Prognostic Value of Circulating Tumor DNA for Recurrence Risk in Stage III Colorectal Cancer: A Systematic Review and Meta-Analysis

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Background:
Circulating tumor DNA (ctDNA) has emerged as a promising biomarker for minimal residual disease (MRD) detection and recurrence risk stratification in colorectal cancer (CRC). However, its prognostic significance in stage III CRC remains incompletely defined. This meta-analysis aimed to evaluate the association between postoperative ctDNA positivity and recurrence risk in patients with stage III CRC.

Methods:
PubMed, Embase, and the Cochrane Library were searched for potentially eligible studies published up to April 2025. Pooled risk ratio (RR) and pooled hazard ratio (HR) were calculated to evaluate recurrence rate and the prognosis of recurrence-free survival (RFS) following CRC surgery and ACT. Meta-analysis was performed using a random-effects model.

Results:
A total of ten studies involving 2461 stage III CRC patients were included. Postoperative ctDNA positivity was significantly associated with an increased risk of recurrence (RR = 4.39, 95% CI: 3.45–5.58, $p < 0.00001$) and a poorer RFS (HR = 6.56, 95% CI: 4.80–8.98, $p < 0.00001$). The pooled analysis showed that ctDNA-positive patients had a significantly higher risk of recurrence following ACT (RR = 4.80, 95% CI: 3.17–7.26, $p < 0.00001$) and a worse RFS (HR = 10.00, 95% CI: 4.84–20.66, $p < 0.00001$).

Conclusion:
Postoperative ctDNA positivity is a strong prognostic marker of recurrence in patients with stage III CRC and could guide individualized surveillance and adjuvant therapy decisions. Further prospective studies are warranted to validate its routine clinical use.

KEYWORDS
Circulating tumor DNA, colorectal cancer, recurrence-free survival

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1. Introduction

Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide, with stage III disease representing a clinically heterogeneous group at significant risk of recurrence despite curative-intent surgery and adjuvant chemotherapy. In 2020 alone, there were over 1.9 million new cases of CRC and nearly 1 million deaths (1). Approximately 30–50% of stage III CRC patients experience relapse, underscoring the limitations of conventional clinicopathological risk stratification in predicting outcomes (2,3). The 8th edition TNM classification further divides stage III into sub-stages (IIIA–IIIC) based on nodal involvement. However, it does not incorporate molecular biomarkers, such as circulating DNA (ctDNA), which may better predict recurrence and molecular heterogeneity driving recurrence risk (4). Despite favorable histopathological features, many patients relapse postoperatively, reflecting limitations of conventional risk stratification (5). Adjuvant chemotherapy (ACT) in stage III CRC is considered the standard; however, overtreatment remains a concern, as a subset derives minimal benefit despite experiencing treatment-related toxicity, while others with aggressive tumor biology may benefit from more intensified regimens (6).

The ctDNA, a non-invasive biomarker shed by tumor cells into the bloodstream, has emerged as a transformative tool for detecting minimal residual disease (MRD) and predicting recurrence with high sensitivity (7–9). Advances in sequencing technologies such as tumor-informed next-generation sequencing (NGS) and digital droplet PCR (ddPCR), enable ctDNA detection at ultralow concentrations, often months before radiographic recurrence (10–12). Despite favorable clinicopathological features, a significant proportion of stage III colon cancer patients still experience postoperative recurrence, underscoring the limitations of current risk stratification systems (13,14). However, its role in guiding clinical decision-making remains investigational, and it is currently being evaluated in prospective trials to determine whether ctDNA-directed therapy can improve outcomes in molecularly defined high-risk subgroups. The ongoing trials, including the DYNAMIC-III and CIRCULATE studies, are now evaluating their initial results regarding ctDNA-guided adjuvant therapy escalation (e.g., oxaliplatin intensification or novel agents) in patients with high molecular risk can improve disease-free survival compared to standard management (15,16). The 5-year outcome of the DYNAMIC trial suggests that using ctDNA to guide ACT in stage II colon cancer is effective. This method could also help further improve risk for ctDNA-positive patients by considering the amount of ctDNA detected and their

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end-of-treatment results. However, critical questions remain unanswered regarding the optimal detection methods (tumor-informed vs. tumor-naïve assays), the timing of testing (perioperative vs. surveillance), and the cost-effectiveness of these methods in routine practice. Furthermore, the development of standardized ctDNA reporting criteria and clinically validated thresholds for intervention is essential before widespread implementation. As evidence from ongoing phase III trials matures, if validated, ctDNA analysis has the potential to refine adjuvant therapy paradigms by enabling risk-adapted, patient-specific approaches that complement conventional clinicopathological staging.

This meta-analysis aims to evaluate the prognostic and predictive role of ctDNA detection following curative-intent surgery and ACT in patients with stage III CRC. Specifically, it investigates the association between postoperative and post-ACT ctDNA positivity and recurrence risk, as well as its potential utility in therapeutic strategies. By synthesizing current evidence from prospective and retrospective studies, this analysis seeks to determine whether ctDNA can serve as a reliable biomarker for MRD and early relapse, thereby refining individualized treatment decisions in stage III CRC management.

2. Materials and Methods

Search Strategy

This systematic review and meta-analysis were conducted in accordance with the Cochrane Handbook for Systematic Reviews of Interventions and adhered to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (17,18). The review protocol was registered in the PROSPERO database (registration number: CRD 420251041175).

We conducted a systematic search of PubMed, Embase, and the Cochrane Library up to April, 2025. The search terms comprised: ("colorectal cancer" OR "colorectal carcinoma" OR "colon cancer" OR "rectal cancer" OR "colorectal neoplasms") AND ("circulating tumor DNA" OR "ctDNA" OR "liquid biopsy" OR "circulating DNA" OR "cell-free DNA" OR "cf-DNA") AND ("recurrence" OR "recurrence risk" OR "relapse" OR "molecular residual disease" OR "minimal residual disease" OR "MRD" OR "recurrence-free survival" OR "RFS" OR "disease-free survival" OR "DFS"). Additional studies were identified by screening references of relevant articles. Only studies published in English were included.

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Inclusion and Exclusion Criteria

We included studies that met the following criteria: (1) patients diagnosed with stage III colorectal cancer; (2) ctDNA was assessed as a biomarker postoperatively or after ACT; (3) reported outcomes related to recurrence or survival, such as recurrence rate, recurrence-free survival (RFS), disease-free survival (DFS), or overall survival (OS), with hazard ratios (HRs) and confidence intervals available or able to be calculated; (4) study designs including randomized controlled trials, cohort studies, or other observational studies with relevant data; and (5) published in English with full text available.

Studies were excluded if they: (1) included mixed stages of colorectal cancer without separate data for stage III; (2) included only rectal cancer patients; (3) lacked clear outcomes related to recurrence or survival; (4) were reviews, editorials, case reports, conference abstracts, duplicate publications, preclinical or animal studies; or (5) were not published in English.

Data Extraction and Assessment of the Quality of Included Studies

Two authors (T. A. and H. S.) independently screened the search results to identify eligible studies, following predefined criteria for study selection, data extraction, and quality assessment. Any disagreements were resolved by consensus between the authors. Data extracted from each study included: (a) study characteristics (first author, year of publication, study design, sample size, and follow-up duration); (b) population baseline characteristics (sex); (c) intervention details (sample origin, ctDNA detection methods, and timing of sample collection); and (d) outcome data (ctDNA positivity after surgery and following completion of ACT). The quality of randomized controlled trials was assessed using Cochrane's Risk-of-Bias tool for randomized trials version 2 (RoB-2) (19,20). For non-randomized studies, the Risk of Bias in Non-Randomized Studies of Interventions (ROBINS-I) tool was used (21). Publication bias was evaluated using funnel plots to explore the relationship between study effect sizes and their precision.

Statistical analysis

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Risk Ratio (RR) with 95% confidence intervals (CIs) was used to evaluate the risk of recurrence, while hazard ratios (HRs) with 95% CIs were employed to assess recurrence-free survival (RFS) across the included studies. Heterogeneity among the included studies was evaluated using Cochrane's Q test and the I^2 statistic. A p-value below 0.10 was considered indicative of significant heterogeneity. I^2 values were interpreted as follows: 0–40% (low), 30–60% (moderate), 50–90% (substantial), and 75–100% (considerable) heterogeneity (3). The DerSimonian and Laird method was used to account for differences between studies. Sensitivity analyses were performed using a leave-one-out approach to detect influential studies or outliers. All statistical tests were two-sided, with a significance threshold of $p < 0.05$. Meta-analyses were conducted, and forest plots generated using Review Manager software (RevMan, version 5.4, The Cochrane Collaboration, Denmark).

3. Results

The database search initially yielded 750 studies. After removing 12 duplicates, 738 articles were screened by their titles and abstracts for relevance. A comprehensive evaluation of 119 full-text articles was carried out, with 109 studies excluded based on eligibility criteria. As a result, 10 studies were included in the final analysis, as detailed in Figure 1.

Of the included studies, nine were prospective cohort studies, and one was a post-hoc analysis of two randomized controlled trials. The publication years ranged from 2019 to 2025. Among ten studies, nine investigated the prognostic value of ctDNA after surgery, six evaluated its role following adjuvant chemotherapy, and three conducted longitudinal ctDNA assessments during follow-up. Most studies (8/10) used a tumor-informed method for ctDNA analysis. The dataset consisted of 2463 patients, with 21.45% patients (448/2089) were positive for ctDNA after surgery. In addition, 15.0% (64/427) of patients were positive for ctDNA in the post-adjuvant setting. Table 1 summarizes the main characteristics and demographics of the studies included in the meta-analysis.

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Table 1. Characteristics of the studies included in this meta-analysis.

Study	Design	N	Male, N (%)	Follow-up†	Sample Origin	Tumor-Informed vs. Tumor-Agnostic	Methods of ctDNA measurement	Timing of ctDNA Collection	ctDNA Positive Rate, n/N (%)
Tie et al., 2019 (22)	Multicentre Prospective Cohort	96	49(51)	28.9 months (11.6-46.4)	Plasma	Tumor-Informed	Safe-SeqS	Post-op (4-10 weeks) Post-ACT (within 6 weeks)	Post-op 20 /96 (21) Post-ACT 15/88 (17)
Henriksen et al., 2022 (23)	Multicentre Prospective Cohort	160	95(59.4)	35 months (13–36)	Plasma	Tumor-Informed	mPCR NGS	Post-op (2–4 weeks) Post-ACT (during and after up to 36 months)	Post-op 20/140 (14)
Li et al., 2022 (24) ChiCTR1800018754	Single-centre Prospective Cohort	151	90(59.6)	33.5 months (33.0-34.2)	Plasma	Tumor-Informed	NGS	Post-ACT (2–4 weeks)	Pre-ACT 24/151(15.9) Post-ACT 13/151(8.9)
Gao et al., 2023 (25)	Single-centre Retrospective Cohort	53	N/A	36.7 months (8.1-55.1)	Plasma	Tumor-Informed	NGS	Post-op (7-10 days)	Post-op 15/17(88.2)
Mo et al., 2023 (26)	Multicentre Prospective Cohort	135	N/A	21 months (8-27)	Plasma	Tumor-Agnostic	mPCR ColonAiQ	Post-op (2.4-12.1 weeks) and every 3 months	Pre-op 110/135(81.5) Post-op 40/121(33)
Frydendahl et al., 2024 (27)	Multicentre	146	86(59)	36 months	Plasma	Tumor-	NovaSeq	Post-op	Pre-op

