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The prognostic role of circulating tumor DNA across breast cancer molecular subtypes: A systematic review and meta-analysis

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ABSTRACT

Objective: Circulating tumor DNA (ctDNA) is increasingly being used as a potential prognostic biomarker in cancer patients. We aimed to assess the prognostic value of ctDNA in different subtypes of breast cancer patients throughout the whole treatment cycle.

Materials and methods: PubMed, Web of Science, Embase, Cochrane Library, Scopus, and clinical trials.gov databases were searched from January 2016 to May 2022. The following search terms were used: ctDNA OR circulating tumor DNA AND breast cancer OR breast carcinoma. Only studies written in English were included. The following pre-specified criteria should be met for inclusion: (i) original articles, conference abstracts, etc.; (ii) patients with breast cancer; (iii) ctDNA measurement; and (iv) clinical outcome data such as recurrence-free survival (RFS) and overall survival (OS). The random-effects model was preferred considering the potential heterogeneity across studies. The main outcomes are ctDNA detection rate and postoperative long-term outcomes (RFS and OS).

Results: A total of 24 studies were screened. At every measurement time, the ctDNA detection rate of the HR+ subgroup was similar to that of the HR- subgroup ($P = 0.075$; $P = 0.458$; $P = 0.744$; and $P = 0.578$), and the ctDNA detection rate of the HER2+ subgroup was similar to that of the HER2- subgroup ($P = 0.805$; $P = 0.271$; $P = 0.807$; and $P = 0.703$). In the HR+ subgroup, RFS and OS of ctDNA positive patients were similar to those of ctDNA negative patients ($P = 0.589$ and $P = 0.110$), while RFS and OS of the ctDNA positive group was significantly shorter than those of the ctDNA negative patients in the HR- subgroup (HR = 4.03, $P < 0.001$; HR = 3.21, $P < 0.001$). According to HER grouping, the results were the same as above. In the triple negative breast cancer (TNBC) subgroup, the RFS and OS of ctDNA-positive patients was significantly shorter than of the ctDNA negative patients before and after surgery.

Conclusions: ctDNA was more predictive of recurrence-free survival and overall survival in the HR- subgroup than in the HR+ subgroup, and the same result was showed in the HER2- subgroup *vs*. HER2+ subgroup. The prognosis of the TNBC subtype is closely related to ctDNA before and after surgery.

1. Introduction

Breast cancer has overtaken lung cancer to become the most common cancer and the fifth leading cause of cancer death worldwide. In 2020, breast cancer accounted for approximately 24.5% of all cancer cases and 15.5% of cancer deaths in women.[1] Monitoring disease progression in patients can help physicians tailor treatment to individual circumstances and improve patient outcomes. The detection of circulating tumor DNA

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(ctDNA) is an emerging non-invasive alternative to tissue biopsy methods that only needs a blood simple.[2] ctDNA is present in the blood of patients at all stages of the disease and carries many of the characteristics of solid tumors. Therefore, ctDNA can be used for screening and early detection, disease surveillance, recurrence prediction, and tumor analysis to inform the order of treatment for solid carcinomas.[3–5] Currently, patients who are asymptomatic after completion of treatment are not routinely assessed for distant recurrence using imaging techniques. In addition, ctDNA monitoring for lung and colorectal cancers has been shown to indicate disease recurrence months before imaging results.[6,7] Therefore, routine ctDNA monitoring may be a useful way to reliably identify patients at risk of aggressive disease progression through early minimal residual disease testing.

Many studies have shown that ctDNA can be used as a biomarker of breast cancer prognosis, and the content of ctDNA is closely related to the outcome indicators of breast cancer, such as recurrence-free survival (RFS), overall survival (OS), etc.[8,9] However, breast cancer is a group of highly heterogeneous diseases, based on estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and other molecular indicators.[10] The biological behavior and prognosis of different subtypes of breast cancer are significantly different.[11–13] Is ctDNA closely related to prognostic indicators of different subtypes of breast cancer? No relevant analysis has been found in the literature. Through meta-analysis and a systematic review, we intend to understand the detection rate of ctDNA in different molecular types of breast cancer and the relationship between ctDNA detection and prognostic indicators, so as to further improve the role of ctDNA in predicting breast cancer prognosis and provide scientific basis for precise treatment.

2. Methods

2.1. Protocol and registration

This systematic review and meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) guidelines[14] and Meta-analysis of Observational Studies in Epidemiology (MOOSE) to identify studies that assessed the association of ctDNA and clinical outcomes in breast cancer patients. The study protocol had been prospectively registered on PROSPERO (CRD 42022331326).

2.2. Search strategy

The electronic databases PubMed, Web of Science, Embase, Cochrane Library, Scopus, and the clinical trials.gov database were searched from January 2016 to May 2022. The detailed search strategy is available in Supplementary material. The titles and abstracts were first screened and then the potential eligible articles were full-text reviewed after removing the duplicates automatically (Endnote X8, Clarivate, Philadelphia, PA) and manually based on the eligibility criteria. This process was performed independently by two authors and any discrepancies were resolved by discussion.

