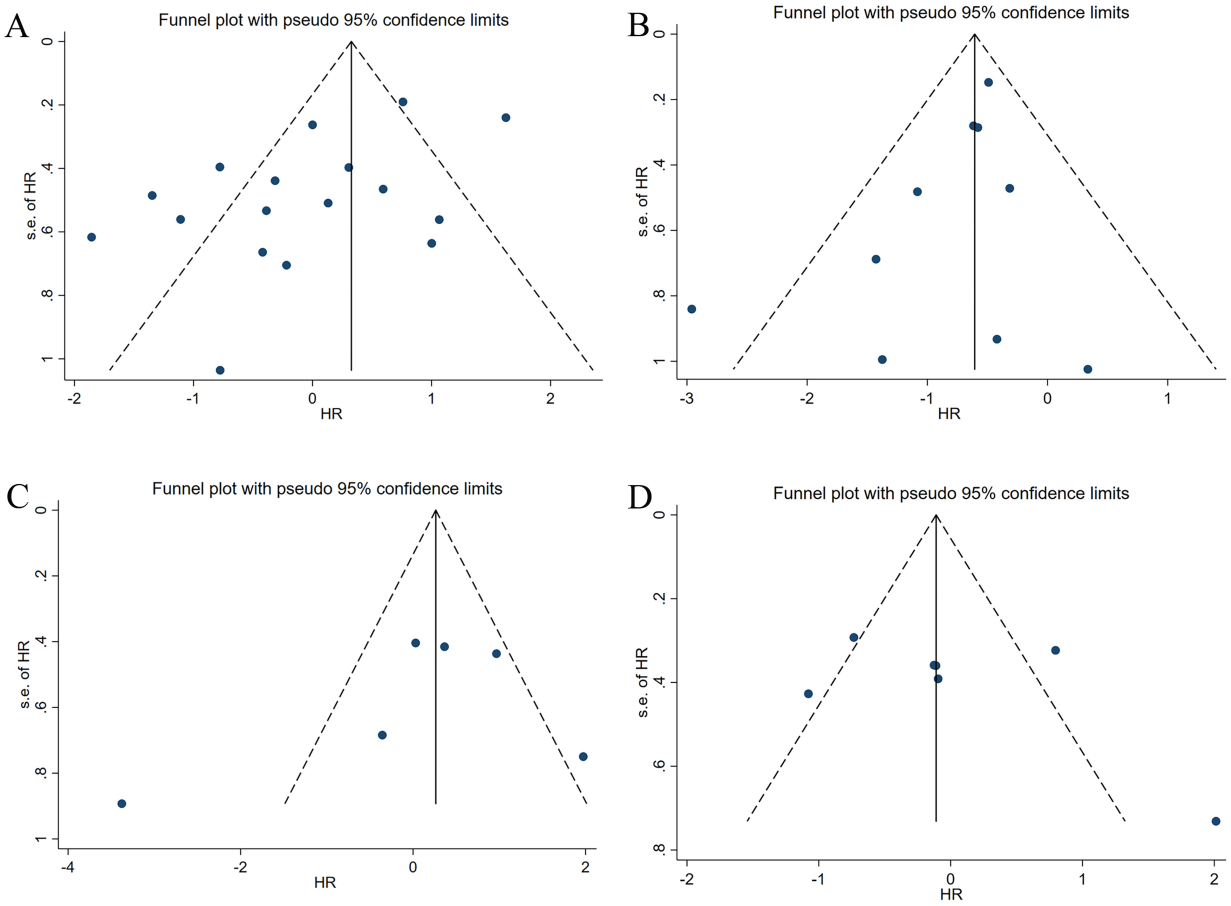

Fig. 5. Funnel plots for OS (A), DSS (B), DFS (C), and RFS (D). OS, overall survival; DSS, disease-specific survival; DFS, disease-free survival; RFS, recurrence-free survival.

events), while the DSS more accurately reflects tumor biological behavior [36]. Fourth, the thresholds for the positivity of FOXP3 varied significantly among studies due to the absence of a standardized test. The site (tumor center vs. invasive front) directly influenced the interpretation of results [19]. Notably, FOXP3 is not exclusively expressed in Tregs but may also be present in certain tumor cells. The inconsistent detection methods employed across included studies—some using dual-stained immunohistochemistry (e.g., CD4+FOXP3+) to localize Tregs, others relying solely on FOXP3 monostaining—may lead to misclassification of cell types (incorporating tumor cell expression), thereby confounding the results. This represents a key technical reason for inter-study heterogeneity and conflicting conclusions. Therefore, future research urgently requires the establishment of detailed, consistent evaluation methods. Fifth, there were additional treatment-related confounding effects, with 31% of studies not specifying whether they controlled for the effect of preoperative chemotherapy and radiotherapy on Treg. Notably, highly toxic radiotherapeutic or chemotherapeutic agents may lead to the induction of Tregs recruitment or activity [37,38]. One of the important growth factors that infiltrate Tregs in OSCC release is TGF-β [39]. The exposure of Tregs to certain anticancer drugs or ionizing radiation might lead to the release of more TGF-β from Tregs [40]. Similarly, the use of combination therapy or some specific inhibitors in cancer treatment might help to deplete Tregs and reduce the molecules secreted by them [41]. These findings suggest that preoperative chemoradiotherapy is a significant influencing factor that needs to be controlled. Moreover, the current study's sample size was limited, with only six studies included in the DSS subgroup (n = 630), and 21.4% of studies in the OS subgroup included fewer than 50 subjects. Additionally, 89.4% of the studies adopted a retrospective design, which could easily lead to biased results.

To further promote the clinical application of FOXP3, a multicenter prospective cohort study is warranted, with a target sample size of $\geq$3000 cases, and a standardized process for FOXP3 detection should be established. Also, it is essential to conduct additional high-quality studies on the mechanism of FOXP3 in OSCC. Spatial transcriptome technology can be utilized to analyze the spatial distribution characteristics of the subpopulation of FOXP3+ cells, so as to explore its specific mechanism of action. In the clinical context, the regulatory factors identified through

regression analysis provide key variables for constructing more precise prognostic models in the future. Future models should focus on integrating FOXP3 expression levels with these clinical characteristics rather than using them as isolated indicators. Additionally, they can incorporate existing prognostic models for immune-related genes in head and neck squamous cell carcinoma [42], to further substantiate the evidence supporting clinical treatment.

Conclusions

This study represents the first systematic evaluation of the prognostic value of FOXP3 in oral squamous cell carcinoma through meta-analysis. Results indicate that higher FOXP3 expression is significantly associated with longer disease-specific survival, supporting its potential as a prognostic biomarker for OSCC. However, its association with overall survival, disease-free survival, and recurrence-free survival did not reach statistical significance. This suggests that its prognostic value may be influenced by factors such as age, introducing uncertainty in clinical interpretation. In summary, this study highlights the relevance of FOXP3 in the prognostic assessment of OSCC. Future large-scale, multicenter prospective studies, combined with standardized detection protocols and mechanistic investigations, are needed to further validate the prognostic role of FOXP3 and its potential significance in precision immunotherapy.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

QYL and XPH designed this study; QYL, KL, XPS, YYS, and XPH jointly carried out the data acquisition, data analysis, and result interpretation. QYL participated in the drafting of the manuscript. All authors have been involved in revising it critically for important intellectual content. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202638204.23.

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