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	Prospective Cohort			(35.6–36.5)		Informed		early (≤14 days) later (≥15 days) and every 3 months up to 36 months Post-ACT (within 3 months)	114/136(84) Post-op 19/111(17) Post-ACT 10/91(11)
Nakamura et al., 2024 (28) UMIN000039205 GALAXY	Multicentre Prospective Cohort	936	N/A	23 months (2-49)	Plasma	Tumor-Informed	NGS (Signatera, Natera)	Post-op (4, 12, 24, 36, 48, 72 and 96 weeks)	Post-op 162/854 (19)
Nakamura et al., 2024 (29) COSMOS-CRC-01 (UMIN000037765)	Multicentre Prospective Cohort	108	N/A	28.4 months (8.9–38)	Plasma	Tumor-Agnostic	Tissue-free epigenomic MRD assay	Post-op (day 28) and every 3 to 6 months for 5 years	Post-op 28/108 (26)
Diergaarde et al., 2025 (30) NCT02842203	Multicentre Prospective Cohort	124	66(53.2)	4.8 years	Plasma	Tumor-Informed	OncoExTra	Post-op (3–12 weeks) and every 3 months up to 36 months Post-ACT (at the end of the therapy)	Post-op 35/88 (40) Post-ACT 15/97 (15)
Taieb et al., 2025 (31)	Post-Hoc analysis of IDEA-France and HORG trials.	554	322(58)	6.7 years	Plasma	Tumor-Informed	NGS Natera, Inc	Post-op (34-50 days)	Post-op 109/554(19.7)

†, Median (range), mPCR, multiplex PCR; ddPCR, multiplex droplet-based digital PCR; NA, not available; NGS, next-generation sequencing; Post-ACT, after adjuvant chemotherapy;

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Recurrence Risk According to Postoperative ctDNA Status

This meta-analysis pooled data from eight studies to evaluate the role of ctDNA in predicting recurrence risk after surgery. The results from a random-effects model showed that ctDNA-positive patients had a significantly higher risk of CRC recurrence (RR = 4.39, 95% CI: 3.45–5.58, $p < 0.00001$; $I^2 = 57\%$), as shown in Figure 2A. In a univariate meta-analysis of nine studies, ctDNA-positive patients demonstrated significantly worse RFS compared to ctDNA-negative patients (HR = 6.56, 95% CI: 4.80–8.98, $p < 0.00001$; $I^2 = 61\%$), as shown in Figure 2B. Additionally, a multivariate meta-analysis of five studies confirmed that ctDNA positivity was associated with lower RFS (HR = 6.69, 95% CI: 5.01–8.92, $p < 0.00001$; $I^2 = 60\%$), also depicted in Figure 2C. In a meta-analysis of three studies involving serial ctDNA measurements during the surveillance period, ctDNA-positive patients exhibited significantly poorer RFS compared to ctDNA-negative patients, with a pooled HR of 26.23 (95% CI: 17.04–40.40, $p < 0.00001$) and no observed heterogeneity ($I^2 = 0$), as shown in Figure 2D.

Recurrence Risk According to Post-ACT ctDNA Status

A total of six studies were included in the analysis to assess the prognostic significance of ctDNA positivity following ACT in CRC stage III. The pooled analysis revealed a significantly higher risk of disease recurrence in patients with detectable ctDNA compared to ctDNA-negative, with a RR of 4.80 (95% CI: 3.17–7.26, $p < 0.00001$; $I^2 = 67\%$), as presented in Figure 3A. In a univariate meta-analysis of six studies, ctDNA-positive patients had significantly worse RFS than ctDNA-negative patients (HR = 10.00, 95% CI: 4.84–20.66, $p < 0.00001$; $I^2 = 76\%$), as shown in Figure 3 B. A multivariate meta-analysis of four studies yielded concordant results, indicating that ctDNA positivity following ACT was associated with inferior RFS (HR = 16.37, 95% CI: 5.11–52.45, $p < 0.00001$; $I^2 = 74\%$) in Figure 3C.

Subgroup analyses

A subgroup analysis was performed to compare tumor-informed and tumor-agnostic ctDNA detection methods in the post-surgical setting. Post-operative ctDNA positivity was significantly associated with an increased risk of recurrence in both the tumor-informed (6 studies, RR = 4.27; 95% CI: 3.24-5.63; $p < 0.00001$; $I^2 = 63\%$) and tumor-agnostic subgroups (2 studies, RR = 5.00;

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95% CI: 2.29-10.93; $p < 0.0001$; $I^2 = 69\%$). No significant difference was found between the two detection methods ($p = 0.71$; $I^2 = 0\%$). Similarly, ctDNA positivity was associated with poorer RFS in both subgroups: tumor-informed (7 studies, HR = 6.17; 95% CI: 4.36–8.75; $p < 0.00001$; $I^2 = 67\%$) and tumor-agnostic (2 studies, HR = 10.12; 95% CI: 5.07–20.20; $p < 0.00001$; $I^2 = 0\%$). The difference between subgroups was not statistically significant ($p = 0.21$; $I^2 = 36\%$).

Early detection of recurrence (lead time)

The studies employing ctDNA detection methods have shown that ctDNA can be identified several months earlier than disease recurrence detected by standard radiological imaging. ctDNA was consistently detected earlier than the diagnosis of recurrence by standard-of-care radiological imaging across three studies. Frydendahl et al. (27) reported a significant lead time between the first detection of ctDNA and radiological confirmation of recurrence, with a median of 8.7 months (4.8–11.5). Similarly, Henriksen et al. (23) observed a median lead time of 10.0 months (8–14), while Diergaarde et al. (30) reported a median of 10.4 months (5.2–10.7).

Publication bias and sensitivity analysis

To evaluate potential publication bias in the analysis of recurrence risk and RFS following surgery and ACT, we performed visual inspection of funnel plots test. For all outcomes, the funnel plots appeared symmetrical, suggesting a low likelihood of publication bias, Figure S2 A,B,C,D. A leave-one-out sensitivity analysis was performed to evaluate the impact of each study on CRC recurrence after surgery. Excluding the GALAXY study (28) from the analysis resulted in a reduction of the effect estimates in postoperative settings, reflected by lower RR values, indicating that the GALAXY study influenced the overall effect size. However, the results remained statistically significant following its exclusion. Furthermore, the decrease in heterogeneity suggests that the GALAXY study contributed to between-study variability, as shown in Figure S3. We also performed sensitivity analyses to evaluate the risk of CRC recurrence after ACT, which showed that no individual study had a significant influence on the overall effect estimate, in Figure S4.

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Quality assessment

The risk of bias for all included studies was evaluated using the ROBINS-I tool. Five studies including Tie et al.(22), Henriksen et al.(23), Lie et al. (24), Frydendahl et al.(27), and Diergaarde et al. (30) showed moderate overall risk, while four studies including Gao et al. (25), Mo et al. (26), GALAXY (28), and Nakamura et al. (29) presented serious risk. Only one study, Taieb et al., was judged to have low overall risk of bias. The full assessment are summarized in Figure S5.

4. Discussion

This systematic review and meta-analysis, encompassing 2,461 patients with stage III CRC, revealed that the presence of detectable ctDNA after curative-intent surgery was significantly associated with an increased risk of recurrence (RR = 4.39; 95% CI: 3.45–5.58) and poorer RFS, as demonstrated in both univariate and multivariate analyses (HR = 6.56, $p < 0.00001$; HR = 6.69, $p < 0.00001$, respectively). Additionally, in patients who treated with ACT, the presence of ctDNA positivity was correlated with a higher risk of disease relapse (RR = 4.80; 95% CI: 3.17–7.26) and inferior RFS in both univariate and multivariate analyses (HR = 10.00, $p < 0.00001$; HR = 16.37, $p < 0.00001$, respectively).

Early prospective studies demonstrated the feasibility of using ctDNA to detect MRD and predict recurrence in patients with stage II and III CRC, highlighting its superior predictive value compared to conventional clinicopathological risk factors (32,33). Meta-analytic data support the notion that postoperative ctDNA positivity is associated with a significantly increased risk of recurrence compared to ctDNA-negative patients, with hazard ratios ranging from 4.0 to 7.0 across studies (34–36). These findings highlight the prognostic superiority of molecular surveillance over traditional histopathological measures. Although subsequent meta-analyses have confirmed the association between ctDNA positivity and adverse outcomes, many of these studies included heterogeneous patient populations across all CRC stages, which may have attenuated the prognostic estimates due to differences in baseline recurrence risk (37). By focusing exclusively on stage III disease, characterized by a substantial risk of relapse, our meta-analysis provides more precise quantification of the prognostic impact of ctDNA in this high-risk

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subgroup. Our results are consistent with previous studies investigating ctDNA in CRC, demonstrating that ctDNA detection at any time point was associated with a high risk of relapse. In both post-op and post-ACT settings, multivariable analyses confirmed that ctDNA remained the most significant prognostic factor.

Serial ctDNA sampling during surveillance is crucial for detecting minimal residual disease and early cancer recurrence. Repeated measurements improve detection accuracy by capturing dynamic tumor changes over time, enabling timely interventions and better patient management. In line with these findings, our meta-analysis of three studies demonstrated that ctDNA-positive patients exhibited significantly reduced RFS, with a pooled HR of 26.23 (95% CI: 17.04–40.40, $p < 0.00001$), highlighting the strong and consistent prognostic value of serial ctDNA monitoring in colorectal cancer surveillance (23,27,30).

ctDNA has demonstrated significant potential to detect cancer recurrence earlier than conventional radiological imaging. Evidence from three studies, which included in our meta-analysis, indicates that ctDNA can identify cancer recurrence a median of 8.7 to 10.4 months earlier than conventional radiologic methods (23,27,30). This consistent lead time underscores the clinical value of incorporating ctDNA analysis into routine surveillance protocols, offering a window for earlier therapeutic intervention and potentially improved patient outcomes. It is still being investigated whether ctDNA-guided surveillance offers enough lead time to impact treatment decisions and improve outcomes. The ongoing randomized IMPROVE-IT2 trial (NCT04084249) is comparing the effectiveness of ctDNA-based monitoring against standard-of-care CT based surveillance in stage III and high-risk stage II colorectal cancer patients treated with adjuvant chemotherapy (38).

Due to the significant prognostic impact of ctDNA, multiple ongoing trials are investigating its utility for treatment decisions at different stages. The DYNAMIC-III study is the first RCT examining ctDNA-informed management in stage III CRC Patients were randomly assigned to ctDNA-guided treatment or SOC (39). If ctDNA was positive, treatment was intensified from single-agent FP to an oxaliplatin-based doublet, extended from 3 to 6 months, or switched to FOLFOXIRI. This confirmed the prognostic significance of ctDNA, revealing that higher ctDNA levels increased recurrence risk. However, intensifying treatment did not improve RFS.

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CIRCULATE-US (NCT05174169) investigates the postoperative dynamics of ctDNA in early-stage colon cancer to assess treatment modifications by ctDNA status (40).

A post hoc analysis of the PRODIGE-GERCOR IDEA-France and HORG-IDEA-Greece trials, with a median follow-up of 6.7 years, reported 5-year OS rates of 52.7% for ctDNA-positive and 89.4% for ctDNA-negative patients. In univariate analysis, ctDNA positivity was associated with worse OS (HR: 5.31; 95% CI: 3.75–7.51; P < .0001), and this association remained significant in multivariable analysis (adjusted HR: 4.84; 95% CI: 3.40–6.89; P < .001) (31). We were unable to perform a pooled analysis of OS due to insufficient data across the included studies.

