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# Prognostic and predictive role of circulating tumor DNA detection in patients with muscle invasive bladder cancer: a systematic review and meta-analysis

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## Abstract

**Background** At present, there is no effective prognostic indicator for muscle invasive bladder cancer (MIBC). A liquid biopsy method, plasma circulating tumor DNA (ctDNA) detection, was evaluated for use in predicting the prognosis of different cancers. This study aims to assess the prognostic value of ctDNA state for muscle-invasive bladder cancer patients.

**Methods** We comprehensively searched three public databases (PubMed, EMBASE, and the Cochrane Library) in December 2023 according to the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) statement. Studies investigating ctDNA and prognostic outcome indicators in patients with MIBC were included in our analysis. The hazard ratios (HRs) with 95% confidence intervals (CIs) were extracted to evaluate the association between ctDNA and the prognosis in patients with MIBC.

**Results** Eleven studies and 1,170 patients diagnosed with muscle-invasive bladder cancer, comprising a total of four retrospective cohort studies and eight prospective cohort studies, included in our meta-analysis, one of which had two different cohorts. The analysis revealed that a positive ctDNA state was associated with poor overall survival (OS), progression-free survival (PFS), and recurrence-free survival (RFS) in patients with MIBC (HR = 4.51, 95% CI: 2.64–7.69, $P < 0.001$; HR = 4.50, 95% CI: 2.77–7.30, $P < 0.001$; HR = 6.56, 95% CI: 4.18–10.30, $P < 0.001$), with significant prognostic effects both pre- and post-treatment. In addition, longitudinal ctDNA analysis proved to be effective in the monitoring of patients with MIBC receiving different treatments (HR = 0.24, 95% CI: 0.14–0.41, $P < 0.001$).

**Conclusions** A positive ctDNA state was associated with poor OS, PFS, and RFS in patients with MIBC pre- and post-treatment. Meanwhile, clearance of ctDNA was associated with improved RFS in patients with MIBC. These findings suggest that the ctDNA state is a predictive and prognostic indicator for patients with MIBC, which can be

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used to monitor recurrence and guide treatment. Thus, ctDNA level detection shows potential for the treatment and prognosis of patients with MIBC.

**Keywords** Muscle invasive bladder cancer, Circulating tumor DNA, Prognosis, Systematic review, Meta-analysis

## Background

Bladder cancer is the fourth most common cancer in men, accounting for 6% of estimated new cancers and 4% of cancer-related deaths [1]. Upon diagnosis, approximately a quarter of bladder cancer cases show invasion in the muscle tissue with a high risk of metastases, with the remainder showing non-muscle-invasive bladder carcinoma (NMIBC) [2]. Approximately 90% of bladder cancer cases are urothelial carcinoma; the remaining are mostly squamous cell carcinoma, adenocarcinoma, or neuroendocrine carcinoma [3–9]. According to the European Association of Urology (EAU) guidelines, radical cystectomy (RC) plus lymph node dissection is the most preferred treatment of muscle invasive bladder cancer (MIBC) [10], with 50% of patients developing distant metastasis after surgery [11]. The prognosis of bladder cancer after surgery is related to the pathological stage pre-surgery [12]. Pathologic complete response (pCR), which indicates the histopathological evaluation of bladder and lymph node specimens after bladder resection to confirm the absence of residual tumor lesions and lymph node metastasis (pT0N0M0) in the bladder, is associated with an increased OS. Recurrence occurs in around 30% of patients at a median of 12 months after cystectomy [1], highlighting the challenges associated with the treatment and management of bladder cancer.

Histopathology, clinical markers, and molecular markers have been extensively studied as prognostic and predictive factors for urinary bladder carcinoma [13–15]. However, traditional biomarkers pose challenges due to the heterogeneity of tumor tissue, as well as the dynamic changes associated with the treatment process, often failing to capture the dynamic nature of tumor progression [16]. In this context, liquid biopsy has emerged as a promising approach owing to its rapid and efficient nature, especially circulating tumor DNA used in various cancers [17], wherein the release of DNA into the circulatory system by dead tumor cells with an estimated half-life of 16 min to 2.5 h which is a prerequisite for its use as a real-time tumor biomarker [18]. The release of ctDNA is tumor-dependent, influenced by factors such as size, metastasis, and stage impacting the amount of ctDNA which could be detected from blood and urine via real-time quantitative polymerase chain reaction, droplet digital polymerase chain reaction (ddPCR) [19], sanger sequencing, and next-generation sequencing (NGS) [20, 21]. Gene mutations and methylation in ctDNA are the most commonly detected alterations and play a crucial role in cancer detection and progression [22]. Collecting blood samples is less invasive, safer, and more efficient than obtaining biological specimens through invasive tissue biopsies [23]. The test has the potential to target the minimal residual disease (MRD) window, with the objective of achieving a cure. It can assist patients in selecting appropriate adjuvant therapies and facilitate disease surveillance for early detection of recurrence during follow-up [24]. Blood specimens have historically been the most extensively studied method in liquid biopsy research. Recently, there has been a growing interest in exploring samples obtained from alternative sources, such as urine [25]. Urine-derived ctDNA sensitivity is potentially significantly higher, especially in cancers that have direct contact with urine. As the field progresses, further refinement and standardization of testing and sample collection methodologies are crucial for the clinical validation of ctDNA. This approach has been evaluated in patients with bladder cancer undergoing chemotherapy and neoadjuvant therapy [16]. Meanwhile, ctDNA has also been associated with disease burden [26], predicting tumor progression and recurrence through elevated levels several months before traditional detection methods [27–29]. Figure 1 presents a diagram illustrating the biology, detection and application of ctDNA.

In recent years, various studies have reported on the impact of the ctDNA state of patients with MIBC on their survival outcomes. In this context, this review aims to explore the predictive and prognostic role of the ctDNA state in patients with MIBC during the perioperative period.

## Methods

This systematic review and meta-analysis was conducted according to the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) guidelines [30] and the PRISMA statement [31]. The protocol used for the meta-analysis has been registered prospectively on the PROSPERO website (CRD42024532671).

