The prognostic and diagnostic value of circulating tumor cells in bladder cancer and upper tract urothelial carcinoma: a meta-analysis of 30 published studies

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ABSTRACT

There are inconsistent conclusions in the association between circulating tumor cells (CTCs) and urothelial cancer (UC). We performed a meta-analysis to assess the prognostic and diagnostic value of CTCs in UC. We search Medline, Embase and Web of science for relevant studies. The study was set up according to the inclusion/exclusion criteria. 30 published studies with a total of 2161 urothelial cancer patients were included. Meta-analysis showed that CTC-positive was significantly associated with tumor stage (≤ II vs III, IV) (OR = 4.60, 95% CI: 2.34–9.03), histological grade (I, II vs III) (OR = 2.91, 95% CI: 1.92–4.40), metastasis (OR = 5.12, 95% CI: 3.47–7.55) and regional lymph node metastasis (OR = 2.47, 95% CI: 1.75–3.49). It was also significantly associated with poor overall survival (OS) (HR = 3.98, 95% CI: 2.20–7.21), progression/disease-free survival (PFS/DFS) (HR = 2.22, 95% CI: 1.80–2.73) and cancer-specific survival (CSS) (HR = 5.18, 95% CI: 2.21–12.13). Overall sensitivity and specificity of CTC detection assays were 0.35 (95% CI: 0.28–0.43) and 0.97 (95% CI: 0.92–0.99) respectively. In summary, our meta-analysis suggests that the presence of CTCs in the peripheral blood is an independent predictive indicator of poor outcomes for urothelial cancer patients. It can also be used as a noninvasive method for the confirmation of cancer diagnosis. More studies are required to further explore the role of this marker in clinical practice.

INTRODUCTION

Bladder cancer (BC) is the most common malignancy of the urinary tract and the ninth most common cancer worldwide. About 95% of bladder cancers are urothelial carcinomas histologically, with rare cases of squamous cell carcinoma and adenocarcinoma [1–3]. Upper tract urothelial carcinoma (UTUC) arising from renal pelvis or ureter is uncommon, accounting for only 5–10% of all urothelial carcinomas [1–3]. Growing evidences have suggested that there are certain significant similarities between BC and UTUC [4]. Furthermore, the behavior of both diseases is identical after adjusting for tumor stage and histological grade [5]. The standard methods for diagnosis of BC and UTUC include cytologic evaluation of urine, imaging tests and cystoscopy [6]. However, the cost for a cystoscopy is considerable expensive and it is an invasive examination with risk of complications. Furthermore, there still lacks effective biomarkers for predicting the prognosis of these patients. Alternative methods which help to diagnose and monitor in real time are urgently needed.

Circulating tumor cells (CTCs) are tumor cells that originate from a primary tumor, flowing through the bloodstream and circulating throughout the body, which may contribute to hematogenous metastasis [7]. The first
report on metastatic tumor cells in the peripheral blood of cancer patients was presented by Ashworth in 1869 [8]. The detection of CTCs focuses on a new method of detecting metastatic disease earlier and being less invasive than currently available conventional methods, such as clinical manifestation and radiographic evaluation. In recent decades, a variety of approaches for detecting CTCs have been developed and applied to clinical settings, including immunocytochemistry (ICC), reverse-transcriptase polymerase chain reaction (RT-PCR), flow cytometry (FCM) and the CellSearch system, which is the only approach approved by the US Food and Drug Administration (FDA) [9]. Recently, many researchers have reached the conclusion that the presence of CTCs is a poor prognostic indicator for breast, colorectal and gastric cancers [10–13]. However, it remains unclear whether this conclusion can also apply to different clinical outcomes from urothelial cancer (UC), a definition which encompasses BC and UTUC. Several studies focusing on UC have showed that CTC-positive was associated with poor prognosis, and the number of CTCs may be associated with tumor stage and therapeutic effects [14, 15]. Whereas others failed to show such association [16–18].

With the aim to further clarify the issue, we performed a meta-analysis of published literatures to quantitatively assess the association of CTC-positive with clinicopathological features and prognosis of patients with UC. A second objective was to pool together and summarize quantitatively the available evidence with regards to diagnostic accuracy of CTC detection in UC.

