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Original Article

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Prognostic Value of ctDNA Detection in Patients With Locally Advanced Rectal Cancer Undergoing Neoadjuvant Chemoradiotherapy: A Systematic Review and Meta-analysis

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Abstract

Background: Circulating tumor DNA (ctDNA) is increasingly used as a biomarker for metastatic rectal cancer and has recently shown promising results in the early detection of recurrence risk.

Methods: We conducted a systematic review and meta-analysis to explore the prognostic value of ctDNA detection in LARC patients undergoing neoadjuvant chemoradiotherapy (nCRT). We systematically searched electronic databases for observational or interventional studies that included LARC patients undergoing nCRT. Study selection according to the PRISMA guidelines and quality assessment of the REMARK tool for biomarker studies. The primary endpoint was the impact of ctDNA detection at different time points (baseline, post-nCRT, post-surgery) on relapse-free survival (RFS) and overall survival (OS). The secondary endpoint was to study the association between ctDNA detection and pathological complete response(pCR) at different time points.

Results: After further review and analysis of the 625 articles initially retrieved, we finally included 10 eligible studies. We found no significant correlation between ctDNA detection at baseline and long-term survival outcomes or the probability of achieving a pCR. However, the presence of ctDNA at post-nCRT was associated with worse RFS (HR = 9.16, 95% CI, 5.48-15.32), worse OS (HR = 8.49, 95% CI, 2.20-32.72), and worse pCR results (OR = 0.40, 95%CI, 0.18-0.89). The correlation between the presence of ctDNA at post-surgery and worse RFS was more obvious (HR = 14.94; 95% CI, 7.48-9.83).

Conclusions: Our results suggest that ctDNA detection is a promising biomarker for the evaluation of response and prognosis in LARC patients undergoing nCRT, which merits further evaluation in the following prospective trials.

Key words: locally advanced rectal cancer; neoadjuvant chemoradiotherapy; circulating tumor DNA; liquid biopsy; prognosis; systematic review.

Implications for Practice

The results of this study suggest that ctDNA detection is a promising biomarker for the evaluation of response and prognosis in patients with locally advanced rectal cancer undergoing neoadjuvant chemoradiotherapy, which merits further evaluation in future prospective trials.

Introduction

Liquid biopsy is a laboratory technique that obtains information about tissues from various body fluids (eg, blood, urine, saliva, pleural fluid, cerebrospinal fluid, etc.) of cancer patients that can be used to detect and analyze biomarkers.[1] Unlike traditional tissue biopsies, liquid biopsies are relatively non-invasive, easy to obtain, and reproducible.[2] Liquid biopsies can detect circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and exosomes and extracellular vesicles in the blood. Among these, ctDNA is tumor DNA derived from apoptotic or necrotic tumor cells of cancer patients, and such DNA fragments may contain tumor-specific mutations that occur in the original cells.[3] Compared to other circulating biomarkers, ctDNA is a potential biomarker because it

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has a higher blood concentration, sensitivity, and specificity. Significant advances have been made in the analysis of ctDNA assays, including polymerase chain reaction (PCR) and next generation sequencing (NGS) based high-throughput detection technology.[4]

Minimal or microscopic residual disease (MRD) is known as a very small number of cancer cells that remain in the body during or after treatment. MRD can lead to distant metastasis or recurrence in patients lacking any clinical or radiographic evidence of metastasis.[5] Researchers initially identified the importance of MRD in hematological malignancies, allowing clinicians to assess patient response to therapy and predict outcomes.[6] A growing number of studies have shown that ctDNA detection can be used to determine the presence of MRD and predict postoperative recurrence in cancer patients.[7]

Recently, several studies have shown that ctDNA detection plays an important role in the prognosis of LARC patients treated with neoadjuvant chemoradiotherapy (nCRT). However, the findings are somewhat controversial due to the small number of patients included in each study.[8] Therefore, we conducted a systematic review and meta-analysis of the prognostic value of ctDNA detection in LARC patients undergoing nCRT.

Method

The work has been reported in line with PRISMA[9] (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) and AMSTAR[10] (Assessing the methodological quality of systematic reviews) Guidelines. We included all studies that were eligible to assess the relationship between the presence of ctDNA and clinical outcomes in LARC patients undergoing nCRT. This meta-analysis has been registered in the international prospective register of systematic reviews (PROSPERO, 2022, CRD42022355971).

Literature Search and Study Selection

To identify eligible studies, we mainly searched electronic databases such as PubMed, Embase, Cochrane Central Register of Controlled Trials, and Web of Science. The main search strategy in the PubMed database are as follows: ("Rectal Neoplasms" [Mesh] OR locally advanced rectal cancer [Title/Abstract] OR LARC [Title/Abstract] OR Neoplasm, Rectal [Title/Abstract] OR Rectal Neoplasm [Title/Abstract] OR Rectum Neoplasms [Title/Abstract] OR Neoplasm, Rectum [Title/Abstract] OR Rectum Neoplasm [Title/Abstract] OR Rectal Tumors [Title/Abstract] OR Rectal Tumor [Title/Abstract] OR Tumor, Rectal [Title/Abstract] OR Neoplasms, Rectal [Title/Abstract] OR Cancer of Rectum [Title/Abstract] OR Rectum Cancers [Title/Abstract] OR Rectal Cancer [Title/Abstract] OR Cancer, Rectal [Title/Abstract] OR Rectal Cancers [Title/Abstract] OR Rectum Cancer [Title/Abstract] OR Cancer, Rectum [Title/Abstract] OR Cancer of the Rectum [Title/Abstract]) AND ("Circulating Tumor DNA" [Mesh] OR DNA, Circulating Tumor [Title/Abstract] OR Tumor DNA, Circulating [Title/Abstract] OR Cell-Free Tumor DNA [Title/Abstract] OR Cell Free Tumor DNA [Title/Abstract] OR DNA, Cell-Free Tumor [Title/Abstract] OR Tumor DNA, Cell-Free [Title/Abstract] OR ctDNA [Title/Abstract] OR ct-DNA [Title/Abstract]). Other databases were searched similarly to the PubMed database. The last search was conducted on August 28, 2022. Two authors (L.C and X.Z) independently reviewed the title and abstract of the citation and obtained the full text of potentially eligible studies, disagreements were resolved by discussion or, if necessary, by a third author (L.H). A reference list review of all retrieved articles was further screened for additional eligible studies.