2.3. Study selection

The following prespecified inclusion criteria were applied: (i) original articles and conference abstracts encompassing observational studies (prospective or retrospective), randomized controlled trials, cross-sectional studies, or case series studies; (ii) studies that reported perioperative breast cancer patients with subtypes; (iii) documented collection and measurement of ctDNA. All methods of ctDNA detection and analysis were allowed, given the lack of a gold standard; (iv) ctDNA detection rate and clinical prognostic outcomes data such as recurrence-free survival (RFS) and overall survival (OS) were reported; and (v) articles written in the English language.

The exclusion criteria were: (i) studies with no primary data (review articles, editorials, comments, or studies with a sample size of 5 or less) or ongoing studies without results; (ii) only the elevated and reduced ctDNA levels or cell-free DNA was measured; (iii) patients with unspecified breast cancer subgroups; (iv) studies focusing on diagnosis or screening outcomes. Besides, studies reporting on similar cohorts within the same time period were also assessed, and the most up-to-date and largest study was chosen. The studies were retrieved with both conference abstracts and full articles, and only the publication as a peer-reviewed full text article was included.

2.4. Data extraction and synthesis

For the purpose of this analysis, ctDNA was considered a binary variable (positive or negative). The following variables were extracted from the selected literature: (i) general information: title, first author, publication year, study design, and country; (ii) population and cancer characteristics: sample size, cancer stage, cancer grade, molecular types, and follow-up duration; (iii) ctDNA information: measurement methods and time points, type of assay, definition of positivity; (iv) outcome measurements: ctDNA detection rate, RFS (composite endpoint including RFS, event-free survival, disease-free survival, etc., depending on the study) and OS.

2.5. Outcomes and measures

The main endpoint of the meta-analysis included ctDNA detection rate in different time points and the prognostic outcomes were RFS and OS. RFS was defined as the time from which a patient achieved complete remission after antineoplastic therapy to the time of relapse or the end of follow-up. OS was defined as the time from the start of a patient's treatment until the patient died from any cause.

2.6. Quality assessment

The RoB 2 tool for randomized controlled trials, the Newcastle-Ottawa Scale (NOS) for observational studies (cohort and case-control study), and the National Institute for Health and Care Excellence (NICE) quality assessment tool for case series studies were used.

2.7. Statistical analysis

The meta-analyses were conducted separately for each time point, including baseline (before any treatment), during neoadjuvant therapy (NAT), after NAT (before surgery), and after surgery. For the meta-analysis of ctDNA detection rate, the pooled estimates and corresponding 95% confidence intervals (CIs) were calculated. To handle extreme proportions, the Freeman-Tukey double arcsine transformation was chosen and the random-effects model was fitted. As for the RFS and OS analysis, the hazard ratio (HR) with 95% CI was calculated. Heterogeneity was assessed and reported using I^2 statistics and Cochran's Q test. When there was significant heterogeneity ($I^2 > 50\%$), a random-effects model was preferred. All reported P values were two-sided, and $P < 0.05$ was considered statistically significant. Funnel plot analysis and Egger's test were performed to detect publication bias. All analyses were performed using R statistical software, version 4.0.0 (R packages *metafor* and *meta*).

3. Results

3.1. Literature search results

A total of 24 records were included, including 14 full-text articles and 10 conference abstracts. The screening process is shown in Fig. 1. The publication years ranged from 2016 to 2022, and 21 studies reported the country of the study population: five studies from China, four from the United Kingdom, three from the United States of America,