RESULTS

Identification of relevant studies

A total of 698 records were identified by initial retrieval, and 473 records were selected after removing duplicates. After screening the titles and abstracts, 418 irrelevant records were excluded. There left 55 full manuscripts for detailed evaluation, of which 26 studies were further excluded for small sample size, poor study design or insufficient data. One additional study was identified by inspection of the bibliographies of previous systematic reviews [19]. Finally, 30 eligible studies were included for meta-analysis [14–43]. The flow diagram of study selection is presented in Figure 1.
Baseline characteristics

A total of 2161 patients from 30 articles were involved. Included studies were conducted in 9 countries and published between 1999 and 2016. Between all the studies, 6 were undertaken among BC and UTUC patients, and the others only referred to BC. There were 20 studies available for the clinicopathological characteristics, 5 studies related with the prognosis and 23 studies implicated in the diagnostic accuracy of CTCs. Detection methods included CellSearch system, RT-PCR, enzyme-linked immunosorbent assay (ELISA) and other ICC. Characteristics of the included studies are shown in Table 1.

Correlation of CTC-positive with clinicopathological parameters

We analyzed 1339 samples from 20 studies to assess whether CTC-positive was associated with UC clinicopathological parameters, including tumor stage, histological grade, metastasis and regional lymph node metastasis. The meta-analysis of all 14 relevant studies on tumor stage indicated a significantly lower incidence of CTCs in the stage ≤ II group relative to the stage III–IV group (OR = 4.60, 95% CI: 2.34–9.03; P < 0.001; random-effect) with moderate heterogeneity (I^2 = 53.8%) (Figure 2A). 12 studies were used to assess the relationship between CTC-positive and histological grade. We found that CTC positivity in grade III is greater than that in grade I–II (OR = 2.91, 95% CI: 1.92–4.40; P < 0.001; fixed-effect) with low heterogeneity (I^2 = 36.7%) (Figure 2B).

The ORs for metastasis were available in 15 studies, and the estimated pooled OR showed a significant relationship between CTC-positive and disease metastasis: OR = 5.12 (95% CI: 3.47–7.55; P < 0.001; fixed-effect) with moderate heterogeneity (I^2 = 47.4%) (Figure 2C). Results were similar for regional lymph node metastasis: OR = 2.47 (95% CI: 1.75–3.49; P < 0.001; fixed-effect) with moderate heterogeneity (I^2 = 49.0%) (Figure 2D). Sensitivity analysis suggested that the results were not altered substantially by individual studies (Figure 3A–3D) except in the case of the effect of one study [30] on the combined OR of metastasis (Figure 3C). No significant publication bias was detected by Begg’s test.

Impact of CTC-positive on survival

Survival analysis according to CTC status was performed in 5 studies accounting for 361 patients. OS was analyzed in 3 studies. The pooled HR showed that

Figure 2: Forest plots of association between the presence of CTCs and (A) TNM staging, (B) histological grade, (C) disease metastasis, (D) regional lymph node metastasis.
CTC-positive was highly correlated with poorer OS and higher risk of death compared with CTC-negative: HR = 3.98 (95% CI: 2.20–7.21; P < 0.001). With regard to PFS/DFS, 4 studies were analyzed that comprised 317 patients. The pooled HR showed that CTC-positive was associated with a significantly increased risk of disease progression: HR = 2.22 (95% CI: 1.80–2.73; P < 0.001). Data on CSS were available only in 2 studies, and we found that CTC-positive was associated with a prognosis of poor CSS: HR = 5.18 (95% CI: 2.21–12.13; P < 0.001). No significant heterogeneity was detected in any analysis (I² < 50%) (Figure 4). Sensitivity analysis suggested that no individual studies significantly affected the pooled HRs.

### Diagnostic accuracy of CTC detection

When all eligible studies and assays were pooled into the diagnostic accuracy meta-analysis, the overall sensitivity and specificity were 0.35 (95% CI: 0.28–0.43) and 0.97 (95% CI: 0.92–0.99) respectively with significant heterogeneity (I² = 89.40% and 89.71%) (Figure 5). Additionally, the pooled Positive Likelihood Ratio (PLR) and Negative Likelihood Ratio (NLR) were 11.2 (95% CI: 4.5–27.5) and 0.67 (95% CI: 0.60–0.76) respectively. The diagnostic odds ratio (DOR) was 17 (95% CI: 6–43). Figure 6 presented the summary receiver operator characteristic (sROC) curve for the included studies, which presents a global summary of

