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Original Article

The diagnostic and prognostic value of CTC enumeration by CellSearch System in prostate cancer: a systematic review and meta-analysis

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Abstract: Background: Prostate cancer (PCa) is the second most common malignancy with high recurrence and progression rate in men. In PCa patients, circulating tumor cells (CTC) played its part in diagnosis and prognosis prediction. CellSearch System (CSS) was the only approved device for clinical application by United States Federal Food and Drug Administration to recognize and enumerate CTCs in peripheral blood. The aim of our study was to perform a systematic review and meta-analysis of the published literatures while investigating the diagnostic and prognostic value of CTC enumeration with CSS in PCa. Methods: Pubmed, Embase and Cochrane Library were searched for trials investigating the diagnostic and prognostic value of CTC enumeration with CSS. Results: Seven articles including 1593 participants with diagnostic accuracy data and twenty-seven articles including 2728 patients with survival data were incorporated. The diagnostic accuracy variables of CTC enumeration were as followed: overall sensitivity, specificity, positive likelihood ratio, negative likelihood ratio and area under the curve were 64.3%, 97.9%, 31.029, 0.365 and 0.91, respectively. The Hazard rate (HR) for the effect of unfavourite CTC enumeration on overall survival (OS), biochemical recurrence-free survival/progression-free survival were 2.68 ($P < 0.001$) and 2.46 ($P < 0.001$), respectively. CTC conversion showed a strong predictive power on OS. A significant correlation was detected between unfavourite CTC enumeration and high Gleason score (OR: 2.57; $P = 0.001$). Conclusions: CTC enumeration by CSS revealed a good diagnostic value in PCa confirmation and prognosis prediction.

Keywords: Prostate cancer, circulating tumor cell, CellSearch System, diagnostic accuracy, prognosis

Introduction

In men, prostate cancer (PCa) is the second most diagnosed malignancy worldwide, with the incidence varies by as much as thirty folds between populations, while the mortality varies about eighteen folds. The highest incidence rate was about 168.3/100,000 in United States (US) Blacks and the highest mortality rate was about 44/100,000 in Trinidad and Tobago [1]. The heterogeneity and long course of PCa make the therapy protocols difficult to fit against over- or under-treatment. Up to 30% localized PCa (LPCa) patients [2] and 45% metastatic PCa (mPCa) patients [3] had biochemical recurrence or tumor progression despite proper treatment. Conventional biomarkers such as prostate-specific antigen (PSA) had only shown limited diagnostic value with a positive predictive value of 47% and limit prognostic value in PCa patients [4]. Better clinical models including pre- and post-therapy factors are eagerly needed in surveillance of the PCa status, in order to earlier diagnose the illness and timely adjust the therapeutic regimen.

CellSearch System (CSS), the only approved device for clinical application by US Federal Food and Drug Administration (US FDA), is able to recognize and enumerate the circulating tumor cells (CTC) in peripheral blood. With this device, a CTC enumeration ≥ threshold may provide us a strong diagnostic and prognostic power in PCa patients.

Methods

Search strategy

National Library of Medicine (Pubmed), Embase and Cochrane Library were searched without

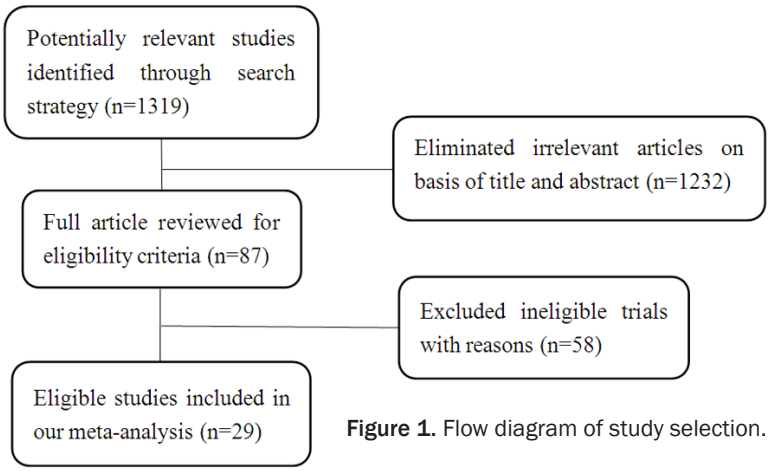


Figure 1. Flow diagram of study selection.

time restrictions using English only on April 10, 2016 to identify relevant publications. Searching strategy was based on combining the following key items: circulating tumor cell, liquid biopsy, prostate cancer and Cellsearch system. No race or age limitations were applied to our searching. We also checked the reference lists to make sure no pertinent literature was missed.

Inclusion and exclusion criteria

Inclusion criterion for our study were as follows: (1) published literature from a peer-reviewed journal; (2) primary cohort of patients with prostate cancer; (3) favorite cohorts or negative controls (patients with CTC counts less than pre-determined cutoff value or healthy volunteers) were clearly identified; (4) CTC enumerated with CellSearch System (CSS); (5) data of the correlations of CTC enumeration with survival, diagnostic accuracy or patients' clinical characteristics (Gleason score, pathological stage (pT stage)) was provided. Literatures with following characteristics were excluded: (1) letters or review articles; (2) trials published as interim reports or in abstract form; (3) articles of mice or other non-human animal's trials.

Date collection

Literature were reviewed and selected by two independent investigators strictly in accordance with the inclusion and exclusion criteria. A pre-designed data form including the basic characteristics, CTC counts and the primary outcomes of included patients and another pre-designed data form including basic characteristics of included studies were used by two independent investigators to extract data. The primary outcomes including different survival outcomes (Hazard ratio (HR) for overall survival (OS), biochemical recurrence-free survival (bRFS) and progression-free survival (PFS)), diagnostic accuracy variables (sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-)), area under the curve (AUC) and correlation of CTC counts with Gleason score and pT stage. When blood samples were withdrawn at different time points in one study, we recorded the results as "baseline (blood sample withdrawn at baseline)", "exact time point (blood sample withdrawn at exact time point after baseline)" or "after treatment (blood sample withdrawn after baseline without exact time point)", as in Scher's study [5]. When the outcomes were measured by different cutoff values in one study, we recorded the results as "exact cutoff value", as in Thalgott's study [6]. When outcomes were separately measured by different tumor types, we recorded the results as "exact tumor type", as in Thalgott's study [6]. Additional data including first author, published year, study size and other clinical characteristics were extracted from the studies.

