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Review Article

Prognostic significance of tumor budding in biliary tract cancer: a systematic review and meta-analysis

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Abstract: Objective: This study was conducted to assess systematically the prognostic value of tumor budding in biliary tract cancer. Methods: A comprehensive literature search was performed in the PubMed, Embase, and Web of Science databases for studies published between March 2021 and March 2025. Two independent reviewers were responsible for study selection, data extraction, and quality assessment. Meta-analysis was performed using RevMan 5.4 software. Results: Tumor budding was significantly associated with worse overall survival by both univariate analysis (HR = 2.55; 95% CI: 1.99-3.26; P < 0.001) and multivariate analysis (HR = 1.75; 95% CI: 1.46-2.09; P < 0.001). Similarly, tumor budding was also linked to reduced disease-free survival, as indicated by both univariate (HR = 1.98; 95% CI: 1.60-2.44; P < 0.001) and multivariate analyses (HR = 1.70; 95% CI: 1.38-2.08; P < 0.001). Moreover, tumor budding was found to be correlated with adverse clinicopathologic features, including poor histologic differentiation, lymph node metastasis, lymphovascular invasion, vascular invasion, and perineural invasion. Conclusion: The findings suggest that tumor budding serves as a robust prognostic indicator for poor survival outcome in patients with cholangiocarcinoma. Its potential as a clinically applicable prognostic biomarker should be further evaluated through the establishment of an internationally recognized consensus based on current evidence.

Keywords: Tumor budding, biliary tract cancer, prognosis, survival outcomes

Introduction

Biliary tract cancer (BTC) is a rare malignant tumor that originates in various parts of the biliary tract system [1, 2]. BTC encompasses intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder cancer/gallbladder duct cancer, and ampulla of Vater cancer. BTC accounts for approximately 3% of all gastrointestinal tumors, with gallbladder cancer (GBC) being the most common, representing 80%-95% of BTC cases, and ranking sixth in global gastrointestinal tumor incidence [3]. Currently, the incidence of BTC is increasing globally, with the highest rates reported from Asian countries [4]. The majority of BTC cases are classified as adenocarcinomas (approximately 95%), characterized by their high invasiveness and tendency to be detected at an advanced stage. At present, the diagnosis of gallbladder cancer primarily relies on imaging-based detection followed by histopathologic confirmation. Abdominal ultrasound serves as the first-line screening tool for identifying suspicious lesions, whereas contrast-enhanced CT and/or MRI are routinely applied for lesion characterization and staging. Definitive diagnosis depends on pathological examination of surgical or biopsy specimens. The prognosis for this condition is extremely poor, with a 5-year survival rate of less than 5% [5]. Conventional prognostic indicators for gallbladder cancer include locally advanced disease, lymph node or hematogenous metastasis, ascites, and poor pathologic differentiation. Despite advancements in diagnostic and treatment modalities, the prognosis for gallbladder cancer remains poor, largely due to the lack of effective early diagnostic biomarkers and prognostic stratification indicators. Although patients are classified based on their clinical stage, significant variations in prognosis persist. This finding highlights the need for the development of novel prognostic indicators that can aid in

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patient stratification and inform treatment decisions.

Tumor budding was first proposed in the 1950s and is considered a key prognostic biomarker for various solid tumors [6-8]. It refers to the presence of single cells or tumor cell clusters consisting of fewer than five cells within the tumor or at the invasive front [9, 10]. Histopathologically, tumor budding is determined on routine hematoxylin-eosin-stained sections by identifying either isolated single tumor cells or small clusters (< 5 cells) located within the main tumor body or at the invasive front. In colorectal cancer, the International Tumor Budding Consensus Conference (ITBCC) has established it as an independent predictor of lymph node metastasis and poor prognosis [11, 12]. The prognostic impact of tumor budding has also been reported in several other malignant tumors, including gastric cancer, esophageal cancer, pancreatic cancer, lung cancer, breast cancer, and extrahepatic cholangiocarcinoma. Increasing evidence suggests that the prognostic value of tumor budding may be extended to other tumor types [13-18]. Across these tumor types, high-grade tumor budding is consistently associated with more aggressive behavior, higher metastatic risk, and worse survival outcome. Some studies have explored the role of tumor budding in cholangiocarcinoma and confirmed that tumor budding is a prognostic factor for hilar and extrahepatic cholangiocarcinoma [19, 20]. The American Joint Committee on Cancer (AJCC) staging manual is the standard for determining BTC treatment plans and prognosis [21]. However, the AJCC staging manual does not include an algorithm for the prognostic significance of tumor budding in BTC.

For the heterogeneous group of BTCs, which include gallbladder cancer (GBC), intrahepatic cholangiocarcinoma (ICC), and extrahepatic cholangiocarcinoma (ECC), previous meta-analyses have suggested that tumor budding may be a prognostic factor, but its clinical application has been hindered by inconsistent scoring systems and limited data. Notably, prior studies and meta-analyses indicated that increased tumor budding burden correlates with poorer overall and disease-free survival in BTC, underscoring its potential value for prognostic stratification. Since the previous meta-analysis, eight new studies have examined the relationship between tumor budding and the prognosis of BTC. The objective of this meta-analysis was to synthesize new evidence to validate the prognostic significance of tumor budding in BTC and assess its association with key clinical and pathologic factors. By doing so, it aims to provide a more comprehensive basis for clinical decision-making.

Materials and methods

Eligibility criteria

This meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and the Meta-Analyses of Observational Studies in Epidemiology (MOOSE) checklist (Supplementary Tables 1 and 2). The research question was defined a priori according to the population, intervention/exposure, comparison and outcome (PICO) framework: the population comprised patients with pathologically confirmed BTC. The exposure of interest was high or positive tumor budding on histopathologic evaluation. The comparison group consisted of patients with low or negative tumor budding; and the prespecified outcomes were overall survival, disease-free/recurrence-free survival and clinicopathologic characteristics. The review protocol was not prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO). Inclusion criteria were: (1) English-language studies; (2) Patients with pathologically confirmed BTC (gallbladder cancer, hilar cholangiocarcinoma, intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, distal cholangiocarcinoma); (3) Studies reporting tumor budding status (low/high or negative/positive) and its association with survival outcomes (overall survival, disease-free survival) or clinicopathologic factors; (4) Studies providing data for both univariate and multivariate analyses. Exclusion criteria include animal studies, reviews, case reports, and studies lacking relevant data.

Search strategy

Search terms: ((budding) OR (sprouting) OR (tumor budding)) AND (((cholangiocarcinoma) OR (bile duct cancer)) OR (gallbladder cancer)) were used to conduct a systematic search in the PubMed, Embase, and Web of Science

databases for relevant studies published between March 2021 and March 2025. The articles were then subjected to a subsequent screening process, which entailed a stepwise evaluation of their abstract and full-text content in accordance with predetermined inclusion and exclusion criteria (Supplementary Table 3).