**Table 1: Baseline characteristics of included studies**

| First author | Year | Country | Patients | Tumor stage (ACJJ) | Methods | Target antigen/target gene | Cut off | Prognostic outcome |
|--------------|------|---------|----------|-------------------|---------|---------------------------|--------|-------------------|
| Winters [23] | 2015 | America | BC+UTUC | II–IV             | CellSearch | EpCAM                    | -      | -                 |
| Alva [24]    | 2015 | America | BC      | II–IV            | IsoFlux | EpCAM | 10 CTCs/7.5 ml | -      | -                 |
| Gazzaniga [29] | 2014 | Italy   | BC      | I                | CellSearch | EpCAM                    | -      | -                 |
| Lu [37]      | 2000 | Japan   | BC+UTUC | 0a–IV           | Nested RT-PCR | UPII               | -      | -                 |
| Retz [18]    | 2001 | Germany | BC     | 0a–IV           | RT-PCR | CK20                      | -      | -                 |
| Kinjo [34]   | 2004 | Japan   | BC     | 0a–IV           | Nested RT-PCR | MUC7               | -      | -                 |
| Flaig [14]   | 2011 | America | BC     | 0a–IV           | CellSearch | EpCAM                    | OS     | -                 |
| Rink [42]    | 2011 | Germany | BC     | 0a–IV           | CellSearch | EpCAM | 1 CTC/7.5 ml | OS/PFS/CSS |
| Li [36]      | 1999 | America | BC     | NR              | RT-PCR | UPII                      | -      | -                 |
| Naoe [15]    | 2007 | Japan   | BC+UTUC | 0a–IV           | CellSearch | EpCAM | 2 CTCs/10 ml | -      | -                 |
| Fujii [28]   | 1999 | Japan   | BC+UTUC | 0a–IV           | Nested RT-PCR | CK20               | -      | -                 |
| Gazzaniga [30] | 2001 | Italy   | BC     | 0a–IV           | RT-PCR | EGFR/UPII/CK19/CK20      | -      | -                 |
| Gudermann [33] | 2000 | Germany | BC+UTUC | 0a–IV           | Nested RT-PCR | CK20               | -      | -                 |
| Okegawa [39] | 2010 | Japan   | BC+UTUC | I–IV            | CellSearch | EpCAM                    | -      | -                 |
| Antoniewicz [25] | 2012 | Poland  | BC     | 0a–IV           | RT-PCR | EGFR/COL1A1               | -      | -                 |
| Ribak [17]   | 2006 | Spain   | BC     | 0a–IV           | Nested RT-PCR | CK20               | -      | -                 |
| Rink [21]    | 2012 | Germany | BC     | 0a–IV           | CellSearch | EpCAM | OS/PFS/CSS |
| Gradilone [20] | 2010 | Italy   | BC     | I                | CELLlection/RT-PCR | EpCAM/Survivin | -      | DFS               |
| Gazzaniga [31] | 2012 | Italy   | BC     | 0a–I            | CellSearch | EpCAM                    | -      | -                 |
| Soria [43]   | 2002 | France  | BC     | 0a–IV           | Telomerase assay | Telomerase activity | -      | -                 |
| Guzzo [16]   | 2012 | America | BC     | 0a–IV           | CellSearch | EpCAM                    | -      | -                 |
| Okegawa [40] | 2004 | Japan   | BC     | 0a–IV           | Nested RT-PCR | UPII/CK20      | -      | DFS               |
| Todenhofer [22] | 2016 | Germany | BC     | 0a–IV           | RT-PCR | HER2/MUC1/EpCAM/ALDH1/ TWIST/akt2/p13Kct | -      | -                 |
| Leotsakos [35] | 2014 | Greece  | BC     | 0a–IV           | RT-PCR | EGFR/CK19/CK20               | -      | -                 |
| Osman [41]   | 2004 | America | BC     | III–IV          | Nested RT-PCR | UPIa/UPIb/UPI/UPII/EGFR   | -      | -                 |
| Meye [38]    | 2002 | Germany | BC     | 0a–IV           | ICC     | CK                        | -      | -                 |
| Gazzaniga [32] | 2005 | Italy   | BC     | I–IV            | RT-PCR | Tenascin U/EGFR          | -      | -                 |
| Desgrandchamps [27] | 1999 | UK      | BC     | 0a–IV           | ICC     | CK                        | -      | -                 |
| Allard [19]  | 2004 | America | BC     | IV              | CellSearch | EpCAM                    | -      | -                 |
| Champelovier [26] | 1999 | France  | BC     | NR              | Nested RT-PCR | CK20               | -      | -                 |