Heterogeneity in assay methodologies, variant allele frequency thresholds, and follow-up durations poses challenges for cross-study synthesis. The included studies employed a range of ctDNA detection methodologies, primarily categorized into tumor-informed and tumor-agnostic assays. A post hoc analysis of the IDEA-France trial, which evaluated ctDNA in 1,010 patients using a tumor-agnostic methylation-based assay, demonstrated its independent prognostic value for TTR and OS (41). However, the latest post-hoc analyses of the IDEA-France and HORG trials suggest that tumor-informed approaches offer superior prognostic accuracy. Notably, the COBRA NRG005 trial in stage II colon cancer was recently halted due to a high false-positive rate with the tumor-agnostic Guardant LUNAR-01 test, raising concerns about the reliability of such assays for detecting MRD after surgery (42). Chan et al. study, which analyzed 127 pre-operative and serial plasma cfDNA samples from 38 CRC patients treated with curative surgery, demonstrated that the tumor-informed ctDNA approach offers higher analytical sensitivity, fewer false positives due to CH mutations, and improved detection of recurrence compared to tumor-agnostic methods (43). Our subgroup analysis showed a significant association of post-operative ctDNA positivity with recurrence risk and poorer RFS in both tumor-informed and tumor-agnostic methods. However, no statistically significant difference between the two approaches was found. It is important to highlight that this comparison is based on indirect evidence from separate studies with notable differences in sample size and weighting. Standardization and harmonization of assay techniques remain critical to facilitate clinical translation.

Limitations

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Several limitations of the current evidence must be acknowledged. The majority of included studies were observational and varied in design, ctDNA assay platforms, timing of blood collection, and adjuvant treatment protocols, contributing to heterogeneity. The studies included in this analysis used different ctDNA detection methods, which led to variability in the size of the gene panels and the sequencing depth across studies. These methodological differences could influence the sensitivity and comparability of ctDNA results. Moreover, standardized thresholds for ctDNA positivity have yet to be established, complicating clinical interpretation. The potential use of RFS rather than OS endpoints also limit the generalizability of conclusions. Additionally, several studies had limited follow-up durations, which may have restricted the ability to evaluate long-term clinical outcomes such as OS or late recurrence.

Conclusion

This meta-analysis confirms that postoperative ctDNA positivity is a robust and independent predictor of recurrence risk and poor RFS in patients with stage III CRC. The detection of ctDNA after surgery and ACT identifies patients at very high risk of relapse, who may benefit from intensified treatment or close monitoring. Conversely, ctDNA-negative patients might safely avoid unnecessary chemotherapy. Incorporating ctDNA testing into routine clinical practice promises to personalize postoperative management and improve outcomes for patients with stage III CRC. Future prospective randomized trials with standardized methodologies are essential to confirm the clinical utility of ctDNA-guided management and its impact on long-term outcomes.

DECLARATIONS

Ethics Approval and Consent to Participate

This study is a meta-analysis and did not involve direct human or animal subjects; therefore, ethics approval and consent to participate were not required.

Consent for Publication

Not applicable.

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Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare no competing interests.

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REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209–49.

2. Benson AB, Venook AP, Adam M, Chang G, Chen YJ, Ciombor KK, et al. Colon Cancer, Version 3.2024, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2024 Jun;22(2 D):e240029.

3. André T, de Gramont A, Vernerey D, Chibaudel B, Bonnetain F, Tijeras-Raballand A, et al. Adjuvant Fluorouracil, Leucovorin, and Oxaliplatin in Stage II to III Colon Cancer: Updated 10-Year Survival and Outcomes According to BRAF Mutation and Mismatch Repair Status of the MOSAIC Study. J Clin Oncol. 2015 Dec 10;33(35):4176–87.

4. Ueno H, Sekine S, Oshiro T, Kanemitsu Y, Hamaguchi T, Shida D, et al. Disentangling the prognostic heterogeneity of stage III colorectal cancer through histologic stromal categorization. Surgery. 2018 Apr;163(4):777–83.

5. Xu W, He Y, Wang Y, Li X, Young J, Ioannidis JPA, et al. Risk factors and risk prediction models for colorectal cancer metastasis and recurrence: an umbrella review of systematic reviews and meta-analyses of observational studies. BMC Med. 2020 Jun 26;18(1):172.

6. Auclin E, Zaanan A, Vernerey D, Douard R, Gallois C, Laurent-Puig P, et al. Subgroups and prognostication in stage III colon cancer: future perspectives for adjuvant therapy. Ann Oncol. 2017 May 1;28(5):958–68.

7. Nader-Marta G, Monteforte M, Agostinetto E, Cinquini M, Martins-Branco D, Langouo M, et al. Circulating tumor DNA for predicting recurrence in patients with operable breast cancer: a systematic review and meta-analysis. ESMO Open. 2024 Mar;9(3):102390.

Preprint not peer reviewed

This preprint research paper has not been peer reviewed. Electronic copy available at: https://ssrn.com/abstract=5364979

8. Lander EM, Aushev VN, Huffman BM, Hanna D, Dutta P, Ferguson J, et al. Circulating Tumor DNA as a Prognostic Biomarker for Recurrence in Patients With Locoregional Esophagogastric Cancers With a Pathologic Complete Response. JCO Precis Oncol. 2024 Dec;8:e2400288.

9. Mi J, Wang R, Han X, Ma R, Zhao D. Treatment stratification and prognosis assessment using circulating tumor DNA in locally advanced rectal cancer: A systematic review and meta-analysis. Cancer Med. 2023 Sep;12(17):17934–44.

10. Reinert T, Henriksen TV, Christensen E, Sharma S, Salari R, Sethi H, et al. Analysis of Plasma Cell-Free DNA by Ultradeep Sequencing in Patients With Stages I to III Colorectal Cancer. JAMA Oncol. 2019 Aug 1;5(8):1124–31.

11. Carethers JM. Assaying Circulating-Tumor DNA To Predict Recurrence of Localized Colon Cancer. Dig Med Res. 2020 Dec;3:112.

12. Garlan F, Laurent-Puig P, Sefrioui D, Siauve N, Didelot A, Sarafan-Vasseur N, et al. Early Evaluation of Circulating Tumor DNA as Marker of Therapeutic Efficacy in Metastatic Colorectal Cancer Patients (PLACOL Study). Clin Cancer Res. 2017 Sep 15;23(18):5416–25.

13. You YN, Rustin RB, Sullivan JD. Oncotype DX(®) colon cancer assay for prediction of recurrence risk in patients with stage II and III colon cancer: A review of the evidence. Surg Oncol. 2015 Jun;24(2):61–6.

14. Yothers G, O'Connell MJ, Lee M, Lopatin M, Clark-Langone KM, Millward C, et al. Validation of the 12-gene colon cancer recurrence score in NSABP C-07 as a predictor of recurrence in patients with stage II and III colon cancer treated with fluorouracil and leucovorin (FU/LV) and FU/LV plus oxaliplatin. J Clin Oncol. 2013 Dec 20;31(36):4512–9.

15. Tie J, Wang Y, N Lo S, Lahouel K, Cohen J, Wong R, et al. Circulating tumor DNA analysis guiding adjuvant therapy in stage II colon cancer: overall survival and updated 5-year results from the randomized DYNAMIC trial. Journal of clinical oncology [Internet]. 2024;42(16). Available from: https://www.cochranelibrary.com/central/doi/10.1002/central/CN-02736459/full

16. Taniguchi H, Nakamura Y, Kotani D, Yukami H, Mishima S, Sawada K, et al. CIRCULATE-Japan: circulating tumor DNA-guided adaptive platform trials to refine adjuvant therapy for colorectal cancer. Cancer science [Internet]. 2021;112(7):2915-2920. Available from: https://www.cochranelibrary.com/central/doi/10.1002/central/CN-02283809/full

17. Higgins J, Thomas J, Chandler J, Cumpston M, Li T, Page M, et al. Cochrane Handbook for Systematic Reviews of Interventions version 6.4. Cochrane; 2023. http://www.training.cochrane.org/handbook.

Preprint not peer reviewed

This preprint research paper has not been peer reviewed. Electronic copy available at: https://ssrn.com/abstract=5364979

18. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021 Mar 29;372:n71.

19. Higgins JPT, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011 Oct 18;343:d5928.

20. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ. 2019 Aug 28;366:l4898.

21. Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. BMJ. 2016 Oct 12;355:i4919.

22. Tie J, Cohen JD, Wang Y, Christie M, Simons K, Lee M, et al. Circulating Tumor DNA Analyses as Markers of Recurrence Risk and Benefit of Adjuvant Therapy for Stage III Colon Cancer. JAMA Oncol. 2019 Dec 1;5(12):1710–7.

23. Henriksen TV, Tarazona N, Frydendahl A, Reinert T, Gimeno-Valiente F, Carbonell-Asins JA, et al. Circulating Tumor DNA in Stage III Colorectal Cancer, beyond Minimal Residual Disease Detection, toward Assessment of Adjuvant Therapy Efficacy and Clinical Behavior of Recurrences. Clin Cancer Res. 2022 Feb 1;28(3):507–17.

24. Li Y, Mo S, Zhang L, Ma X, Hu X, Huang D, et al. Postoperative circulating tumor DNA combined with consensus molecular subtypes can better predict outcomes in stage III colon cancers: A prospective cohort study. Eur J Cancer. 2022 Jul;169:198–209.

25. Gao Z, Huang D, Chen H, Yang Y, An K, Ding C, et al. Development and validation of postoperative circulating tumor DNA combined with clinicopathological risk factors for recurrence prediction in patients with stage I-III colorectal cancer. J Transl Med. 2023 Jan 30;21(1):63.

26. Mo S, Ye L, Wang D, Han L, Zhou S, Wang H, et al. Early Detection of Molecular Residual Disease and Risk Stratification for Stage I to III Colorectal Cancer via Circulating Tumor DNA Methylation. JAMA Oncol. 2023 Jun 1;9(6):770–8.

27. Frydendahl A, Nors J, Rasmussen MH, Henriksen TV, Nesic M, Reinert T, et al. Detection of circulating tumor DNA by tumor-informed whole-genome sequencing enables prediction of recurrence in stage III colorectal cancer patients. Eur J Cancer. 2024 Nov;211:114314.

28. Nakamura Y, Watanabe J, Akazawa N, Hirata K, Kataoka K, Yokota M, et al. ctDNA-based molecular residual disease and survival in resectable colorectal cancer. Nat Med. 2024 Nov;30(11):3272–83.

29. Nakamura Y, Tsukada Y, Matsuhashi N, Murano T, Shiozawa M, Takahashi Y, et al. Colorectal Cancer Recurrence Prediction Using a Tissue-Free Epigenomic Minimal Residual Disease Assay. Clin Cancer Res. 2024 Oct 1;30(19):4377–87.

Preprint not peer reviewed

This preprint research paper has not been peer reviewed. Electronic copy available at: https://ssrn.com/abstract=5364979

30. Diergaarde B, Young G, Hall DW, Mazloom A, Costa GL, Subramaniam S, et al. Circulating Tumor DNA as a Marker of Recurrence Risk in Stage III Colorectal Cancer: The α-CORRECT Study. J Surg Oncol. 2025 Jan 25;

31. Taieb J, Souglakos J, Boukovinas I, Falcoz A, Pages F, Messaritakis I, et al. Combined Analyses of Circulating Tumor DNA and Immunoscore in Patients With Stage III Colon Cancer: A Post Hoc Analysis of the PRODIGE-GERCOR IDEA-France/HORG-IDEA-Greece Trials. J Clin Oncol. 2025 May;43(13):1564–77.

32. Tie J, Wang Y, Tomasetti C, Li L, Springer S, Kinde I, et al. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med. 2016 Jul 6;8(346):346ra92.

33. Reinert T, Henriksen TV, Christensen E, Sharma S, Salari R, Sethi H, et al. Analysis of Plasma Cell-Free DNA by Ultradeep Sequencing in Patients With Stages I to III Colorectal Cancer. JAMA Oncol. 2019 Aug 1;5(8):1124–31.

34. Wang R, Zhao A, Cao N, Li Z, Zhang G, Liu F. The value of circulation tumor DNA in predicting postoperative recurrence of colorectal cancer: a meta-analysis. Int J Colorectal Dis. 2020 Aug;35(8):1463–75.

35. Zheng J, Qin C, Wang Q, Tian D, Chen Z. Circulating tumour DNA-Based molecular residual disease detection in resectable cancers: a systematic review and meta-analysis. EBioMedicine. 2024 May;103:105109.