### Literature search

Using "cell tumor DNA" and "bladder cancer" as keywords, we searched three databases up until December 2023: PubMed, EMBASE and the Cochrane Library. The references of relevant reviews and meta-analyses were also searched in case of omissions. Further details of the search strategies employed are provided in Additional File 1. The literature search process was completed independently by two reviewers (Wenqiang Qi and Xindong Gao).

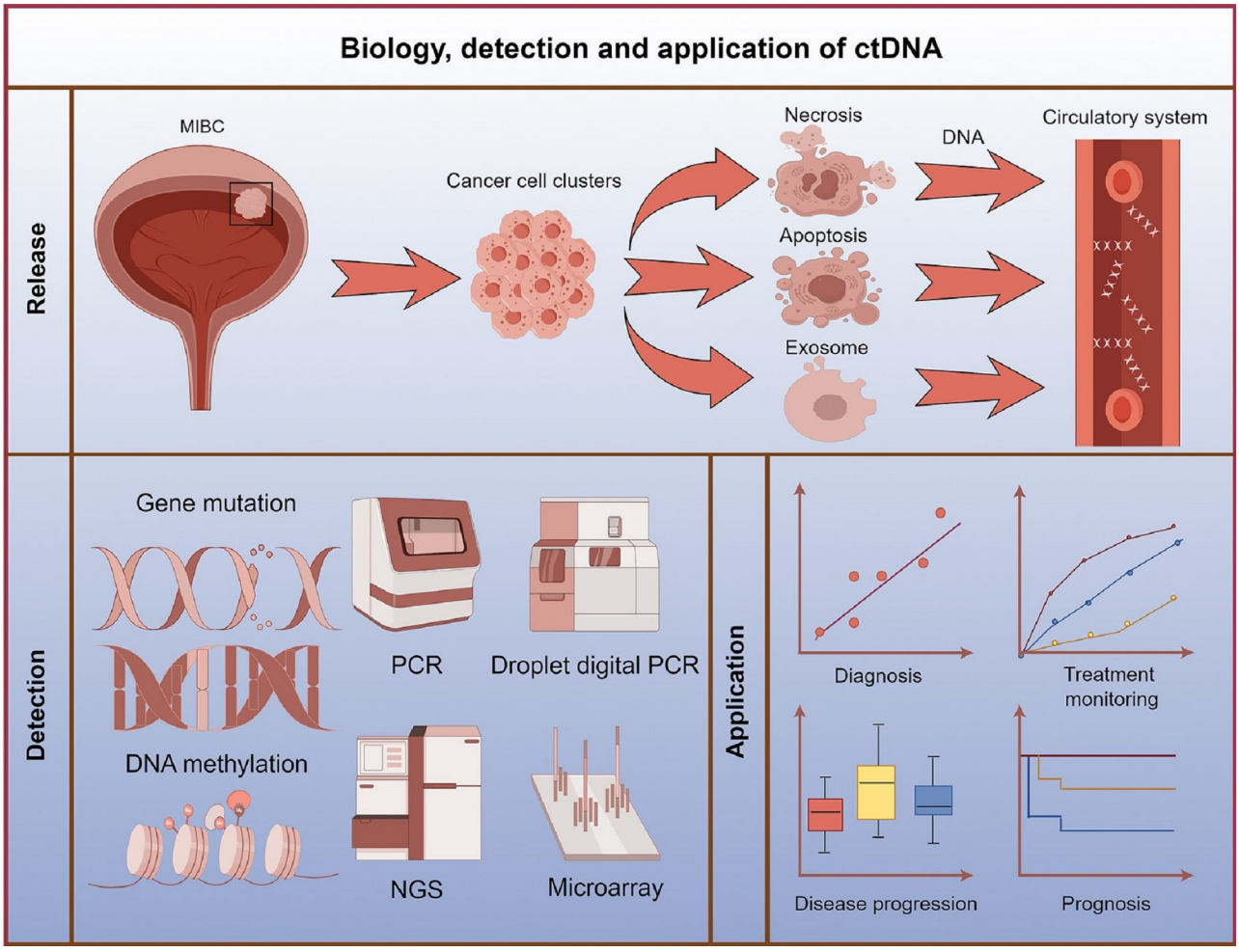


**Fig. 1** Characteristics of ctDNA. By Figdraw. ctDNA, circulating tumor DNA; MIBC, muscle invasive bladder cancer; PCR, polymerase chain reaction; NGS, Next-generation sequencing

### Inclusion and exclusion criteria

The inclusion criteria were as follows: (i) studies that included patients diagnosed with MIBC who received systematic treatment, including neoadjuvant chemotherapy plus radical cystectomy (NAC + RC), radical cystectomy (RC), or radical cystectomy plus adjuvant chemotherapy (RC + AC); (ii) studies that included data on the reported hazard ratios (HRs), corresponding 95% confidence intervals (CIs) for the ctDNA state, and survival outcomes of the patients; (iii) studies published in English. The exclusion criteria were as follows: (i) non-conforming article types, such as case reports, reviews, and conference abstracts; (ii) articles with no results of interest.

The two evaluators (Wenqiang Qi and Xindong Gao) reviewed the literature independently and excluded any studies unrelated to the topic (e.g. other disease types). By reading the abstracts of potential studies, the evaluators were able to identify those that met the exclusion criteria. The evaluators then reviewed the literature included in this initial round to agree on the final set of studies to be included in the meta-analysis. A third reviewer (Junxian Li) was tasked with resolving any discrepancies.

### Quality assessment

The quality assessment of the included cohort studies was evaluated using the Newcastle-Ottawa Quality Assessment Scale [23]. This scale assigns scores out of nine, splitting every three scores into distinct levels. The higher the score, the better the quality. Studies with an NOS score equivalent to or exceeding seven were included in our meta-analysis.

### Data extraction

Two reviewers (Xindong Gao and Wenqiang Qi) independently extracted relevant data from the included studies using a prefabricated table; any difference in opinions was resolved by discussion. From each study, the following data was extracted: (i) publication data: the name of first author, time of publication; (ii) cohort data: patient

source (country), study design, study period, tumor type, the type of treatment, sample size, age, ctDNA state, outcome, follow-up time. Furthermore, HRs with 95% CIs were extracted as the form of outcome variables. For studies with only survival curves, Engauge Digitizer V4.1 (Markmitch, Goteborg, Sweden) was used to extract data from the figures in the articles [32].