BC: Bladder Cancer; UTUC: Upper Tract Urothelial Carcinoma; CTCs: Circulation Tumor Cells; RT-PCR: Quantitative Reverse Transcription Polymerase Chain Reaction; OS: Overall Survival; PFS: Progression-Free Survival; DFS: Disease-Free Survival; CSS: Cancer-Specific Survival.
test performance. CTCs yielded an area under the curve (AUC) of 0.70 (95% CI: 0.66–0.74), indicating a moderate accuracy of the diagnostic test. According to the Deek’s funnel plot asymmetry test, the P value was 0.76 for the slope coefficient, which showed there was no significant publication bias (Figure 7).

To explore the potential source of heterogeneity, we conducted subgroup analysis stratified by geographical location, control type, sample size and method. The pooled sensitivity, specificity, PLR, NLR, and DOR for each subgroup are listed in Table 2.

**DISCUSSION**

CTCs are tumor cells with specific biomarkers circulating in the peripheral blood, which can be detected in blood samples from most patients with solid tumors but rarely from healthy individuals. CTCs may have several valuable roles in monitoring disease progress and predicting treatment response of patients with malignant tumors [44]. From a clinical perspective, disease assessment by CTCs detection in the peripheral blood, with the merit of time- and cost-saving, appears acceptable to patients, and may be readily repeated as a monitoring tool. To date, encouraging results concerning the association between CTC-positive and clinical outcomes in patients with breast, prostate, and colorectal cancer have been recently published [45–47]. However, there are currently few studies on the clinical relevance of CTC-positive with UC that have included data synthesis. The present study is the first meta-analysis to systematically evaluate the associations between CTC markers and clinicopathological parameters as well as prognosis in UC patients.

The results of our study showed that the CTC-positive in peripheral blood was correlated with tumor stage, histological grade, metastasis and regional lymph node metastasis. This phenomenon indicates CTCs are more easily detected in more advanced stages (TNM) of cancer. Therefore, it is reasonable to correlate these clinicopathological characteristics with the risk of migration of malignant cells, shed from the primary tumor to peripheral circulation, which is probably an important source of metastasis directly relating to a worse prognosis. Our subsequent analysis which indicated that patients in the CTC-positive group showed poorer PFS/DFS, CSS and OS than those in the CTC-negative group was consistent with this hypothesis. From this perspective, taking the results from the present meta-analysis as references, CTCs may serve as a connecting element that bridges certain
Figure 4: Meta-analysis of HRs for the association of the presence of CTCs with CSS, OS and DFS/PFS.

Figure 5: Forest plot showing study-specific (right-axis) and mean sensitivity and specificity with corresponding heterogeneity statistics.
clinicopathological characteristics with progress-related and recurrence-related outcomes. The “seed and soil” theory may provide an explanation for the relationship between CTC and metastasis: tumor cells enter the blood circulation after detaching from the primary tumor and can migrate to reach distant organs, where they can implant themselves and give rise to metastasis [48]. However, additional studies with larger sample sizes and more comprehensive data about the CTCs and survival are needed to confirm this hypothesis. We also evaluated the diagnostic value of CTC in UC. However, because of several methodological limitations, the diagnostic

Figure 6: Summary ROC curve with confidence and prediction regions around mean operating sensitivity and specificity point.