Study selection and data extraction

Two reviewers independently evaluated the data based on authors, year, country, sample size, tumor subtype, tumor budding criteria, survival outcomes, and clinicopathological associations. Inconsistencies were resolved on the assessment by the corresponding author.

Outcome of interest

Data were extracted for the survival outcomes for 1) Overall survival: pooled hazard ratios from both univariate and multivariate analyses; 2) Disease-free survival/recurrence-free survival: pooled hazard ratios from both univariate and multivariate analyses; and 3) Clinicopathologic associations: significant clinical and pathologic factors related to tumor budding status.

Quality assessment

Quality assessment was performed using the Newcastle-Ottawa Scale (NOS), with a score of ≥ 6 indicating a low risk of bias [22]. Scoring criteria were based on the selection of study groups, the comparability of groups, and the ascertainment of either exposure or outcome.

Statistical analysis

Statistical analysis and meta-analysis were performed using the Cochrane Collaboration's RevMan 5.4. The pooled hazard ratio (HR) and 95% confidence interval (CI) were calculated to assess the association between tumor budding and survival outcomes. A P value < 0.05 was considered significant between the two groups. The I^2 statistic was employed to evaluate heterogeneity. The heterogeneity of the data was categorized as low, moderate, or high based on the I-squared value. In instances of low heterogeneity, a fixed-effects model was implemented, while a random-effects model was employed in cases of high heterogeneity. The presence of publication bias was assessed by using funnel plots. Sensitivity analyses were conducted by sequentially excluding results with significantly high heterogeneity.

Results

Study selection and characteristics

A comprehensive literature search was conducted based on inclusion and exclusion criteria, yielding 101 studies in the initial screening. After successive screening, eight studies were selected, involving a total of 1,518 patients (**Figure 1**). Baseline characteristics of all the included studies are reported in **Table 1**. All patients in the included studies underwent radical surgical resection. Tumor budding was identified in postoperative histopathological examination of the resected specimens. All included studies were retrospective studies. Two of the studies were from Japan, two from South Korea, two from China, and the remaining two from the United States and Germany, respectively. Tumor subtypes included intrahepatic cholangiocarcinoma (n = 4), extrahepatic cholangiocarcinoma (n = 1), hilar cholangiocarcinoma (n = 1), distal cholangiocarcinoma (n = 3), and mixed-type biliary tract cancer (n = 1). The studies by Diana et al. and Yoshida et al. included two comparisons, reported in our study as the ICC study and ECC study by Diana et al., and the PHCC study and DCC study by Yoshida et al., respectively. Ultimately, we identified eight studies containing ten comparisons in this study. The criteria for budding were primarily classified as "low" (< 5 lesions) and "high" (≥ 5 lesions), with some studies using a three-tiered classification (low/moderate/high). The NOS scores for the included studies ranged from 6 to 8, and none of the manuscripts were considered to have a high risk of bias (Supplementary Table 4). The summary of the results of each included study is reported in **Table 2**.

Survival outcomes

The hazard ratios (HR) and confidence intervals (CI) for overall survival from univariate analyses were pooled from six studies involving seven comparisons. The meta-analysis results showed that patients with tumor budding-positive or high tumor budding had significantly poorer overall survival compared to those with

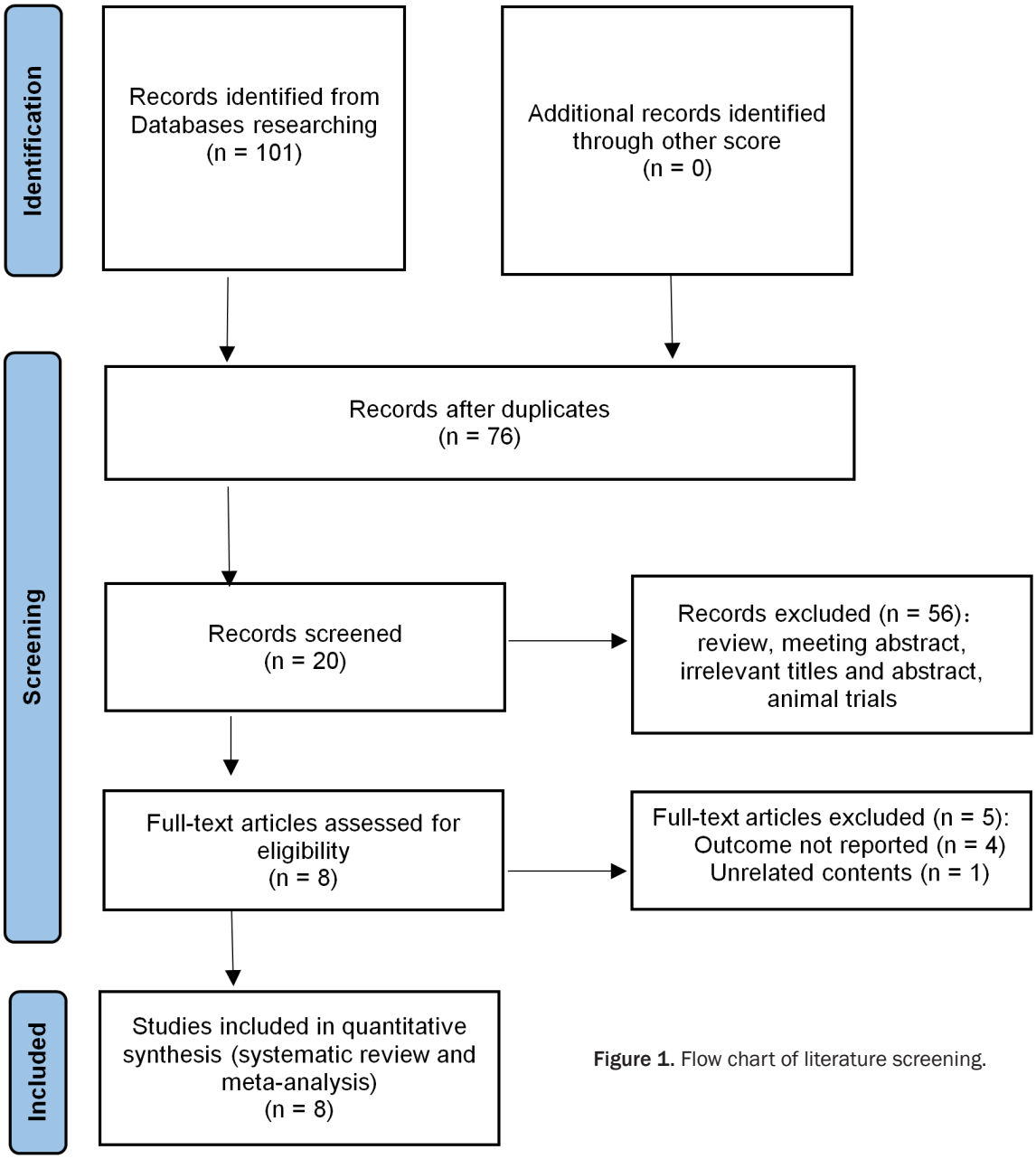

Figure 1. Flow chart of literature screening.

