

# Predictive value of circulating cell-free DNA in the survival of breast cancer patients

## A systemic review and meta-analysis

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### Abstract

**Purpose:** Circulating cell-free DNA (cfDNA) has been reported to predict outcomes in patients with various types of cancer. However, its prognostic value in patients with breast cancer is not well established still now. In this meta-analysis, we evaluated the prognostic role of cfDNA in breast cancer patients.

**Methods:** We performed systematic searches in electronic databases to identify studies that evaluated the prognostic value of cfDNA in breast cancer patients. The end points were progression-free survival (PFS) and overall survival (OS). The hazard ratios (HRs) and their 95% confidence intervals (95% CIs) were extracted to assess the prognostic significance of cfDNA. Subgroup analyses were also conducted.

**Results:** A total of 11 publications involving 1467 patients were included in this meta-analysis. cfDNA was shown to be significantly associated with PFS (HR 2.02, 95% CI 1.51–2.72,  $P < .001$ ,  $I^2 = 82\%$ ) and OS (HR 1.75, 95% CI 1.01–3.05,  $P < .001$ ,  $I^2 = 92\%$ ). The results of subgroup analyses also revealed that cfDNA was a good predictor of prognosis in breast cancer patients.

**Conclusion:** Our meta-analysis indicated that cfDNA was associated with poor PFS and OS, thus it may help to predict outcomes of patients with breast cancer. However, further studies are needed to confirm our results.

**Abbreviations:** CA = carbohydrate antigen, cfDNA = cell-free DNA, CIs = confidence intervals, HR = hazard ratio, HRs = hazard ratios, LOH = loss of heterozygosity, NOS = Newcastle–Ottawa Quality Assessment Scale, OS = overall survival, PFS = progression-free survival, TNM stage = tumor-node-metastasis stage.

**Keywords:** breast cancer, cell-free DNA, meta-analysis, plasma DNA, prognosis

## 1. Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer-related death among women in the worldwide.<sup>[1]</sup> Although radical surgery, radiotherapy, and drug therapy have significantly reduced the risk of relapse and improved overall survival of breast cancer patients, a certain percentage of patients still develop early tumor recurrent or progression.<sup>[2,3]</sup> Thus, estimating relapse and monitoring

metastases could contribute to a better outcome and improve quality of life for breast cancer patients.<sup>[4]</sup> In clinical practice, serum markers such as carbohydrate antigen (CA) 15-3, BR 27.29, mucin-like cancer-associated antigen, CA549, and carcinoembryonic antigen are the most common prognostic factors for monitoring patients and predicting their risk of relapse.<sup>[5]</sup> However, these serum biomarkers have limited usefulness due to their low sensitivity and specificity.<sup>[6]</sup> Therefore, new prognostic biomarkers are urgently needed to monitor the progression of breast cancer.

Circulating cell-free DNA (cfDNA), a type of cell-free nucleic acids, is defined as extracellular DNA in the blood.<sup>[7]</sup> The occurrence of circulating cfDNA is thought to be related to the apoptosis and necrosis of cells.<sup>[8]</sup> High concentrations and genetic alterations of cfDNA are revealed to be more likely present in cancer patients as compared to healthy controls.<sup>[9,10]</sup> Furthermore, many studies have reported the prognostic value of cfDNA in various kinds of solid neoplasm, including lung cancer, pancreatic cancer, prostate cancer, hepatocellular cancer, renal cell cancer, colorectal cancer, and breast cancer.<sup>[11–17]</sup> In addition, several studies have indicated that the levels of circulating cfDNA, methylation of cfDNA, loss of heterozygosity (LOH) of cfDNA, and cfDNA integrity are associated with disease outcome of breast cancer patients. However, such studies are conflicting in their results, and have not been systematically reviewed. The aim of this meta-analysis was to clarify the predictive and prognostic role of cfDNA in patients with breast cancer.

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JY, LC, and XM contributed equally to this work.

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## 2. Methods

### 2.1. Search strategy and study selection

As this literature is a meta-analysis, ethical approval is not necessary. We searched relevant articles in PubMed, Embase, Cochrane, and the Ovid Medline database up to July 2017 without language or date restrictions. The search strategy included (“cfDNA” or “cell-free DNA” or “cell free DNA” or “plasma DNA” or “serum DNA” or “circulating DNA”) and (“breast cancer” or “breast neoplasm” or “breast tumor”) and (“prognosis” or “prognostic” or “predictive” or “prediction” or “outcome”). Moreover, related articles and reference lists of these articles were also reviewed to identify all available studies.

### 2.2. Inclusion and exclusion criteria

Two investigators independently evaluated the eligibility of studies (Jing Yang and Jing Zhang). A study would be included if it met the following inclusion criteria: All patients enrolled in the study were diagnosed with breast cancer; Studies investigated the prognostic value of cfDNA in breast cancer patients; Endpoints included progression-free survival (PFS) or overall survival (OS), and sufficient data were presented for extracting hazard ratio (HR) value; The latest or the most informative one was to include whether studies assessed the same patient population; and English language articles. The following exclusion criteria were applied: nonresearch publications such as case reports, editorials, reviews, letters, comments; and duplicate articles.