Quality evaluation

The quality of each literature was assessed and further scored by two investigators independently. The quality assessment of diagnostic accuracy studies-2 (QUADAS-2) which included "patient selection", "index test", "reference standard" and "flow and timing" was used to assess the quality of literature which provided diagnostic accuracy data. Based on the Newcastle-Ottawa quality assessment scale (NOS) which included "selection", "comparability" and "outcomes", the literature quality was assessed for the cohort study which provided survival data. NOS score ≥ 6 stars was considered as high quality. Any disagreement about

Table 1. Characteristics of the included studies and patients

Authors and published time	Country	Patient number	Tumor type	Cutoff value	Blood drawing time	NOS (star)
Meyer 2016 [13]	Germany	152	LPCa	1/7.5 ml	BL	8
Thalgott 2015 (1) [31]	Germany	15	LPCa	1/20 ml	BL	7
Thalgott 2015 (2) [15]	Germany	33	mCRPC	5/7.5 ml	BL, 1st, 4th, 10th (treatment cycle)	8
Thalgott 2015 (3) [16]	Germany	33	mCRPC	5/7.5 ml	BL, 1st, 4th, 10th (treatment cycle)	8
Marin 2015 [17]	Spain	43	mCRPC	5/7.5 ml	BL, 6th, 12th, 24th (month)	7
Lowes 2015 [14]	Canada	55	PCa	1/7.5 ml	BL, 6th, 12th, 24th (month)	7
Chang 2015 [18]	China	70	mCRPC	5/7.5 ml	BL	8
Bitting 2015 [19]	America	89	mCRPC	5/7.5 ml	BL	7
Okegawa 2014 [20]	Japan	57	mCRPC	5/7.5 ml	BL	7
Goldkorn 2014 [21]	America	263	mCRPC	5/7.5 ml	BL	7
Fossa 2014 [22]	Norway	41	mCRPC	5/7.5 ml	BL, 2nd-3rd (month)	8
Amato 2013 [23]	America	202	PCa	5/7.5 ml	BL	6
Ligthart 2013 [7]	Netherlands	489	mCRPC	1, 5/7.5 ml	BL, aftertreatment	7
Thallgott 2013 [6]	Germany	80	mCRPC+mTRPC	1, 3, 5/7.5 ml	BL	6
Rsesel 2012 [8]	Spain	86	mHSPC	1, 4/7.5 ml	BL	6
Goodman 2011 [32]	America	33	mHSPC	3/7.5 ml	BL	8
Danila 2011 [24]	America	48	mCRPC	5/7.5 ml	BL, 4th (week)	8
Ligthart 2011 [9]	Netherlands	253	mCRPC	1, 5/7.5 ml	BL, after treatment	7
Strijbos 2010 [25]	Netherlands	162	mCRPC	5/7.5 ml	BL, after treatment	8
Okegawa 2010 [11]	Japan	96	mCRPC	2, 5/7.5 ml	BL	7
Scher 2009 [5]	America	164	mCRPC	5/7.5 ml	BL, 4th, 8th, 12th (week)	7
Olmos 2009 [26]	England	119	mCRPC	5/7.5 ml	BL	8
Okegawa 2009 [27]	Japan	64	mCRPC	5/7.5 ml	BL	7
Goodman 2009 [33]	America	96	mCRPC+LCRPC	4/7.5 ml	BL	8
Okegawa 2008 [28]	Japan	80	mHSPC	5/7.5 ml	BL	6
de Bone 2008 [29]	England	231	mCRPC	5/7.5 ml	BL, 2nd-5th, 6th-8th, 9th-12th, 13th-20th (week)	8
Davis 2008 [10]	America	122	LPCa	1/22.5 ml	BL, 6th (week)	-
Danila 2007 [30]	America	120	mCRPC	5/7.5 ml	BL	7
Allard 2004 [12]	America	467	mPCa	2/7.5 ml	BL	-

NOS: Newcastle-Ottawa scale; LPCa: localized prostate cancer; BL: baseline; mCRPC: metastatic castration-resistant prostate cancer; PCa: prostate cancer; mTRPC: metastatic taxane-refractory prostate cancer; mHSPC: metastatic hormone-sensitive prostate cancer; LCRPC: localized castration-resistant prostate cancer; mPCa: metastatic prostate cancer.

data extraction, data synthesis and literature quality evaluation was settled by the correspondence author.