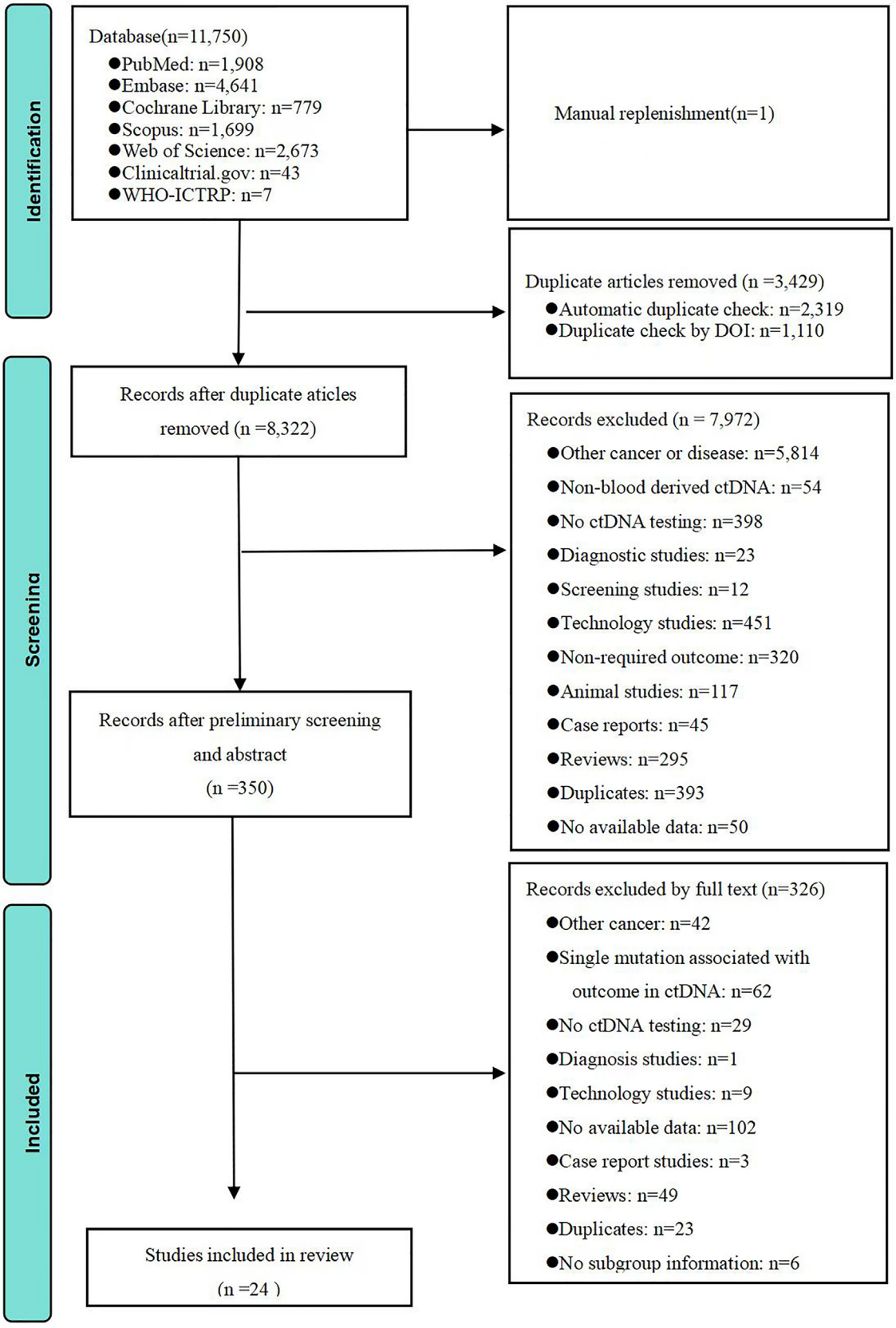

Fig. 1. Flow chart of records inclusion and exclusion.

Table 1
Basic information of the included studies.

Author	Article type	Registration No.	Samples, n	Country	Multi center	Prospective
L Cavallone,[15] 2020	Article	NCT01276899 Q-CROC-03 trial	26	Canada and USA	Y	–
I Garcia-Murillas,[16] 2019	Article	ChemoNEAR or plasma DNA study	170	UK	Y	Y
S Li,[17] 2020	Article	NCT03260192	44	China	N	Y
MJM Magbanua,[18] 2020	Article	NCT01042379 I-SPY 2 TRIAL	84	–	Y	N
E Ortolan,[19] 2019	Article	–	31	France	–	Y
M Radovich,[20] 2020	Article	NCT02101385 BRE12–158	142	USA	Y	–
RC Coombes,[21·] 2019	Article	EBLIS	49	UK	Y	Y
F Riva,[22] 2016	Article	NCT02220556 CTC–CEC DNA study	36	France	–	Y
F Rothe,[23] 2019	Article	NeoALTTO	69	Belgium	Y	–
H Takahashi,[24] 2016	Article	–	87	Japan	–	Y
PH Lin,[25] 2021	Article	–	95	China	–	–
T Yoshinami,[26] 2020	Article	–	62	Japan	N	–
Q Zhou,[27] 2022	Article	ABCSG-34 trial	142	Austria	Y	Y
W Janni,[28] 2022	Conference abstract	BRandO BiO registry study	38	Germany	–	–
E Agostinetto,[29] 2022	Conference abstract	–	38	Belgium	N	–
MJM Magbanua,[30] 2021	Conference abstract	I-SPY 2 TRIAL	132	USA	–	–
J Lan,[31] 2022	Article	–	20	China	N	–
X Zhang,[32·] 2019	Article	–	102	China	N	–
DM Carraro,[33] 2020	Conference abstract	–	16	–	–	–
N Turner,[34] 2022	Conference abstract	c-TRAK TN trial	161	UK	Y	Y
RJ Cutts,[35] 2021	Conference abstract	ChemoNEAR	22	UK		N
F Lynce,[36] 2022	Conference abstract	OXEL	33	USA		–
F Ma,[37] 2018	Conference abstract	NCT02041338	31	China	N	Y
M Fedyanin,[38] 2020	Conference abstract	–	66	–	N	Y

Abbreviations: -, not reported; N, no; No., number; UK, United Kingdom; USA, United States of America; Y, yes.

two each from France, Belgium, and Japan, and one each from Germany, Australia, and Canada (Table 1).[15–38] According to the time of ctDNA measurements, 14 studies reported at baseline, five reported during NAT, eight reported after NAT and before surgery, and 11 reported after surgery. By reported outcomes, 12 studies reported RFS outcomes, five reported OS outcomes, and 22 reported ctDNA positive detection rates. Twenty studies reported clinical follow-up periods, ranging from 12 months to 4.8 years. Fifteen reported the HR+ subtype, 22 reported the HR- subtype, 13 reported the HER2+ subtype, and 21 reported the HER2- subtype (Table 2).