### Statistical and meta-analysis

The 95% CI of the HRs was calculated to evaluate the association between the ctDNA state and survival outcomes using Review Manager software (RevMan) (version 5.4) (Cochrane Collaboration). The heterogeneity levels were quantified using Cochran's Q-test and the $I^2$-square index: 0–25%, 25–50%, and 50–75% represented low, moderate, and considerable heterogeneity, respectively. A fixed-effect model was used to estimate the pooled effect size in cases of low or moderate heterogeneity. For significant heterogeneity, a random-effects model was employed to reduce potential biases. In addition, sensitivity analysis was conducted using a one-by-one elimination method to detect the stability of meta-analysis using STATA (version 17; StataCorp LLC, University of Texas Station, USA). A value of $p < 0.05$ for the bilateral test was defined as statistically significant.

## Results

### Literature screening

A total of 987 articles were initially identified for inclusion: 494 from PubMed, 401 from EMBASE, and 92 from the Cochrane Library database. After removing duplicates and browsing the full text, 11 papers [33–43] met the inclusion criteria and were included in the subsequent systematic review and meta-analysis. All patients were diagnosed with MIBC and received systematic treatment, including NAC + RC or RC or RC + AC. A flowchart of the research selection process is provided in Fig. 2.

### Characteristics of the studies and patient cohorts

Among the 11 studies included in the systematic review, one (Lindskrog et al.) included two different cohorts [36]. "Lindskrog* et al." was used to represent the prospective cohort study, while "Lindskrog et al." denoted the retrospective one. Thus, a total of four retrospective cohort studies [34, 36, 40, 43] and eight prospective cohort studies [33, 35–39, 41, 42] were included in the meta-analysis. Powles et al. [43] and Powles et al. [40] followed the same group of people at different period of time. The baseline characteristics and major survival outcomes of the included studies are summarized in Table 1. Six studies [33, 35–37, 41, 42] were conducted in Europe, one [34] in North America and four [38–40, 43] in other countries. The publication dates of the articles included in the analysis were between 2017 and 2023, and the follow-up time ranged from 18 months to 15 years. The sample sizes ranged from 17 to 581. A total of 1,170 patients were included in our meta-analysis.

### Research quality assessment

A quality assessment of the included studies was conducted using the Newcastle-Ottawa Quality Assessment Scale [23], based on a score out of nine, splitting every three scores into three levels. The higher the score, the better the quality. The NOS scores for each study are shown in the final column of Table 1, corresponding to scores that were ≥ 7 points, indicative of an acceptable research quality. The specific scores of each study included in our meta-analysis are presented in Additional File 2.

### Impact of ctdna state on OS

Five studies [34, 36, 37, 40, 43] with six cohorts, comprising a total of 876 patients, reported a relationship between the ctDNA state and overall survival (OS) in patients with MIBC. Vandekerkhove et al. [34] evaluated patients who were administered cisplatin or carboplatin or PD-1 before surgery, while Lindskrog* et al. [36] evaluated patients who received NAC but did not specify the type of treatment method. Powles et al. [43] evaluated patients who received NAC with atezolizumab. The other three cohorts [36, 37, 40] evaluated patients who received no adjuvant therapy. Blood sample collection was conducted after neoadjuvant chemotherapy and radical cystectomy. Despite significant heterogeneity ($I^2$ = 80%, $P < 0.001$), the analysis results showed that patients with MIBC with a ctDNA-positive state showed poor OS (HR = 4.51, 95% CI [2.64,7.69], $P < 0.001$) (Fig. 3), and it can be inferred that ctDNA-positive state is correlated with an unfavorable prognosis.

### Impact of ctdna state on PFS

Four studies [35, 37, 39, 41] involving 206 patients with MIBC reported on the association between the ctDNA state and progression-free survival (PFS). Carrasco et al. [35] evaluated patients, among which some were administered NAC or AC with cisplatin. Papadimitriou et al. [37] evaluated patients who did not receive adjuvant therapy. Van Dorp et al. [39] evaluated patients who received NAC with ipilimumab and nivolumab before surgery. Carrasco et al. [41] evaluated patients who received NAC or AC treatment, but they did not specify the type of treatment method. Blood samples were collected after neoadjuvant chemotherapy and radical cystectomy. A significant association was observed between a positive ctDNA state and poor PFS (HR = 4.50, 95% CI [2.77,7.30], $P < 0.001$), with low heterogeneity between the studies ($I^2$ = 0%, $P = 0.55$) (Fig. 4), which highly probably indicates

