Prognostic significance of TOP2A expression in bladder cancer: Systematic review and meta-analysis

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ABSTRACT

Introduction: Elevated topoisomerase IIA (TOP2A) expression is consistently linked to poor prognosis, driving cancer progression through its role in DNA unlinking. Although TOP2A is well-established in other cancers, its prognostic value in bladder cancer remains unclear. This study was conducted to assess the prognostic significance of TOP2A for patients with bladder cancer.
Methods: PubMed, EMBASE (Ovid), and the Cochrane Library were systematically searched to identify studies published up to August 8, 2024. Published studies reporting the prognosis of patients with bladder cancer stratified by TOP2A expression were included. A random-effect model was used to pool the standardized mean difference (SMD) with 95% confidence interval (CI) for tumor stage and grade and hazard ratio (HR) with 95% CI for cancer-specific survival (CSS). The Newcastle-Ottawa Quality (NOS) was used to assess the risk of bias in the included studies.
Results: Nine studies with 1764 eligible patients with bladder cancer were identified. TOP2A expression was found in 32% patients with bladder cancer. High TOP2A expression was significantly correlated with higher tumor grade (SMD 1.61, 95% CI -0.02−3.23, p=0.05), higher tumor stage (SMD 1.09, 95% CI 0.01−2.17, p=0.05), and worse CSS (HR 1.19, 95% CI 1.05−1.36, p=0.008) in patients with bladder cancer.

Conclusions: TOP2A expression shows potential as a prognostic biomarker in bladder cancer; however, significant heterogeneity in study design, measurement methods, and analytical approaches limits the strength of the current evidence. Further prospective studies with standardized biomarker assessment are warranted to validate its clinical utility.

INTRODUCTION

In 2023, bladder cancer represented the fourth most frequently diagnosed malignancy among men, contributing to roughly 6% of all new cancer cases and 4% of cancer-related mortality.[1] Metastatic disease is detected in about 5% of patients at the time of their initial diagnosis.[1] Approximately 50% of patients will experience cancer recurrence following radical cystectomy.[2] Current risk stratification relies on clinicopathological factors and are often inadequate for capturing the complexity and heterogeneous behavior of bladder cancer.[3] Hence, novel biomarkers would be a valuable adjunct in risk-stratifying patients with bladder cancer which can further aid in guiding treatment decisions and improving outcomes.[4]

DNA topoisomerase IIA (TOP2A) plays a pivotal role in cancer development, progression, invasion, and prognosis.[5] Cancer arises from genomic instability and impaired DNA repair mechanisms, which lead to the accumulation of DNA damage over time. While the DNA double helix is stabilized by hydrogen bonds, essential cellular processes such as replication and transcription require its unwinding, introducing topological strain. This strain is alleviated by topoisomerases, a class of enzymes that transiently cleave and rejoin DNA strands to maintain genomic integrity. Among them, type II topoisomerase (TOP2) functions as a homodimer, introducing ATP-dependent double-strand breaks to facilitate the passage of intact DNA segments, while protecting the cleaved ends until resealing. Emerging evidence has highlighted the complex role of TOP2A, a gene encoding a TOP2 isoform. Beyond its fundamental cellular function, TOP2A has been implicated in oncogenesis and tumor progression. Elevated TOP2A expression is consistently linked to poor prognosis in various cancers, driving cancer progression through its role in DNA unlinking.[6–10]

By regulating the cell cycle and apoptosis, it promotes tumor growth and serves as a critical target for chemotherapy. Although multiple studies have reported that elevated TOP2A expression (both at the gene and protein levels) is associated with higher tumor grade, advanced stage and worse tumor prognosis, some studies have shown that high TOP2A protein is associated with better disease-free survival. Association between TOP2A protein and tumor outcome are needed to evaluate as more than 50% of bladder cancer cases have TOP2A protein expression. In this study, we conducted a systematic review and meta-analysis as hypothesis-generating synthesis to evaluate TOP2A role protein in bladder cancer prognosis.

METHODS

Protocol registration and PRISMA guideline

The protocol of this systematic review was registered on PROSPERO on August 18th, 2024, with the registration number CRD42024576668. The systematic review and meta-analysis were performed and written following the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline .[11]

Search strategy and eligibility criteria

A systematic literature search was carried out on PubMed, EMBASE (Ovid), and the Cochrane Library from inception until August 8th, 2024 to identify studies on TOP2A expression as a prognostic indicator for bladder cancer. Keywords such as "bladder cancer", "TOP2A", "survival", "recurrence", and "progression," along with their synonyms as defined by subject headings, were used to search for relevant articles (Supplementary Table 1).