Inclusion and Exclusion Criteria

Studies in this article must meet the following inclusion criteria: (1) all observational studies and randomized controlled trials; (2) human studies; (3) any nCRT methods were accepted; (4) documented continuous ctDNA collection and clinical outcome, including pathological complete response (pCR) and survival data [recurrence-free survival (RFS), metastases-free survival (MFS), disease-free survival (DFS), local recurrence-free survival (LRFS), progression-free survival (PFS), distant metastasis-free survival (DMFS), and overall survival (OS)]; and (5) the results of ctDNA are binary variables, and all ctDNA detecting and analysis methods are accepted. The main exclusion criteria included: (1) reviews, letters, case reports, and conference abstracts; (2) animal experiments, in vitro studies, and ongoing studies; (3) studies with less than 10 patient samples.

In addition, studies that reported incomplete data were excluded after careful examination of available Supplemental Data and email requests to the corresponding authors of the original studies.

Quality Assessment

Two authors (L.C and X.Z) conducted independent assessments according to the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK)[11] checklist. Disagreements were resolved by discussion or by a third author (L.H). No studies were excluded on this basis. According to the Cochrane Collaboration's Risk of Bias tool and the assessment of Risk Of Bias In Systematic reviews tool (ROBIS),[12,13] we comprehensively assessed the risk of bias in the eligible studies.

Data Extraction and Synthesis

Two reviewers (L.C and X.Z) extracted data individually using predefined data extraction tables. Disagreements were resolved by discussion or by a third author (L.H). We extracted the following data from the literature eligible for inclusion in this study and its Supplementary Information: authors, clinical trial registration number, publication year, patient characteristics, number of patients, ctDNA analysis method, pCR, RFS [according to the study (Tables 1 and 2), composite endpoints included RFS, MFS, DFS, LRFS, PFS, and DMFS] and OS. We excluded the conference abstracts and included only peer-reviewed full-text publications.

In our analysis, ctDNA was treated as a binary variable (detected *vs.* not detected).

The odds ratio (OR) and 95% CI for pCR analysis were calculated to summarize the overall effect. The hazard ratio (HR) with 95%CI was calculated for each study to obtain overall estimates for RFS and OS analyses. *P*-values for pooled HRs and ORs were not reported.

In all analyses, we calculated and reported the heterogeneity estimation (by I^2 and statistical test methods to

Table 1. Study characteristics of the studies included in the systematic review and meta-analysis.

Author	Clinical trial registration number	Year	Patient characteristics	Study description	Types of neoadjuvant chemoradiotherapy	Stage distribution	Quality assessment*
Jeanne Tie	ACTRN12612000327886	2019	Rectal adenocarcinoma, pre-treatment MRI (or endorectal ultrasound if MRI was contraindicated) staging which demonstrated locally advanced disease ($cT_{3-4}N_0$ or $cT_{any}N_{1-2}$)	Prospective multicentre study in patients with LARC treated with nCRT	Long course fluoropyrimidine-based chemoradiotherapy (n = 159).	Stage II (n = 35) stage III (n = 124)	31
Shelize Khakoo	NCT00825110	2020	LARC (cT_{3-4} and/or node positive) confirmed on histology and absence of metastases on imaging	A single center study in patients with LARC were prospectively recruited	Capecitabine 1650 mg/m^2/day for 6 weeks alongside 50.4-54 Gy radiotherapy (n = 47).	Stages I-II (n = 6) stage III (n=41)	29
Satoshi Murahashi	_	2020	LARC with clinical stage II or III ($cT_{3-4}N_0$, or $cT_{any}N_+$)	Prospective study enrolled patients with LARC	Standard CRT [50.4 Gy in 28 fractions over 5 weeks and concurrent Tegafur/Gimeracil/Oteracil (80-120 mg/m^2/day) orally administered over 4 weeks] (n = 33); SRT [25 Gy administered in 5 fractions over 1 week] (n = 9); Others (n = 43).	Stage II (n = 27) stage III (n = 58)	32
Filip Pazdirek	_	2020	Patients with locally advanced (stages II, III) rectal cancer	Prospective study included patients with LARC	50.4 Gy of radiation and concomitant administration of Xeloda (capecitabine) at a dose of 825 mg/m^2.Irradiation was carried out by 25 fractions with initial boost of 5.4 Gy (n = 36).	Stage II (n = 11) stage III (n = 25)	21
Susan G. R. McDuff	_	2021	Patients with newly diagnosed LARC	Retrospective study	Long-course chemoradiation [45 Gy to the pelvis followed by a boost to the mesorectum to 50.4 Gy in 1.8 Gy fractions with concurrent capecitabine or infusional fluorouracil (5-FU)]. Three of the patients who received infusional 5-FU also received midostaurin on a separate interventional protocol (n = 29).	Stage II (n = 6) stage III (n = 23)	26
Joana Vidal	GEMCAD1402	2021	Rectal adenocarcinoma, with an inferior margin distal border below the peritoneal reflection. High-risk LARC was considered on the basis of high-resolution MRI clinical (c) staging.	A phase II randomized, multicentric clinical trial	Arm A (induction chemotherapy with aflibercept plus mFOLFOX6,n = 36) or arm B (induction chemotherapy with mFOLFOX6 alone, n = 26). Both schemes were administered for 6 cycles, followed by 5 weeks of CRT with capecitabine.	NA	30
Jiaolin Zhou	NCT03042000	2021	Rectal adenocarcinoma and pre-treatment MRI (or endorectal ultrasonography if MRI was contraindicated) identified clinical T_4Nany (cT_4N_{any}) or $cT_{any}N_{1b-2}$ disease or LARC with adverse factors.	Prospective multicenter trial	Long-term neoadjuvant radiotherapy (45-50 Gy/25 fractions/5 weeks) with 3 cycles of neoadjuvant chemotherapy (nCT), in which the first 2 cycles were concomitant with radiation. Single-agent capecitabine was given orally at a dose of 1650-2000 mg/m^2/day divided in 2 doses from days 1 to 14 every 3 weeks. The CapeOx regimen consisted of a 2-h intravenous infusion of oxaliplatin 85-100 mg/m^2 on day 1 and capecitabine with the same protocol as the single-agent regimen (n = 104).	Stage II (n = 5) stage III (n = 99)	31