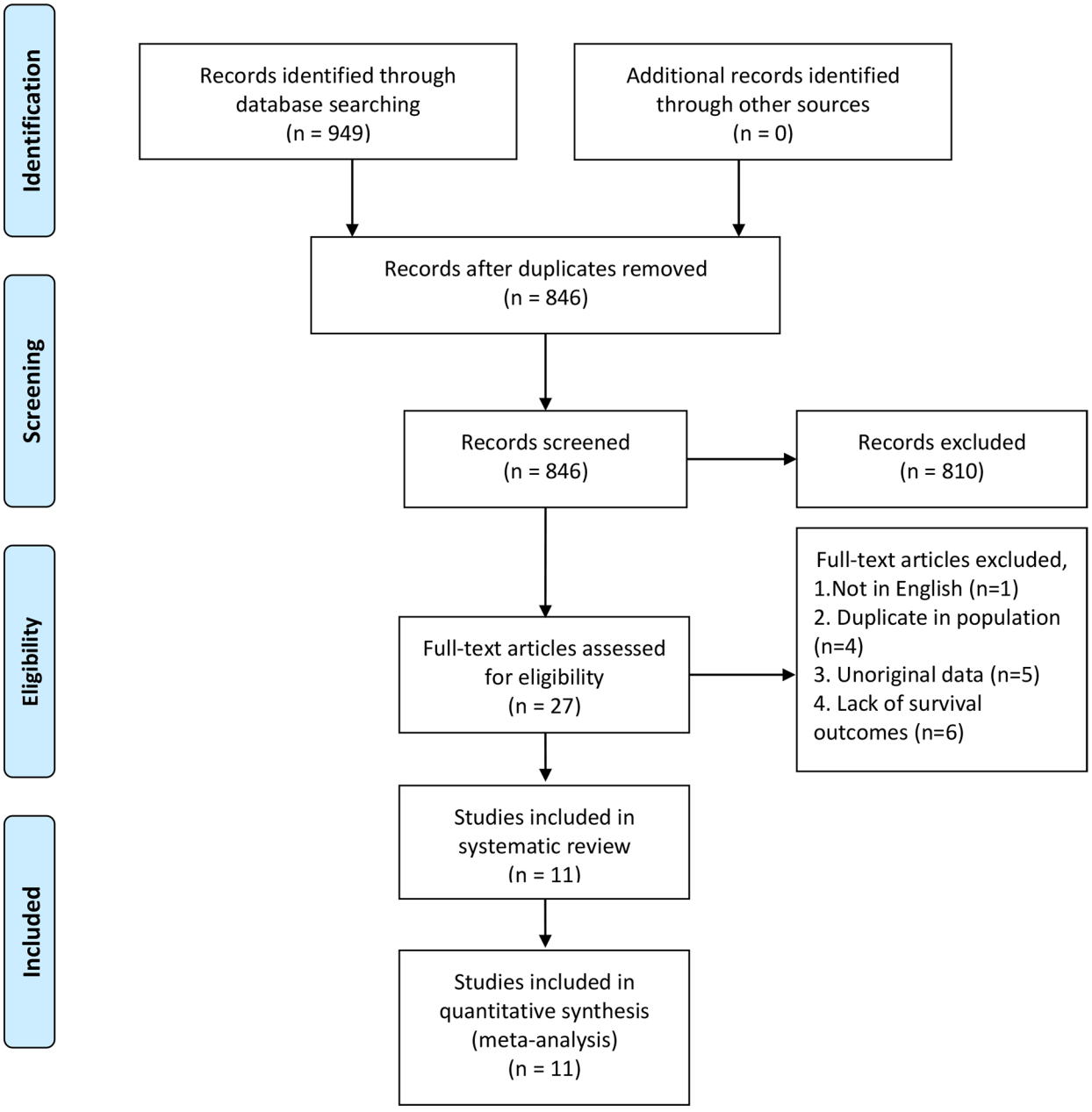


**Fig. 2** PRISMA flow diagram of literature retrieval. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses

a significant association between positive ctDNA state and reduced progression-free survival in patient with MIBC.

### Impact of ctdna state on RFS

Five studies [33, 34, 36, 38, 43] and six cohorts, comprising a total of 902 patients, reported a relationship between the ctDNA state and recurrence-free survival (RFS) in patients with MIBC. Patel et al. [33] evaluated patients among which some received NAC or AC with cisplatin. Vandekerkhove et al. [34] evaluated patients who received NAC with cisplatin or carboplatin or PD-1 before surgery. Lindskrog* et al. [36] evaluated patients who received NAC, but they did not specify the type of treatment method. Szabados et al. [38] evaluated patients among which some received NAC with atezolizumab. Powles et al. [43] evaluated patients among which some received NAC or AC with atezolizumab. Lindskrog et al. [36] evaluated patients who did not receive adjuvant chemotherapy. Blood samples were collected after

**Table 1** Baseline characteristics of included studies

| Author (year) | patient source(country) | Study design | Study period | Treatment | Sample size | Age | ctDNA state | Outcome | Follow-up (months) | NOS SCORE |
|---|---|---|---|---|---|---|---|---|---|---|
| Carrasco et al. 2023 [35] | Spain | PCS | 2019–2022 | NAC + RC/RC + AC | 42 | 67(50–80) | Positive/Negative | PFS | 21(6–37) | 8 |
| Lindskrog et al.2023 [36] | Denmark | RCS | 2001–2016 | RC | 102 | 69{62–75} | Positive/Negative | RFS/OS | 72 | 8 |
| Papadimitriou et al.2023 [37] | Athens | PCS | NA | RC | 86 | 70 | High/Low** | OS/MFS | 41[36.2–45.8] | 7 |
| van Dorp et al. 2023 [39] | Multi-country | PCS | 2017.12-2021.1 | NAC + RC | 41 | 70{65–73} | Positive/Negative | PFS | 11.7 | 8 |
| Patel et al. 2017 [33] | Netherlands | PCS | 2014.3-2015.10 | NAC + RC/CR/RAD | 17 | 59 | Positive/Negative | RFS | 24.7(16–32) | 9 |
| Lindskrog* et al. 2023 [36] | Denmark | PCS | 2013–2017 | NAC + RC | 68 | 67{59–71} | Positive/Negative Clearance/Remnant | RFS/OS | 68 | 8 |
| Szabados et al. 2022 [38] | Multi-country | PCS | 2016.5-2020.6 | NAC + RC | 95 | 73(53–87) | Positive/Negative | RFS | 25[25–26] | 8 |
| Vandekerkhove et al. 2021 [34] | Canada | RCS | 2014.12-2018.11 | NAC + RC | 39 | 67(37–88) | Positive/Negative | OS/RFS | 8.4 (0.3–33) | 7 |
| Powles et al. 2021 [40] | Multi-country | RCS | 2015.10-2019.11 | NAC + RC + AC/ RC + AC/AC | 581 | 67(31–88) | Positive/Negative | DFS/OS | 21.9(16–45) | 9 |
| Powles et al. 2023 [43] | Multi-country | RCS | 2015.10-2021.11 | NAC + RC + AC/ RC + AC/AC | 581 | 67(31–88) | Positive/Negative | DFS/OS | 46.8{36.1–53.6} | 9 |
| Christensen et al. 2022 [42] | Denmark | PCS | 2013–2019 | NAC + RC | 92 | NR | Clearance/Remnant | RFS | 41.3 | 7 |
| Carrasco et al. 2022 [41] | Spain | PCS | 2018–2021 | NAC + RC/RC + AC | 37 | 71(51–85) | Positive/Negative | PFS | 36 | 8 |