low tumor budding or no tumor budding (HR: 2.55; 95% CI: 1.99-3.26; P < 0.00001) (fixed-effect model; I^2 = 29%; P = 0.20) (**Figure 2A**). Similarly, the hazard ratio (HR) and confidence interval (CI) for overall survival from multivariate analysis were pooled from eight studies containing ten comparisons. The meta-analysis results showed that tumor budding-positive or high tumor budding patients were a significant prognostic factor (HR: 1.75; 95% CI: 1.46-2.09; P < 0.00001) (fixed-effect model; I^2 = 42%; P = 0.08) (**Figure 2B**). Through sensitivity analysis of OS results in the multivariate analysis by sequentially excluding one study at a time, we identified one study that may have contributed to heterogeneity.

Additionally, the hazard ratios (HR) and confidence intervals (CI) for disease-free survival (DFS) in the univariate and multivariate analyses were derived from 5 studies with 6 comparisons and 6 studies with 7 comparisons. The meta-analysis results for DFS in the univariate analysis showed that patients with tu-

Table 1. Baseline characteristics of included studies

Author	Year	Country	Tumor type	TB grade	Number of patients
Agostini-Vulaj et al. [23]	2021	USA	ICC, ECC	low/intermediate/high	ICC: 44/10/4 ECC: 31/13/10
Budau et al. [24]	2022	Germany	ICC	low/intermediate/high	31/35/23
Nakayama et al. [25]	2022	Japan	DBDC	low/high	12/53
Yoshida et al. [26]	2023	Japan	PHCC, DCC	low/high	PHCC: 39/135 DCC: 28/81
Kim et al. [27]	2023	Korea	CCA	low/high	198/21
Jun et al. [28]	2024	Korea	DBDC	low/high	172/238
Liu et al. [29]	2024	China	ICC	low/high	62/139
Wu et al. [30]	2024	China	ICC	negative/positive (low grade was determined negative)	70/69

AAC: ampullary adenocarcinoma; TB: tumor budding; BTC: biliary tract cancer.

mor budding positive or high tumor budding had significantly worse DFS than those with low or no tumor budding (HR: 1.98; 95% CI: 1.60-2.44; P < 0.00001) (fixed-effect model; I^2 = 10%; P = 0.35) (**Figure 2C**). The results of the meta-analysis of RFS in the multivariate analysis showed that patients with tumor budding positive or high tumor budding had significantly worse disease-free survival than those with low tumor budding or no tumor budding (HR: 1.70; 95% CI: 1.38-2.08; P < 0.001) (fixed-effect model; I^2 = 0%; P = 0.46) (**Figure 2D**). The assessment of publication bias from the funnel plot showed a symmetrical distribution (Supplementary Figure 1).

Association between tumor budding and clinicopathology

We identified well-known clinicopathologic predictors of poor survival associated with tumor budding in BTC in each included study. Significantly higher clinicopathologic factors in the tumor budding group are shown in **Table 2**. A meta-analysis was performed on these factors reported in at least four included studies. Low histologic differentiation was significantly higher in patients with positive tumor budding or high tumor budding (OR: 3.22; 95% CI: 2.08-4.97; P < 0.00001) (fixed-effect model; I^2 = 12%; P = 0.34) (**Figure 3A**). There was no significant difference in advanced T stage (T3 stage) between tumor budding-positive or high tumor budding patients (OR: 1.35; 95% CI: 0.95-1.90; P = 0.09) (fixed-effect model; I^2 = 55%; P = 0.05) (**Figure 3B**). In 6 comparisons across 5 studies, patients with tumor budding had a significantly higher incidence of lymph node metastasis (OR: 1.57; 95% CI: 1.16-2.13; P = 0.003) (fixed-effect model; I^2 = 49%; P = 0.08) (**Figure 3C**). After excluding one study that may have contributed to heterogeneity in lymph node metastasis outcomes, we found that tumor budding was significantly associated with an increased risk of lymph node metastasis (OR: 1.44; 95% CI: 1.06-1.97; P = 0.02) (fixed-effect model; I^2 = 23%; P = 0.26) (**Figure 3E**). There was no statistically significant association between tumor budding and positive surgical margins (OR: 1.25; 95% CI: 0.81-1.91; P = 0.31) (fixed-effect model; I^2 = 0%; P = 0.45) (**Figure 3D**).

Tumor budding was also associated with a significantly higher risk of lymphatic invasion (OR: 2.54; 95% CI: 1.57-4.11; P = 0.0001) (fixed-effect model; I^2 = 0%; P = 0.54) (**Figure 4A**), vascular invasion risk (OR: 1.57; 95% CI: 1.15-2.13; P = 0.004) (fixed-effect model; I^2 = 43%; P = 0.14) (**Figure 4B**), and was associated with the risk of perineural invasion (OR = 3.04; 95% CI: 2.09-4.42; P < 0.00001) (fixed-effect model; I^2 = 0%; P = 0.67) (**Figure 4C**).

A study demonstrated a significant association between tumor budding and an elevated risk of distant metastasis [23]. In univariate analysis, other clinicopathologic factors that demonstrated a significant association with tumor budding included growth pattern [28], histologic pattern [28], intrahepatic metastasis [23], pancreatic infiltration [28], and ZEB1 positivity