All studies included in the analysis met the specified inclusion criteria: 1) the study involved human participants age ≥18 years old diagnosed with bladder cancer (muscle invasive and non-muscle invasive), 2) the study investigated the association between TOP2A expression and oncological outcomes of bladder cancer, such as tumor grade, stage, overall survival, disease-free survival and progression-free survival, and 3) the study used immunohistochemistry (IHC) method to determine TOP2A protein expression. Conversely, the exclusion criteria for this study are: 1) overlapping study, 2) study with insufficient or low-quality data, 3) unsuitable article types such as meeting reports, reviews, or non-human studies, 4) unavailable full-text publication, and 5) full-text article not in English.

Selection of studies and data extraction

The articles identified through the initial search were gathered, and duplicates found across multiple searches were removed. The titles and abstracts of the remaining articles were then evaluated based on the predetermined inclusion and exclusion criteria. The comprehensive search was conducted by two independent reviewers (BT and TF), and any discrepancies in study inclusion were resolved through detailed discussion with a third reviewer (AHM).

Two reviewers (BT and TF) performed the data extraction independently using a standardized data extraction spreadsheet. The following data was extracted from each eligible publication: the first author's name, publication year, study design, country, patient population, age, method with cut-off value, stage, grade, high/low TOP2A expression, treatment, follow-up period, and outcome.

Given the variability in IHC scoring systems and cut-off values across studies, a centralized cut-off for high TOP2A expression was not applied. Instead, high versus low TOP2A expression was defined according to each study's original criteria.

Risk of bias assessment

The risk of bias in the included studies was independently assessed by 2 reviewers (BL and ADMP) using the Newcastle-Ottawa Scale (NOS).[12] The three primary categories of bias in NOS are selection, comparability, and outcome. "Good" quality was defined as a score of 7 points or higher, "Fair" quality as 2 to 6 points, and "Poor" quality as ≤1 point.

Data synthesis and statistical analysis

Statistical analysis was conducted using RevMan 5.4. The summary measures were SMD with 95%CI for tumor stage and grade and Hazard Ratio (HR) with 95%CI for CSS. The random-effect model was used to pool effect size with 95%CI, and the DerSimonian and Laird estimator was applied to estimate between-study variance. For time to event data, HR were log-transformed before pooling and subsequently back-transformed for interpretation.

Statistical significance was considered if the two-sided p-value was less than 0.05. Heterogeneity among individual studies was measured by Cochran's Q test and the I2 index. Significant heterogeneity was observed when $P < 0.10$, while $I2 > 50\%$ indicated the presence of substantial heterogeneity. Publication bias was assessed by constructing a funnel plot and examining it for asymmetry.[13]

RESULTS

Literature search process

A total of 156 studies were retrieved from the database searches, following which duplicates were removed, leaving 112 articles remaining. Only 9 studies met the pre-defined eligibility criteria (Figure 1). The first study reporting TOP2A protein expression in bladder cancer was published in 2001.[14] By interpreting staining results from IHC, authors reported both high and low expression of TOP2A protein and its association with oncological outcomes which are key themes that require further discussion.

Risk of bias assessment

The risk of bias assessment in each study is summarized in Supplementary Table 2. Based on NOS, we rated the overall risk of bias to be fair in 3 studies[15–17] and low in 6 studies. [8–10,14,18,19] Most of the included studies exhibited good methodological quality and a low risk of bias.