3.2. The detection rate of ctDNA

At baseline, there was no significant difference within either the HR subtype group (P = 0.075) or the HER2 subtype group (P = 0.805). There results were the same at the other three measurement time points (during NAT, after NAT before surgery, and after surgery), as shown in Table 3.

3.3. ctDNA and RFS

3.3.1. ctDNA and RFS in subgroups

The following results were based on combined data from all measurement times. In the HR+ subgroup, RFS of ctDNA positive patients was similar to that of ctDNA negative patients (P = 0.589), while RFS of the ctDNA positive group was significantly shorter than that of the ctDNA negative patients in the HR- subgroup (HR = 4.03, P < 0.001). In the HER2+ subgroup, RFS of ctDNA positive patients was similar to that of ctDNA negative patients (P = 0.199), and RFS of ctDNA positive patients was significantly shorter than that of ctDNA negative patients in the HER2- subgroup (HR = 4.69, P < 0.001) (Table 4).

3.3.2. ctDNA and RFS in subgroups at different measurement time points

In the HR- subgroup, the RFS of ctDNA positive patients was significantly shorter than that of the ctDNA negative patients at baseline, after NAT before surgery, and after surgery (HR = 5.11, P = 0.04; HR = 3.03, P = 0.015; HR = 6.27, P < 0.001). In the HR+ subgroup, RFS of ctDNA positive individuals was similar to that of ctDNA negative individuals at baseline (HR = 1.75, P = 0.589) (Table 5). In the HER2- subgroup, the RFS of ctDNA positive patients was significantly shorter than of ctDNA negative patients after NAT before surgery and after surgery (HR = 3.03, P = 0.015; HR = 6.27, P < 0.001). In the HER2+ subgroup, RFS of ctDNA positive individuals was similar to that of ctDNA negative individuals at baseline and during NAT (HR = 0.91, P = 0.890; HR = 1.40, P = 0.625) (Table 5). In the TNBC (HR-/HER2-) subgroup, the RFS of ctDNA positive patients was significantly shorter than that of ctDNA negative patients after NAT before surgery and after surgery (HR = 2.62, 95% CI: 1.25–5.48; HR = 3.53, 95% CI: 2.55–4.90) (Fig. 2).

3.4. ctDNA and OS

3.4.1. ctDNA and OS in subgroups

The following results were based on combined data from all measurement times. In the HR+ subgroup, OS of ctDNA positive patients was similar to that of ctDNA negative patients (P = 0.110), while OS of ctDNA positive patients was significantly shorter than that of ctDNA negative patients in the HR- and HER2- subgroup (HR = 3.21, P < 0.001; HR = 2.97, P < 0.001) (Table 6).

3.4.2. ctDNA and OS in subgroups at different measurement times

In the HR- subgroup, OS of ctDNA positive patients was significantly shorter than that of ctDNA negative patients at baseline (HR = 5.46, P = 0.034). In the HR+ subgroup, OS of ctDNA positive individuals was similar to that of ctDNA-negative individuals after NAT before surgery (HR = 1.72, P = 0.119), but shorter than of the ctDNA negative patients at baseline (HR = 2.20, P = 0.016) (Table 7). In the TNBC (HR-/HER2-) subgroup, OS of ctDNA positive patients was significantly shorter than that of ctDNA negative patients after NAT before surgery and after surgery (HR = 3.70, 95% CI:1.03–13.23; HR = 2.80, 95% CI:1.43–5.47), except during NAT (HR = 2.86, 95% CI:0.74–11.11) (Fig. 3).

Table 2
Indicators and outcome information of the included studies.