Figure 7: Deeks’ funnel plot with regression line.
Table 2: Subgroup analysis of diagnostic accuracy of CTCs

| Variables          | SEN (95% CI) | SEP (95% CI) | PLR (95% CI) | NLR (95% CI) | DOR (95% CI) | AUC  |
|--------------------|-------------|-------------|-------------|-------------|-------------|------|
| Overall            | 0.34 (0.27, 0.42) | 0.97 (0.93, 0.99) | 11.8 (4.7, 29.5) | 0.68 (0.60, 0.76) | 17 (7, 46) | 0.70 (0.66, 0.74) |
| Geographical location | | | | | | |
| American           | 0.38 (0.21, 0.59) | 0.85 (0.62, 0.95) | 2.5 (0.9, 7.0) | 0.73 (0.52, 1.02) | 3 (1, 13) | 0.65 (0.61, 0.69) |
| European           | 0.35 (0.26, 0.46) | 0.98 (0.92, 0.99) | 14.5 (4.4, 47.5) | 0.66 (0.57, 0.78) | 22 (6, 76) | 0.74 (0.70, 0.78) |
| Asian              | 0.28 (0.22, 0.35) | 0.99 (0.75, 1.00) | 33.6 (0.9, 1307.1) | 0.72 (0.66, 0.79) | 46 (1, 1870) | 0.46 (0.42, 0.50) |
| Control type       | | | | | | |
| Healthy            | 0.41 (0.29, 0.53) | 0.99 (0.94, 1.00) | 44.3 (6.8, 290.7) | 0.60 (0.49, 0.73) | 74 (11, 492) | 0.79 (0.76, 0.83) |
| Mixed              | 0.30 (0.21, 0.39) | 0.93 (0.84, 0.97) | 4.0 (1.8, 9.0) | 0.76 (0.67, 0.87) | 5 (2, 13) | 0.61 (0.57, 0.66) |
| Method             | | | | | | |
| Immunology-based assay | 0.48 (0.23, 0.74) | 0.98 (0.91, 0.99) | 20.6 (5.2, 81.8) | 0.54 (0.31, 0.91) | 38 (8, 192) | 0.97 (0.95, 0.98) |
| PCR-based assay    | 0.32 (0.26, 0.40) | 0.96 (0.90, 0.98) | 7.5 (3.1, 18.0) | 0.71 (0.64, 0.78) | 11 (4, 27) | 0.61 (0.57, 0.65) |
| Sample size        | | | | | | |
| < 100              | 0.37 (0.28, 0.46) | 0.99 (0.94, 1.00) | 27.5 (5.6, 134.3) | 0.64 (0.56, 0.74) | 43 (8, 217) | 0.70 (0.66, 0.74) |
| ≥ 100              | 0.27 (0.18, 0.38) | 0.93 (0.85, 0.97) | 3.7 (2.2, 6.1) | 0.79 (0.71, 0.88) | 5 (3, 8) | 0.68 (0.63, 0.72) |

SEN: Sensitivity; SEP: Specificity; PLR: Positive Likelihood Ratio; NLR: Negative Likelihood Ratio; DOR: Diagnostic Odds Ratio; AUC: Area Under the sROC Curve.
Data extraction

We recorded the following information from each eligible paper: name of first author, year of publication, country, methods for detecting CTCs, cutoff value, numbers of subjects in different clinical and pathological parameters, numbers of subjects found to be positive or negative for CTC and prognostic outcomes of interest. When more than one marker was used to detect CTCs, we recorded all of these results as independent data sets. In cases where multiple blood samples were collected, we only investigated baseline (preoperative or pretreatment) value of CTC in the analysis.

Statistical analyses

Statistical analyses were performed with Stata software (version 12.0, College Station, TX). ORs with 95% confidence intervals were used to estimate the association between CTC-positive and clinicopathological characteristics. A P-value less than 0.05 was considered to be statistically significant. To statistically evaluate the prognostic effect of CTC, we extracted HRs and 95% CIs on OS, PFS/DFS or CSS from multivariable analysis. If these statistical variables were not explicitly provided in the original studies, we calculated the necessary statistics on the basis of available reported data with Excels tools developed by Tierney et al. [53]. By convention, an observed HR > 1 implied a worse prognosis in the CTC-positive group in comparison to negative group. Pooled analysis of the diagnostic accuracy of CTC was also conducted.

Heterogeneity among studies was checked with the Chi-square based on Q statistical test and I². Where P ≤ 0.1 or I² > 50% indicated significant heterogeneity among studies, a random-effects model was used. Otherwise, a fixed-effects model was adopted. In order to evaluate the influence of single studies on the pooled results, we performed a sensitivity analysis using the leave-one-out approach. In addition, publication bias was evaluated by Begg’s rank correlation or Deek’s funnel plot asymmetry test [54].