Characteristics of included studies

A total of nine studies explored the relationship between TOP2A and bladder cancer outcomes, involving 1,764 bladder cancer patients. Of these studies, four were conducted in Europe [10,14,18,19], three in Asia [8,9,15], and two in Africa.[16,17] Among these patients, 1,356 had NMIBC, while 408 had MIBC. However, variations existed in how tumor grade was described across the included studies. Based on WHO 2004 grading system classification [9], 109 patients were low grade and 351 were high grade [9,15,16]. According to the WHO 1973 classification [20], 149 patients were categorized as G1, 558 as G2, and 539 as G3. Patients' age ranged from 33-95 years old with follow-up time ranged from 1 – 236 months. Only five of the nine studies reported administering intravesical chemotherapy instillation. [9,10,14,17,19] The included studies utilized BCG [10,17,19], doxorubicin [17], and pirarubicin [9], while one study did not specify the type of chemotherapy used.[14] In the included studies, 32% of cases were classified as having high TOP2A expression.[8–10,14,16–19] However, the criteria for determining high TOP2A expression varied across the included studies, with differences not only in the methods used but also in the cutoff values applied. The cutoff values used varied, including an immunoreactive score (IRS score) greater than 4 [17] and a score greater than 8 [9], both calculated by multiplying the staining area by intensity. Other cutoffs were based on area (TII-α Index) or intensity, including > 10% [16], $\geq$ 10% [14], > 30% [10], > 35% [15], median [19], > 67% [8], and $\geq$ 70% [18]. Based on included studies, high TOP2A expression was linked to worse CSS in four studies [8,14,18], worse Overall Survival (OS) in one study [9], worse Relapse-Free Survival (RFS) in two studies [8,15], higher recurrence rate in three studies [15–17], and worse Progression-Free Survival (PFS) in one study.[8] However, one study found that high TOP2A expression was associated with lower recurrence rates [9], and another study observed improved Disease-Free Survival (DFS) with high TOP2A expression and no association between TOP2A expression and OS or PFS.[10] Table 1 summarizes the characteristics of the included studies.

Association of TOP2A expression with tumor stage and grade of bladder cancer

High TOP2A expression was correlated with higher tumor grade (SMD: 1.61; 95%CI: -0.02-3.23 ; p=0.05) in two studies [8,15] (Figure 2) and higher tumor stage (SMD: 1.09; 95%CI: 0.01-2.17; p=0.05) in two studies [8,15] (Figure 3). The Cochran's Q test and the I2 index indicated significant heterogeneity for tumor grade (I2 = 83%, p = 0.003) and tumor stage (I2 = 81%, p = 0.02). Funnel plots indicated that one study [15] exceeded the pseudo 95% confidence interval for both tumor grade and stage (Supplementary Figure 1). Statistical test for funnel plot asymmetry was not performed because of the limited number of studies and the significant heterogeneity among them.[13]

Association of TOP2A expression with bladder cancer prognosis

Eight out of nine studies used different analytical approaches to report bladder cancer survival as an endpoint.[8–10,14–16,18,19] High TOP2A expression was associated with worse CSS in four studies (Figure 4; HR: 1.19; 95% CI: 1.05-1.36; p = 0.008) [8,14,15,18], worse RFS in two studies [8,19], worse PFS in one study [8], and worse OS in one study.[9] Two studies found there was no correlation between TOP2A concentration with OS [10,16]. Only one study found that high TOP2A expression was associated with better DFS.[10] Statistical test for funnel plot asymmetry in CSS was not performed because of the limited number of studies and the significant heterogeneity among them.[13] Zeng et al. [8] found that bladder cancer patients with high TOP2A demonstrated significantly worse CSS (21.3 months vs. 41.1 months, p = 0.004), shorter RFS (11.6 vs. 37.3 months, p = 0.004) and shorter PFS (16.8 months vs. 33.6 months; p=0.008) in patients with bladder cancer compared to low TOP2A expression. Zeng et al. [8] also observed a trend toward shorter RFS in patients with high TOP2A mRNA expression, although it did not reach statistical significance (27.8 vs. 36.8 months, p = 0.135). Liu et al. [9] observed a difference in median overall survival time between patients with high TOP2A and low TOP2A protein expression (48 months vs. 66 months; $p < 0.05$). Kruger et al. [19] reported significantly shorter RFS in patients with elevated TOP2A expression (RR 2.14, p = 0.031). Additionally, three studies found high TOP2A expression associated with cancer recurrence in three studies.[15–17] Koren et al. found that higher TOP2A expression in recurrent bladder cancer compared to healthy patients after TURBT (Mean TII-α index 19.5 vs 14.3).[15] Two other studies, Elkady et al. (p = 0.01) [17] and Abdelkrim et al. (p = 0.009) [16], also supported the association between tumor recurrence and high TOP2A protein. On the other hand, two studies found that high TOP2A expression was associated with lower cancer recurrence [9] or better DFS.[10] Liu et al. reported that elevated TOP2A expression was linked to decreased cancer recurrence (p = 0.037).[9] Raspollini et al. [10] showed that higher TOP2A protein levels were associated with longer DFS (RR 0.978, p = 0.029).