Author	Measurement time point	Outcome	Subtype	Follow-up time, median
L Cavallone,[15] 2020	During NAT; after NAT and before surgery	RFS, OS, ctDNA rate	HER2-. HR-	63 months post-diagnosis/ 55 months post-surgery
I Garcia-Murillas,[16] 2019	Baseline; after surgery	RFS, OS, ctDNA rate	HR+. HR-. HER2+. HER2-	36.3 (range 4.1–73.2) months
S Li,[17] 2020	Baseline; after NAT and before surgery	DFS, OS, ctDNA rate	HR+. HR-. HER2+. HER2-	46 (range 11–68) months
MJM Magbanua,[18] 2020	Baseline; during NAT; after NAT and before surgery	DRFS, ctDNA rate	HR+. HR-. HER2-	4.8 (range 0.5–6.3) years
E Ortolan,[19] 2019	After NAT and before surgery; after surgery	EFS, ctDNA rate	HER2-. HR-	3 (range 0.5–6.5) years
M Radovich,[20] 2020	After surgery	DFS, DDFS, OS, ctDNA rate	HER2-. HR-	17.2 (range 0.1–58.3) months
RC Coombes,[21·] 2019	After surgery	RFS, ctDNA rate	HR+. HR-. HER2+. HER2-	Up to 4 years
F Riva,[22] 2016	Baseline; after NAT and before surgery; After surgery	ctDNA rate	HER2-. HR-	24 (range 9–36) months
F Rothe,[23] 2019	Baseline; during NAT; before surgery	EFS, ctDNA rate	HR+. HR-	6.64 (range 0.003–7.94) years
H Takahashi,[24] 2016	Baseline; after surgery	ctDNA rate	HR+. HR-. HER2+. HER2-	After surgery 23 (range 3–33) months
PH Lin,[25] 2021	Baseline; after surgery	RFS, ctDNA rate	HR+. HR-. HER2+. HER2-	5.1 years
T Yoshinami,[26] 2020	Baseline	DDFS, ctDNA rate	HR+. HR-. HER2+. HER2-	–
Q Zhou,[27] 2022	Baseline; during NAT; after NAT and before surgery	ctDNA rate	HR+. HR-. HER2-	–
W Janni,[28] 2022	After surgery	–	HR+. HR-. HER2+. HER2-	3 years
E Agostinetto,[29] 2022	Baseline; after NAT and before surgery; after surgery	EFS, ctDNA rate	HR+. HR-. HER2+. HER2-	3.30 (range 0.39–5.85) years
MJM Magbanua,[30] 2021	–	–	HR+. HR-. HER2-	Median 2.8 years
J Lan,[31] 2022	After surgery	ctDNA rate	HR+. HR-. HER2+. HER2-	–
X Zhang,[32] 2019	Baseline; after surgery	ctDNA rate	HR+. HER2+. HER2-	At least 5 years or until the end of a patient's life
DM Carraro,[33] 2020	Baseline; during NAT	ctDNA rate	HR-. HER2-	–
N Turner,[34] 2022	After NAT and before surgery	ctDNA rate	HR-. HER2-	12–24 months
RJ Cutts,[35] 2021	After surgery	ctDNA rate	HR+. HR-. HER2+. HER2-	24.6 months
F Lynce,[36] 2022	Baseline	ctDNA rate	HR-. HER2-	12 months
F Ma,[37] 2018	Baseline	ctDNA rate	HR-. HER2+	–
M Fedyanin,[38] 2020	After surgery	ctDNA rate	HER2+	2 years

Abbreviations: -, not reported; ctDNA, circulating tumor DNA; DDFS, distant disease-free survival; DFS, Disease-free survival; DRFS, distant relapse-free survival; EFS, Event-free survival; HER2+, human epidermal growth factor receptor 2 positive; HER2-, human epidermal growth factor receptor 2 negative; HR+, hormone receptor positive; HR-, hormone receptor negative; NAT, neoadjuvant therapy; RFS, recurrence-free survival; OS, overall survival.

Table 3
ctDNA detection rate of subgroups at different measurement time points.

Measurement time point	Molecular subgroup	Included studies, n	Samples, n	I^2, %	$P_{heterogeneity}$ value	Model	ctDNA detection rate,%	95% CI
Baseline	HR ($P = 0.075$)							
	HR-	12	344	82.8	<0.010	Random-effects	65.68	52.69–77.66
	HR+	7	366	91.7	<0.010	Random-effects	45.34	27.85–63.42
	HER2 ($P = 0.805$)							
	HER2-	10	395	89.0	<0.010	Random-effects	60.59	45.23–75.01
	HER2+	6	186	83.8	<0.010	Random-effects	57.57	38.30–75.79
After NAT before surgery	HR ($P = 0.744$)							
	HR-	6	143	83.5	0.001	Random-effects	27.44	10.74–47.83
	HR+	5	283	86.4	<0.010	Random-effects	32.12	16.26–50.29
	HER2 ($P = 0.807$)							
	HER2-	7	258	86.7	<0.010	Random-effects	28.34	13.85–45.33
	HER2+	4	95	90.9	<0.010	Random-effects	22.59	0.00–64.02
After surgery	HR ($P = 0.578$)							
	HR-	9	512	93.3	<0.010	Random-effects	27.46	13.02–44.45
	HR+	6	258	61.9	0.015	Random-effects	22.00	13.41–31.78
	HER2 ($P = 0.703$)							
	HER2-	12	719	90.4	<0.010	Random-effects	25.16	15.35–36.25
	HER2+	5	109	61.4	0.035	Random-effects	28.53	13.68–45.75
During NAT	HR ($P = 0.458$)							
	HR-	4	132	84.7	<0.010	Random-effects	33.85	13.06–58.30
	HR+	2	108	52.9	0.145	Random-effects	24.07	12.37–38.03
	HER2 ($P = 0.271$)							
	HER2-	3	98	89.2	<0.010	Random-effects	37.79	9.73–70.97
	HER2+	1	65	–	–	–	20.00	11.10–31.77

Abbreviations: -, not applicable; CI, confidence interval; ctDNA, circulating tumor DNA; HER2+, human epidermal growth factor receptor 2 positive; HER2-, human epidermal growth factor receptor 2 negative; HR+, hormone receptor positive; HR-, hormone receptor negative; NAT, neoadjuvant therapy; n, number.