*, this study has two different cohorts that can be included in the meta-analysis. PCS, prospective cohort study; RCS, retrospective cohort study; MIBC, muscle invasive bladder cancer; NAC, neoadjuvant chemotherapy; AC, adjuvant chemotherapy; RC, radical cystectomy; CR, chemoradiotherapy; RAD, radiotherapy; [], Confidence Interval; (), range; {}, interquartile range; NR, Not Reported; **, we treat High/Low as Positive/Negative when conducting analysis. OS, overall survival; PFS, progression-free survival; RFS, recurrence-free survival; MFS, metastasis-free survival; DFS, disease-free survival; More than 90% of patients included in studies conducted by Powles were diagnosed with MIBC and < 10% were diagnosed with nMIBC, so these studies were therefore assigned to the MIBC group

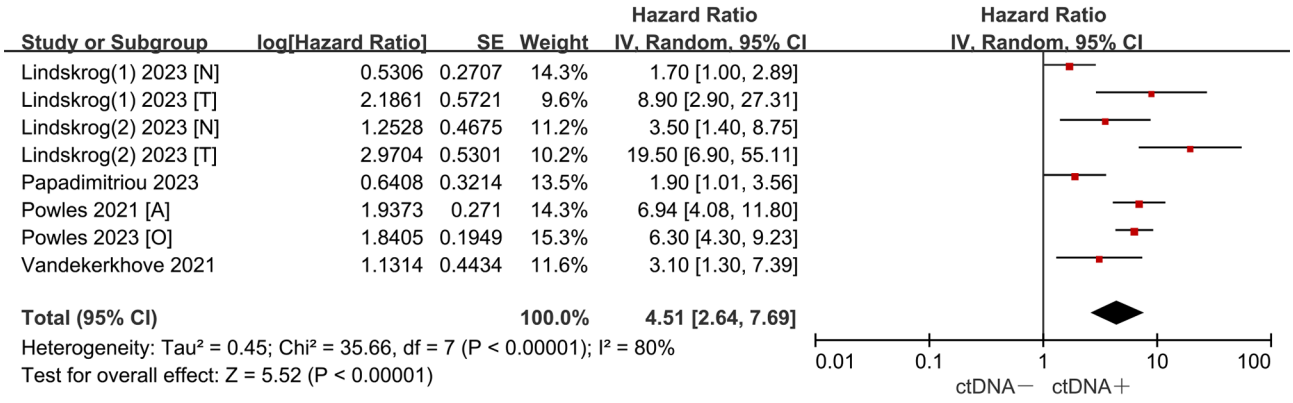


**Fig. 3** Forest plot of the association between ctDNA and OS in MIBC patients. ctDNA, circulating tumor DNA; OS, overall survival; MIBC, muscle invasive bladder cancer; (1), before treatment; (2), after treatment; [N], NAC-naïve cohort; [T], NAC-treated cohort; [A], atezolizumab group; [O], observe group

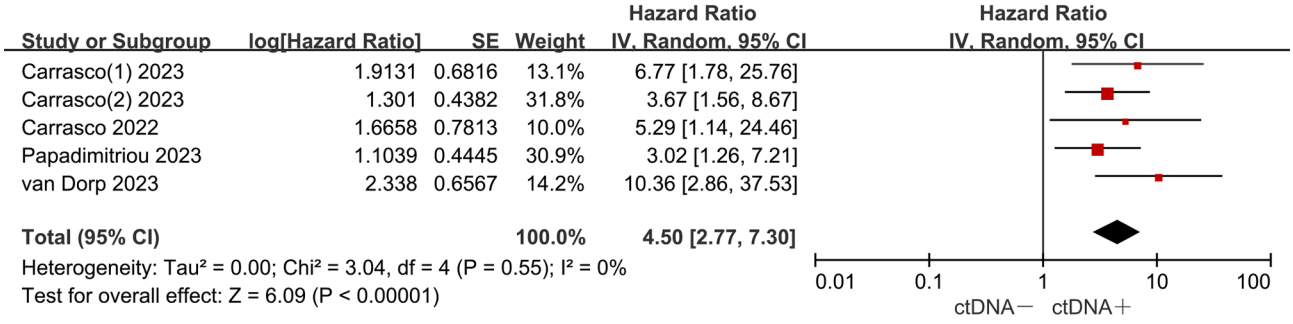


**Fig. 4** Forest plot of the association between ctDNA and PFS in MIBC patients. PFS, progression-free survival; MIBC, muscle invasive bladder cancer; (1), before treatment; (2), after treatment

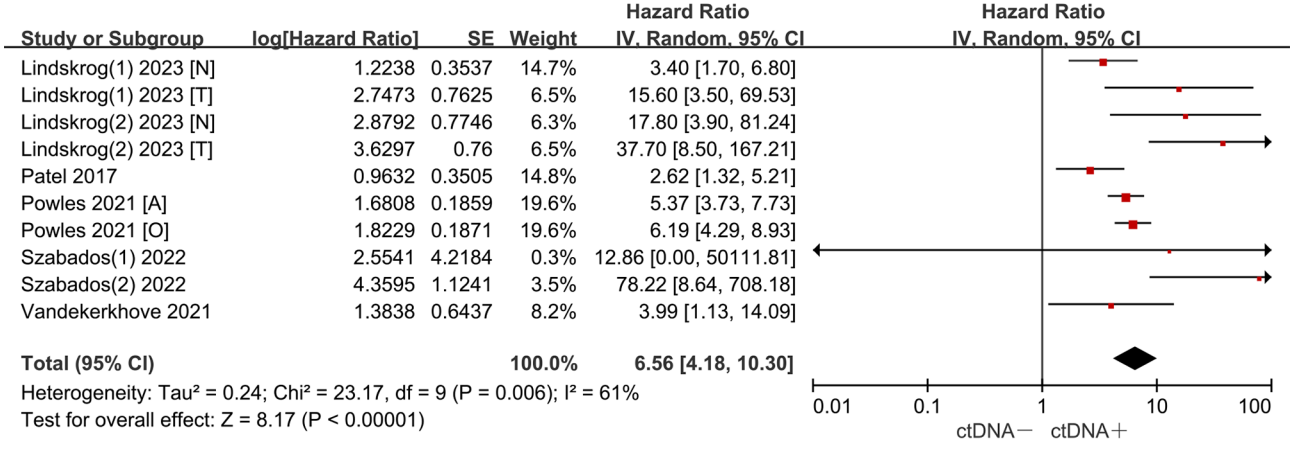


**Fig. 5** Forest plot of the association between ctDNA and RFS in MIBC patients. RFS, recurrence-free survival; MIBC, muscle invasive bladder cancer; (1), before treatment; (2), after treatment; [N], NAC-naïve cohort; [T], NAC-treated cohort; [A], atezolizumab group; [O], observe group