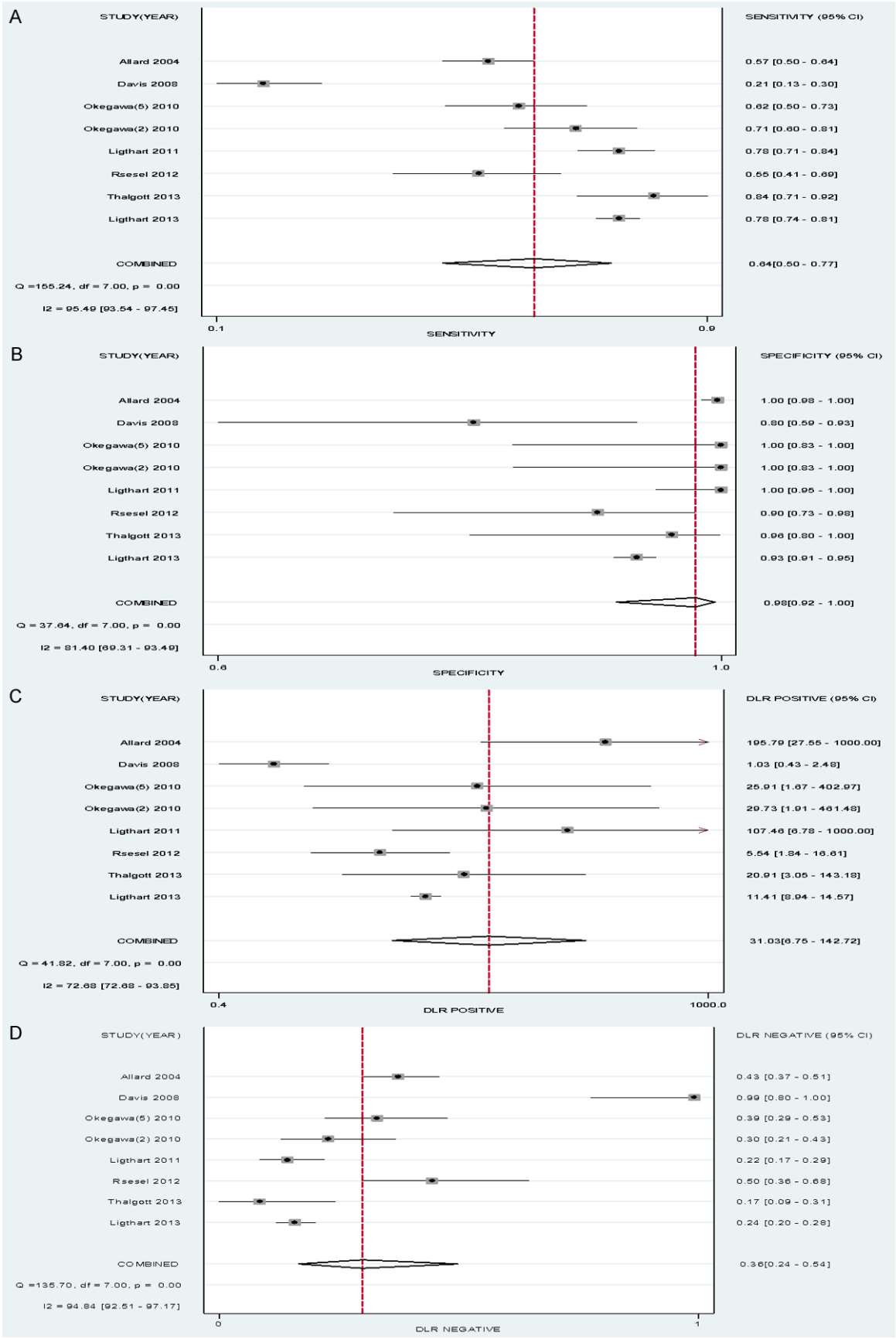
Statistical analysis

STATA 12.0 was implemented in all the statistical analysis. *P* value less than 0.05 was considered significantly different in statistics. 95% confidence intervals (CI) were recorded with all the statistical outcomes. Forest plots were used to estimate the heterogeneity among all the trials included. Both $I^2 > 50\%$ and $P < 0.05$ (Cochrane's Q) suggest a statistically significant heterogeneity. Fixed effect model was used for analysis when the heterogeneity was not statistically significant and random effect model was used for analysis when the heterogeneity was statistically significant. Potential publication bias was evaluated by funnel plots. The publication bias of diagnostic accuracy literature was further examined by Deek's asymmetry test and publication bias of prognostic literature was further tested by egger's and begg's test.

Results

Characteristics of included studies

A total of 1319 potentially relevant literature were yielded in our search and the selection process was listed in **Figure 1**. Seven articles [6-12] including 1593 participants which pro-


A
STUDY(YEAR)
SENSITIVITY (95% CI)
Allard 2004
0.57 [0.50 - 0.64]
Davis 2008
0.21 [0.13 - 0.30]
Okegawa(5) 2010
0.62 [0.50 - 0.73]
Okegawa(2) 2010
0.71 [0.60 - 0.81]
Ligthart 2011
0.78 [0.71 - 0.84]
Rsesel 2012
0.55 [0.41 - 0.69]
Thalgott 2013
0.84 [0.71 - 0.92]
Ligthart 2013
0.78 [0.74 - 0.81]
COMBINED
0.64[0.50 - 0.77]
Q =155.24, df = 7.00, p = 0.00
I2 = 95.49 [93.54 - 97.45]
0.1
0.9
SENSITIVITY
B
STUDY(YEAR)
SPECIFICITY (95% CI)
Allard 2004
1.00 [0.98 - 1.00]
Davis 2008
0.80 [0.59 - 0.93]
Okegawa(5) 2010
1.00 [0.83 - 1.00]
Okegawa(2) 2010
1.00 [0.83 - 1.00]
Ligthart 2011
1.00 [0.95 - 1.00]
Rsesel 2012
0.90 [0.73 - 0.98]
Thalgott 2013
0.96 [0.80 - 1.00]
Ligthart 2013
0.93 [0.91 - 0.95]
COMBINED
0.98[0.92 - 1.00]
Q = 37.64, df = 7.00, p = 0.00
I2 = 81.40 [69.31 - 93.49]
0.6
1.0
SPECIFICITY
C
STUDY(YEAR)
DLR POSITIVE (95% CI)
Allard 2004
195.79 [27.55 - 1000.00]
Davis 2008
1.03 [0.43 - 2.48]
Okegawa(5) 2010
25.91 [1.67 - 402.97]
Okegawa(2) 2010
29.73 [1.91 - 461.48]
Ligthart 2011
107.46 [6.78 - 1000.00]
Rsesel 2012
5.54 [1.84 - 16.61]
Thalgott 2013
20.91 [3.05 - 143.18]
Ligthart 2013
11.41 [8.94 - 14.57]
COMBINED
31.03[6.75 - 142.72]
Q = 41.82, df = 7.00, p = 0.00
I2 = 72.68 [72.68 - 93.85]
0.4
1000.0
DLR POSITIVE
D
STUDY(YEAR)
DLR NEGATIVE (95% CI)
Allard 2004
0.43 [0.37 - 0.51]
Davis 2008
0.99 [0.80 - 1.00]
Okegawa(5) 2010
0.39 [0.29 - 0.53]
Okegawa(2) 2010
0.30 [0.21 - 0.43]
Ligthart 2011
0.22 [0.17 - 0.29]
Rsesel 2012
0.50 [0.36 - 0.68]
Thalgott 2013
0.17 [0.09 - 0.31]
Ligthart 2013
0.24 [0.20 - 0.28]
COMBINED
0.36[0.24 - 0.54]
Q =135.70, df = 7.00, p = 0.00
I2 = 94.84 [92.51 - 97.17]
0
1
DLR NEGATIVE