Table 2. Summary of the outcomes in included studies

Author	Tumor budding criteria	Significant clinicopathologic factors associated with tumor budding	OS HR [95% CI]	DFS or RFS HR [95% CI]	Conclusion
Agostini-Vulaj et al. [23]	low: 0-4 intermediate: 5-9 high: ≥ 10	ICC: sex, poor histologic differentiation ECC: poor histologic differentiation, lympho-vascular invasion	Univariate: ICC: 1.93 [0.90-4.14] ECC: 2.67 [1.16-6.16] Multivariate: ICC: 2.12 [0.91-4.93] ECC: 2.34 [0.79-6.92]	Univariate: ICC: 1.66 [0.76-3.63] ECC: 2.84 [1.08-7.47] Multivariate: ICC: N/A ECC: 1.83 [0.64-5.21]	ICC: High TB was not associated with significantly OS or RFS in either univariate or multivariate analysis ECC: High TB was associated with significantly OS and DFS in univariate analysis but was non-significant in multivariate analysis
Budau et al. [24]	low: 0-4 intermediate: 5-9 high: ≥ 10	N/A	Univariate: Intermediate or high: 3.49 [2.19-5.54] Multivariate: Intermediate or high: 3.54 [2.15-5.83]	Univariate: Intermediate or high: 1.92 [1.356-2.73] Multivariate: Intermediate or high: 1.94 [1.34-2.81]	High TB was associated with significantly poor OS in both univariate and multivariate analysis for ICC
Nakayama et al. [25]	low: < 5 high: ≥ 5	poor histologic differentiation, the invasion depth, lymph node metastasis, venous invasion, perineural invasion, disease stage, ZEB1 expression	Univariate: 4.48 [1.58, 12.7] Multivariate: 3.91 [1.15, 13.3]	N/A	High TB was associated with significantly poor OS in both univariate and multivariate analysis for patients with DCBC
Yoshida et al. [26]	low: < 5 high: ≥ 5	N/A	Univariate: PHCC: Significant *p*-value but HR [95% CI] data not reported DCC: Significant *p*-value but HR [95% CI] data not reported Multivariate: PHCC: 1.46 [0.89-2.38] DCC: 1.21 [0.58-2.50]	Univariate: PHCC: Significant *p*-value but HR [95% CI] data not reported DCC: Significant *p*-value but HR [95% CI] data not reported Multivariate: PHCC: 1.10 [0.65-1.87] DCC: 1.52 [0.68-3.38]	PHCC: High TB was associated with significantly OS and DFS in univariate analysis but was non-significant in multivariate analysis DCC: High TB was associated with significantly OS and DFS in univariate analysis but was non-significant in multivariate analysis
Kim et al. [27]	low: < 9 high: ≥ 10	poor histologic differentiation, lymphatic invasion, and higher N category	Univariate: 3.134 [1.291, 7.608] Multivariate: 1.428 [0.494, 4.128]	Univariate: 5.755 [2.133, 15.529] Multivariate: 3.969 [1.375, 11.455]	High TB was associated with significantly poor DFS in both univariate and multivariate analysis, but was associated with significantly poor OS in univariate analysis
Jun et al. [28]	low: < 5 high: ≥ 5	growth pattern, Histologic subtype, poor histologic differentiation, Lymphovascular invasion, Perineural invasion, Pancreatic invasion, Duode l invasion, T category, Stage grouping	Univariate: Significant *p*-value but HR [95% CI] data not reported Multivariate: 1.406 [1.051, 1.882]	N/A	High TB was associated with significantly OS in both univariate and multivariate analysis
Liu et al. [29]	low: < 5 high: ≥ 5	N/A	Univariate: 1.586 [1.010, 2.492] Multivariate: 1.549 [0.977, 2.455]	Univariate: 1.849 [1.214, 2.816] Multivariate: 1.636 [1.060, 2.525]	High TB was associated with significantly poor RFS in univariate and multivariate analysis, but high TB was associated with significantly poor OS in univariate analysis but was nonsignificant in multivariate analysis for patients with ICC
Wu et al. [30]	negative: < 5 positive: ≥ 5	perineural invasion	Univariate: 3.491 [1.611, 7.567] Multivariate: 3.005 [1.333, 6.775]	Univariate: 1.772 [1.126, 2.789] Multivariate: 1.748 [1.085, 2.816]	Positive TB associated with significantly poor OS and RFS in both univariate and multivariate analysis for patients with ICC

Int: intermediate; BTC: biliary tract cancer; AAC: ampullary adenocarcinoma; LN: lymph node; DFS: disease free survival; RFS: recurrence free survival; OS: overall survival.