neoadjuvant chemotherapy and radical cystectomy. The results showed in large probability that patients with MIBC with a ctDNA-positive state had a poor RFS (HR = 6.56, 95% CI [4.18,10.30], $P < 0.001$), with a high heterogeneity ($I^2 = 61\%$, $P = 0.006$) (Fig. 5).

### Subgroup analysis

According to Vandekerkhove et al. [33, 34, 36, 38, 43], the ctDNA state and level may change during treatment. Furthermore, the ctDNA state post-treatment was found to be significantly lower than that in samples collected before or during treatment (Kruskal–Wallis $P = 2.4^* e^{-7}$

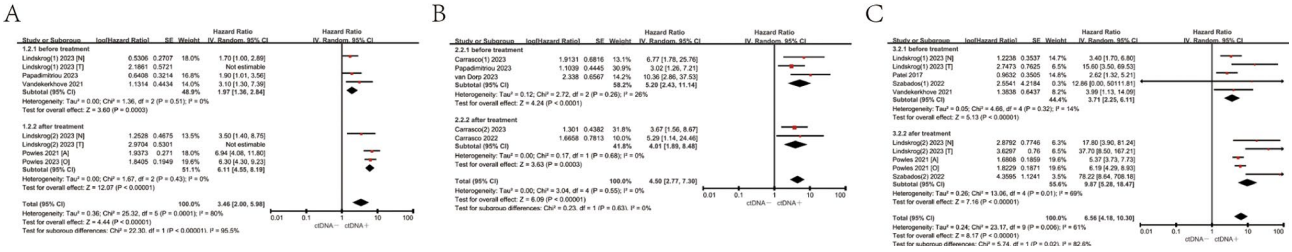


**Fig. 6** Forest plot of the association between dynamic ctDNA and RFS in MIBC patients. RFS, recurrence-free survival; MIBC, muscle invasive bladder cancer. [T], NAC-treated cohort; [A], atezolizumab group; [O], observe group

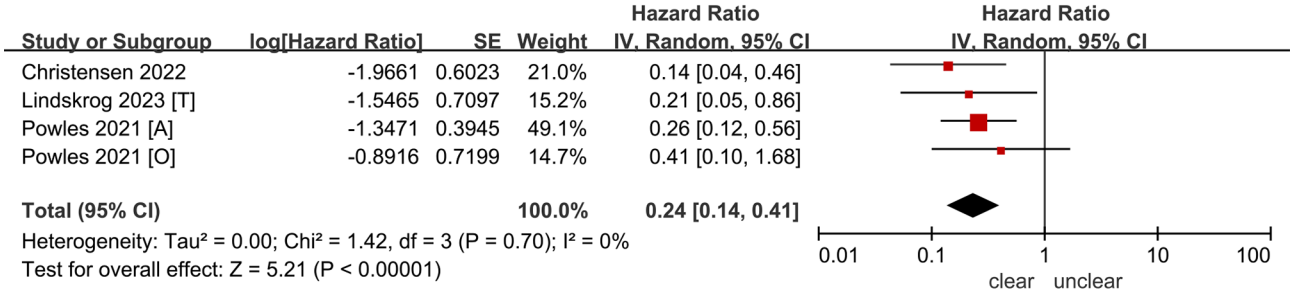


**Fig. 7** Subgroup analyses of (A) OS, (B) PFS and (C) RFS according to time. OS, overall survival; PFS, progression-free survival; RFS, recurrence-free survival; (1), before treatment; (2), after treatment; [N], NAC-naïve cohort; [T], NAC-treated cohort; [A], atezolizumab group; [O], observe group

), indicating the importance of this factor in future clinical work. As a result, the patient cohorts in of the studies included in our meta-analysis were divided into two subgroups based on the time of treatment: pre-treatment and post-treatment. The results of subgroup analysis indicated that a positive ctDNA state was probably significantly associated with a poorer OS, PFS, and RFS in patients with MIBC both pre- and post-treatment (Fig. 6).

### Impact of dynamic ctdna state on RFS

Three studies, comprising a total of 741 patients [36, 42, 43], reported on the relationship between the dynamic ctDNA state and recurrence-free survival (RFS) in patients with MIBC. Powles et al. [43] evaluated patients among which some were administered NAC or AC with atezolizumab. The other two studies [36, 42] evaluated patients who received NAC, but they did not specify the type of treatment method. Blood samples were collected after neoadjuvant chemotherapy and radical cystectomy. As shown in Fig. 7, there was no heterogeneity ($I^2$= 0, $P$ = 0.70) among the studies, and the results of our analysis indicated that patients with MIBC who exhibited clearance of ctDNA post-treatment had a better RFS (HR = 0.24, 95% CI [0.14,0.41], $P$ < 0.001), which means that clearance of ctDNA is likely to be an indicator of curative effect and guide treatment.

### Sensitivity analysis

Sensitivity analyses were conducted on the results of the studies included in this review to evaluate the stability of the meta-analysis models. Changes in the overall HR estimates for these survival outcomes were not significant, indicating that our meta-analysis results were stable (Fig. 8).