Table 4
ctDNA and RFS in subgroups based on combined data from all measurement time points.

Molecular subgroup	Included studies, n	Samples, n	I^2, %	$P_{heterogeneity}$ value	Model	HR	95% CI	*P* value
HR+	1	29	–	–	–	1.75	0.23–13.35	0.589
HR-	7	321	44	0.097	Fixed-effect	4.03	2.63–6.18	<0.001
HER2+	2	120	84	0.014	Random-effects	4.62	0.45–47.83	0.199
HER2-	6	306	53	0.059	Random-effects	4.69	2.34–9.42	<0.001

Abbreviations: -, not applicable; CI, confidence interval; ctDNA, circulating tumor DNA; HER2+, human epidermal growth factor receptor 2 positive; HER2-, human epidermal growth factor receptor 2 negative; HR-, hormone receptor negative; n, number; RFS, recurrence-free survival.

Table 5
ctDNA and RFS in subgroups at different measurement time points.

Measurement time point	Molecular subgroup	Included studies, n	Samples, n	I^2, %	*P* heterogeneity value	Model	HR	95% CI	*P* value
Baseline	HR ($P = 0.412$)								
	HR+	1	29	–	–	–	1.75	0.23–13.39	0.589
	HR-	1	15	–	–	–	5.11	1.08–24.18	0.040
	HER2								
	HER2+	1	69	–	–	–	0.91	0.24–3.50	0.890
During NAT	HR								
	HR-	1	21	–	–	–	3.12	0.89–10.92	0.074
	HER2 ($P = 0.393$)								
	HER2-	1	21	–	–	–	3.12	0.91–11.11	0.074
	HER2+	1	65	–	–	–	1.40	0.37–5.50	0.625
Before surgery	HR								
	HR-	2	46	0	0.772	Fixed-effect	3.03	1.24–7.40	0.015
	HER2								
	HER2-	2	46	0	0.772	Fixed -effect	3.03	1.24–7.40	0.015
After surgery	HR								
	HR-	4	282	70	0.018	Random-effects	6.27	2.12–18.54	<0.001
	HER2 ($P = 0.314$)								
	HER2-	4	282	70	0.018	Random-effects	6.27	2.12–18.54	<0.001
	HER2+	1	55	–	–	–	15.20	4.00–58.10	<0.001

Abbreviations: -, not applicable; CI, confidence interval; ctDNA, circulating tumor DNA; HER2+, human epidermal growth factor receptor 2 positive; HER2-, human epidermal growth factor receptor 2 negative; HR+, hormone receptor positive; HR-, hormone receptor negative; n, number; NAT, neoadjuvant therapy.

4. Discussion

Breast cancer can be divided into different molecular subtypes according to the different expression levels of HR, HER2, and Ki-67. In common clinical classifications, the molecular subtypes of HER2+ (positive or negative HR) and HR+ (positive HR and negative HER2) have a relatively good prognosis, while the triple-negative subtype (HR-/HER2-) had a relatively poor prognosis.[39] In order to utilize the maximum data information, we analyzed the ctDNA detection rates according to HR+/HR- and HER2+/HER2- subtypes respectively.

In this study, according to the HR type, the ctDNA detection rates in the HR+ group were similar to those in the HR- group. However, in the