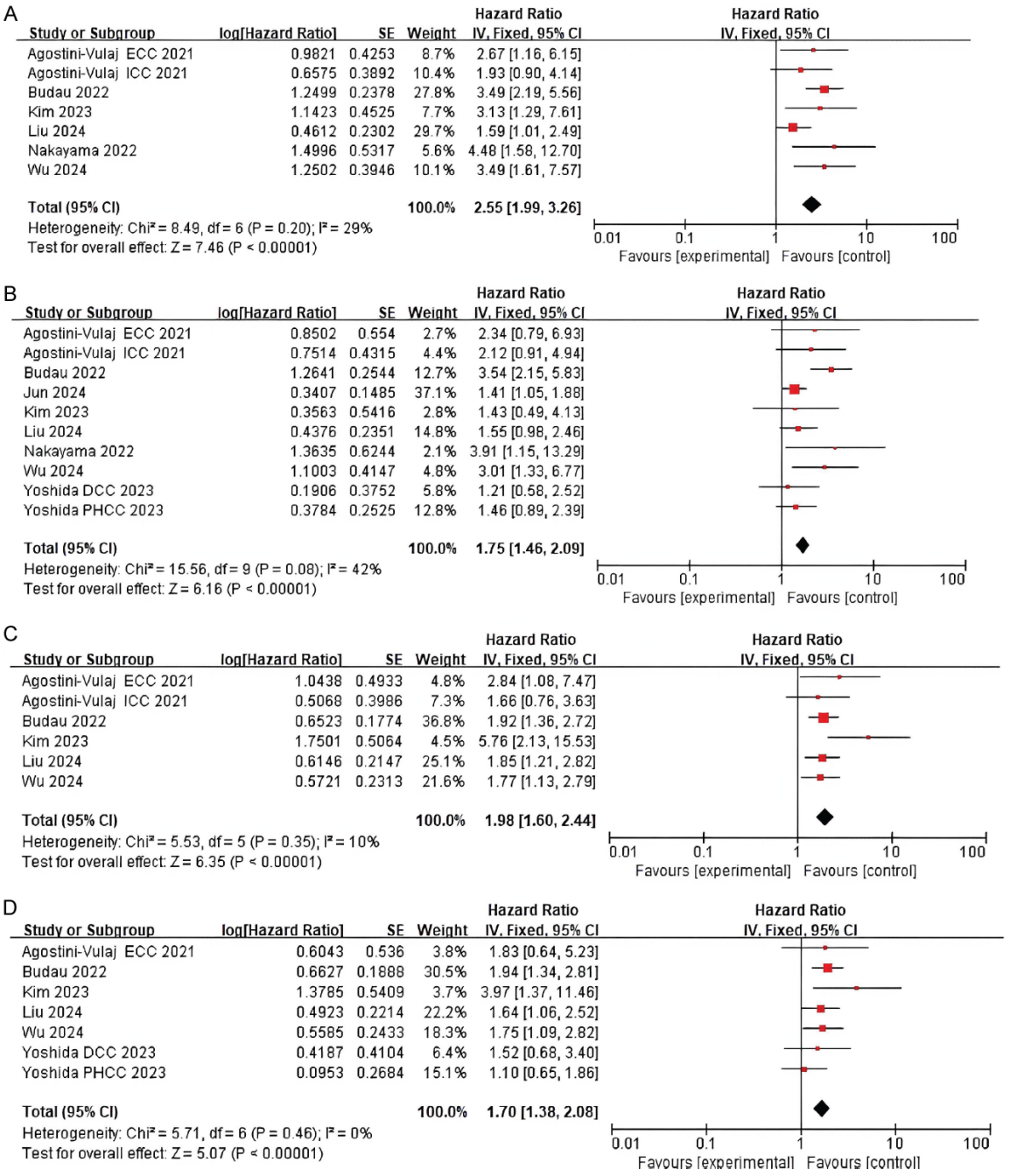

Figure 2. Forest plot for survival outcomes. A: Meta-analysis of overall survival from the results of the univariate analysis. B: Meta-analysis of overall survival from the results of the multivariate analysis. C: Meta-analysis of disease-free survival from the results of the univariate analysis. D: Meta-analysis of disease-free survival from the results of the multivariate analysis.

[25]. A body of research has been conducted on the effect of neoadjuvant therapy on tumor budding. The findings, as reported by Ito et al. and Wu et al., suggested a correlation between tumor budding and unfavorable outcomes, irrespective of the administration of neoadjuvant therapy [19, 30].

Discussion

The present meta-analysis demonstrated that tumor budding is significantly associated with both overall survival (OS) and disease-free survival (DFS) in patients with BTC, and remains an independent prognostic factor by univariate

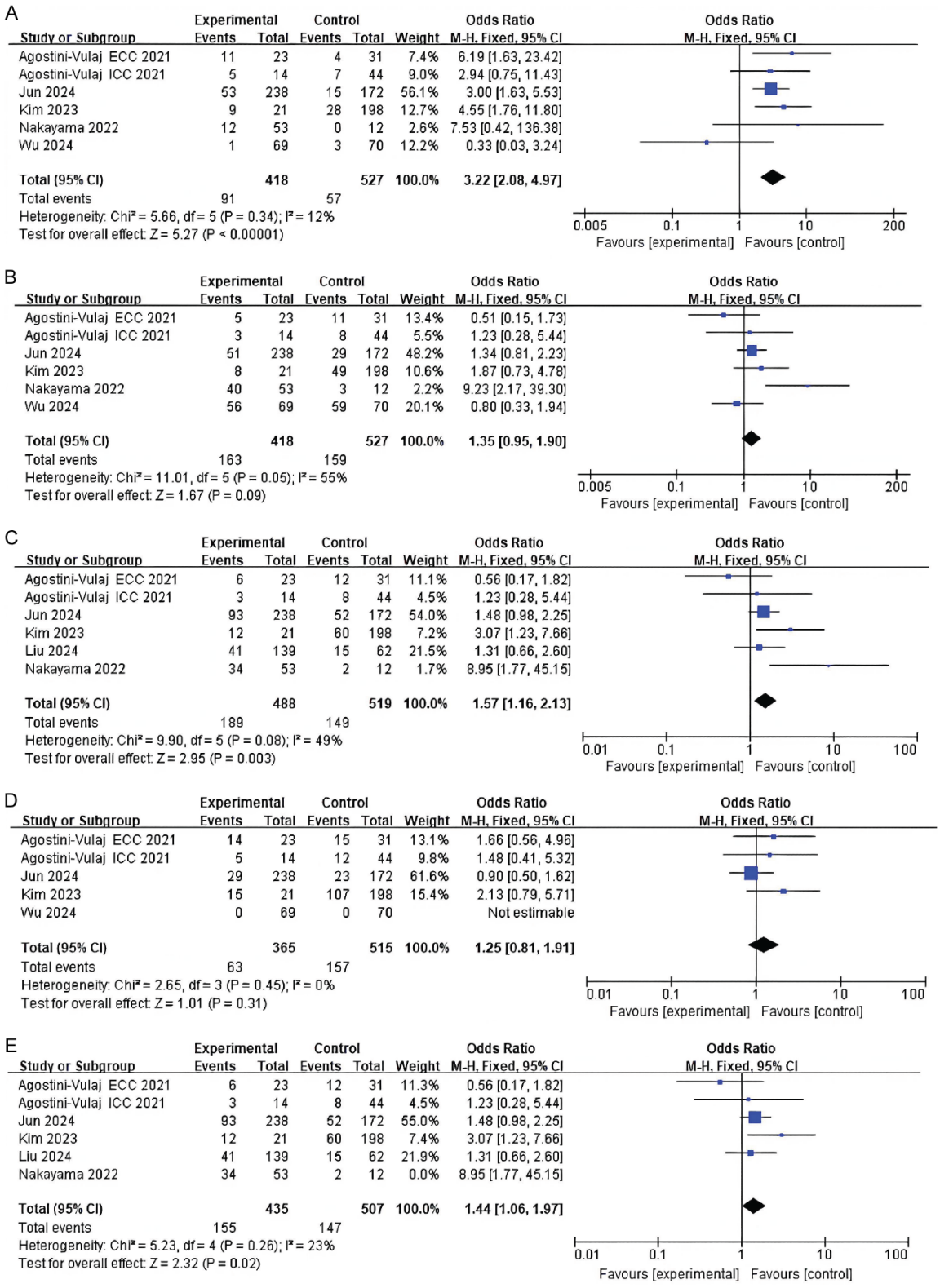

Figure 3. Relationship of tumor budding with clinicopathologic findings. A: Poor histologic differentiation. B: Advanced T-stage. C: Lymph node metastasis. D: Positive surgical margin. E: Lymph node metastasis after removing one study for potential heterogeneity.