## Discussion

Bladder cancer is the 10th most common cancer in the world, with number of deaths caused by this disease increasing each year [44]. At present, the main prognostic indicators for patients with MIBC are histopathological indicators, such as tumor stage and lymph node state, for which molecular markers serve as auxiliary predictive indicators. The ctDNA, that is, the DNA released into the blood by necrotic and apoptotic cancer cells, has diagnostic, therapeutic, and prognostic value in clinical practice [45]. To the best of our knowledge, this review is the first to assess the role of ctDNA monitoring in predicting the prognosis of patients with MIBC. Ultimately, 11 studies were included in our meta-analysis, comprising a total of 1,170 patients. First, we found that a positive ctDNA state among patients with MIBC was positively correlated with a lower OS, PFS, and RFS. In addition, we analyzed the dynamic changes in the ctDNA state of patients with MIBC, concluding that patients with ctDNA clearance after treatment had a better RFS. The results of subgroup analysis showed that the ctDNA state pre- and post-treatment was significantly correlated with

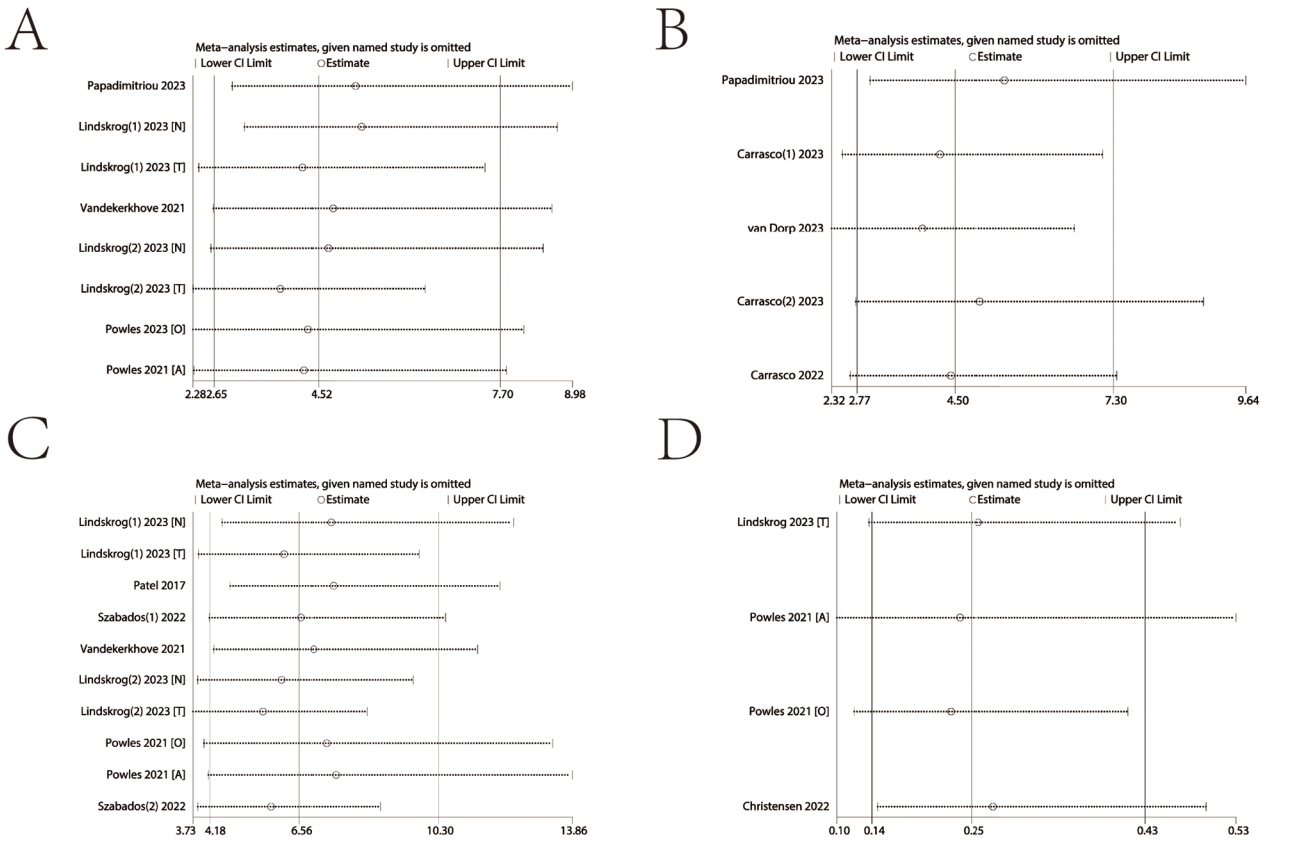


**Fig. 8** Sensitivity analysis of results for (**A**) OS, (**B**) PFS and (**C**) RFS in MIBC patients OS, overall survival; PFS, progression-free survival; RFS, recurrence-free survival; MIBC, muscle invasive bladder cancer; (1), before treatment; (2), after treatment; [N], NAC-naïve cohort; [T], NAC-treated cohort; [A], atezolizumab group; [O], observe group

prognosis. Furthermore, the treatment methods included in our analysis were not entirely consistent, indicating that the prognostic and predictive role of ctDNA has a wide range of applicable populations.

At present, the relationship between the ctDNA state and prognosis of patients with bladder cancer remains unclear. Crupi et al. [16] conducted a systematic review including a total of six studies and 845 patients with MIBC. Their review confirmed the significant prognostic role of ctDNA state after cystectomy and identified the potential predictive benefits of NAC and preoperative immunotherapy environment, serving as a basis for patient stratification and the development of personalized treatment plans in clinical practice. However, due to patient heterogeneity, different endpoints, and the time points of ctDNA analysis, statistical analysis was not conducted. A meta-analysis conducted by Gögenur et al. [46] included several different cancers and reached a similar conclusion: ctDNA detection at different time points of patients with new adjuvant therapy is significantly correlated with recurrence. Building on Crupi's foundation, the present study introduces several recent studies [35–37, 39, 40], all of which included patients with MIBC. Unlike previous studies, several retrospective studies [34, 36, 40, 43] were also included in this study, and a meta-analysis was conducted, allowing for a more intuitive analysis to be conducted compared to a simple review.

Meanwhile, a considerable number of studies have discussed the relationship between ctDNA state and other cancers, including meningioma, esophageal cancer, lung cancer, and colorectal cancer [45, 47–49]. These studies have shown that a high or positive ctDNA state is associated with a poor prognosis. According to Chen et al. [48], the transition of the ctDNA state during the perioperative period may be indicative of the therapeutic effect, acting as a guide for postoperative treatment. Therefore, we believe that the transition of the ctDNA state in patients with MIBC shows potential as a topic of research in postoperative adjuvant therapy.