36. El Messaoudi S, Mouliere F, Du Manoir S, Bascoul-Mollevi C, Gillet B, Nouaille M, et al. Circulating DNA as a Strong Multimarker Prognostic Tool for Metastatic Colorectal Cancer Patient Management Care. Clin Cancer Res. 2016 Jun 15;22(12):3067–77.

37. Mi J, Han X, Wang R, Ma R, Zhao D. Circulation tumour DNA in predicting recurrence and prognosis in operable colorectal cancer patients: A meta-analysis. Eur J Clin Invest. 2022 Dec;52(12):e13842.

38. Nors J, Henriksen T, Gotschalck K, Juul T, Søgaard J, Iversen L, et al. IMPROVE-IT2: implementing noninvasive circulating tumor DNA analysis to optimize the operative and postoperative treatment for patients with colorectal cancer - intervention trial 2. Study protocol. Acta oncologica (Stockholm, Sweden) [Internet]. 2020;59(3):336-341. Available from: https://www.cochranelibrary.com/central/doi/10.1002/central/CN-02100257/full

39. Tie J, Cohen J, Wang Y, Ginestet P, Wong R, Shapiro J, et al. Circulating tumor DNA analysis informing adjuvant chemotherapy in locally advanced rectal cancer: the randomized AGITG DYNAMIC-Rectal study. Journal of clinical oncology [Internet]. 2024;42(3). Available from: https://www.cochranelibrary.com/central/doi/10.1002/central/CN-02666033/full

40. Dasari A, Lin Y, Kopetz S, Jacobs S, Lucas P, Sahin I, et al. Colon adjuvant chemotherapy based on evaluation of residual disease (CIRCULATEUS): NRG-GI008. Journal of clinical

Preprint not peer reviewed

This preprint research paper has not been peer reviewed. Electronic copy available at: https://ssrn.com/abstract=5364979

oncology [Internet]. 2022;40(16). Available from: https://www.cochranelibrary.com/central/doi/10.1002/central/CN-02454716/full

41. Taieb J, Taly V, Vernerey D, Bourreau C, Bennouna J, Faroux R, et al. Analysis of circulating tumour DNA (ctDNA) from patients enrolled in the IDEA-FRANCE phase III trial: prognostic and predictive value for adjuvant treatment duration. Annals of oncology [Internet]. 2019;30:v867. Available from: https://www.cochranelibrary.com/central/doi/10.1002/central/CN-02095921/full

42. Bachet JB, Laurent-Puig P, Meurisse A, Bouché O, Mas L, Taly V, et al. Circulating tumour DNA at baseline for individualised prognostication in patients with chemotherapy-naïve metastatic colorectal cancer. An AGEO prospective study. Eur J Cancer. 2023 Aug;189:112934.

43. Chan HT, Nagayama S, Otaki M, Chin YM, Fukunaga Y, Ueno M, et al. Tumor-informed or tumor-agnostic circulating tumor DNA as a biomarker for risk of recurrence in resected colorectal cancer patients. Frontiers in Oncology [Internet]. 2023;Volume 12-2022. Available from: https://www.frontiersin.org/journals/oncology/articles/10.3389/fonc.2022.1055968

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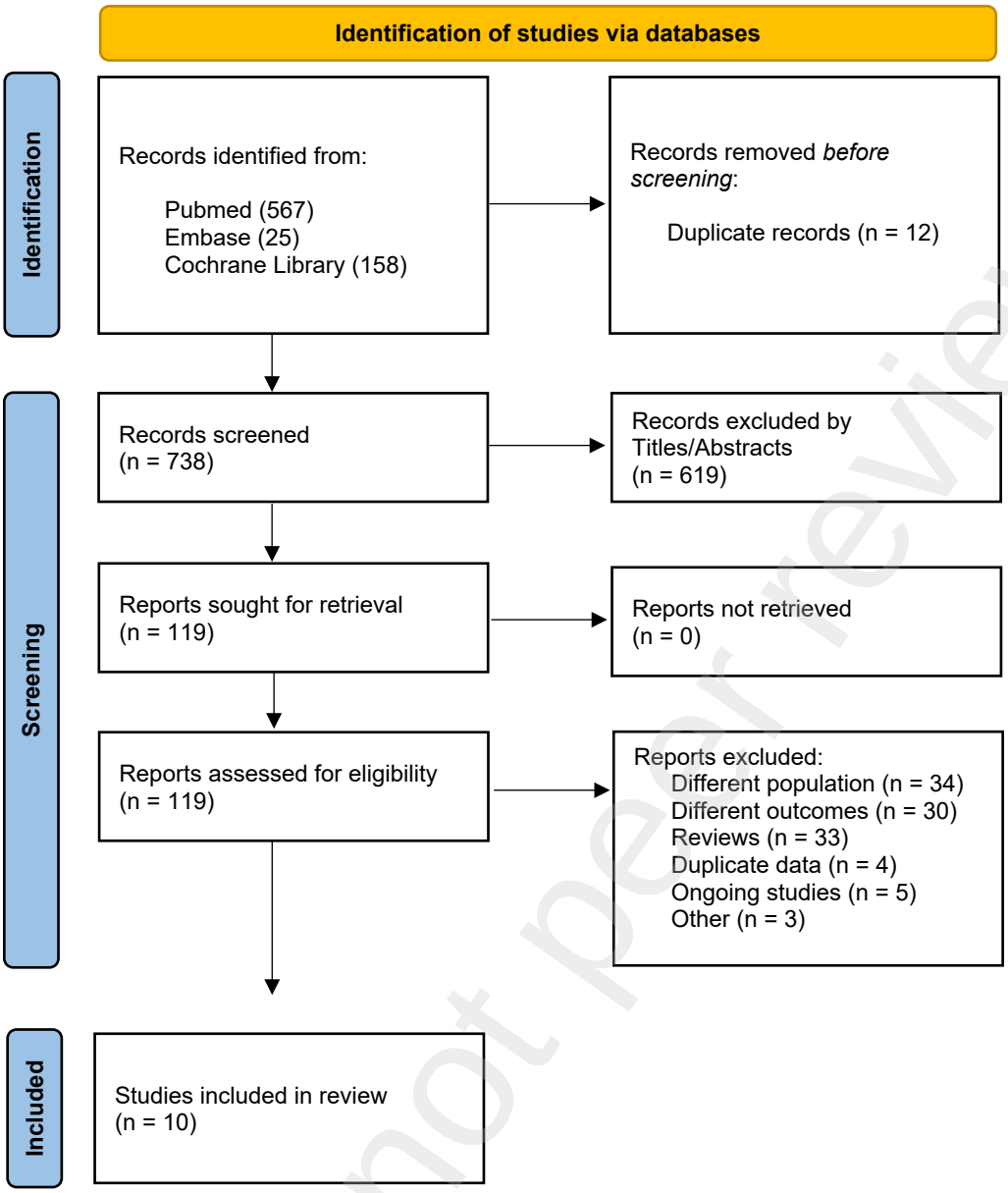


Figure 1. PRISMA flowchart for the literature searching strategy and procedure for study selection.

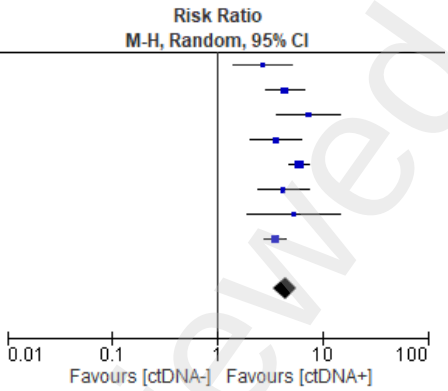
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A

Study or Subgroup	ctDNA+ Events	ctDNA+ Total	ctDNA- Events	ctDNA- Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Tie 2019	10	20	14	76	9.1%	2.71 [1.42, 5.17]	2019
Henriksen 2022	16	20	22	120	14.0%	4.36 [2.82, 6.75]	2022
Mo 2023	29	40	8	81	8.4%	7.34 [3.70, 14.56]	2023
Nakamura 2024	9	13	18	93	11.0%	3.58 [2.06, 6.21]	2024
GALAXY 2024	117	162	82	683	21.1%	6.02 [4.81, 7.53]	2024
Frydendahl 2024	13	19	15	92	10.9%	4.20 [2.41, 7.31]	2024
Diergaarde 2025	14	35	4	53	4.5%	5.30 [1.90, 14.79]	2025
Taieb 2025	74	109	86	445	21.0%	3.51 [2.79, 4.42]	2025
Total (95% CI)		**418**		**1643**	**100.0%**	**4.39 [3.45, 5.58]**	
Total events	282		249				

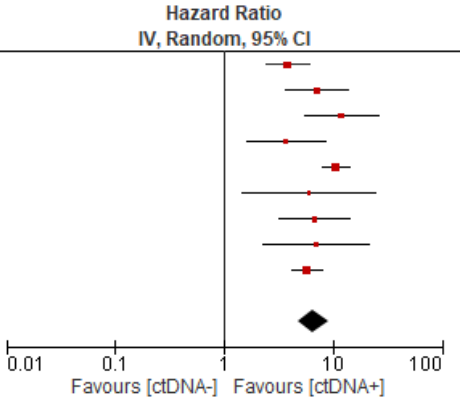
Heterogeneity: Tau² = 0.06; Chi² = 16.34, df = 7 (P = 0.02); I² = 57%
Test for overall effect: Z = 12.03 (P < 0.00001)



B

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI	Year
Tie 2019	1.335	0.2345	14.9%	3.80 [2.40, 6.02]	2019
Henriksen 2022	1.9555	0.3302	11.3%	7.07 [3.70, 13.50]	2022
Mo 2023	2.4799	0.4048	9.1%	11.94 [5.40, 26.40]	2023
Gao 2023	1.3091	0.4275	8.5%	3.70 [1.60, 8.56]	2023
GALAXY 2024	2.358	0.146	18.6%	10.57 [7.94, 14.07]	2024
Nakamura 2024	1.7901	0.7202	4.0%	5.99 [1.46, 24.57]	2024
Frydendahl 2024	1.9095	0.384	9.7%	6.75 [3.18, 14.33]	2024
Diergaarde 2025	1.9459	0.5702	5.8%	7.00 [2.29, 21.40]	2025
Taieb 2025	1.7492	0.1603	18.0%	5.75 [4.20, 7.87]	2025
Total (95% CI)			**100.0%**	**6.56 [4.80, 8.98]**	

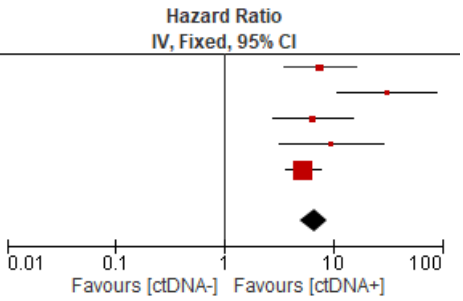
Heterogeneity: Tau² = 0.12; Chi² = 20.28, df = 8 (P = 0.009); I² = 61%
Test for overall effect: Z = 11.76 (P < 0.00001)



C

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Fixed, 95% CI	Year
Tie 2019	2.0149	0.3889	14.4%	7.50 [3.50, 16.07]	2019
Henriksen 2022	3.433	0.5456	7.3%	30.97 [10.63, 90.23]	2022
Frydendahl 2024	1.8703	0.4381	11.3%	6.49 [2.75, 15.32]	2024
Diergaarde 2025	2.2618	0.5605	6.9%	9.60 [3.20, 28.80]	2025
Taieb 2025	1.6506	0.19	60.1%	5.21 [3.59, 7.56]	2025
Total (95% CI)			**100.0%**	**6.69 [5.01, 8.92]**	

Heterogeneity: Chi² = 10.13, df = 4 (P = 0.04); I² = 60%
Test for overall effect: Z = 12.90 (P < 0.00001)