DISCUSSION

Our findings demonstrated that elevated TOP2A expression was linked to poorer CSS, RFS and DFS in bladder cancer.[8,9,14–19] This reflects current understanding on the biology of TOP2A, a key enzyme that helps manage the topology of DNA during processes like replication,

transcription, and repair, essential for proper cellular function. Overexpression of TOP2A results in increased cellular proliferation and genomic instability, which in turn promotes mutations and chromosomal aberrations that drive cancer progression. Additionally, TOP2A overexpression has been linked to an immunosuppressive environment, with increased infiltration of regulatory T cells suppressing cytotoxic CD8+ T-cells, thereby promoting tumor progression. Further supporting our finding of the poor prognostic value of TOP2A expression in bladder cancer is finding that knockdown of TOP2A significantly suppressed the proliferation, migration, and invasion potential of bladder cancer cells.[8] Despite exhibiting more aggressive biological characteristics, patients with amplification of TOP2A, respond better to anthracycline-based chemotherapy.[21] Chan et al. identified TOP2A overexpression as an independent predictor of success in anthracycline-based therapy [22], lending support to one of our included studies by Raspollini et al.[10] Liu et al. also observed that in bladder cancer patients with high TOP2A expression, treatment with anthracycline-class drugs led to reduced tumor recurrence but worse overall survival.[9] Around 75% of bladder cancer patients with high TOP2A expression showed no recurrence when treated with anthracycline-based chemotherapy.[9] The discrepancy in response could be explained by several reasons including but not limited to inter-individual heterogeneity in tumor microenvironment, development of anthracycline resistance and the role of other biomarkers such as HER-2, ERCC1 and RRM1.[9]

TOP2A plays a crucial role in stratifying bladder cancer patients based on disease prognosis, particularly given its association with poorer outcomes. In non-muscle-invasive bladder cancer (NMIBC), high TOP2A expression may warrant more aggressive treatment strategies, such as adjuvant intravesical chemotherapy or the inclusion of immunotherapy, especially if other clinicopathological factors indicate elevated risk. Similarly, for patients with muscle-invasive bladder cancer (MIBC), high TOP2A levels could support the recommendation for adjuvant chemotherapy or immunotherapy to enhance overall survival chances. By incorporating TOP2A expression into risk assessment, clinicians can make more informed treatment decisions tailored to individual patient profiles, ultimately aiming for improved clinical outcomes.

This review systematically evaluates the relationship between immunohistochemically detectable TOP2A protein and the prognosis of bladder cancer patients. While previous studies have explored TOP2A in other types of cancer, this review highlights its potential clinical relevance in bladder cancer and offers a foundation for future research and therapeutic decision-making. Our study has several limitations. Most of the included studies were retrospective in design with small sample sizes, and bladder cancer classifications between NMIBC and MIBC were not separately evaluated in many of them.[15–19] Only a few studies incorporated IHC along with other methods like Polymerase Chain Reaction (PCR) and fluorescence in situ hybridization (FISH) for comprehensive analysis.[8] Treatment regimens varied widely across the included studies [9,10,14,19], and some studies did not provide details on the treatments.[15,16,18] Moreover, despite a comprehensive search, only nine studies met the inclusion criteria, and only two studies

contributed data to the stage and grade meta-analyses. This limited evidence base reduces the statistical power of the analyses and may compromise the stability and robustness of the pooled estimates. Due to insufficient data, we were unable to calculate a summary HR for other important survival outcomes except CSS, making it necessary to confirm the prognostic value of these markers in future studies. While IHC was the primary method used to assess biomarker expression, the cut-off points and reference groups differed significantly, contributing to heterogeneity. For instance, some studies used tumors with 5% to 67% or more than 10% positive cells as a reference, while others applied scores above 8. The criteria for determining biomarker expression levels were inconsistent. In addition to IHC, methods such as PCR and FISH should be employed to better identify prognostic biomarkers. A more thorough investigation into standardized approaches for defining prognostic biomarkers in bladder cancer is needed. Significant heterogeneity in biomarker analysis may be attributed to differences in factors such as patient gender, age, tumor stage and grade, and varying cut-off values. Furthermore, the lack of standardization in staging and grading systems across the included studies may have introduced methodological variability, thereby contributing to the high degree of heterogeneity observed in the results. Additionally, the asymmetrical funnel plot suggests the presence of potential publication or selection bias, which may have influenced the overall conclusions of this meta-analysis. Furthermore, differences in intravesical treatment across the included studies may have affected the prognostic impact of TOP2A, as subgroup analyses based on treatment modality were limited or not feasible.