In addition to plasma ctDNA, other molecular markers have also been widely used in the prediction and prognosis of bladder cancer. Abe et al. [50] demonstrated the predictive and prognostic value of urine granule DNA in recurrent bladder cancer and found that the monitoring

of the urine pellet DNA variant allele frequency (UpDNA VAF) has reasonable clinical efficacy in patients with bladder cancer. According to Wang et al. [13], snoRNA and lincRNA play an important role in the occurrence and development of bladder cancer, which may be related to the prognosis of cancer.

The mechanism by which ctDNA plays a role in patient prognosis is not yet clear. The most widely accepted view is that ctDNA carries genetic characteristics related to tumor cells, such as gene mutations, methylation, amplification, or rearrangement, and can serve as an important indicator for tumor screening, diagnosis, treatment efficacy evaluation, and prognostic risk stratification. The level of ctDNA generally shows dynamic changes and is influenced by multiple factors. Among these, factors such as the pathological type, location, and stage of tumor tissue may affect the release of ctDNA. A large amount of DNA from other sources may interfere with the monitoring of ctDNA. Since the half-life of ctDNA is relatively short (generally < 2 h), the use of different sampling times, preservation methods, and detection methods may lead to differing results. Some chemotherapy drugs or immunosuppressants can also affect ctDNA content. These are among some of the obstacles currently hindering the continued application of ctDNA detection.

The pathological response is closely related the ctDNA state. Due to limited data available and the different outcome variables, quantitative analysis cannot be conducted. Carrasco et al. [41] found that the ctDNA state during bladder resection was directly related to a higher pathological stage in patients with MIBC; in other words, patients with positive ctDNA tended to have more advanced disease. Christensen et al. [51] found that the presence and dynamics of ctDNA during chemotherapy were associated with decreased pathological staging. Lindskrog et al. [36] found that the ctDNA state and dynamics during NAC were highly correlated with pathological staging ($P < 0.0001$). In addition, in predicting the treatment efficacy and patient prognosis after RC, the ctDNA state before RC and the ctDNA dynamics during NAC were found to be superior to pathological staging. Papadimitriou et al. [37] summarized the cell free DNA (cfDNA) level as being closely related to a higher degree of pathological tumor staging ($P < 0.05$). Van Dorp et al. [39] observed no correlation between preoperative urinary ctDNA deletion and pathological reactions ($P = 0.39$). Despite its relevance and importance, a meta-analysis on the correlation between the ctDNA state and the pathological response in patients treated with a neoadjuvant strategy cannot be conducted at present due to different outcome variables.

The systematic review and meta-analysis presented in this study has several limitations that warrant consideration. Firstly, the geographical distribution of the included studies may introduce biases related to racial or regional factors, potentially affecting the generalizability of the findings. Secondly, regarding sample size and follow-up duration, only two studies featured large sample sizes, while the majority had smaller samples; notably, one study had a particularly short follow-up period. Additionally, the number of studies included in each analysis was limited to fewer than ten, which complicates the interpretation of funnel plot asymmetry. Furthermore, due to the relatively small overall sample size, it was not feasible to conduct a robust assessment of publication bias [52, 53]. This limitation is significant and may contribute to observed heterogeneity. Moreover, variations in therapeutic approaches, timing of sample collection, and methods of sample testing could also be sources of heterogeneity. Therefore, future prospective clinical studies with larger sample sizes are necessary to more accurately evaluate the relationship between ctDNA levels and patient prognosis in MIBC, as well as to validate our findings.

## Conclusions

This systematic review and meta-analysis found that a positive ctDNA state in patients with MIBC was associated with a poor OS, PFS, and RFS, while ctDNA clearance post-treatment was associated with a longer RFS. These results indicate that ctDNA state shows promise as a prognostic factor for patients with MIBC, suggesting that the use of ctDNA could facilitate the early diagnosis and treatment of bladder cancer to some extent.

### Abbreviations

| | |
|---|---|
| MIBC | Muscle invasive bladder cancer |
| CtDNA | Circulating tumor DNA |
| PRISMA | Preferred Reporting Items for Systematic Review and Meta-analysis |
| HR | Hazard ratios |
| CI | Confidence intervals |
| OS | Overall survival |
| PFS | Progression-free survival |
| RFS | Recurrence-free survival |
| NMIBC | Non-muscle-invasive bladder carcinoma |
| EAU | European Association of Urology |
| RC | Radical cystectomy |
| Pcr | Pathologic complete response |
| ddPCR | Droplet digital polymerase chain reaction |
| NGS | Next-generation sequencing |
| MRD | Minimal residual disease |
| MOOSE | Meta-Analysis of Observational Studies in Epidemiology |
| NAC | Neoadjuvant chemotherapy |
| AC | Adjuvant chemotherapy |
| NOS | Newcastle-Ottawa Quality Assessment Scale |
| UpDNA VAF | Urine pellet DNA variant allele frequency |
| cfDNA | Cell free DNA |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12935-025-03707-z.

Supplementary Material 1: Additional file 1: Title of data: Search strategy. Description of data: Complete retrieval strategy in the PubMed, EMBASE,

and the Cochrane Library.

Supplementary Material 2: Additional file 2: Title of data: Detailed quality assessment of cohort study. Description of data: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability. Studies rates ≥ 6 are eligible. NOS, Newcastle-Ottawa Scale. *, this study has two different cohorts that can be included in the meta-analysis. PCS, prospective cohort study. A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability. Studies rates ≥ 6 are eligible. Xa not qualified study. NOS, Newcastle-Ottawa Scale.

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Not applicable.

**Author contributions**
XDG and WQQ contributed to the design and BKS and XWJ provided administrative support. XDG, WQQ, PZD, DYG, JXL and YYX collected and extracted data, and conducted statistical analysis. The Manuscript writing and final approval of the manuscript are jointly undertaken by all authors.

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**Data availability**
No datasets were generated or analysed during the current study.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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## Publisher's note