Table 1. Continued

Author	Clinical trial registration number	Year	Patient characteristics	Study description	Types of neoadjuvant chemoradiotherapy	Stage distribution	Quality assessment*
Wenyang Liu	NCT02533271	2022	Patients with LARC ($cT_{3,4}N_0$ or $cT_{any}N_{1,2}$)	Multicentre, open-label, prospective phase II/III randomized trial	Short-course preoperative radiotherapy (SCPRT, 5 Gy × 5 alone) with neoadjuvant chemotherapy (NCT) (4 cycles of capecitabine plus oxaliplatin regimen) (n = 29) and preoperative long-course chemoradiotherapy (2 Gy × 25 with capecitabine)(n = 31).	NA	32
Raffaello Roesel	NCT03699410	2022	Rectal adenocarcinoma, a clinical staging of a LARC	Prospective study on patients treated for LARC	Long course 5-fluorouracil based chemotherapy (5-FU) plus 50.4 Gy radiotherapy (n = 25).	NA	24
Yaqi Wang	NCT02605265	2021	LARC ($cT_{3,4}/N_{0-2}, M_0$) patients	Prospective cohort study	[50 Gy/25 fractions; concurrent capecitabine + irinotecan chemotherapy] and 1 cycle of interval chemotherapy (CAPIRI, capecitabine + irinotecan) (n = 119).	Stage II (n = 2) stage III (n = 80) stage III (n = 37)	34

*Quality assessment based on the REMARK score. Maximum score 40.
Abbreviations: CRT, chemoradiotherapy; LARC, locally advanced rectal cancer; MRI, magnetic resonance imaging; NA, not available. nCRT, neoadjuvant chemoradiotherapy; SRT, Short-course radiotherapy.

assess the null hypothesis of homogeneity across studies). Regardless of the results of the statistical tests for homogeneity, (when $P > .05$ the null hypothesis of homogeneity was not rejected). Both fixed and random effects models were accepted (when $P > .05$, the null hypothesis of homogeneity was not rejected). Using the inverse variance method for pooling to calculate the overall HR assuming a common effect for fixed effects analyses. Adversely, using the DerSimonian-Laird method to account for heterogeneity for random effects analyses. To detect publication bias, funnel plot analysis and Egger's test were used. Using R statistical software version 4.2.1 (meta package) to conduct all analyses.

Main Study Outcomes and Measures

The primary endpoint of this study was to investigate the ctDNA detection on RFS and OS at different time points (baseline, post-nCRT, post-surgery) effect. We included outcome measures from studies reporting RFS, MFS, DFS, LRFS, PFS, and DMFS to estimate RFS.

The secondary endpoint was to explore the association of ctDNA detection with pCR at different time points (baseline, post-nCRT).

Result

Study Selection

Through a systematic literature search, we retrieved a total of 625 literatures. After removing duplicate literatures, we excluded irrelevant literatures by checking titles and abstracts one by one. In this systematic review and meta-analysis, 10 eligible articles were finally included. The flow chart of selecting articles according to PRISMA guidelines is shown in Fig. 1.

Study and Patient Description

A total of 10 studies were included in the meta-analysis. Nine were prospective studies that collected ctDNA at different time points,[14-19] and 3 of them did not collect post-surgery ctDNA data.[20-22] The remaining one was a retrospective study.[23] All studies included nCRT therapy.

In randomized controlled trials, regardless of the treatment group, study outcomes are reported together. In 4 studies,[17,20,21,23] RFS and OS outcomes were not fully reported in publications but were calculated using published data.

The main methods for ctDNA analysis include (1) multiplex PCR[22]; (2) real-time PCR & NGS[19]; (3) droplet digital PCR (ddPCR)[15,23]; (4) NGS[17,18,21]; (5) sequencing[14,16]; (5) more agnostic methods, such as a two-level approach (first a low-cost detection method of denaturing capillary electrophoresis, then followed by examination of initially negative samples by a high-sensitivity BEAMING assay).[20]

The main characteristics of the studies included in this meta-analysis, the different time points of ctDNA collection, and the details of the follow-up of patients are summarized in Tables 1 and 2; Supplementary Table S1 summarizes the definition of endpoints for each trial and study included in the meta-analysis. The number of patients with evaluable blood samples and ctDNA detection at baseline, post-nCRT, and post-surgery can be found in Supplementary Table S2.

Table 2. Number of patients at different timepoints analyzed in the meta-analysis, ctDNA collections related to nCRT and survival endpoint characteristics.