Collectively, these methodological constraints underscore the need for cautious interpretation of the results. While our findings suggest that TOP2A expression may be associated with adverse prognosis, definitive conclusions regarding its clinical utility cannot yet be drawn. Future large-scale, well-designed prospective studies using standardized biomarker assessment and reporting frameworks are required to validate the prognostic value of TOP2A and to clarify its role within multiparametric risk stratification models for bladder cancer. Moving forward, noting that there are various genes that are involved in bladder cancer progression, it is likely that the integration of various key proteins including TOP2A, RRM1, HER2, and ERCC1, in a multiparametric assay is required to risk stratify bladder cancer and guide treatment decisions.

CONCLUSIONS

This study is the first meta-analysis to evaluate the relationship between TOP2A expression and bladder cancer prognosis. The results suggest that high TOP2A expression is linked to high grade tumor and MIBC. Shorter CSS was observed in patients with high TOP2A expression. Overall, TOP2A shows potential as a biomarker for clinical prediction. Despite promising findings, heterogeneity across studies and inconsistent methodologies limit the generalizability of results. To validate the prognostic and therapeutic significance of TOP2A in bladder cancer, larger prospective studies with standardized methods are essential. Further validation is needed before clinical application can be considered.

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FIGURES AND TABLES

Figure 1. PRISMA diagram.