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D

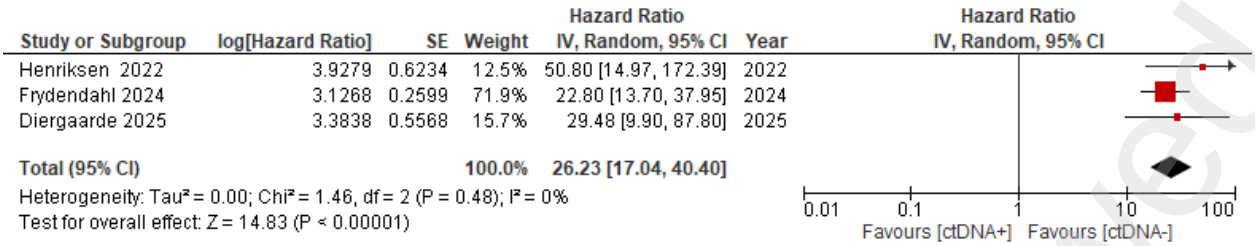
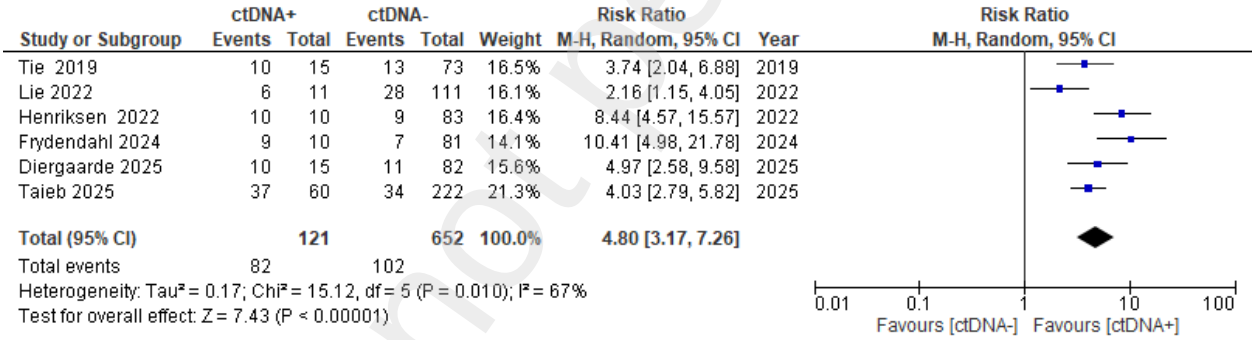
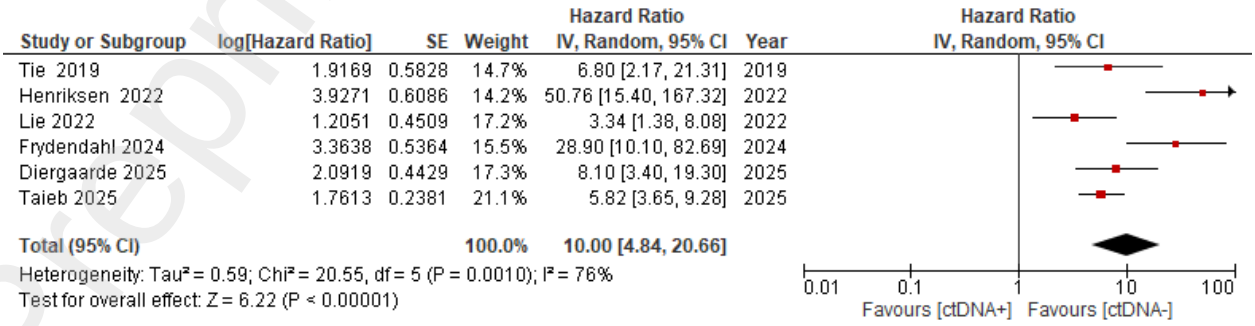


Figure 2. Forest plots of the pooled effect on recurrence risk and RFS in stage III CRC patients after surgery: (A) Forest plot of recurrence risk in ctDNA-positive versus ctDNA-negative patients; (B) Forest plot of univariate analysis of RFS; (C) Forest plot of multivariate analysis of RFS; (D) Forest plot of RFS based on serial ctDNA samples during the surveillance time period.

A



B



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C

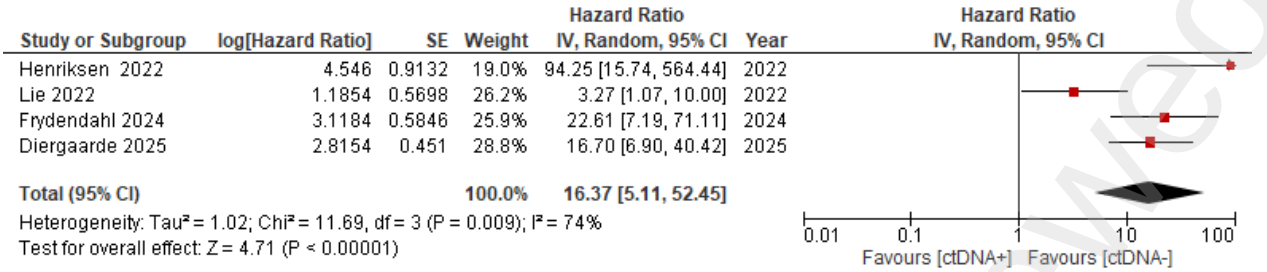
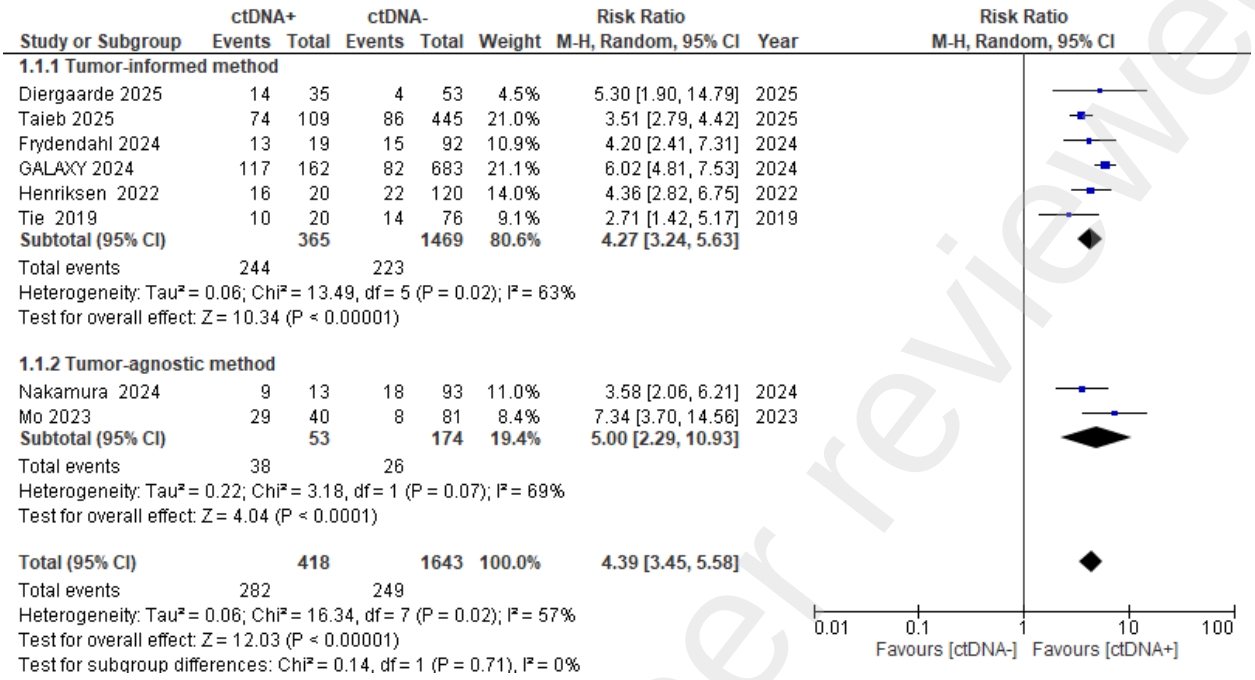


Figure 3. Forest plots of the pooled effect on recurrence risk and RFS in stage III CRC patients after ACT: (A) Forest plot of recurrence risk in ctDNA-positive versus ctDNA-negative patients; (B) Forest plot of univariate analysis of RFS; (C) Forest plot of multivariate analysis of RFS.

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B

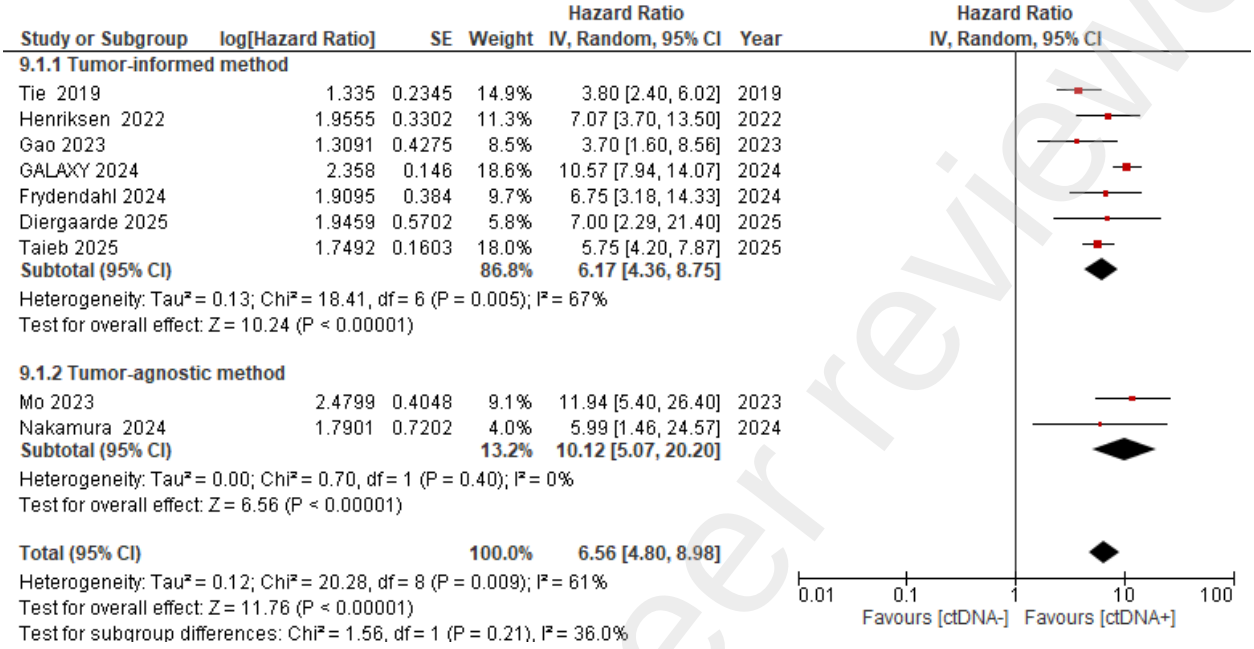
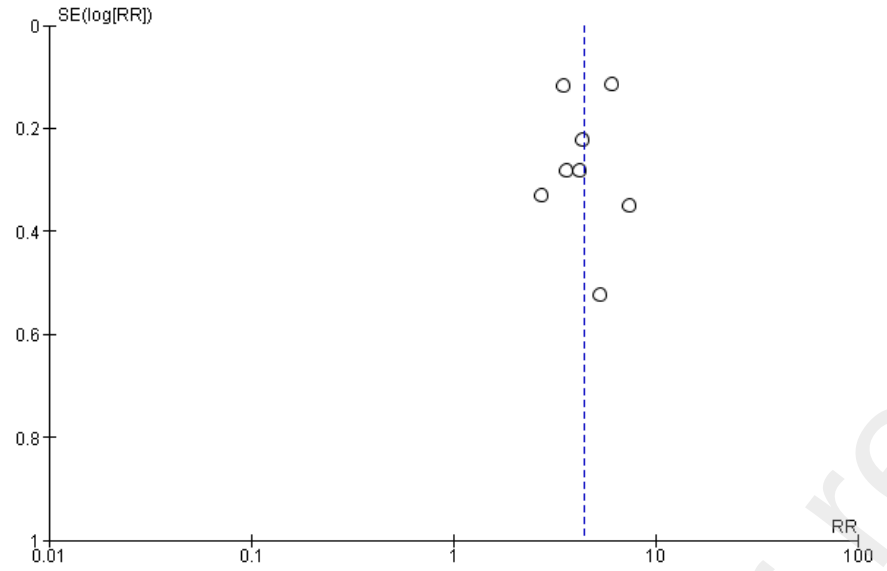


Figure S1. (A) Forest plot of recurrence risk associated with post-operative ctDNA positivity, stratified by detection method (tumor-informed vs. tumor-agnostic); (B) Forest plot of RFS associated with post-operative ctDNA positivity, stratified by detection method (tumor-informed vs. tumor-agnostic).