Authors’ contributions

Zheng Zhang and Wei Fan conceived of and designed the study. Zheng Zhang and Qiaoling Deng performed the literature search, data collection, statistical analysis. Shihui Tang, Ping Wang and Peipei Xu wrote the paper. Mingxia Yu and June Wang revised the manuscript. Mingxia Yu directed the project.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Humphrey PA, Moch H, Cubilla AL, Ulbright TM, Reuter VE. The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs-Part B: Prostate and Bladder Tumours. Eur Urol. 2016; 70:106–19. doi: 10.1016/j.euro.2016.02.028.
2. Antoni S, Ferlay J, Soerjomataram I, Znaor A, Jemal A. Detection of circulating tumor cells in metastatic and clinically localized urothelial carcinoma. Urology. 2011; 78:863–7. doi: 10.1016/j.urology.2011.05.045.
3. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012; 62:10–29. doi: 10.3322/caac.20138.
4. Green DA, Rink M, Xylinas E, Matin SF, Stenzl A, Roupert M, Karakiewicz PI, Scherr DS, Shariat SF. Urothelial carcinoma of the bladder and the upper tract: disparate twins. J Urol. 2013; 189:1214–21. doi: 10.1016/j.juro.2012.05.079.
5. Zhang Z, Forge KA, Yang XJ, Teh BT, Hansel DE. Comparative gene expression profiling analysis of urothelial carcinoma of the renal pelvis and bladder. BMC Med Genomics. 2010; 3:58. doi: 10.1186/1755-8794-3-38.
6. Mbeutcha A, Lucca I, Mathieu R, Lotan Y, Shariat SF. Current Status of Urinary Biomarkers for Detection and Surveillance of Bladder Cancer. Urol Clin North Am. 2016; 43:47–62. doi: 10.1016/j.ucr.2015.08.005.
7. Massague J, Obenauf AC. Metastatic colonization by circulating tumour cells. Nature. 2016; 529:298–306. doi: 10.1038/nature17038.
8. Ashworth T. A case of cancer in which cells similar to those in the tumors were seen in the blood after death. Aust Med J. 1869; 14:146–9.
9. Harouaka R, Kang Z, Zheng SY, Cao L. Circulating tumor cells: advances in isolation and analysis, and challenges for clinical applications. Pharmacol Ther. 2014; 141:209–21. doi: 10.1016/j.pharmthera.2013.10.004.
10. Huang X, Gao P, Sun J, Chen X, Song Y, Zhao J, Xu H, Wang Z. Clinicopathological and prognostic significance of circulating tumour cells in patients with gastric cancer: a meta-analysis. Int J Cancer. 2015; 136:21–33. doi: 10.1002/ijc.28954.
11. Lv Q, Gong L, Zhang T, Ye J, Chai L, Ni C, Mao Y. Prognostic value of circulating tumor cells in metastatic breast cancer: a systematic review and meta-analysis. Clin Transl Oncol. 2016; 18:322–30. doi: 10.1007/s12094-015-1372-1.
12. Rahbari NN, Aigner M, Thorlund K, Molland B, Motschall E, Jensen K, Diener MK, Buchler MW, Koch M, Weitz J. Meta-analysis shows that detection of circulating tumor cells indicates poor prognosis in patients with colorectal cancer. Gastroenterology. 2010; 138:1714–26. doi: 10.1053/j.gastro.2010.01.008.
13. Wang S, Zheng G, Cheng B, Chen F, Wang Z, Chen Y, Wang Y, Xiong B. Circulating tumor cells (CTCs) detected by RT-PCR and its prognostic role in gastric cancer: a meta-analysis of published literature. PLoS One. 2014; 9: e99259. doi: 10.1371/journal.pone.0099259.
14. Flag TW, Wilson S, van Bokhoven A, Varella-Garcia M, Wolfe P, Maroni P, Genova EE, Morales D, Lucia MS. Detection of circulating tumor cells in metastatic and clinically localized urothelial carcinoma. Urology. 2011; 78:863–7. doi: 10.1016/j.urology.2011.05.045.
15. Naoe M, Ogawa Y, Morita J, Omori K, Takeshita K, Shichijyo T, Okumura T, Igarashi A, Yanaihara A, Iwamoto S, Fukagai T, Miyazaki A, Yoshida H. Detection of circulating urothelial cancer cells in the blood using the CellSearch System. Cancer. 2007; 109:1439–45. doi: 10.1002/cncr.22543.
16. Guzzo TJ, McNeil BK, Bivalacqua TJ, Elliott DJ, Sokoll LJ, Schoenberg MP. The presence of circulating tumor cells does not predict extravesical disease in bladder cancer patients prior to radical cystectomy. Urol Oncol. 2012; 30:44–8. doi: 10.1016/j.urolonc.2009.10.008.
17. Ribal MJ, Mengual L, Marin M, Algaba F, Aris E, Fernandez PL, Oliva R, Villavicencio H, Alcaraz A. Molecular staging of bladder cancer with RT-PCR assay for CK20 in peripheral blood, bone marrow and lymph nodes: comparison with standard histological staging. Anticancer Res. 2006; 26:411–9.