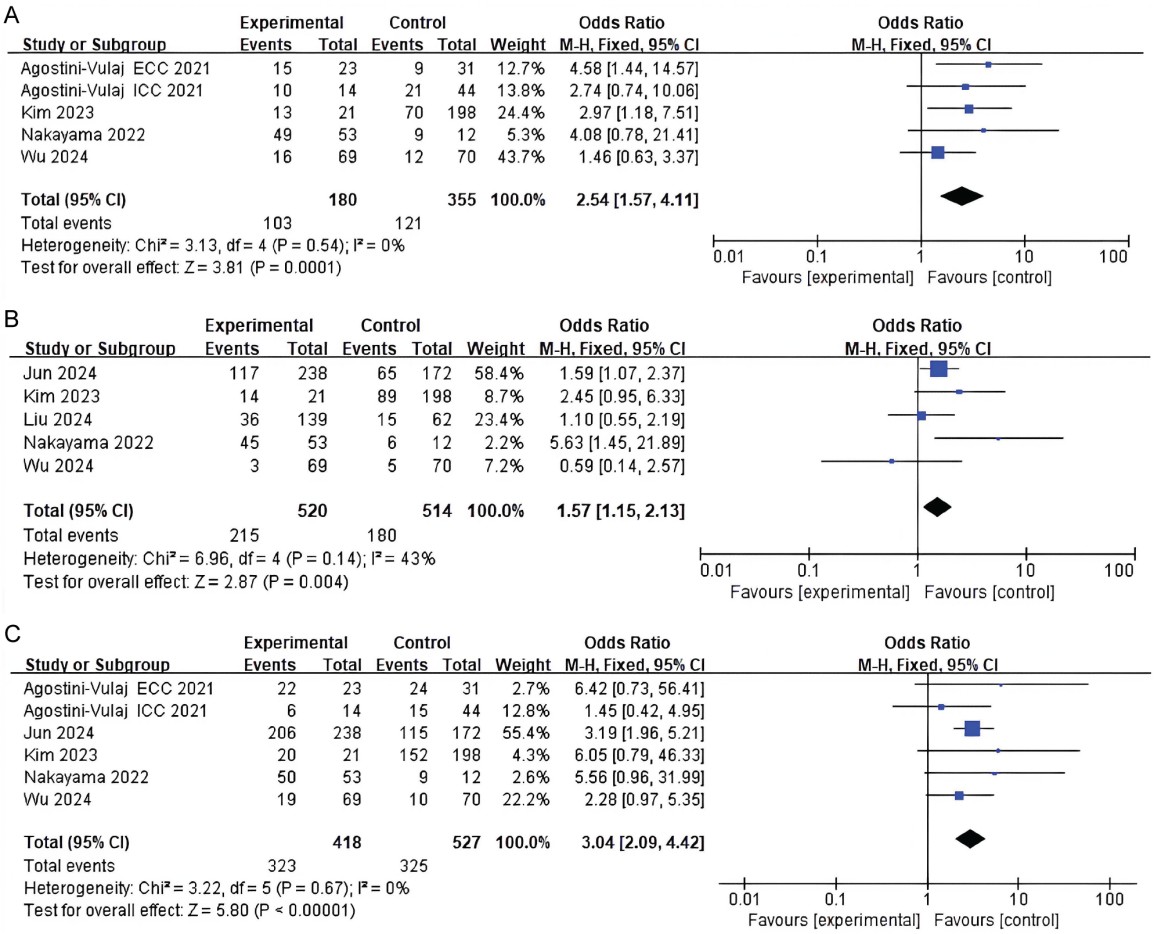

Figure 4. Relationship of tumor budding with clinicopathologic findings. A: Lymphatic invasion. B: Vascular invasion. C: Perineural invasion.

and multivariate analyses. These findings are highly consistent with the meta-analysis by Regmi et al. [31], which likewise reported markedly shorter OS and DFS in patients with high-grade tumor budding, with this association persisting after sensitivity analyses that excluded heterogeneous studies. Collectively, the concordant evidence supports tumor budding as a robust prognostic indicator in BTC. Taken together, these consistent findings suggest that tumor budding is a reliable histopathologic biomarker with substantial clinical relevance for BTC prognosis, and in our view it reflects an intrinsic biological aggressiveness that is not fully captured by traditional staging systems.

From a clinical standpoint, we believe the value of tumor budding lies in capturing aggressive biological behavior that is not fully reflected by conventional staging. Tumor budding may therefore function as a microscopic "invasion signature", enabling finer prognostic stratification among patients within the same TNM stage. This interpretation is supported by the strong associations observed between tumor budding and malignant clinicopathologic features, including poor histological differentiation, lymph-node metastasis, lymphatic invasion, vascular invasion, and perineural invasion. These factors are well-established determinants of BTC prognosis and are integral to AJCC TNM staging [21]. Prior studies consistently linked tumor budding to poor differentiation and nodal metastasis [23, 25, 27, 28], both of which are themselves associated with inferior survival outcomes [32, 33]. In addition, lymphatic, vascular, and perineural invasion predicted unfavorable prognosis in BTC [34]. In our synthesis, tumor budding in bile duct cancer was also correlated with a higher risk of microvascular invasion [35]. Taken together,

these clinicopathologic links reinforce the view that tumor budding reflects a high-metastatic-potential phenotype. Accordingly, incorporating standardized tumor budding evaluation into pathologic reporting alongside AJCC staging could improve postoperative risk assessment and help identify resected BTC patients who warrant closer surveillance or intensified adjuvant strategies.

Interestingly, no significant association was detected between tumor budding and positive surgical margin rates across included studies, which contrasts with Regmi et al. [31]. This discrepancy may be attributable to differences in tumor-subtype composition, margin definitions, and budding-grading systems among studies. We interpret this non-association cautiously and do not consider it biologically contradictory, because margin status is influenced not only by tumor behavior but also by surgical technique and anatomic constraints, whereas tumor budding is a purely biological marker. Standardized definitions for margin assessment and tumor budding, together with prospective BTC-subtype-specific analyses, are therefore required to clarify this relationship.

Beyond conventional pathology, the interaction between tumor budding and the immune microenvironment deserves further attention. Tumor budding may contribute to immune evasion by modulating infiltration of immune cells such as tumor-associated macrophages (TAMs) and T cells [36]. The density and spatial distribution of these immune populations could correlate with budding intensity, thereby affecting metastatic propensity and prognosis. Given the limited evidence currently available, future studies should investigate budding-immune crosstalk to provide mechanistic support for immunotherapy strategies in BTC [37]. In parallel, combining tumor budding with molecular biomarkers (e.g., p53, Ki-67, VEGF) may improve prognostic accuracy. Emerging data suggest potential synergistic effects between budding and these markers, which may enhance prediction of survival and treatment response and facilitate multi-biomarker risk models for precision management [38].

Another notable finding was the lack of a significant association between tumor budding and advanced T stage, which differs from Regmi et al. [31]. This may reflect substantial heterogeneity among included studies (I^2 = 55%, P = 0.05), arising from mixed BTC subtypes, subtype-specific T-stage definitions, and non-uniform budding thresholds. Biologically, T staging predominantly reflects the extent of local tumor invasion, whereas tumor budding more directly indicates invasive and metastatic potential. We interpret this as evidence that tumor budding and T stage represent two distinct but complementary biological dimensions - macroscopic invasion versus microscopic dissemination. Such behavioral divergence could dilute an overall correlation, suggesting that tumor budding might be particularly useful for detecting tumors with high metastatic potential even in relatively early T stages [39].