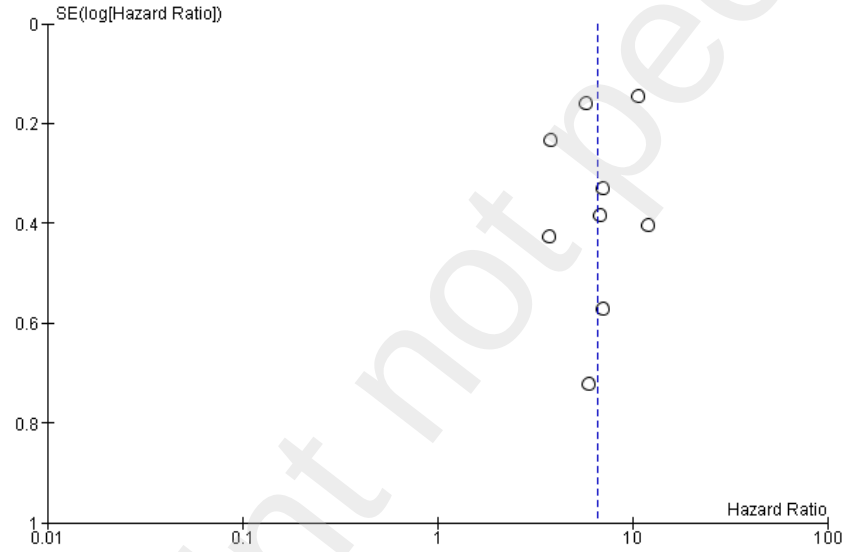
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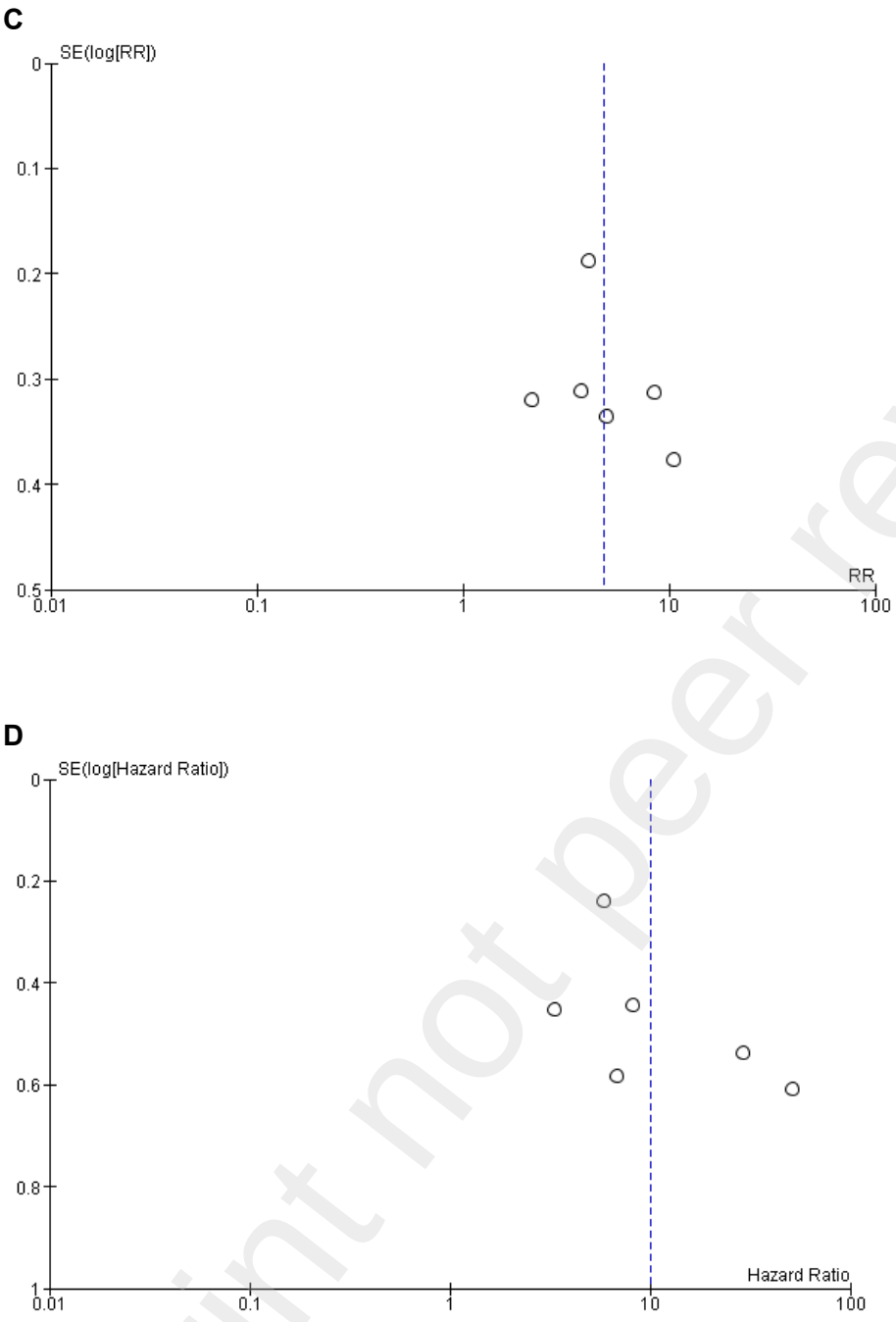


Figure S2. Funnel plots for publication bias assessment of recurrence outcomes in stage III CRC patients: (A) Funnel plot of recurrence risk after surgery; (B) Funnel plot of RFS after surgery; (C) Funnel plot of recurrence risk ACT; (D) Funnel plot of RFS after ACT.

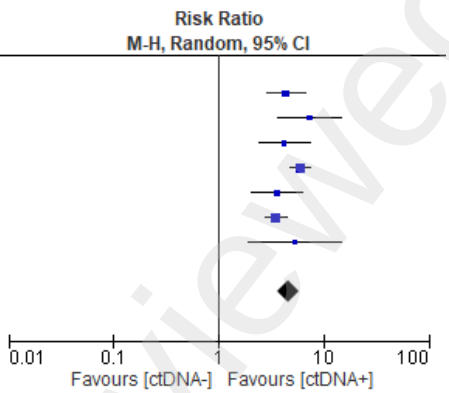
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A

Study or Subgroup	ctDNA+ Events	ctDNA+ Total	ctDNA- Events	ctDNA- Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Tie 2019	10	20	14	76	0.0%	2.71 [1.42, 5.17]	2019
Henriksen 2022	16	20	22	120	15.3%	4.36 [2.82, 6.75]	2022
Mo 2023	29	40	8	81	9.0%	7.34 [3.70, 14.56]	2023
Frydendahl 2024	13	19	15	92	11.8%	4.20 [2.41, 7.31]	2024
GALAXY 2024	117	162	82	683	23.7%	6.02 [4.81, 7.53]	2024
Nakamura 2024	9	13	18	93	11.9%	3.58 [2.06, 6.21]	2024
Taieb 2025	74	109	86	445	23.5%	3.51 [2.79, 4.42]	2025
Diergaarde 2025	14	35	4	53	4.8%	5.30 [1.90, 14.79]	2025
Total (95% CI)		**398**		**1567**	**100.0%**	**4.60 [3.60, 5.89]**	
Total events	272		235				

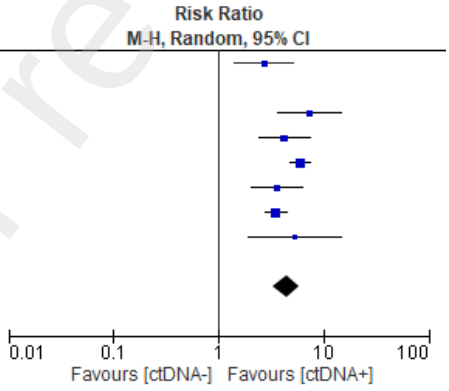
Heterogeneity: Tau² = 0.05; Chi² = 13.95, df = 6 (P = 0.03); I² = 57%
Test for overall effect: Z = 12.15 (P < 0.00001)



B

Study or Subgroup	ctDNA+ Events	ctDNA+ Total	ctDNA- Events	ctDNA- Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Tie 2019	10	20	14	76	11.2%	2.71 [1.42, 5.17]	2019
Henriksen 2022	16	20	22	120	0.0%	4.36 [2.82, 6.75]	2022
Mo 2023	29	40	8	81	10.4%	7.34 [3.70, 14.56]	2023
Frydendahl 2024	13	19	15	92	13.2%	4.20 [2.41, 7.31]	2024
GALAXY 2024	117	162	82	683	23.1%	6.02 [4.81, 7.53]	2024
Nakamura 2024	9	13	18	93	13.3%	3.58 [2.06, 6.21]	2024
Taieb 2025	74	109	86	445	23.0%	3.51 [2.79, 4.42]	2025
Diergaarde 2025	14	35	4	53	5.9%	5.30 [1.90, 14.79]	2025
Total (95% CI)		**398**		**1523**	**100.0%**	**4.39 [3.31, 5.81]**	
Total events	266		227				

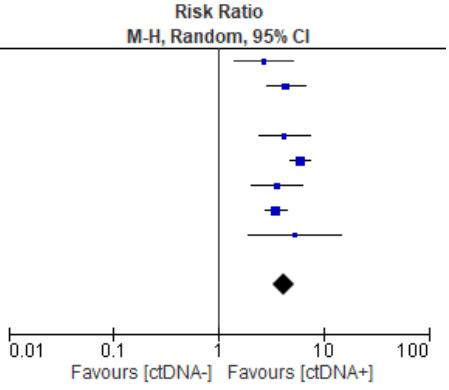
Heterogeneity: Tau² = 0.08; Chi² = 16.32, df = 6 (P = 0.01); I² = 63%
Test for overall effect: Z = 10.31 (P < 0.00001)



C

Study or Subgroup	ctDNA+ Events	ctDNA+ Total	ctDNA- Events	ctDNA- Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Tie 2019	10	20	14	76	9.7%	2.71 [1.42, 5.17]	2019
Henriksen 2022	16	20	22	120	15.2%	4.36 [2.82, 6.75]	2022
Mo 2023	29	40	8	81	0.0%	7.34 [3.70, 14.56]	2023
Frydendahl 2024	13	19	15	92	11.7%	4.20 [2.41, 7.31]	2024
GALAXY 2024	117	162	82	683	23.5%	6.02 [4.81, 7.53]	2024
Nakamura 2024	9	13	18	93	11.8%	3.58 [2.06, 6.21]	2024
Taieb 2025	74	109	86	445	23.2%	3.51 [2.79, 4.42]	2025
Diergaarde 2025	14	35	4	53	4.8%	5.30 [1.90, 14.79]	2025
Total (95% CI)		**378**		**1562**	**100.0%**	**4.19 [3.28, 5.36]**	
Total events	253		241				