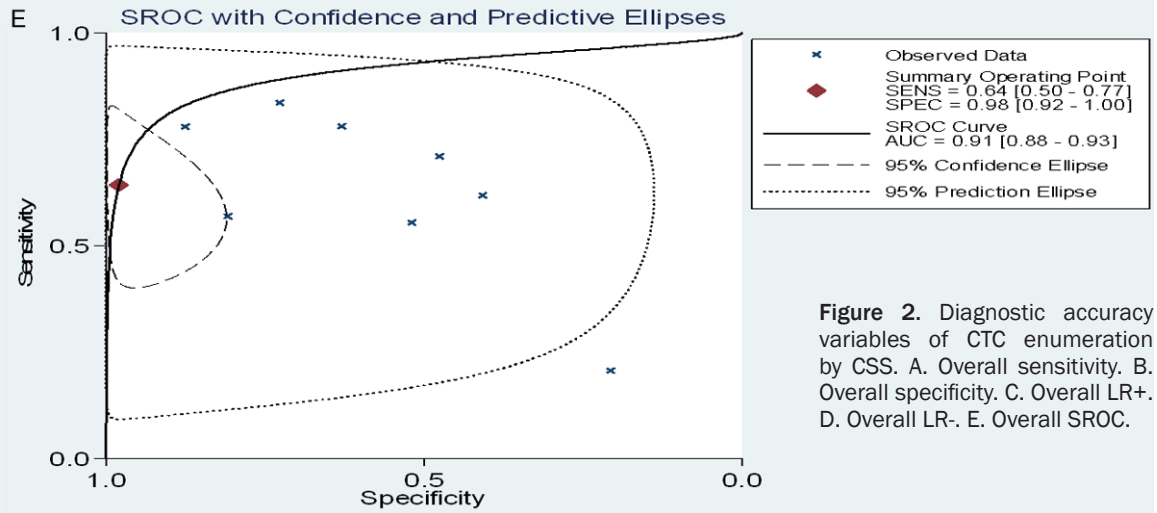


Figure 2. Diagnostic accuracy variables of CTC enumeration by CSS. A. Overall sensitivity. B. Overall specificity. C. Overall LR+. D. Overall LR-. E. Overall SROC.

Table 2. Subgroup of diagnostic accuracy variables

Subgroup	Sensitivity (95% CI; Heterogeneity: I^2)	Specificity (95% CI; Heterogeneity: I^2)	Positive likelihood ratio (95% CI; Heterogeneity: I^2)	Negative likelihood ratio (95% CI; Heterogeneity: I^2)	Area under the curve (95% CI)
Cut off value as 1/7.5 ml	0.753 (0.655-0.830; 81.89%)	0.951 (0.858-0.984; 47.75%)	15.254 (4.856-47.921; 0%)	0.260 (0.179-0.379; 85.49%)	0.93 (0.90-0.95)
mPCa patient	0.732 (0.660-0.793; 85.95%)	0.979 (0.912-0.995; 88.02%)	34.476 (8.636-137.626; 65.40%)	0.274 (0.219-0.343; 85.00%)	0.87 (0.84-0.90)

vided diagnostic accuracy data and twenty-seven [5-9, 12-33] articles including 2728 patients which provided survival data were incorporated in our systematic review. CTC enumeration was defined as unfavourite CTC enumeration (UCE) and favorite CTC enumeration (FCE) base on a pre-designed cutoff value. A number ≥ cutoff value was regarded as unfavourite and a number < cutoff value was regarded as favorite. According to the UCE and FCE, patients were divided into unfavourite group (UG) and favorite group (FG) respectively. Four diagnostic accuracy studies [6-9] pre-defined the cutoff value as 1/7.5 ml and other three studies [11-13] pre-defined the cutoff value as 1/22.5 ml [10], 2/7.5 ml [11, 13] or 5/7.5 ml [11]. Two prognostic studies [13, 14] pre-defined the cutoff value as 1/7.5 ml and Twenty-one studies [6, 7, 9, 11, 15-30] pre-defined the cutoff value as 5/7.5 ml. In these prognostic studies, most thresholds were defined as 1/7.5 ml with LPCa patients and as 5/7.5 ml with mPCa patients. The characteristics of the included studies and patients were presented in **Table 1**.

Quality assessment

In the seven diagnostic accuracy literature, five [7-11] of them demonstrated low risk of bias with high applicability concerns, Allard's study [12] presented high risk of bias with high applicability concern and Thalgott's study [6] showed high risk of bias with low applicability concern. As demonstrated in **Table 1**, the qualities of included prognostic studies were all ≥ 6, which were considered to be high.

Diagnostic accuracy of CTC enumeration by CSS

Seven studies [6-12] reported the diagnostic accuracy variables of CTC enumeration. The total number of true positive, false positive, false negative and true negative blood sample were 900, 82, 374 and 1413 respectively. Our meta-analysis revealed the overall sensitivity was 64.3% (95% CI 49.6%-76.7%; I^2 = 92.49%; **Figure 2A**). Overall specificity, LR+ and LR- were 97.9% (95% CI 91.6%-99.5%; I^2 = 92.49%; **Figure 2B**), 31.029 (95% CI 6.746-142.719; I^2 = 72.68%; **Figure 2C**) and 0.365 (95% CI 0.245-0.544; I^2 = 94.84%; **Figure 2D**) respectively. The summary receiver operating characteristic curve (SROC) showed the area under the curve (AUC) was 0.91 (95% CI 0.88-0.93) (**Figure 2E**). Subgroups of the diagnostic accuracy were presented in **Table 2**. When the cutoff value was