18. Retz M, Lehmann J, Roder C, Weichert-Jacobsen K, Loch T, Romahn E, Luhl C, Kalthoff H, Stockle M. Cytokeratin-20 reverse-transcriptase polymerase chain reaction as a new tool for the detection of circulating tumor cells in peripheral blood and bone marrow of bladder cancer patients. Eur Urol. 2001; 39:507–15. doi: 52496.
19. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Wolfe P, Maroni P, Genova EE, Morales D, Lucia MS. Detection of circulating tumor cells (CTCs) detected in peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res. 2004; 10:6897–904. doi: 10.1158/1078-0432.ccr-04-0378.
20. Gradilone A, Petracca A, Nicolazzo C, Gianni W, Cortesi E, Naso G, Vincenzi B, Cristini C, De Berardinis E, Di Silverio F, Agliano AM, Gazzaniga P. Prognostic significance of survivin-expressing circulating tumour cells in T1G3 bladder cancer. BJU Int. 2010; 106:710–5. doi: 10.1111/j.1464-410X.2009.09130.x.
21. Rink M, Chun FK, Dahlem R, Soave A, Minner S, Hansen J, Stoupiec M, Coth C, Kluth LA, Ahyai SA, Friedrich MG, Shariat SF, Fisch M, et al. Prognostic role and HER2 expression of circulating tumor cells in peripheral blood of patients prior to radical cystectomy: a prospective study. Eur Urol. 2012; 61:810–7. doi: 10.1016/j.eururo.2012.01.017.
22. Todenhöfer T, Hennenlotter J, Donner K, Kuhs U, Außerklamm S, Rausch S, Bier S, Mischinger J, Schellbach D, Hauch S, Feniuk N, Bedke J, Gakis G, et al. Transcripts of circulating tumor cells detected by a breast cancer-specific platform correlate with clinical stage in bladder cancer patients. J Cancer Res Clin Oncol. 2016; 142:1013–20. doi: 10.1007/s00432-016-2129-0.
23. Winters B, James A, Lee J, Kho J, Morrissey C, Wright J. Chemotherapeutic effects on circulating tumor cells in bladder cancer. Int J Urol. 2015; 22:612–3. doi: 10.1111/iju.12758.
24. Alva A, Friedlander T, Clark M, Huebner T, Daignault S, Winters B, James A, Lee J, Kho J, Morrissey C, Wright J. Chemotherapeutic effects on circulating tumor cells in bladder cancer patients. Int Urol Nephrol. 2012; 44:761–7. doi: 10.1007/s11255-011-0102-z.
25. Antoniewicz AA, Paziewska A, Mikula M, Goryca K, Dabrowska M, Poletajew S, Borowka A, Ostrowski J. Lack of evidence for increased level of circulating urothelial cells in the peripheral blood after transurethral resection of bladder tumors. Int Urol Nephrol. 2012; 44:761–7. doi: 10.1007/s11255-011-0102-z.
26. Champelovier P, Mongelard F, Seigneurin D. CK20 gene expression: technical limits for the detection of circulating tumor cells. Anticancer Res. 1999; 19:2073–8.
27. Desgrandchamps F, Teren M, Dal Cortivo L, Marolleau JP, Bertheau P, Villette JM, Cortesse A, Teillac P, Le Duc A, Hamdy FC. The effects of transurethral resection and cystoprostatectomy on dissemination of epithelial cells in the circulation of patients with bladder cancer. Br J Cancer. 1999; 81:832–4. doi: 10.1038/sj.bjc.6690771.
28. Fujii Y, Kageyama Y, Kawakami S, Kihara K, Oshima H. Detection of disseminated urothelial cancer cells in peripheral venous blood by a cytokeratin 20-specific nested reverse transcriptase-polymerase chain reaction. Jpn J Cancer Res. 1999; 90:753–7.
29. Gazzaniga P, de Berardinis E, Busetto GM, Raimondi C, Gandini O, Nicolazzo C, Petrocchi A, Vincenzi B, Farcomeni A, Gentile V, Cortesi E, Frati L. Prognostic value of circulating tumor cells in nonmuscle invasive bladder cancer: a CellSearch analysis. Ann Oncol. 2012; 23:2352–6. doi: 10.1093/annonc/mdr619.
30. Gazzaniga P, Gandini O, Giuliani L, Magnanti M, Gradilone A, Silvestri I, Gianni W, Gallucci M, Frati L, Agliano AM. Detection of epidermal growth factor receptor mRNA in peripheral blood: a new marker of circulating neoplastic cells in bladder cancer patients. Clin Cancer Res. 2001; 7:577–83.
Detection of circulating tumour cells in peripheral blood of patients with advanced non-metastatic bladder cancer. BJU Int. 2011; 107:1668–75. doi: 10.1111/j.1464-410X.2010.09562.x.