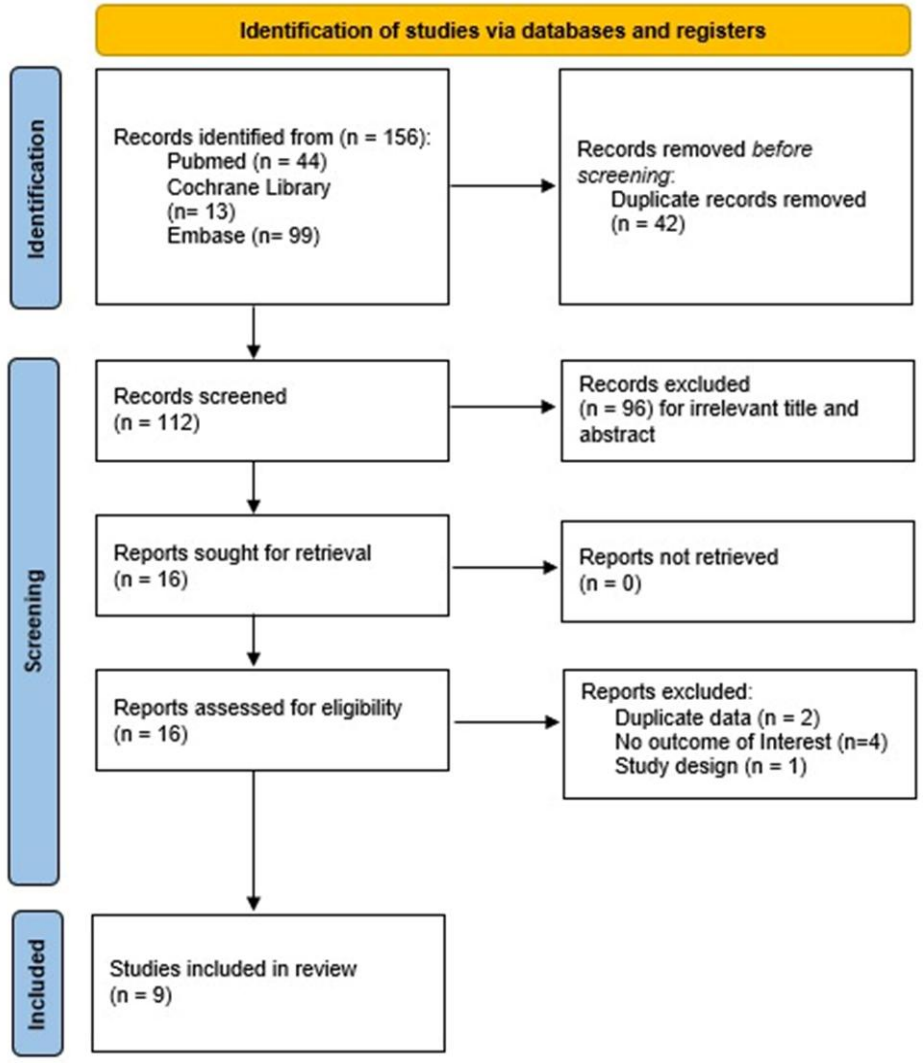

Figure 2. Forest plot of studies assessing standardized mean difference for tumor grade comparing high vs. low TOP2A expression. CI: confidence interval; IV: inverse variance; SD: standard deviation.

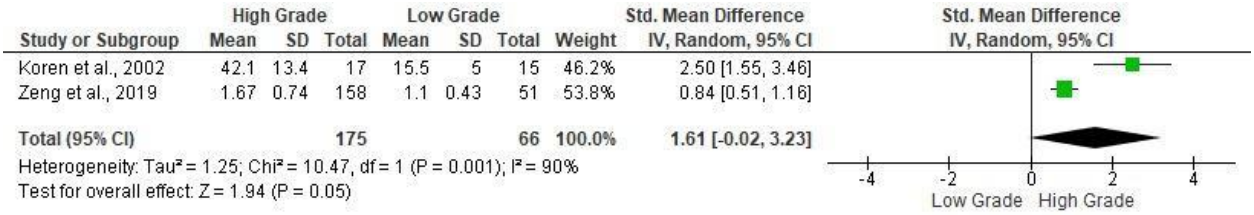

Figure 3. Forest plot of studies assessing standardized mean difference for tumor stage comparing high vs. low TOP2A expression. CI: confidence interval; IV: inverse variance; MIBC: muscle-invasive bladder cancer; NMIBC: non-muscle-invasive bladder cancer; SD: standard deviation.

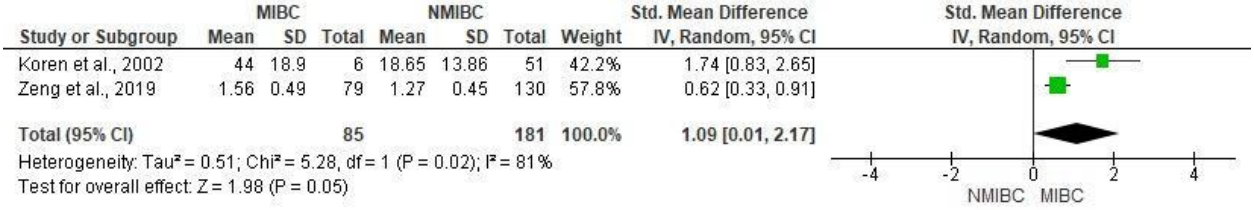

Figure 4. Forest plot of studies assessing hazard ratio (HR) for cancer-specific survival (CSS) comparing high vs. low TOP2A expression. CI: confidence interval; IV: inverse variance; SE: standard error; TOP2A: topoisomerase Iiα.