Author	Clinical trial registration number	Year	Number of patients (*N*)	Patients with evaluable ctDNA at baseline (*N*)	Patients with evaluable ctDNA post-nCRT (*N*)	Patients with evaluable ctDNA post-surgery (*N*)	ctDNA collections timepoints related to the meta-analysis outcomes	Survival endpoints collected	Duration of follow up	Method for ctDNA analysis
Jeanne Tie	ACTRN12612000327886	2019	200	159	144	159	Pre-nCRT Post-nCRT Post-surgery	RFS	Median 24 months	A personalised Safe-SeqS assay
Shelize Khakoo	NCT00825110	2020	47	47	47	23	Pre-nCRT Mid-nCRT Post-nCRT Post-surgery	MFS, DFS, LRFS, OS	Median 26.4 months	ddPCR
Satoshi Murahashi	—	2020	85	82	81	59	Pre-nCRT Post-nCRT Post-surgery	RFS	NA	Molecular barcoded amplicon-based deep sequencing
Filip Pazdirek	—	2020	36	36	—	—	Pre-nCRT Mid-nCRT	DFS, OS	At least 3 years after surgery	A two-level approach (first a low-cost detection method of denaturing capillary electrophoresis was used followed by examination of initially negative samples by a high-sensitivity BEAMING assay)
Susan G. R. McDuff	—	2021	29	—	26	21	Pre-nCRT Post-nCRT Post-surgery	PFS	Median 20 months	ddPCR
Joana Vidal	GEMCAD 1402	2021	62	52	45	—	Pre-nCRT Post-nCRT	DFS,OS	Median 38 months	A single-sample next generation sequencing–based in vitro diagnostic assay
Jiaolin Zhou	NCT03042000	2021	104	104	95	89	Pre-nCRT Mid-nCRT Post-nCRT Post-surgery	MFS	Median 18.8 months	Next-generation sequencing
Wenyang Liu	NCT02533271	2022	60	42	60	—	Pre-nCRT Mid-nCRT Post-nCRT	RFS, LRFS, DMFS, OS	Median 33.25 months	Multiplex PCR
Raffaello Roesel	NCT03699410	2022	25	23	24	23	Pre-nCRT Mid-nCRT Post-nCRT Post-surgery	NA	Median 14 months	Real-time PCR and NGS
Yaqi Wang	NCT02605265	2021	119	119	103	103	Pre-nCRT Mid-nCRT Post-nCRT Post-surgery	RFS	Median 21.5months	Customized NGS

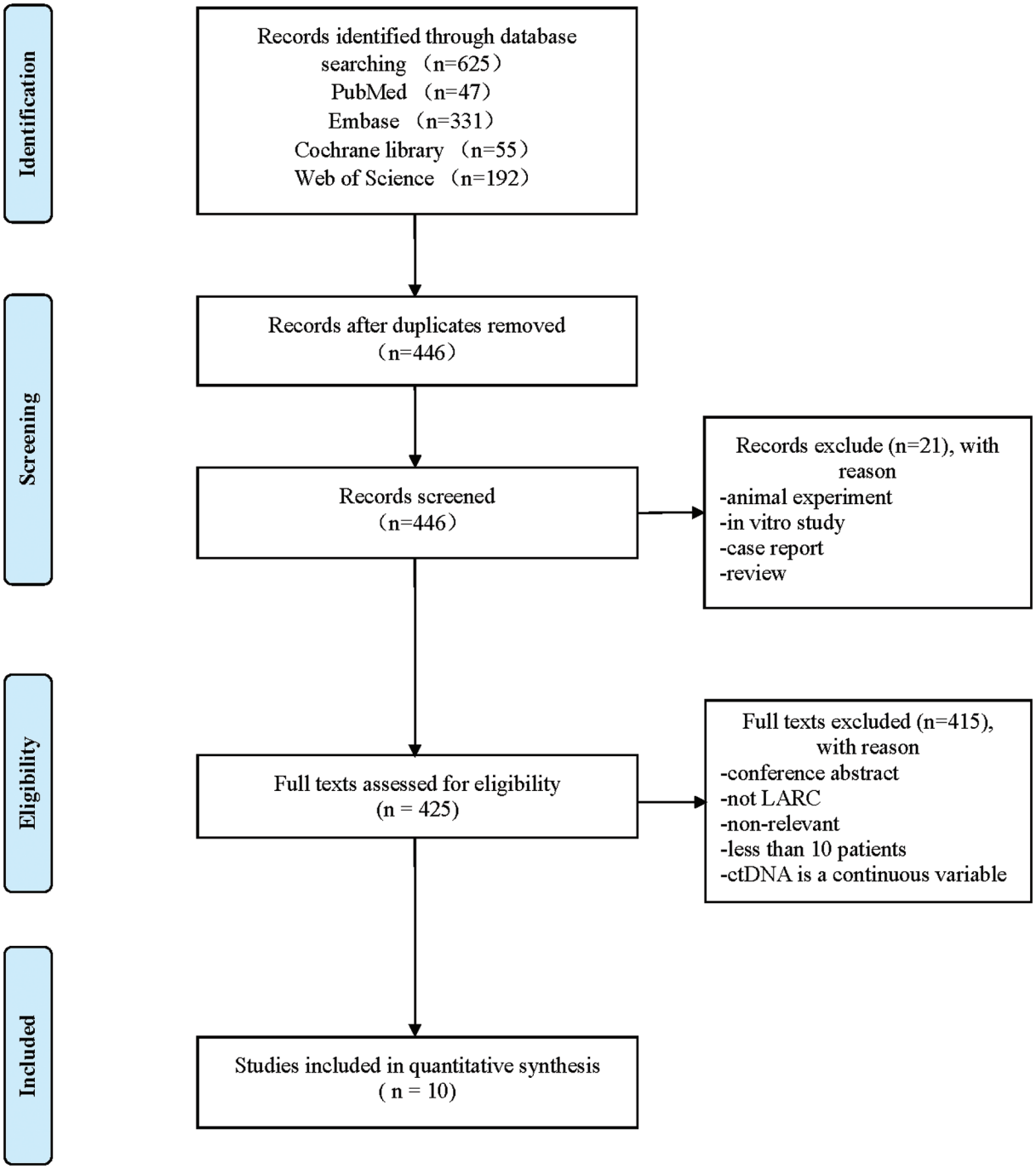


Figure 1. Literature search and study selection according to PRISMA 2020 flow diagram for systematic review.

Primary Endpoint: Association of ctDNA With Survival Outcomes

ctDNA at Baseline

Six studies (n = 455) reported baseline ctDNA assay data and outcomes.[14,15,17,20-22] A total of 342/455 (75.16%) patients included in the RFS analysis and 85/135 (62.96%) patients included in the OS analysis can detect ctDNA at baseline. Overall, baseline ctDNA presence and statistically significantly worse RFS (HR = 1.11;95% CI, 0.47-2.60; Fig. 2A) and OS (HR = 0.71; 95% CI, 0.23-2.18; Fig. 2B) are not relevant. Evidence of heterogeneity was found in HR estimates from the included studies (I^2 = 53%; I^2 = 81%; respectively).