Mechanistically, tumor budding has been closely linked to epithelial-mesenchymal transition (EMT) [40]. Nakayama et al. [25] reported that high-grade budding was significantly associated with ZEB1 positivity, a key EMT transcription factor that suppresses epithelial markers such as E-cadherin, promotes loss of polarity and adhesion, and facilitates detachment into single cells or small clusters with enhanced invasive capacity. Although none of the included studies evaluated tumor budding in preoperative biopsy specimens, the ITBCC-endorsed “hot-spot” method used for colorectal cancer [41] may be applicable to BTC biopsies in future practice. Exploring its feasibility in BTC biopsy specimens could enable earlier prognostic evaluation and influence preoperative decision-making.

Several limitations merit consideration. First, all included studies were retrospective, which may introduce selection bias, and randomized trials are lacking. Second, subtype-specific analyses were not feasible, limiting conclusions about the differential role of tumor budding across BTC subtypes. Third, the predictive value of tumor budding for treatment response remains unclear, including whether high-budding tumors derive greater benefit from neoadjuvant or intensified adjuvant therapy. Nevertheless, we believe these findings provide a strong rationale for incorporating tumor budding assessment into future clinical trials and multidisciplinary protocols. Future work should therefore focus on (1) establishing BTC-tailored standardized budding criteria aligned with ITBCC principles to reduce interstudy variabili-

ty; (2) conducting large, multicenter prospective studies to validate its prognostic and decision-guiding utility; and (3) integrating budding with molecular characteristics to refine prognostic modeling and identify potential therapeutic targets. Overall, our results support tumor budding as a practical and biologically meaningful histologic marker of BTC aggressiveness that complements TNM staging and holds promise for broader clinical translation once standardized and prospectively validated.

Conclusion

The effect of tumor budding on the prognosis of various tumors has been well-established. The results of this study further confirm that tumor budding can serve as a reliable predictor of poor survival in patients with BTC. Therefore, after establishing a standardized international consensus based on existing evidence, it will be necessary to consider including tumor budding as a prognostic marker for BTC.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. PRISMA guidelines

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	The prognostic significance of tumor budding in Biliary tract cancer: a systematic review and meta-analysis.	1
ABSTRACT			
Structured summary	2	Structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings are included in the manuscript.	1
INTRODUCTION			
Rationale	3	Rationale for the review in the context of what is already known was reported in the introduction.	1
Objectives	4	To perform a systematic review and meta-analysis of survival outcomes based on the results of univariate and multivariate analysis in previously published literatures to identify the prognostic significance of tumor budding in BTC. This was reported in last paragraph of introduction.	2
METHODS			
Protocol and registration	5	Protocol registration was not done for this Meta-analysis.	2
Eligibility criteria	6	Inclusion and Exclusion criteria are reported in methods section of the manuscript.	2
Information sources	7	PubMed, Embase, and Web of Knowledge were systematically searched for relevant studies from March 2021 to March 2025. Contact with authors was not done for additional information.	3
Search	8	Electronic search strategy all three databases were done by using search term ((budding) OR (sprouting)) AND (((((cholangiocarcinoma) OR (bile duct cancer)) OR (bile duct carcinoma)) OR (gallbladder cancer)) OR (gallbladder carcinoma)).	3
Study selection	9	Process for selecting studies is described in PRISMA flow chart in **Figure 1**.	**Figure 1**
Data collection process	10	Two reviewers independently evaluated the data for quality. Inconsistencies were resolved on the assessment by the corresponding author.	3
Data items	11	Outcomes of interest for data collection are reported in the manuscript.	3
Risk of bias in individual studies	12	Newcastle Ottawa Scale (NOS) was used to check the quality of included studies.	3
Summary measures	13	Hazard ratio (HR) for survival outcomes was calculated.	3
Synthesis of results	14	The fixed effect (Mantel-Haenszel) model was used when there was no significant heterogeneity and the random effects (Inverse variance) model was used for those with significant heterogeneity. Heterogeneity was defined as low, moderate, and high based on/square value (< 40%: low; 30%-60%: moderate; 50%-90%: substantial > 75%: high). Heterogeneity with a high/square value > 50% was considered statistically significant.	3
Risk of bias across studies	15	Publication bias assessed by visual analysis of funnel plot.	3
Additional analyses	16	Leave-one out sensitivity analysis for outcomes with statistically significant heterogeneity.	3
RESULTS			
Study selection	17	Explained in PRISMA flow diagram.	**Figure 1**
Study characteristics	18	Characteristics of the included studies are described in **Table 1**.	**Table 1**
Risk of bias within studies	19	All of the included studies scored at least 6 in the Newcastle Ottawa Scale (NOS) (0 to 9 points) for cohort studies.	Supplementary Table 4
Results of individual studies	20	Results of all outcomes from each study are reported in **Table 2**.	**Table 2**
Synthesis of results	21	Results of meta-analysis for each outcome are reported in results section and Figures.	**Figures 2-4**

Risk of bias across studies	22	Funnel plot was used to evaluate the publication bias across studies.	Supplementary Figure 1
DISCUSSION			
Summary of evidence	23	Summary of the main findings including the strength of evidence for each main outcome and their relevance to key groups was reported in discussion section.	5-6
Limitations	24	Limitation of study is reported in the discussion section.	6
Conclusions	25	The prognostic significance of tumor budding is backed by strong evidence for multiple tumors. Results of our study showed that tumor budding is strong predictor of poor survival for BTC. Clinical utility of tumor budding as a prognostic marker for BTC should be considered after developing a standard international consensus based on the current evidence.	6
FUNDING			
Funding	26	This study was funded by the Medical Research Project of Chengdu (No: 2025436).	7