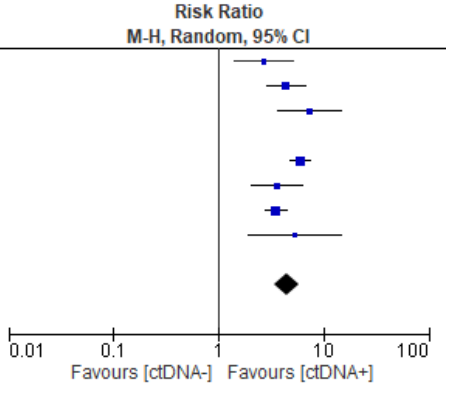
Heterogeneity: Tau² = 0.05; Chi² = 14.10, df = 6 (P = 0.03); I² = 57%
Test for overall effect: Z = 11.42 (P < 0.00001)



D

Study or Subgroup	ctDNA+ Events	ctDNA+ Total	ctDNA- Events	ctDNA- Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Tie 2019	10	20	14	76	10.7%	2.71 [1.42, 5.17]	2019
Henriksen 2022	16	20	22	120	15.9%	4.36 [2.82, 6.75]	2022
Mo 2023	29	40	8	81	9.9%	7.34 [3.70, 14.56]	2023
Frydendahl 2024	13	19	15	92	0.0%	4.20 [2.41, 7.31]	2024
GALAXY 2024	117	162	82	683	22.7%	6.02 [4.81, 7.53]	2024
Nakamura 2024	9	13	18	93	12.7%	3.58 [2.06, 6.21]	2024
Taieb 2025	74	109	86	445	22.6%	3.51 [2.79, 4.42]	2025
Diergaarde 2025	14	35	4	53	5.5%	5.30 [1.90, 14.79]	2025
Total (95% CI)		**399**		**1551**	**100.0%**	**4.41 [3.36, 5.78]**	
Total events	269		234				

Heterogeneity: Tau² = 0.07; Chi² = 16.28, df = 6 (P = 0.01); I² = 63%
Test for overall effect: Z = 10.73 (P < 0.00001)



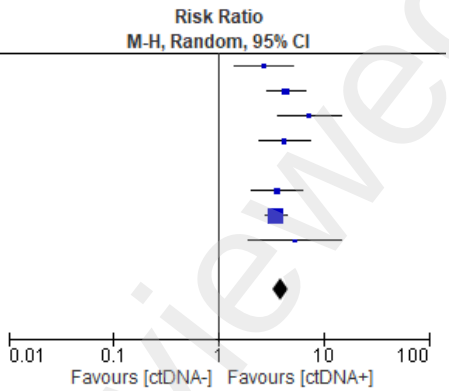
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E

Study or Subgroup	ctDNA+ Events	ctDNA+ Total	ctDNA- Events	ctDNA- Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Tie 2019	10	20	14	76	7.2%	2.71 [1.42, 5.17]	2019
Henriksen 2022	16	20	22	120	15.2%	4.36 [2.82, 6.75]	2022
Mo 2023	29	40	8	81	6.4%	7.34 [3.70, 14.56]	2023
Frydendahl 2024	13	19	15	92	9.6%	4.20 [2.41, 7.31]	2024
GALAXY 2024	117	162	82	683	0.0%	6.02 [4.81, 7.53]	2024
Nakamura 2024	9	13	18	93	9.7%	3.58 [2.06, 6.21]	2024
Taieb 2025	74	109	86	445	49.1%	3.51 [2.79, 4.42]	2025
Diergaarde 2025	14	35	4	53	2.9%	5.30 [1.90, 14.79]	2025
Total (95% CI)		**256**		**960**	**100.0%**	**3.85 [3.24, 4.59]**	
Total events	165		167				

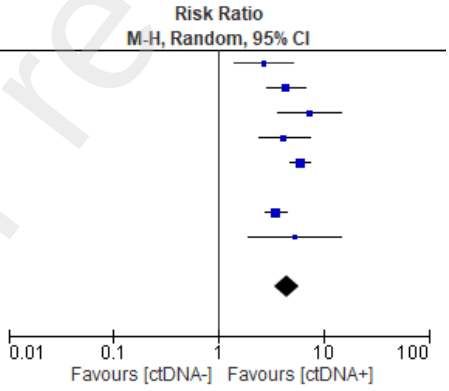
Heterogeneity: Tau² = 0.00; Chi² = 6.23, df = 6 (P = 0.40); I² = 4%
Test for overall effect: Z = 15.15 (P < 0.00001)



F

Study or Subgroup	ctDNA+ Events	ctDNA+ Total	ctDNA- Events	ctDNA- Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Tie 2019	10	20	14	76	10.5%	2.71 [1.42, 5.17]	2019
Henriksen 2022	16	20	22	120	15.8%	4.36 [2.82, 6.75]	2022
Mo 2023	29	40	8	81	9.7%	7.34 [3.70, 14.56]	2023
Frydendahl 2024	13	19	15	92	12.5%	4.20 [2.41, 7.31]	2024
GALAXY 2024	117	162	82	683	23.1%	6.02 [4.81, 7.53]	2024
Nakamura 2024	9	13	18	93	0.0%	3.58 [2.06, 6.21]	2024
Taieb 2025	74	109	86	445	22.9%	3.51 [2.79, 4.42]	2025
Diergaarde 2025	14	35	4	53	5.4%	5.30 [1.90, 14.79]	2025
Total (95% CI)		**405**		**1550**	**100.0%**	**4.50 [3.45, 5.87]**	
Total events	273		231				

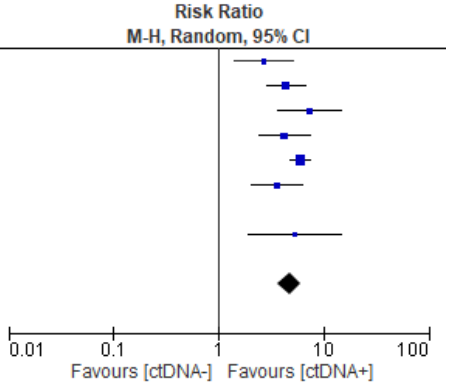
Heterogeneity: Tau² = 0.07; Chi² = 15.58, df = 6 (P = 0.02); I² = 61%
Test for overall effect: Z = 11.10 (P < 0.00001)



G

Study or Subgroup	ctDNA+ Events	ctDNA+ Total	ctDNA- Events	ctDNA- Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Tie 2019	10	20	14	76	10.6%	2.71 [1.42, 5.17]	2019
Henriksen 2022	16	20	22	120	17.8%	4.36 [2.82, 6.75]	2022
Mo 2023	29	40	8	81	9.7%	7.34 [3.70, 14.56]	2023
Frydendahl 2024	13	19	15	92	13.2%	4.20 [2.41, 7.31]	2024
GALAXY 2024	117	162	82	683	30.5%	6.02 [4.81, 7.53]	2024
Nakamura 2024	9	13	18	93	13.3%	3.58 [2.06, 6.21]	2024
Taieb 2025	74	109	86	445	0.0%	3.51 [2.79, 4.42]	2025
Diergaarde 2025	14	35	4	53	5.0%	5.30 [1.90, 14.79]	2025
Total (95% CI)		**309**		**1198**	**100.0%**	**4.71 [3.69, 6.01]**	
Total events	208		163				

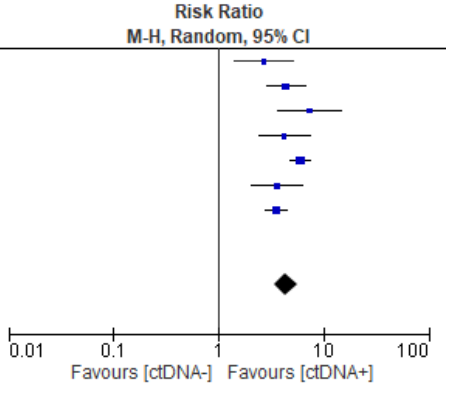
Heterogeneity: Tau² = 0.04; Chi² = 9.60, df = 6 (P = 0.14); I² = 37%
Test for overall effect: Z = 12.45 (P < 0.00001)



H

Study or Subgroup	ctDNA+ Events	ctDNA+ Total	ctDNA- Events	ctDNA- Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Tie 2019	10	20	14	76	9.8%	2.71 [1.42, 5.17]	2019
Henriksen 2022	16	20	22	120	14.7%	4.36 [2.82, 6.75]	2022
Mo 2023	29	40	8	81	9.1%	7.34 [3.70, 14.56]	2023
Frydendahl 2024	13	19	15	92	11.7%	4.20 [2.41, 7.31]	2024
GALAXY 2024	117	162	82	683	21.6%	6.02 [4.81, 7.53]	2024
Nakamura 2024	9	13	18	93	11.8%	3.58 [2.06, 6.21]	2024
Taieb 2025	74	109	86	445	21.4%	3.51 [2.79, 4.42]	2025
Diergaarde 2025	14	35	4	53	0.0%	5.30 [1.90, 14.79]	2025
Total (95% CI)		**383**		**1590**	**100.0%**	**4.34 [3.36, 5.61]**	
Total events	268		245				

Heterogeneity: Tau² = 0.07; Chi² = 16.21, df = 6 (P = 0.01); I² = 63%
Test for overall effect: Z = 11.26 (P < 0.00001)

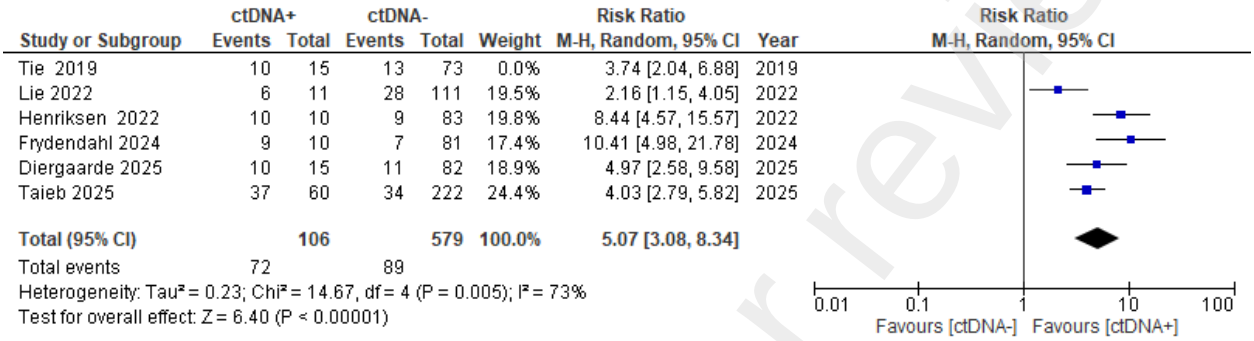


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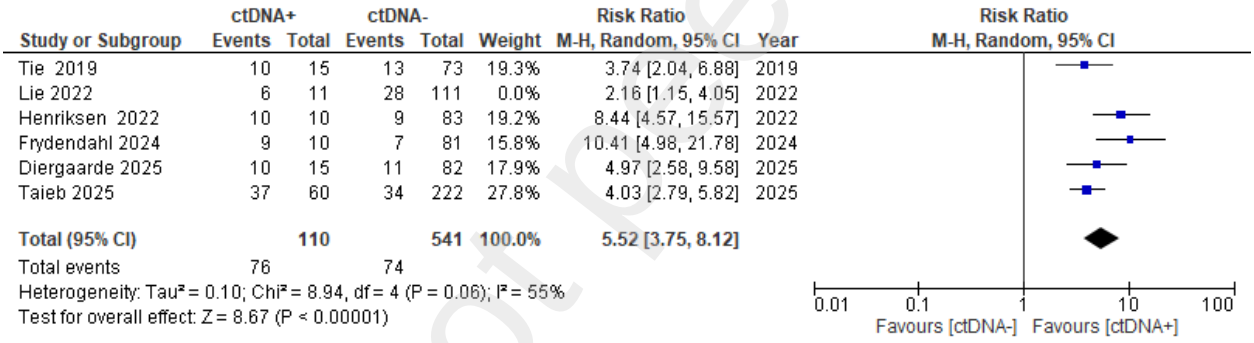
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Figure S3. Leave-one-out sensitivity analysis for overall survival. Results obtained when each study is left out of each analysis: A) Tie et al; B) Henriksen et al; C) Mo et al; D) Frydendahl et al; E) GALAXY; F) Nakamura et al; G) Taieb et al; H) Diergaarde et al.