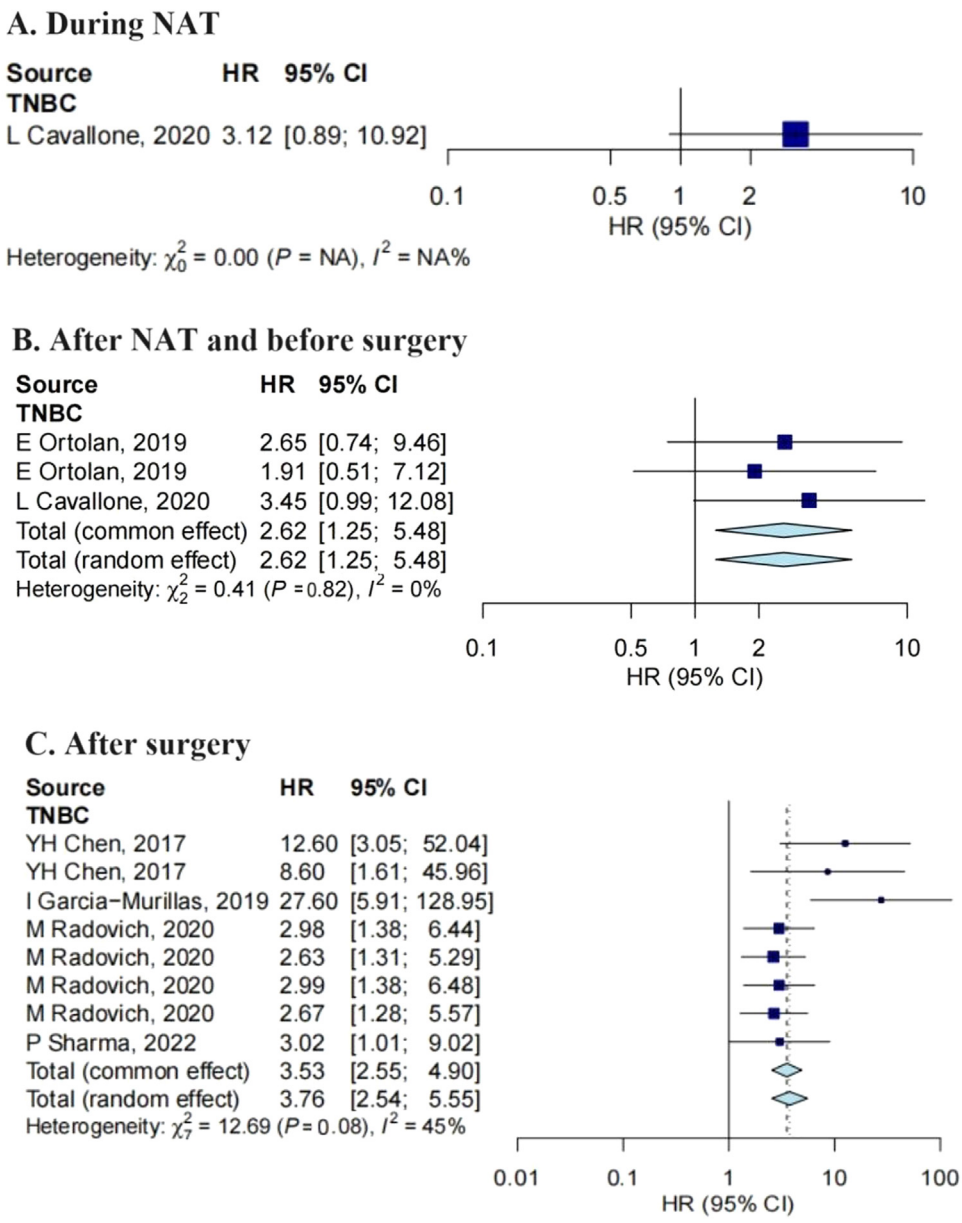

Fig. 2. Association between circulating tumor DNA (ctDNA) and recurrence-free survival (RFS) in triple-negative breast cancer (TNBC) patients. CI, confidence interval; HR, hazard ratio; NAT, neoadjuvant therapy.

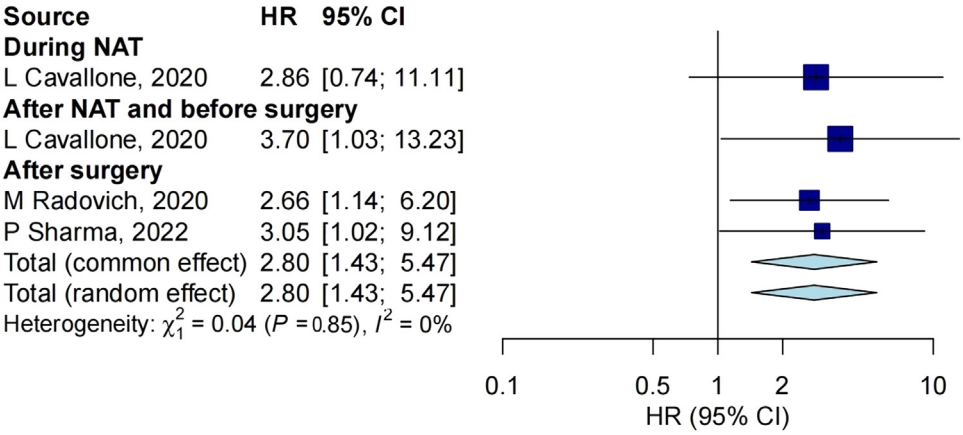

Fig. 3. Association between circulating tumor DNA (ctDNA) and overall survival (OS) in triple-negative breast cancer (TNBC) patients. CI, confidence interval; HR, hazard ratio; NAT, neoadjuvant therapy.

Table 6
ctDNA and OS in subgroup based on combining data from all measurement time points.

Molecular subgroup	Included studies, n	Samples, n	I^2, %	$P_{heterogeneity}$ value	Model	HR	95% CI	*P* value
HR+	2	109	0	0.982	Fixed-effect	1.71	0.88–3.32	0.110
HR-	4	227	0	0.876	Fixed-effect	3.21	1.84–5.59	<0.001
HER2-	3	212	0	0.913	Fixed-effect	2.97	1.64–5.38	<0.001

Abbreviations: CI, confidence interval; ctDNA, circulating tumor DNA; HR+, hormone receptor positive; HR-, hormone receptor negative; HER2-, human epidermal growth factor receptor 2 negative; OS, overall survival.