Supplementary Table 2. MOOSE checklist

Item No	Recommendation	Brief description of how the criteria were satisfied
Reporting of background should include		
1	Problem definition	Review of literature revealed heterogeneous results and this is referenced in introduction.
2	Hypothesis statement	To perform a systematic review and meta-analysis of survival outcomes based on the results of univariate and multivariate analysis in previously published literatures to identify the prognostic significance of tumor budding in BTC. This is reported in last paragraph of introduction.
3	Description of study outcome(s)	Meta-analysis of prognostic outcomes based on results of univariate and multivariate analysis.
4	Type of exposure or intervention used	Significance of tumor budding in biliary tract cancer.
5	Type of study designs used	Retrospective cohort studies.
6	Study population	All patients with tumor budding in histologic analysis.
Reporting of search strategy should include		
7	Qualifications of searchers	Credentials of authors listed in author list.
8	Search strategy, including time period included in the synthesis and key words	PubMed, Embase, and Web of Knowledge were systematically searched for relevant studies from March 2021 to March 2025 using the search term ((budding) OR (sprouting) OR (tumor budding)) AND (((((cholangiocarcinoma) OR (bile duct cancer)) OR (bile duct carcinoma)) OR (gallbladder cancer)) OR (gallbladder carcinoma)).
9	Effort to include all available studies, including contact with authors	All studies meeting inclusion criteria were included, and the data presented in these studies was sufficient for meta-analysis without need to contact authors.
10	Databases and registries searched	PubMed, Embase, and Web of Knowledge.
11	Search software used, name and version, including special features used (e.g., explosion)	No special search software was used.
12	Use of hand searching	Hand searching of references cited in final 8 included studies was performed.
13	List of citations located and those excluded, including justification	Number of records located and reasons for exclusion at each step are diagrammed in PRISMA style flow diagram in **Figure 1**.
14	Method of addressing articles published in languages other than English	Articles published in non-English language were excluded.
15	Method of handling abstracts and unpublished studies	Abstracts and unpublished studies were not included in this study.
16	Description of any contact with authors	No contact with authors was made.

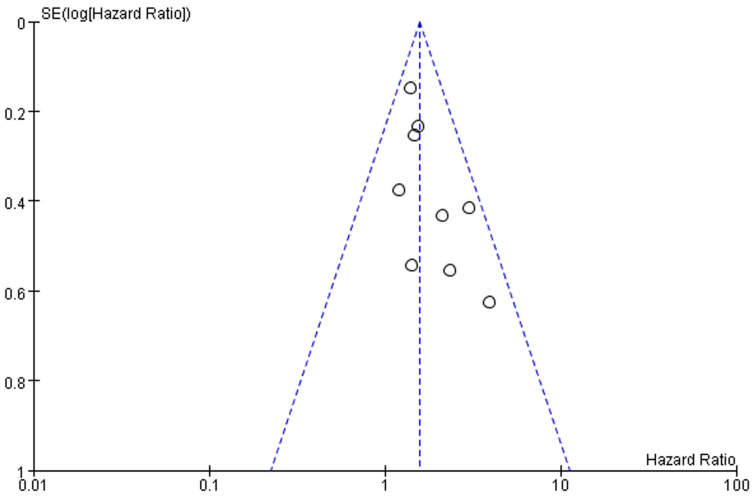
Reporting of methods should include		
17	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	All included studies reported at least one or several intraoperative, postoperative, and long-term oncological outcomes.
18	Rationale for the selection and coding of data	Any variable that was reported by 2 or more studies was selected and analysed.
19	Documentation of how data were classified and coded	Data was extracted by one reviewer and verified. Data was classified coded to be consistent with the methodology and definition used by the original study.
20	Assessment of confounding	When the extracted data was found in median and range, the mean and standard deviation was estimated as described by Luo et al. and Wan et al.
21	Assessment of study quality, including blinding of quality assessors, stratification or regression on possible predictors of study results	Study quality was assessed by using the Newcastle-Ottawa scale (0-9 points) and reported in Supplementary Table 4.
22	Assessment of heterogeneity	Individual variable heterogeneity was assessed by I^2 and visual assessment of the Forest plot.
23	Description of statistical methods in sufficient detail to be replicated	Survival outcomes were analyzed by estimation of Hazard Ratio (HR) with a 95 percent (%) confidence interval (CI). The fixed effect (Mantel-Haenszel) model was used when there was no significant heterogeneity and the random effects (Inverse variance) model was used for those with significant heterogeneity.
Reporting of results should include		
24	Graphic summarizing individual study estimates and overall estimate	Forest Plots for each outcome are provided in **Figures 2**-**4**.
25	Table giving descriptive information for each study included	Reported in **Table 1**.
26	Results of sensitivity testing (e.g., subgroup analysis)	Leave-one out sensitivity analysis performed for outcomes with high heterogeneity. This is described in the methods and results section.
27	Indication of statistical uncertainty of findings	95% confidence intervals are presented with all estimates of overall effect (hazard ratio).
Reporting of discussion should include		
28	Quantitative assessment of bias	The discussion sectioned contained summary of bias in reporting of multivariable results.
29	Justification for exclusion	Studies that did not provide meaningful data were excluded as described in methods as they were of low utility.
30	Assessment of quality of included studies	Quality of methodology and data analysis for variables analysed was included in the discussion.
Reporting of conclusions should include		
31	Consideration of alternative explanations for observed results	When studies reached opposite conclusions, this was described in the discussion and reasons for the discrepancies provided.
32	Generalization of the conclusions	The studies included and data sources utilized are representative of prognostic significance of tumor budding in biliary tract cancer.
33	Guidelines for future research	As mentioned in the discussion, future studies should focus on the prognostic significance of tumor budding in individual types of BTC. Moreover, we should focus on identifying the risk of postoperative adjuvant therapy failure based on the presence or absence of tumor budding in BTC.
34	Disclosure of funding source	This study was funded by the Medical Research Project of Chengdu (No: 2025436).

Supplementary Table 3. Search strategy

Search	Terms
#1	(budding) OR (sprouting) OR (tumor budding)
#2	((((cholangiocarcinoma) OR (bile duct cancer)) OR (bile duct carcinoma)) OR (gallbladder cancer)) OR (gallbladder carcinoma)
#1&#2	((budding) OR (sprouting)) AND (((((cholangiocarcinoma) OR (bile duct cancer)) OR (bile duct carcinoma)) OR (gallbladder cancer)) OR (gallbladder carcinoma))
	PubMed: 20 Embase: 36 Web of Knowledge: 45

Supplementary Table 4. Newcastle-Ottawa quality assessment scale for cohort studies

Authors	Represent-ativeness of the exposed cohort	Selection of the non exposed cohort	Ascertain-ment of exposure	Demonstra-tion that outcome not present at start	Compara-bility of cohort	Assessment of outcome	Follow-up length adequate for outcome to occur	Adequacy of Follow-up of cohorts	Total score
Agostini-Vulaj et al.	1	1	1	1	1	1	1	1	8
Budau et al.	0	0	1	1	1	1	1	1	6
Nakayama et al.	0	1	1	1	1	1	1	1	7
Yoshida et al.	1	1	1	1	1	1	1	1	8
Kim et al.	0	1	1	1	2	1	1	1	8
Jun et al.	0	0	1	1	1	1	1	1	6
Liu et al.	0	1	1	1	2	1	1	1	8
Wu et al.	1	1	1	1	2	1	1	0	8



Supplementary Figure 1. Funnel plot for publication bias.