Pazdirek et al. can largely explain this heterogeneity,[20] the only study to suggest that the presence of ctDNA at baseline is associated with poorer RFS and OS. This may be due to the small number of patients they included in the study, in addition, they adopted a two-level approach to analysis ctDNA. The random effect model was used to account for heterogeneity between studies.

ctDNA at Post-nCRT

Six studies (n = 494) reported ctDNA detection data after completion of nCRT.[14,15,17,18,21,22] A total of 65/494 (13.16%) patients can detect ctDNA at post-nCRT and be associated

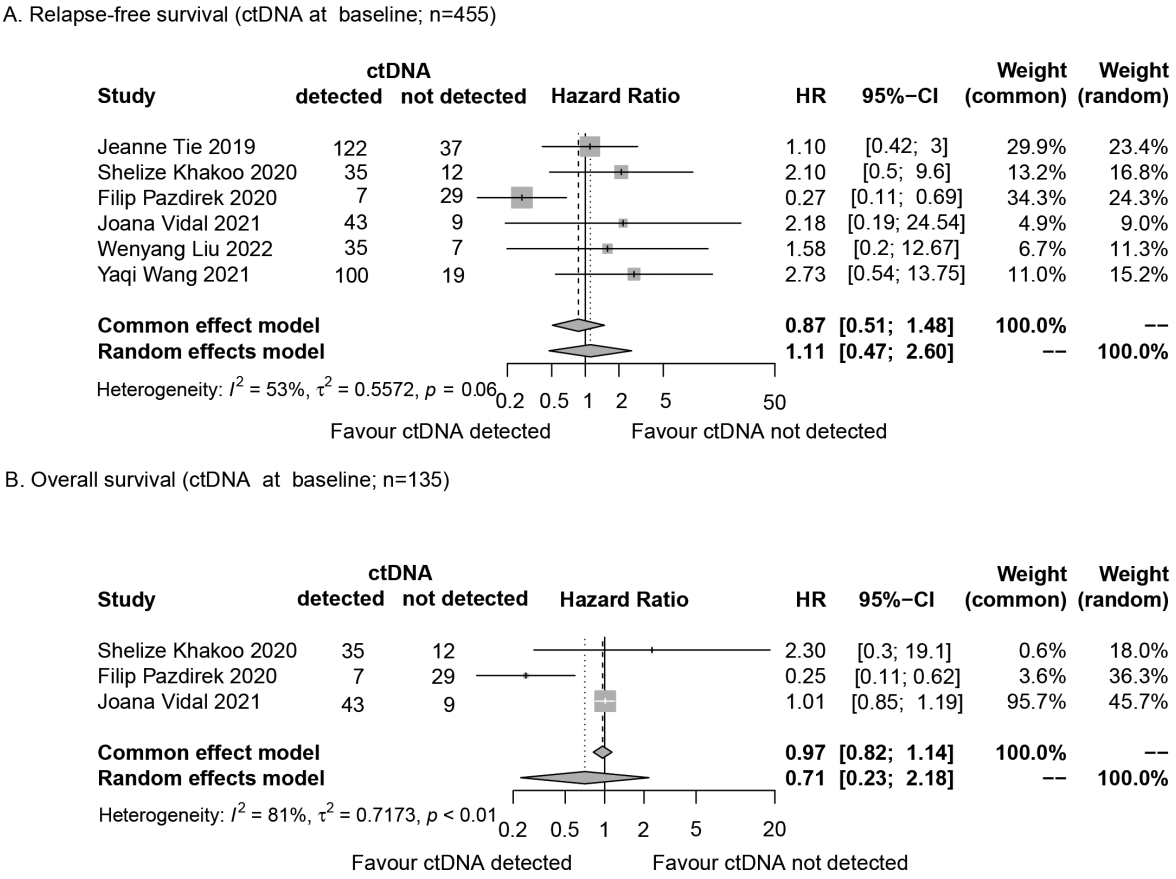


Figure 2. Forest plot of the impact of ctDNA on RFS and OS at baseline.

with a statistically significantly higher risk of recurrence (HR = 9.16; 95% CI, 5.48-15.32; Fig. 3A). Three studies (n = 152) provided data on OS.[15,21,22] ctDNA was detected after completion of nCRT in 31/152 (20.39%) patients and was associated with worse OS (HR = 8.49; 95% CI, 2.20-32.72; Fig. 3B).

ctDNA at Post-Surgery

Five studies (n = 351) reported the association between ctDNA detection and survival outcomes post-surgery.[14-16,18,23] ctDNA was detected in 54/351 (15.38%) patients at post-surgery and was associated with poorer RFS (HR = 14.94; 95% CI, 7.48-29.83; Fig. 4).

Secondary Endpoints: ctDNA and pCR

ctDNA at Baseline

To assess the odds of achieving pCR at baseline, seven studies (n = 581) were combined.[14,16-19,21] The random effect model showed that there was no association between ctDNA status and pCR outcomes at baseline (OR = 0.67; 95% CI, 0.42-1.04; Fig. 5A). In the OR estimates of the included studies did not find evidence of heterogeneity (I^2 = 0%).

Overall, detected ctDNA at baseline was proved to be a poor predictor of pCR (overall accuracy = 0.68; 95% CI, 0.60-0.75; Fig. 5B).

ctDNA at Post-nCRT

Seven studies (n = 475) assessed the odds of achieving pCR in the presence of ctDNA at post-nCRT.[14,16,18,19,21-23] The fixed effect model showed an association between ctDNA presence and poor pCR outcomes at post-nCRT (OR = 0.40; 95% CI, 0.18-0.89; Fig. 6A). No evidence of heterogeneity was found in the OR estimates of the included studies (I^2 = 0%). Overall, detected ctDNA at post-nCRT was proved to be a worse predictor of pCR (overall accuracy = 0.35; 95% CI, 0.31-0.39; Fig. 6B).

Quality Assessment and Risk of Bias Analysis

According to the REMARK list, all studies were scored with a range of 21-34, with 40 being the highest score. Supplementary Fig. 1 summarized graphically with a funnel plot the risks of publication bias. Although less certain, pCR analysis of ctDNA at post-nCRT had some potential publication bias (P = .0588).