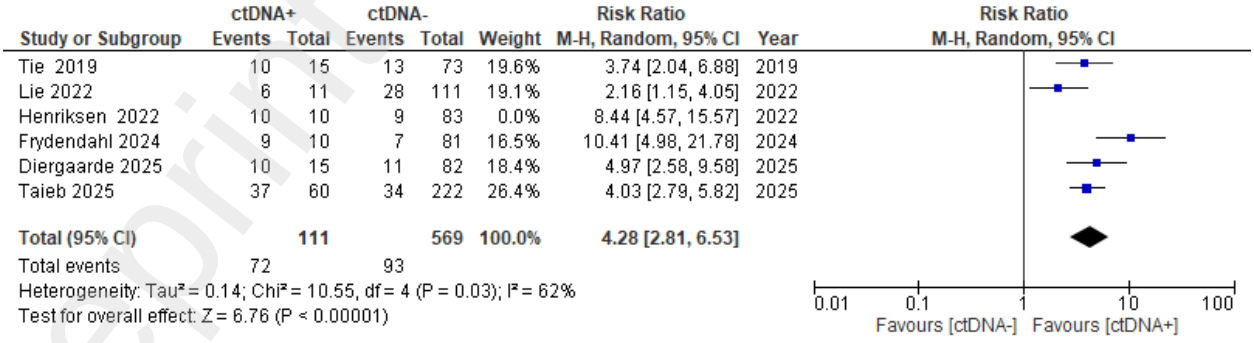
A



B



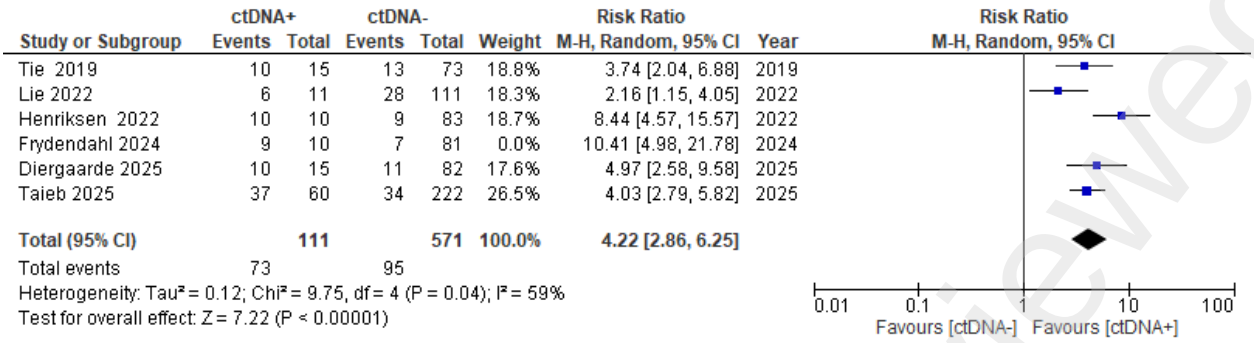
C



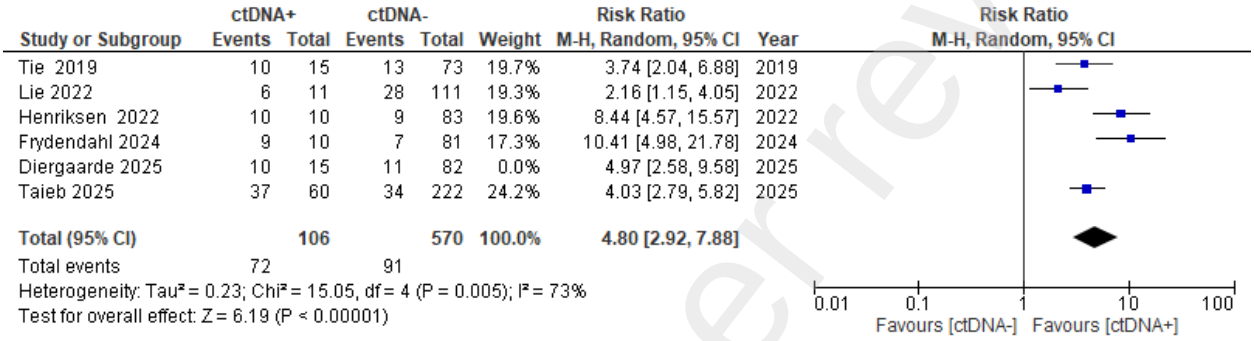
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D



E



F

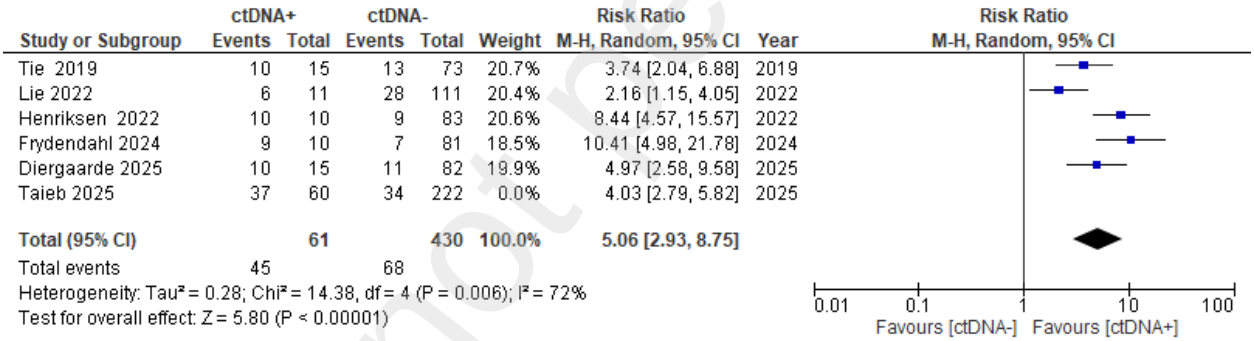


Figure S4. Leave-one-out sensitivity analysis for overall survival. Results obtained when each study is left out of each analysis: A) Tie et al; B) Lie et al; C) Henriksen et al; D) Frydendahl et al; E) Diergaarde et al; F) Taieb et al.

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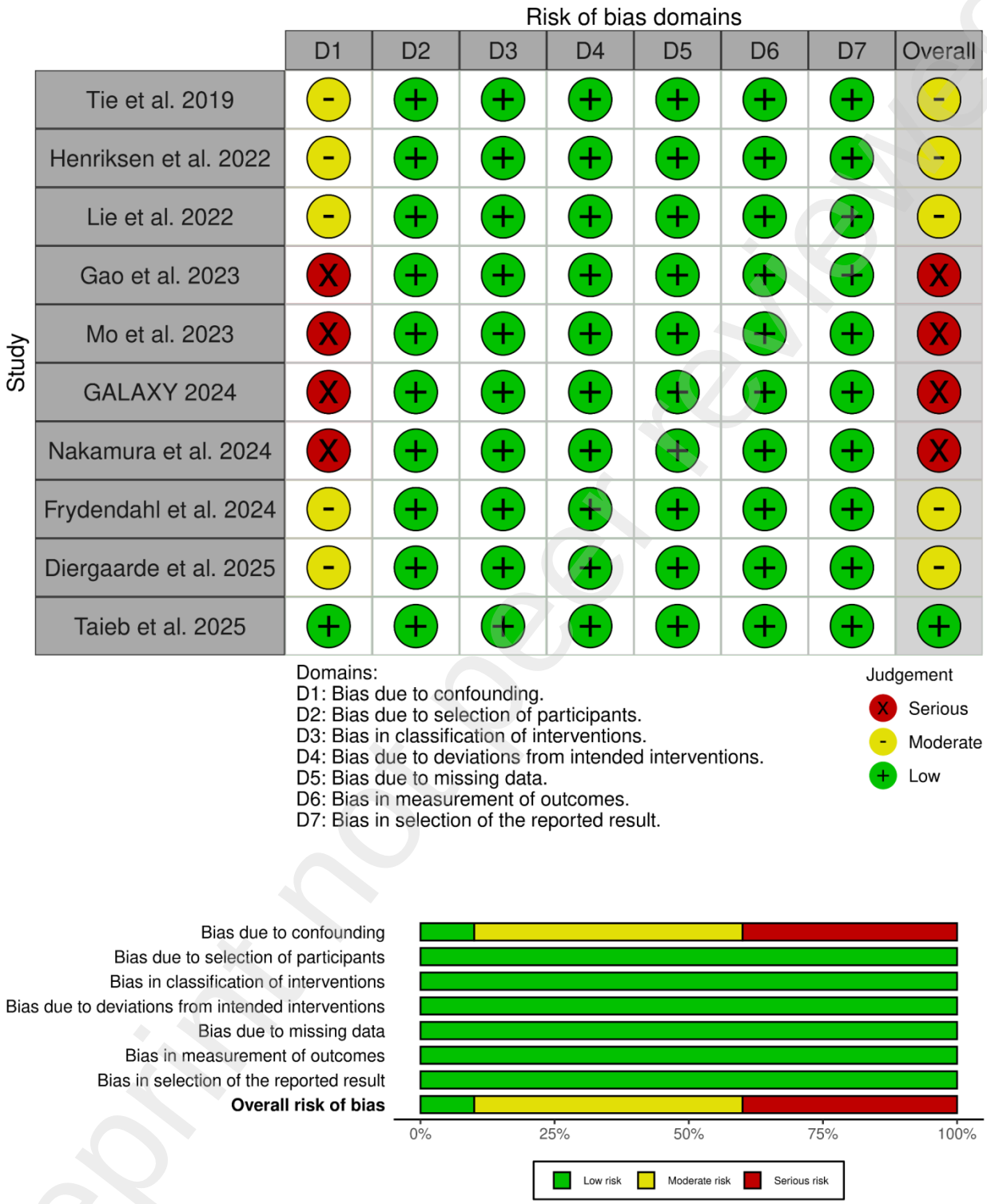


Figure S5. ROBINS-I Risk of Bias Assessment Across Included Studies.

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Author's Contributions

Turkan Aliyeva (T.A.) conceived the research idea, led the project administration and supervision, conducted literature search, full manuscript screening, data curation and formal analysis, registered the protocol in PROSPERO, contributed to writing and reviewing the manuscript and served as the corresponding author.
Hiba Siddiqui (H.S.) contributed to the literature search, full manuscript screening, data extraction and writing.
Julia Natche (J.N.) wrote the introduction, methodology sections, results and abstract.
Yumna Ahmad Al-Wraikat (Y.A.A.) contributed to initial screening and extraction, and prepared Table 1.
Farah Mahzabin Hossain (F.M.H.) contributed to writing the discussion and conclusion sections.
Imane El-Amri (I.E.) performed the risk of bias assessment.

All authors critically reviewed and approved the final manuscript.

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Not applicable.

Declaration of Competing Interests

The authors hereby declare that they have no financial, personal, or professional conflicts of interest that could have influenced the work reported in this study. There are no competing interests to disclose.

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