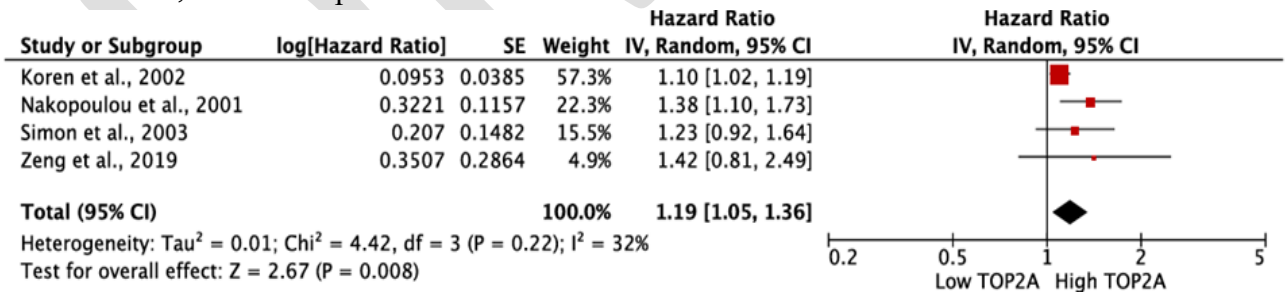

Table 1. Characteristics of included studies[8-10,14-19]												
Study, year	**Country**	**Study design**	**No of pts**	**Age (median, range in months)**	**Methods**	**IHC cutoff value**	**High/Low TOP2A protein expression**	**Stage (n)**	**Grade (n)**	**Treatment**	**Follow-up (median, range in months)**	**Correlation of High TOP2 expression with oncological outcomes**
Nakopoulou et al, 2001[14]	Greece	Retrospective cohort	94	NA	IHC	TII-αI index >10%*	19/75	T1 (51) T2−T4 (43)	G1 (17) G2 (30) G3 (47)	Ta: TURBT/fulguration T1: TURBT and adjuvant intravesical T2-T4: Total cystectomy/Radiotherapy	36 (10−88)	Worse CSS (S)
Koren et al, 2002[15]	Israel	Prospective cohort	57	69.0 (37−95)	IHC	TII-αI index >35%*	NA	Ta (25) T1(26) T2 (6)	Low-grade (15) High-grade (17)	NA	NA (NA-70)	Worse CSS and higher CR (S)
Simon et al, 2003[18]	Switzerland	Retrospective cohort	1073	NA	IHC	>70%	313/886	Ta (480) T1 (313)	G1 (132) G2 (495) G3 (452)	NA	42 (1−236)	Worse CSS (S)

								T2−T4 (280)				
Kruger et al, 2005[19]	Germany	Prospective cohort	73	67 (37−85)	IHC	Median	25/26	T1 (73)	G2 (33) G3 (40)	TURBT with intravesical instillation (BCG)	NA	Worse RFS (S)
Abdelkrim et al, 2014[16]	Tunisia	Cross-sectional Study	71	63.1 (39−88)	IHC	>10%	28/43	Ta (39) T1 (32)	Low-grade (17) High-grade (15)	NA	28 (3−77)	Higher CR (S) OS (NS)
Raspollini et al, 2016[10]	Italy	Prospective cohort	92	72.2 (70.2−74.2)	IHC	>30%	62/30	T1 (92)	High-grade (92)	TURBT with intravesical instillation (BCG)	NA (13−170)	Better DFS (S) OS, and PFS (NS)
Elkady et al, 2018[17]	Egypt	Cross-Sectional Study	50	60.4 (59−70)	IHC	IRS score >4**	9/41	Ta (32) T1 (18)	Low-grade (26) High-grade (24)	TURBT in all patients with intravesical doxorubicin in low-risk and BCG instillation in high-risk patients	NA	Higher CR (S)

Zeng et al, 2019[8]	China	Prospective cohort	209	69 (58.6−79.4)	IHC	>67%	99/110	T1 (130) T2−T4 (79)	Low-grade (51) High Grade (158)	Radical cystectomy and TURBT	27.9 (10−95)	Worse CSS, PFS, and RFS (S)
Liu et al, 2022[9]	China	Prospective cohort	45	67.87 (33−88)	IHC	Score >8***	32/13	NMIBC (45)	High-grade (45)	Pirarubicin	53.81 (6−108)	Worse OS and lower CR (S)

*Tα-II Index: percentage of positively stained tumor cells in the area with greatest staining. **Immunoreactive score (IRS score): 0−2 was considered negative, 2−3 mild, 4−6 moderate, and 7−12 strong; IRS score is calculated by the intensity of staining and percentage of positive cases. ***Score: staining intensity score x frequency score of positive area; Intensity score is defined as 0−3 (negative, weak, moderate, strong); Frequency score is defined as 0−4 (<5%; 5–25%; 26–50%; 51–75%; and >75%). Staining intensity was rated as: 0=negative, 1=low, 2=moderate, and 3=strong. Stained cell percentages were: 0=negative, 1<10%, 2=10–50%, 3=51–80%, and 4 >80%. BCG: bacillus Calmette-Guérin; CR: cancer recurrence; CSS: cancer-specific Survival; DFS: disease-free survival; IHC: immunohistochemistry; NA: not available; NS: non-significant; OS: overall survival; PCR: polymerase chain reaction; PFS: progression-free survival; RFS: recurrence-free survival; S: significant; TURBT: transurethral removal of bladder tumor.