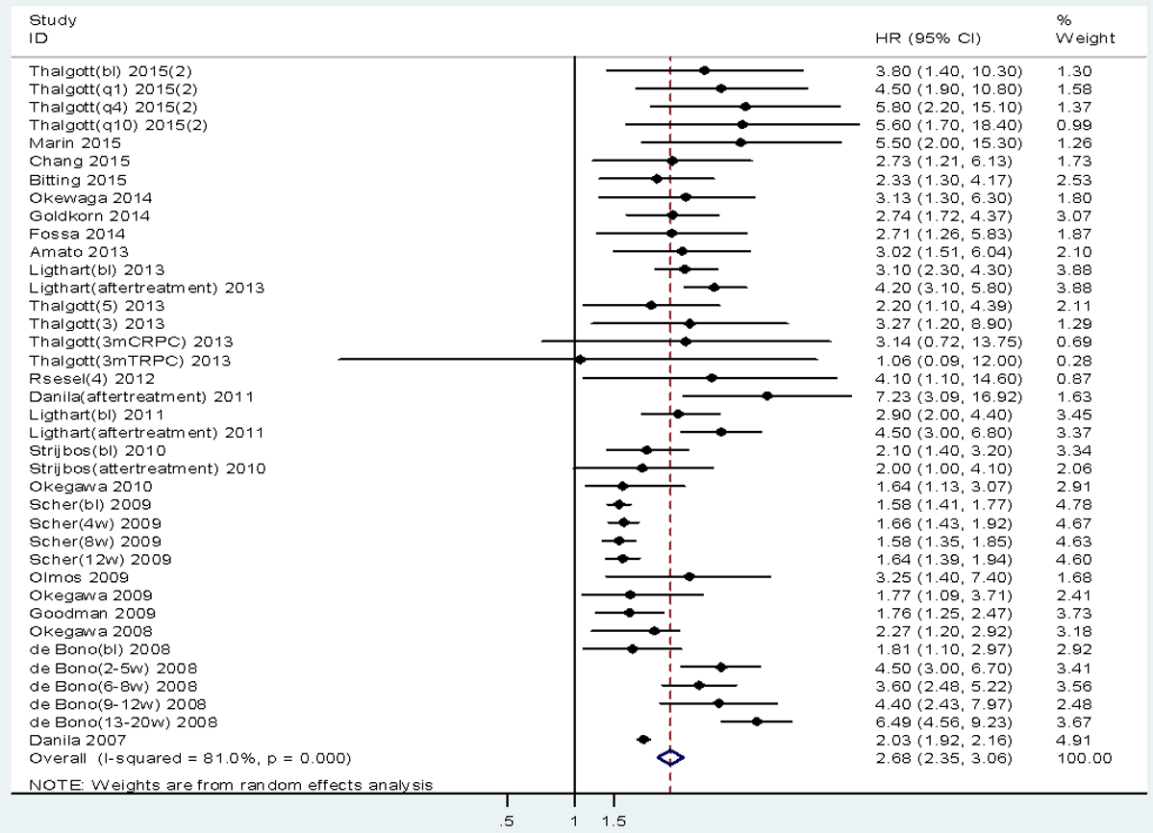


Figure 3. Forrest plots of combined hazard ratios for Overall Survival.

predefined as 1/7.5 ml, CTC enumeration showed a good sensitivity (78.5%) with high specificity (95.1%). The threshold "1/7.5 ml" revealed a good diagnostic value (AUC = 0.93) in PCa.

CTC enumeration by CSS predicted OS

Our meta-analysis showed a statistically significant prognostic effect on OS of CTCs detected by CSS in the blood samples. The estimated HR for the effect of UCE detected by CSS on OS was 2.68 (95% CI 2.35-3.06; P < 0.001) with random effect model. The heterogeneity was statistically significant (I^2 = 81.0%, P < 0.001) (**Figure 3**). The subgroups of HR for OS were presented in **Table 3**. Estimated HR in all the subgroups were statistically significant (P < 0.001). In the subgroup which blood samples were collected after a period of therapy, we found a significant higher HR (3.41; 95% CI 2.53-4.61; P < 0.001) than the baseline withdrawn subgroup (2.00; 95% CI 1.90-2.09; P < 0.001). It might indicate that UCE after therapy could be more strongly predictable to a poorer OS.

CTC enumeration conversion predicted OS

We further explored the predictive value of CTC enumeration by CSS conversion on OS. Patients were divided into four groups (Group 1: CTC enumeration < 5 at all draws; Group 2: CTC enumeration ≥ 5 at BL but < 5 after therapy; Group 3: CTC enumeration < 5 at BL but ≥ 5 after therapy; Group 4: CTC enumeration ≥ 5 at all draws). We meta-analyzed the estimate HR of OS between each groups (**Table 4**). Group 2 showed no statistical correlation with poorer OS compared with group 1 (P = 0.055). Group 3 revealed strongest correlation with poorer OS compared with group 1 (HR: 2.96; 95% CI 1.93-4.55; P < 0.001). Group 4 presented a relatively weak statistical correlation with poorer OS compared with group 3 (HR: 1.43; 95% CI 1.02-2.02; P = 0.04). As showed in **Table 4**, all the patients with UCE at last draw presented a statistically significant correlation with poorer OS compared with patients with FCE at last draw.

CTC enumeration by CSS predicts bRFS/PFS

Our study meta-analyzed the estimated HR for the effect of UCE detected by CSS on bRFS/

Table 3. Subgroup for overall survival

Subgroup	HR	95% CI		*P* value	Heterogeneity (I^2; p)	Effect model
		Lower limit	Upper limit			
Cutoff value as 5/7.5 ml	2.72	2.36	3.12	< 0.001	83.3%; < 0.001	Random
mPCa patient	2.72	2.37	3.12	< 0.001	81.9%; < 0.001	Random
mCRPC patient	2.75	2.38	3.18	< 0.001	84.3%; < 0.001	Random
For mHSPC	2.42	1.59	3.68	< 0.001	0%; 0.397	Fixed
Blood withdrawn at baseline	2.00	1.90	2.09	< 0.001	48.7%; 0.004	Fixed
Blood withdrawn after treatment	3.41	2.53	4.61	< 0.001	91.2%; < 0.001	Random
Blood withdrawn at baseline and Cutoff value as 5/7.5 ml	2.26	1.98	2.59	< 0.001	56.7%; 0.001	Random
Blood withdrawn from mCRPC patient at baseline with Cutoff value as 5/7.5 ml	2.26	1.95	2.61	< 0.001	62.3%; < 0.001	Random
Blood withdrawn from mCRPC patient at baseline	2.28	1.97	2.63	< 0.001	58.8%; 0.001	Random
Blood withdrawn from mPCa patient at baseline	2.26	1.99	2.58	< 0.001	51.1%; 0.003	Random
In America	1.86	1.63	2.12	< 0.001	77.5%; < 0.001	Random
In Europe	3.56	3.19	3.98	< 0.001	43.6%; 0.014	Fixed
In Asia	2.10	1.62	2.72	< 0.001	0%; 0.602	Fixed