Table 7
ctDNA and OS in subgroup at different measurement time points.

Measurement time point	Molecular subgroup	Included studies, n	Samples, n	I^2, %	*P* value	Model	HR	95% CI	*P* value
Baseline	HR ($P = 0.292$)								
	HR+	2	109	0	0.840	Fixed-effect	2.20	1.16–4.18	0.016
	HR-	1	15	–	–	–	5.46	1.14–26.10	0.034
During NAT	HR								
	HR-	1	21	–	–	–	2.86	0.74–11.11	0.130
	HER2								
	HER2-	1	21	–	–	–	2.86	0.74–11.11	0.130
After NAT before surgery	HR								
	HR+	1	80	–	–	–	1.72	0.87–3.39	0.119
	HR-	1	23	–	–	–	3.70	1.03–13.23	0.044
	HER2								
	HER2-	1	23	–	–	–	3.70	1.03–13.23	0.044
After surgery	HR								
	HR-	2	189	0	0.846	Fixed-effect	2.80	1.43–5.47	0.003
	HER2								
	HER2-	2	189	0	0.846	Fixed-effect	2.80	1.43–5.47	0.003

Abbreviations: -, not applicable; CI, confidence interval; ctDNA, circulating tumor DNA; HER2+, human epidermal growth factor receptor 2 positive; HER2-, human epidermal growth factor receptor 2 negative; HR+, hormone receptor positive; HR-, hormone receptor negative; n, number; NAT, neoadjuvant therapy; OS, overall survival.

HR+ group, the RFS of the ctDNA positive cases was similar to that of the ctDNA negative cases. In contrast, the RFS of the ctDNA positive cases was significantly lower than that of the ctDNA negative cases. ctDNA monitoring has been shown to indicate disease recurrence months before imaging results.[40] This suggests that ctDNA testing is more instructive for later treatment adjustment in the HR- group than in the HR+ group. For the HR- group, further analysis of the results at different time periods showed that ctDNA detection results after NAT (before surgery and after surgery) were more predictive of disease progression than those at baseline and during NAT.

For ctDNA and OS in the HR tested cases, the OS of the ctDNA positive cases was significantly shorter than that of the ctDNA negative cases. Similarly, ctDNA test results after NAT (before surgery and after surgery) are more indicative of the OS. In this study, the number of HER2 tested cases was less than that of HR tested cases, but the association between ctDNA testing and disease prognosis was similar in HER2 tested and HR tested groups.

Due to the limited amount of data, the analysis of the correlation between ctDNA and prognosis of different subtypes was not classified according to HER2+, HER2-/HR+, or TNBC (HR-/HER2-), which are of clinical concern. However, we separately analyzed the association between ctDNA and the prognosis of TNBC subtypes, which is of greater clinical interest. TNBC is a typically heterogeneous disease characterized by high aggressiveness, multiple metastases, and a lack of drug targets.[41] Our data suggest that ctDNA is more predictive of the progression of HR- or HER2-subtypes than HR+ or HER2+, and that the prognosis of TNBC subtypes is closely related to ctDNA before and after surgery, so ctDNA has potential application value in the treatment of TNBC. However, a large number of prospective studies are still needed to confirm its clinical effectiveness and practicality.

The detection of ctDNA reflects the tumor burden of the body and is closely related to the prognosis.[42] It can be seen that the tumor burden of different molecular types (HR+ *vs.* HR-; HER2+ *vs.* HER2-) of breast cancer is similar at the same stage of tumor development. So why does the ctDNA test indicate disease progression for HR- subtype and HER2- subtype, while it does not indicate disease progression for the HR+ and HER2+ subtypes? The different expression levels of HR and HER2 in different subtypes of breast cancer may be caused by different mutation points of tumor cells. ctDNA is mainly derived from the apoptosis of tumor cells and carries genetic characteristics associated with tumor cells, reflecting the genomic changes and heterogeneity of tumors.[43–45] Therefore, while doing the detection and quantitative analysis of ctDNA, we may pay attention to the genomic mutation information carried by ctDNA to more accurately illustrate the prognosis of different subtypes, so as to provide data support for tumor type diagnosis and the formulation of targeted treatment plans.

This study is a meta-analysis of data from published articles, and its conclusions should be further verified in clinical studies. The number of articles involving different molecular subtypes at different time points is small, which may have an impact on the stability of the meta-analysis results.

5. Conclusions

Compared to the HR+ and HER2+ subgroups, ctDNA was more predictive of RFS and OS in the HR- and HER2- subgroups. The prognosis of the TNBC subtype is closely related to ctDNA detection before and after surgery.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

N.G. conducted the data curation and wrote the original draft. Q.Z. performed the formal analysis and investigation. Q.Z., M.Z., X.C., B.Z., S.W., H.Z., M.W., F.M. and F.S. reviewed and edited the manuscript, S.W. and H.Z supervised the writing and validated the data, F.S. was responsible for the conceptualization, funding acquisition, and project administration.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jncc.2024.04.005.

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