43. Soria JC, Morat L, Durdux C, Housset M, Cortez A, Blaise R, Sabatier L. The molecular detection of circulating tumor cells in bladder cancer using telomerase activity. J Urol. 2002; 167:352–6.

44. Friedlander TW, Premasekharan G, Paris PL. Looking back, to the future of circulating tumor cells. Pharmacol Ther. 2014; 142:271–80. doi: 10.1016/j.pharmthera.2013.12.011.

45. Bork U, Rahbari NN, Scholch S, Reissfelder C, Kahlert C, Buchler MW, Weitz J, Koch M. Circulating tumour cells and outcome in non-metastatic colorectal cancer: a prospective study. Br J Cancer. 2015; 112:1306–13. doi: 10.1038/bjc.2015.88.

46. Thalgott M, Rack B, Horn T, Heck MM, Eiber M, Kuhler H, Retz M, Gschwend JE, Andergassen U, Nawroth R. Detection of Circulating Tumor Cells in Locally Advanced High-risk Prostate Cancer During Neoadjuvant Chemotherapy and Radical Prostatectomy. Anticancer Res. 2015; 35:5679–85.

47. Turker I, Uyeturk U, Sonmez OU, Oksuzoglu B, Helvaci K, Arslan UY, Budakoglu B, Alkis N, Aksoy S, Zengin N. Detection of circulating tumor cells in breast cancer patients: prognostic predictive role. Asian Pac J Cancer Prev. 2013; 14:1601–7.

48. Fidler IJ. The pathogenesis of cancer metastasis: the ‘seed and soil’ hypothesis revisited. Nat Rev Cancer. 2003; 3:453–8. doi: 10.1038/nrc1098.

49. Msaouel P, Koutsilieris M. Diagnostic value of circulating tumor cell detection in bladder and urothelial cancer: systematic review and meta-analysis. BMC Cancer. 2011; 11:336. doi: 10.1186/1471–2407-11-336.

50. Chou R, Gore JL, Buckley D, Fu R, Gustafson K, Griffin JC, Grusing S, Selph S. Urinary Biomarkers for Diagnosis of Bladder Cancer: A Systematic Review and Meta-analysis. Ann Intern Med. 2015; 163:922–31. doi: 10.7326/m15-0997.

51. D’Costa JJ, Goldsmith JC, Wilson JS, Bryan RT, Ward DG. A Systematic Review of the Diagnostic and Prognostic Value of Urinary Protein Biomarkers in Urothelial Bladder Cancer. Bladder Cancer. 2016; 2:301–17. doi: 10.3233/blc-160054.

52. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Bmj. 2009; 339:b2535. doi: 10.1136/bmj.b2535.

53. Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials. 2007; 8:16. doi: 10.1186/1745-6215-8-16.

54. Deeks JJ, Macaskill P, Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. J Clin Epidemiol. 2005; 58:882–93. doi: 10.1016/j.jclinepi.2005.01.016.