Table 4. CTC conversions predicted OS

Subgroup	HR	95% CI		*P* value	Heterogeneity (I^2; p)	Effect model
		Lower limit	Upper limit			
Group 2 vs. Group 1	2.00	0.99	4.05	0.055	61.1%; 0.025	Random
Group 3 vs. Group 1	2.96	1.93	4.55	< 0.001	2.4%; 0.393	Fixed
Group 4 vs. Group 1	2.83	2.10	3.81	< 0.001	40.6%; 0.150	Fixed
Group 4 vs. Group 3	1.43	1.02	2.02	0.04	16.0%; 0.313	Fixed
Group 4 vs. Group 2	2.15	1.59	2.91	< 0.001	18.8%; 0.292	Fixed
Group 3 vs. Group 2	2.15	1.43	3.24	< 0.001	13.2%; 0.330	Fixed

Group 1: CTC enumeration < 5 at all draws; Group 2: CTC enumeration ≥ 5 at BL but < 5 after therapy; Group 3: CTC enumeration < 5 at BL but ≥ 5 after therapy; Group 4: CTC enumeration ≥ 5 at all draws.

PFS. UCE was a strong indicator to poor bRFS/PFS (HR: 2.46; 95% CI 1.90-3.17) with statistical significance (P < 0.001), and the heterogeneity is relatively low (I^2 = 38.6%). Subgroups were also performed. As shown in **Table 5**, UCE was not a statistically significant factor indicating poorer OS in LPCa patients (P = 0.087; I^2 = 0%) but a statistically significant factor indicating poorer OS in mPCa patients (HR: 3.00; 95% CI 1.92-4.69; P < 0.001; I^2 = 55.2%). Both 1/7.5 ml (HR: 2.47; 95% CI 1.05-5.81; P = 0.038; I^2 = 0%) and 5/7.5 ml (HR: 2.07; 95% CI 1.55-2.77; P < 0.001; I^2 = 25.5%) could be a good cutoff value divided patients into UC and FC for poorer bRFS/PFS prediction.

Association of CTC enumeration with GS and pT stage

Statistical pooling of all the eligible studies demonstrated that patients with UCE were significantly more likely to have higher (≥ 8) GS compared with patients in FG (OR: 2.57; 95% CI 1.48-4.47; P = 0.001). A relatively high heterogeneity was detected among the studies (I^2 = 50.1%). Subgroups were also performed and presented in **Table 6**. In LPCa patients, the risk for higher GS between UG and FG was not statistically different (P = 0.242). While in mPCa patients, those whom in UG were statistically more likely to have a higher GS compared with others in FG (OR: 3.03; 95% CI 1.25-7.35; P = 0.014; I^2 = 74.9%). 5/7.5 ml (OR: 2.86; 95% CI 1.41-5.83; P = 0.004; I^2 = 69.3%) was a better cutoff value in predicting higher GS compared with 1/7.5 ml (OR: 2.41; 95% CI 0.61- 9.57; P = 0.211; I^2 = 0%).

Interestingly, our study found UCE was correlated with earlier pT stage (< pT3a) with a low OR (0.58; 95% CI 0.32-1.07; I^2 = 14.4%), but the difference was not statistical significant (P = 0.079).

Publication bias assessment

As shown in **Figure 4**, funnel plots were used to examine all the publication bias. For further assessment, Deek's asymmetry test was used in diagnostic accuracy literature and begg's

Table 5. Subgroup for bRFS/PFS

Subgroup for PFS/bRFS	HR	95% CI		*P* value	Heterogeneity (I^2; p)	Effect model
		Lower limit	Upper limit			
Cutoff value as 5/7.5 ml	2.07	1.55	2.77	< 0.001	25.5%; 0.235	Fixed
Cutoff value as 1/7.5 ml	2.47	1.05	5.81	0.038	0%; 0.817	Fixed
mPCa patient	3.00	1.92	4.69	< 0.001	55.2%; 0.022	Random
mCRPC patient	2.00	1.47	2.72	< 0.001	33.9%; 0.182	Fixed
LPCa patient	2.26	0.89	5.76	0.087	0%; 0.945	Fixed
mHSPC patient	4.88	2.80	8.53	< 0.001	26.5%; 0.257	Fixed
Blood drawing at baseline	2.24	1.69	2.96	< 0.001	45%; 0.069	Fixed
Blood drawing at baseline with cutoff value as 5/7.5 ml	1.74	1.25	2.41	0.001	0%; 0.541	Fixed
For bRFS	2.42	1.10	5.33	0.029	0%; 0.965	Fixed
For PFS	3.00	1.92	4.69	< 0.001	55.2%; 0.022	Random

Table 6. Subgroup of GS prediction

Subgroup for Gleason score	OR	95% CI		*P* value	Heterogeneity (I^2; p)	Effect model
		Lower limit	Upper limit			
LPCa patient	1.89	0.65	5.49	0.242	0%; 0.419	Fixed
mPCa patient	3.03	1.25	7.35	0.014	74.9%; 0.003	Random
Cutoff value as 1/7.5 ml	2.41	0.61	9.57	0.211	0%; 0.752	Fixed
Cutoff value as 5/7.5 ml	2.86	1.41	5.83	0.004	69.3%; 0.006	Random

test was used in prognostic literature. No publication bias was revealed by the overall analysis of diagnostic accuracy literature (P = 0.827) (**Figure 4A**). There were no publication bias among OS literature (P = 0.291) (**Figure 4B**) and among bRFS/PFS literature (P = 0.631) either.