Discussion

Although after nCRT, pCR was associated with a low risk of recurrence, some patients still relapse.[24] On the contrary, not all patients with residual after nCRT experienced disease recurrence,[23] which strongly suggests the need for additional biomarkers to more accurately stratify the risk of recurrence.[24]

Our systematic review and meta-analysis included patients with LARC who received nCRT. And we strengthened the

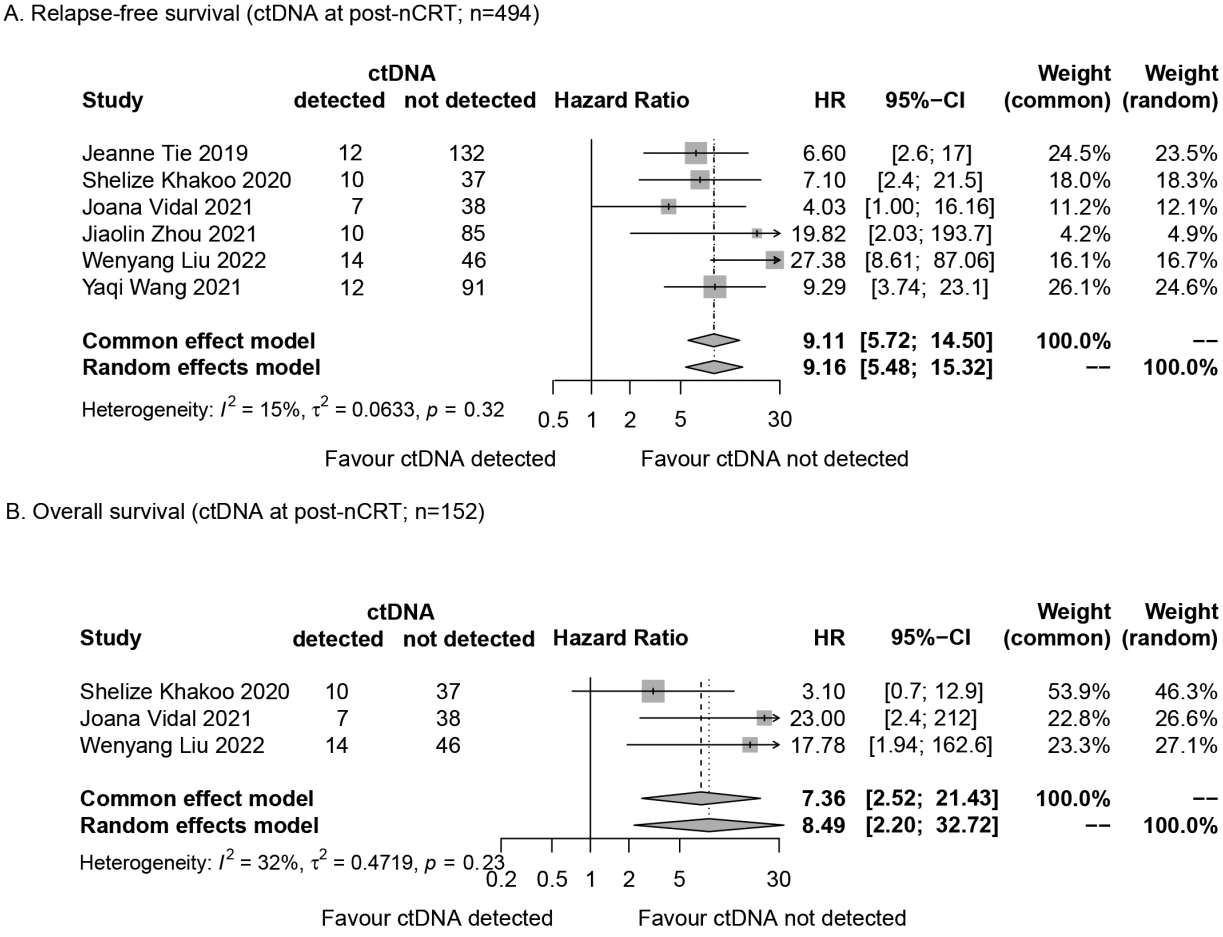


Figure 3. Forest plot of the impact of ctDNA on RFS and OS at post-nCRT.

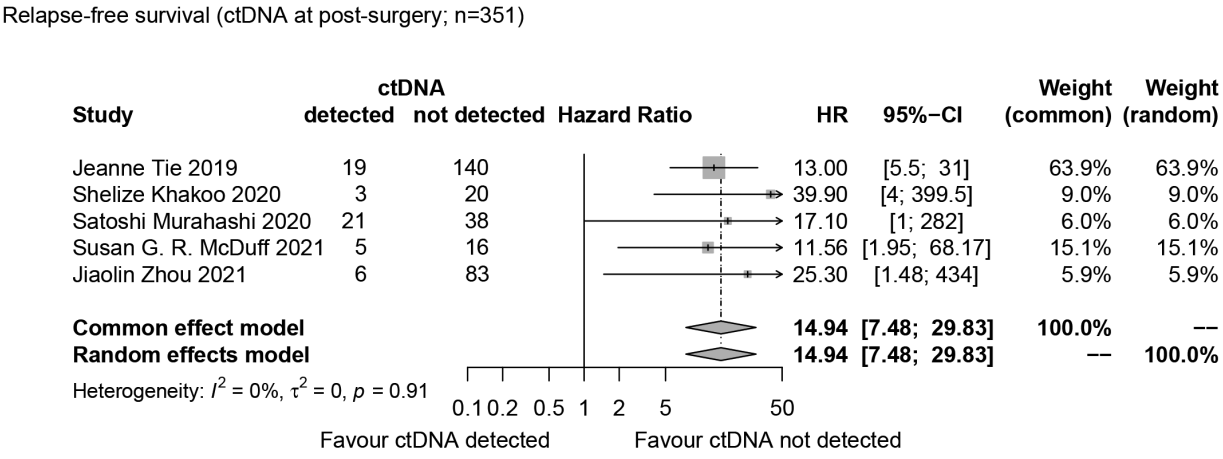
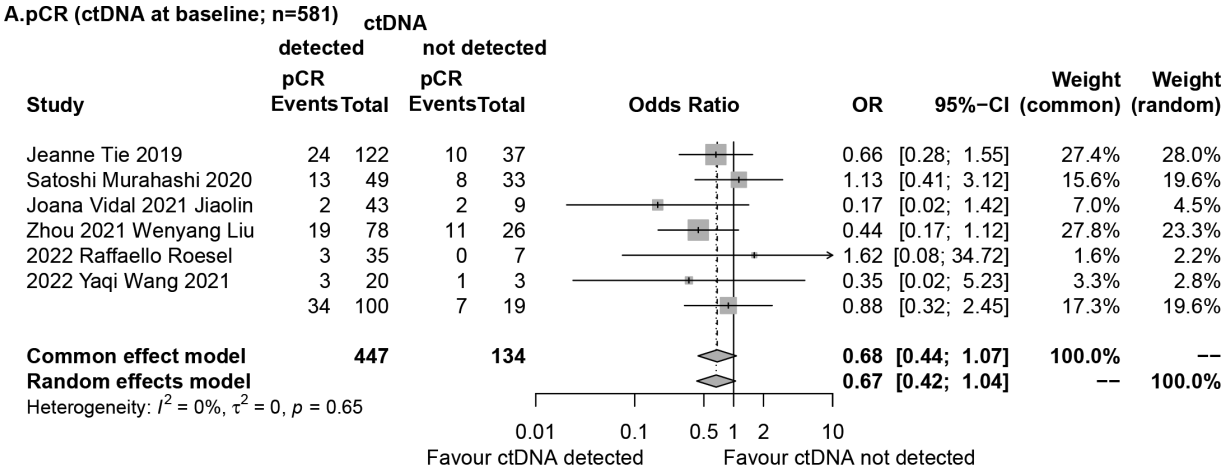


Figure 4. Forest plot of the impact of ctDNA on RFS at post-surgery.

potential role of ctDNA detection as a prognostic biomarker in LARC. Baseline ctDNA status was not significantly associated with recurrence risk, whereas the presence of ctDNA at post-nCRT and post-surgery was significantly associated with higher recurrence risk. Tie et al. believed that ctDNA detected at baseline was released by the primary tumor and could not be used as a prognostic indicator, but only as a diagnostic indicator, while the presence of ctDNA could reflect MRD and potential recurrence risk after tumor resection.[14]

Some studies have pointed out that the dynamic changes of ctDNA in the process of undergoing nCRT also have a certain significance. Zhou et al.[18] found that the presence of ctDNA 2-3 weeks after nCRT was significantly associated with a



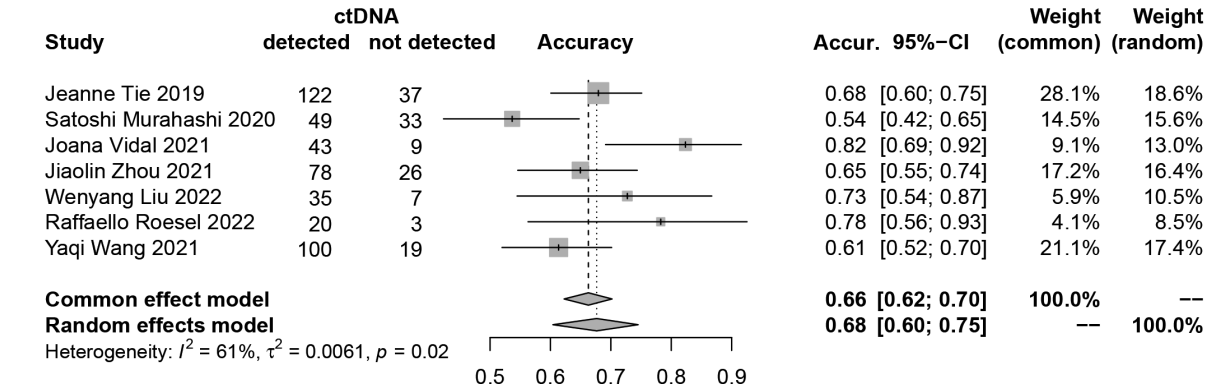


Figure 5. Forest plot of the prediction of pCR based on ctDNA status at baseline.

higher risk of distant metastasis. However, there is no fully unified view of the time point at which ctDNA is detected during nCRT, so we did not sum up the pCR outcomes and risk of recurrence for patients at this time point. Therefore, more research is needed to explore the optimal time point for the detection of ctDNA during nCRT to reduce the financial burden on patients and maximize the benefits.

Our meta-analysis combined data from studies on the association of ctDNA with OS.[15,20-22] Our study showed that post-nCRT and post-surgery ctDNA detection were associated with recurrence risk and that post-nCRT ctDNA detection was associated with OS. Only the study by Khakoo et al.[15] reported the association of post-surgery ctDNA detection with OS, who suggested that postoperative detection of ctDNA was associated with a poorer OS (but not reaching statistical significance), which may be related to the immaturity of OS data related.

Our study showed that the detection of ctDNA at baseline was not associated with the probability of acquiring pCR. This result is well explained by Tie et al.,[14] the presence of baseline ctDNA can only diagnose the presence of the disease and cannot predict the probability of obtaining pCR after surgery. However, this also requires further prospective studies with larger samples to verify. The status of ctDNA at post-nCRT correlated with the probability of acquiring pCR. This is because after receiving nCRT, a subset of patients benefited from the treatment, which resulted in the elimination of ctDNA released by the primary tumor tissue, resulting in pCR. At this time, detected ctDNA was associated with poorer prognosis, instructing clinicians to pay more attention to these patients in the subsequent course of treatment.