Discussion

CTCs were migrant cells generated in the multistep process of tumor metastasis [34], which have been suggested a good prognostic value in several malignancies such as breast cancer [35] and colorectal cancer [36]. But the diagnostic value was still controversial. CSS was the only device approved for clinical application by US FDA to recognize and enumerate CTCs in peripheral blood. Previous study [37] reported better prognostic value of immunomagnetic based CSS compared with other techniques such as immunohistochemistry and Reverse Transcription-Polymerase Chain Reaction (RT-PCR). For the first time, we performed a comprehensive and detailed meta-analysis to reveal the diagnostic and prognostic value of CTC enumeration by CSS in PCa as well as the associations between CTC enumeration and Clinical characteristics of these patients.

In CSS, cells with epithelial cell adhesion molecule (EpCAM) were trapped by specific antibody-coated magnetic beads and then fluorescent-stained by 4',6-diamidino-2-phenylindole. These cells were further labeled by leukocyte specific CD45-allophycocyan and epithelial cell specific cytokeratins-8, 18, 19 phycoerythrin. CTCs would be finally recognized as EpCAM positive, CD45 negative, cytokeratin positive cells by Cell-Spotter [38].

Our results suggested that CTC enumeration by CSS had limited diagnostic sensitivity (64.3%). The EpCAM based CSS failed to identify CTCs after the process of epithelial-mesenchymal transition (EMT) which characterized by epithelial cells reduction and mesenchymal cells increase might account for this. Combination of multiple biomarkers or techniques in the CTC detection could improve the diagnostic sensitivity. Fizazi [39] combined telomerase activity detection with immunomagnetic detection, and observed a sensitivity of 76%. Okegawa [11] combined CSS with methylated DNA status analysis by PCR and reached a sensitivity of 92%. On the other hand, CTC enumeration by CSS demonstrated a high specificity (97.9%) and large AUC (0.91) in our study. Therefore CTC detection by CSS might be a good diagnostic method used in PCa diagnosis confirming instead of first-line screening. However, a further device combined EpCAM with other bio-

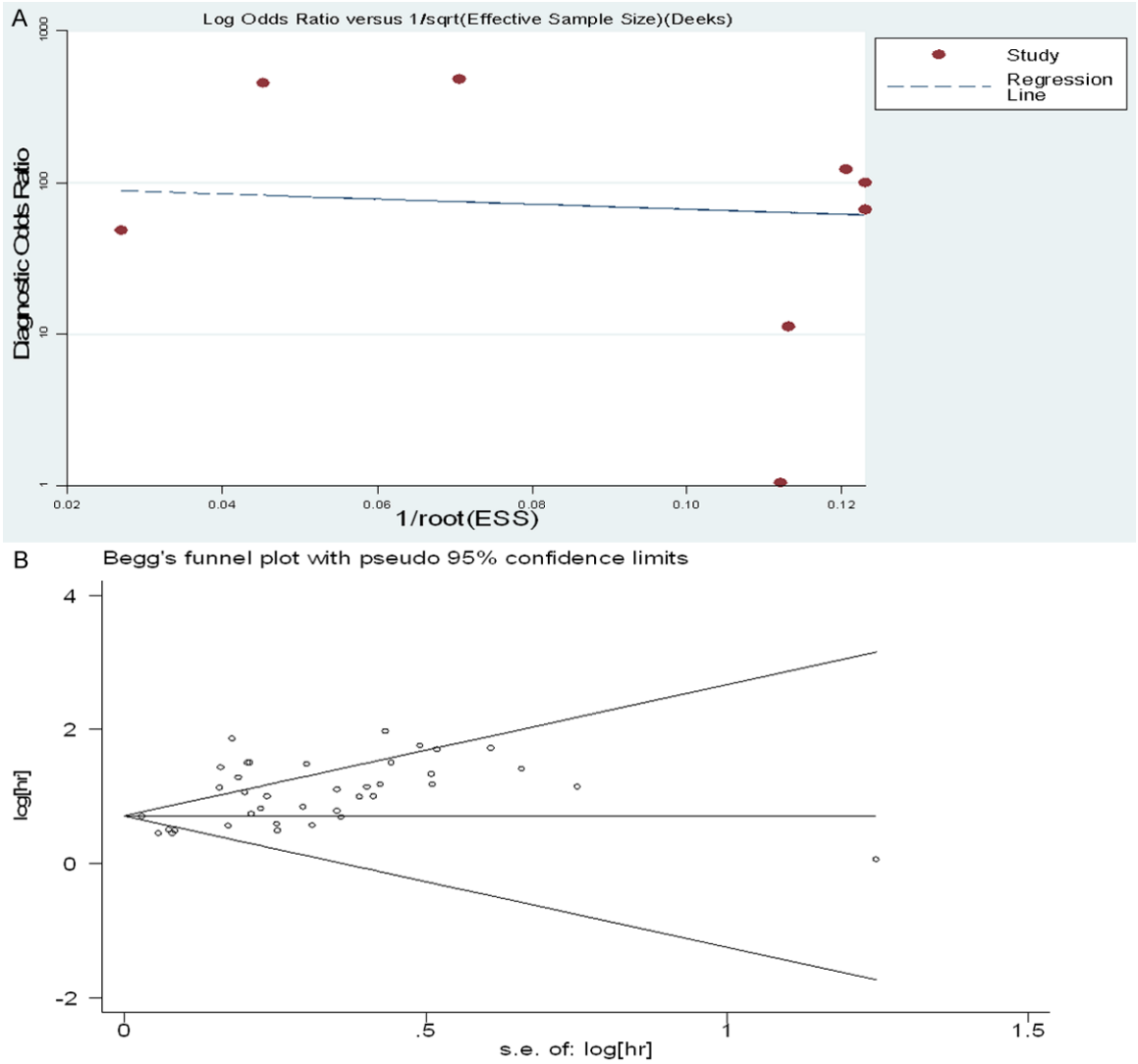


Figure 4. Funnel plots of publication bias. A. Deek's funnel plot of overall diagnostic accuracy literature publication bias. B. Begg's funnel plot of overall OS literature publication bias.

markers expressed on mesenchymal cells or device combined immunomagnetic technique with other techniques would be eagerly desired to improve the sensitivity of CTC detection without decreasing of specificity.