Our study also has certain limitations, which mainly had a bearing on the heterogeneity of the included studies. First, in our study, ctDNA was treated as a binary variable (detectable/not detectable). We excluded a subset of studies that considered ctDNA as a continuous variable, data that may also have determined the outcome, but were unresolvable in our study. Second, the selection of endpoint events for each study is not uniform, including RFS, MFS, DFS, LRFS, PFS, and DMFS. We finally chose RFS as the endpoint of this composite according to different studies (Tables 1 and 2), but admittedly, there are differences between them. Third, among the studies we included, the analysis methods of ctDNA also varied. And some studies used custom panels for analysis, and these data may also have some influence on our findings. It is generally known that the sensitivity of ctDNA detected by different detection and analysis methods is different, so a unified ctDNA detection and analysis method is urgently needed to judge the significance of ctDNA detection for prognosis. Finally, the time points for detecting ctDNA also differ in different studies. We mainly observed 3 time points: baseline, post-nCRT, and post-surgery. In fact, the detection of ctDNA during nCRT also has an important impact on prognosis, but due to the inclusion of the study differences in the specific collection time points during nCRT, so we did not analyze the

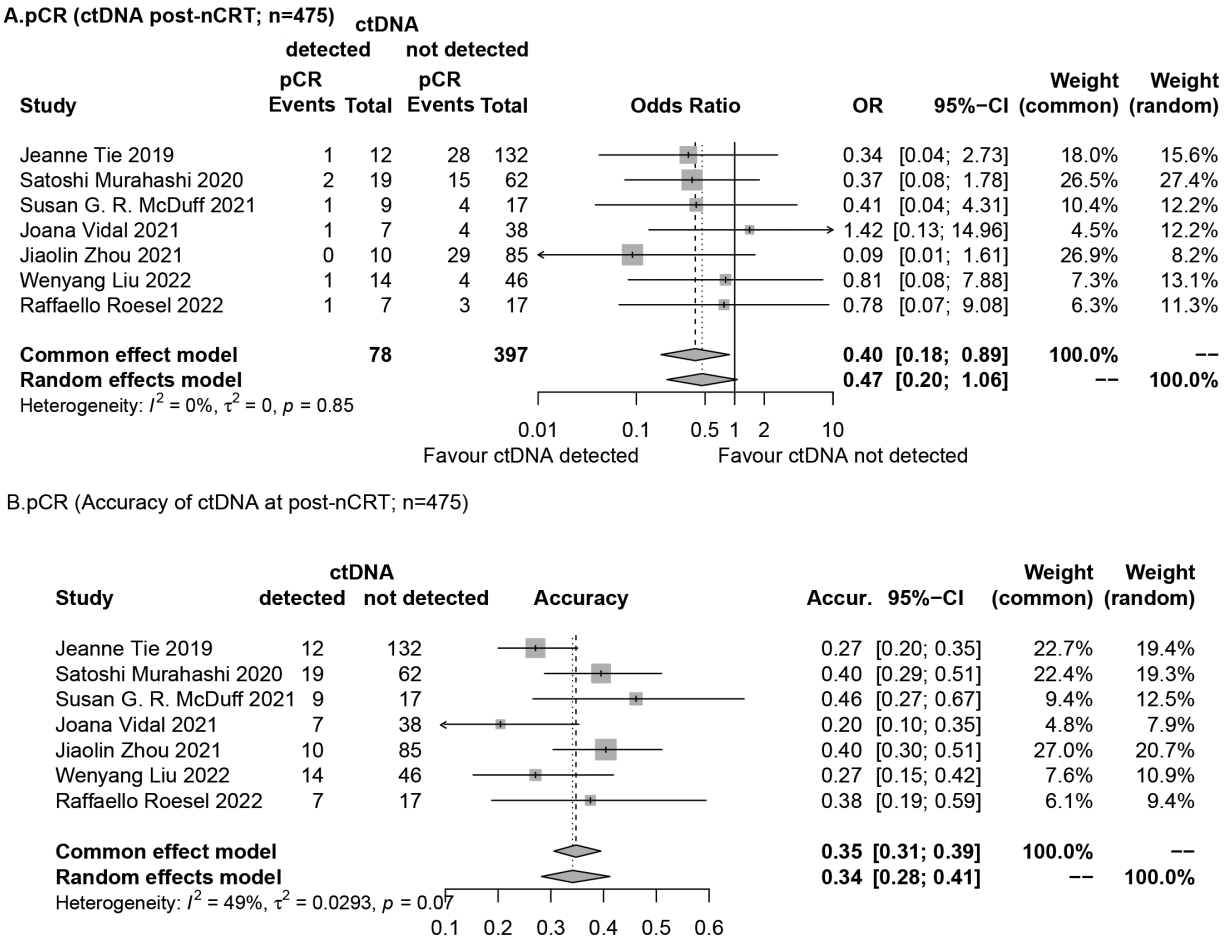


Figure 6. Forest plot of the prediction of pCR based on ctDNA status at post-nCRT.

significance of ctDNA detection during nCRT for prognosis, which guides us to pay attention to ctDNA detection during nCRT in future studies.

In conclusion, this meta-analysis showed that the presence of ctDNA detectable at baseline was not associated with long-term outcomes of LARC nor pCR outcomes. However, the presence of ctDNA was associated with poorer pCR and long-term survival outcomes after receiving nCRT. This suggests that the inclusion of ctDNA in the assessment of LARC patients undergoing nCRT is of great significance for individual recurrence risk stratification and the formulation of patient treatment regimens. Although the heterogeneity of the included studies has a certain impact on our study, our study points out some directions for the problems that should be solved in the prospective trial of ctDNA in LARC patients.

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Conflict of Interest

The authors declare that there were no commercial or financial relationships that could be interpreted as potential conflicts of interest in this research.

Author Contributions

Conception/design: L.C. Provision of study material or patients: L.C., X.Z., L.H. Collection and/or assembly of data: L.C, X.Z, L.H., Q.M, T.F, C.J, Z.M, Q.L. Data analysis and interpretation: L.C, X.Z, L.H., Q.M, T.F, C.J, Z.M, Q.L. Conceptualization, methodology, and validation: C.W. Supervision, conceptualization, resources, and writing: J.T. Manuscript writing and final approval of manuscript: All authors.

Data Availability

Data, code, and other materials available on request from the authors.

Supplementary Material

Supplementary material is available at *The Oncologist* online.

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