As previous study reported [40], a prognostic factor with a risk ratio (RR) larger than 2 was considered clinically practical. Most HRs in our meta-analysis were above 2, it suggested that CTC enumeration by CSS was not only statistically significant but also clinically significant in the prognosis of PCa patients.

Twenty-two studies were incorporated in our OS meta-analysis. The overall estimate HR (2.68; P < 0.01) demonstrated a statistically significant correlation between UCE detected by CSS and poorer OS. In the subgroup analysis, interestingly, studies conducted in Europe (HR: 3.56; P < 0.001) demonstrated significantly higher estimate HR compared with other locations such as America (HR: 1.86; P < 0.001) and Asia (HR: 2.10; P < 0.001). This finding should be further explored by multi-center trials conducted with unified standard. Besides, our study found risk for poorer OS significantly higher in patients with UCE after a period of therapy (HR: 3.41; P < 0.001) than patients with UCE at baseline (HR: 2.00; P < 0.001), which indicated that UCE after treatment might be able to guide us in the therapeutic regimen regulation.

Our study further explored the impact of CTC enumeration by CSS conversion on OS. CTC conversion from UCE to FCE was not statisti-

cally significant in predicting poorer OS (Group 2 versus Group 1: HR = 2.00; P = 0.055). Group 4 presented no clinically significant poorer OS compared with Group 3 (HR: 1.43; P = 0.04), which demonstrated that a final UCE indicated the poorer OS despite the baseline CTC enumeration. Other comparison between groups revealed that CTC conversion from FCE to UCE predicted significant poorer OS.

Meanwhile, UCE was also a statistically and clinically significant indicator to poorer bRFS/PFS (HR: 2.46; P < 0.001), which would be able to help us in timely adjusting the therapeutic regimen with real-time supervision.

Gleason score and pT stage were convincing factors in prognostic predicting and therapy decision making for PCa patients. Our study revealed a statistically significant correlation between UCE and high Gleason score (OR: 2.57; P = 0.001), but no statistical correlation between UCE and high pT stage (OR: 0.58; P = 0.079). In fact, three included studies [10, 13, 14] reported a negative OR (< 1) between UCE and high pT stage, while only one study [31] reported a positive OR (≥ 1). This might due to the frequency of CTC with EMT makers were more likely to rise in cancer patients with metastasis [41] and patients who were resistant to treatment [42], and these CTCs were easily mis-detected by CSS.

Owning to that, the cutoff value of CTC enumeration by CSS should be defined lower with more blood sample in diagnostic application, and should be defined higher or with less blood sample in prognostic application, especially in LPCa prognostic prediction. Generally, the cutoff value for different tumor types and for different purposes should be further explored by more detailed and specialized trials.

Moreover, earlier mice studies demonstrated that intravenously injected CTC clusters presented more powerful ability in starting tumor metastasis than single CTC [43, 44], and the existence of CTC clusters in metastatic cancer patients were strongly correlated with poorer prognosis [45]. Cluster-Chip, a recently developed device by Sarioglu [46], might be able to fill the gap of CTC cluster detection in current techniques. Combination of single CTC and CTC cluster detection would substantially improve the detection sensitivity of CTC.

In addition to intact CTC, apoptotic CTC, circulating free tumor DNA (cfDNA) and circulating tumor RNA (ctRNA) shed from solid tumor masses into peripheral blood could also be source of liquid biopsy material in PCa diagnosis and prognosis prediction. These "liquid biopsies" were relatively non-invasive and allowed for real-time supervision of the ever-changing molecular aberrations within various PCa cell sub-populations [47].

With exponential improving of technologies, CTC detection and other liquid biopsies will soon replace solid tumor biopsy in PCa management for sure.

Certain limitations existed in our meta-analysis. Several characteristics could influence the detection of CTCs, such as tumor metastatic status, tumor hormonesensitivity and detection threshold definition. Diagnostic sensitivity was higher in non-metastasis patients than in patients with distant metastasis in Pantel's study [41], and in Kallergi's study [42], CTCs were more likely to be detected in patients who were resistant to treatment. Except for CTC enumeration, many other factors could contribute to patient prognosis. Different ages, tumor types, metastatic sites and therapeutic regimens existed among studies incorporated in our meta-analysis. The number of diagnostic accuracy studies was limited, while the heterogeneity is statistically significant. Moreover, some included studies presented high risk of bias and low applicability concern. Meta-regression was not performed to identify factors contributed to the high heterogeneity, concerning for the limited study number and mixed variables such as different end points, patient characteristics and study designs. Multivariate analysis of logHR and SE were combined with univariate analysis of logHR and SE extracted from survival curve, which reduced the power to make associations of CSS CTC enumeration with the outcomes.

Conclusions

Our study is the first comprehensive and detailed meta-analysis to reveal the diagnostic and prognostic value of CTC enumeration by CSS in PCa as well as the associations between CTC enumeration and clinical characteristics of these patients. Generally, the result of our study manifested that CTC enumeration by CSS

is a clinically useful method to diagnose and predict prognosis in PCa patients. With proper use of CSS, we may be able to confirm PCa diagnosis without invasion and adjust the therapeutic regimen timely under real-time supervision. However, due to the variability in study design and end point definition which contribute to the high heterogeneity, more high quality, multi-center studies with unified standard will be needed to further address the diagnostic and prognostic value of CTC enumeration by CSS. Meanwhile, better equipment with higher sensitivity and more accurate thresholds for different applications of CSS should be further explored.

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Disclosure of conflict of interest

None.

Authors' contribution

Ruochen Zhang and Xin Qin selected the literature; Ruochen Zhang and Zijun Zhao collected data; Zijun Zou and Liangyou Tang assessed the quality of each literature; Yiping Lu settled the disagreements. Ruochen Zhang analyzed data and wrote the paper.

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