### 2.3. Data extraction

Two investigators independently reviewed all eligible studies and extracted the data (Jing Yang and Linyan Chen). Information retrieved from these studies included author, year of publication, country, population source, number of enrolled patients, characteristics of patients (age, sex, stage), method of cfDNA assessment, origin of cfDNA (serum or plasma), follow-up, and survival data. We recorded all the data by using standard electronic tables.

### 2.4. Statistical analysis

HRs and its 95% confidence intervals (95% CIs) were adopted to clarify the prognostic value of cfDNA. HRs >1 indicated an elevated risk of disease progression or death. For studies in which HRs and 95% CIs were not available, we extracted survival rates from Kaplan–Meier curves by using Engauge Digitizer version 4.1.<sup>[18]</sup> To make sure the accuracy, 2 investigators (Jing Yang and Li Cheng) independently performed this process. In addition, data of multivariate analysis were preferable if both HRs of univariate and multivariate were retrievable. Log HR and its standard error for each study were retrieved following the method described by other authors.<sup>[18]</sup>

To assess the heterogeneity among studies, pooled HRs were initially calculated by using a fixed effects model. If there was significant heterogeneity among studies ( $P \leq .1$ ,  $I^2 > 50\%$ ), random effects model was adopted.<sup>[19]</sup> We used Cochrane’s  $Q$  statistics and  $I^2$  statistics to assess the homogeneity of the studies;  $I^2 > 50\%$  and  $P < .05$  were considered significant for heterogeneity.

Subgroups were stratified by tumor-node-metastasis (TNM) stage (I–III, IV, I–IV), assay indicators (quantification or mutation), origin of cfDNA, sampling time (before treatment,

after treatment, before and after treatment), and method of cfDNA assessment. We did not carry out the subgroup analysis if the number of study was not more than 1. We performed subgroup analysis to further investigate the potential factors associated with survival of breast cancer patients. Statistical analysis was conducted with Review Manager Version 5.2 (The Cochrane Collaboration). All  $P$  values are 2-tailed. The methods adopted in this study have been used in the previous study.<sup>[20]</sup>

### 2.5. Quality assessment

The quality of the included studies was assessed by using the Newcastle–Ottawa Quality Assessment Scale (NOS).<sup>[21]</sup> The NOS consists of 8 items, which were categorized into 3 main parts, including selection, comparability, and outcome. A star system is used to assess study quality. Studies with higher stars indicate higher quality. NOS 5 to 9 stars were considered to be high quality in this meta-analysis.<sup>[20]</sup>

### 2.6. Publication bias

Publication bias was evaluated by inspecting the symmetry of the funnel plot and tested with Begg and Egger adjusted rank correlation test.<sup>[22]</sup> Publication bias assessment was conducted with STATA version 12.1 (Stata Corporation, College Station, TX).

## 3. Results

### 3.1. Literature search results

We retrieved 7124 articles, of which 1744 were duplicates and 346 were non-English publication. By reviewing title and abstract, we found 2 review articles, 1 comments article, 1 editorials article, and 4989 articles out of the scope of our meta-analysis. Thirty-nine studies were recognized as potentially relevant publication, all of which were included in detailed assessment. After obtaining and reading the full articles, we excluded 18 articles because they did not estimate PFS or OS and without follow data; moreover, 9 articles were excluded because these studies did not provide sufficient data for extracting HRs value; 1<sup>[23]</sup> of 2 studies from Takeshita et al<sup>[23,24]</sup> was excluded for overlapping of study population. Therefore, a total of 11<sup>[24–34]</sup> studies met our inclusion criteria and were finally included in this meta-analysis. Figure 1 shows the study selection process of our meta-analysis.

### 3.2. Study characteristics and quality

The basic characteristics of studies are summarized in Table 1. Eleven studies published between 2009 and 2017 were eligible for our meta-analysis. Study sample sizes ranged from 14 to 541 (median 100), with a total of 1467 patients. Among the 11 studies, 3 studies were from Germany (379 patients, 25.9%),<sup>[29,32,33]</sup> 3 were from America (273 patients, 18.6%),<sup>[28,30,34]</sup> 2 were from Japan (133 patients, 9.0%),<sup>[24,31]</sup> 1 was from India (86 patients, 5.9%),<sup>[27]</sup> and 1 was from Britain (55 patients, 3.8%)<sup>[26]</sup>; in addition, the samples of 1 study were obtained from participants of a phase3 clinical trial in which the patients were from 24 countries (541 patients, 36.8%).<sup>[25]</sup> Three studies (675 patients, 46.0%) collected samples before treatment,<sup>[25,30,32]</sup> 3 studies (291 patients, 19.8%) collected samples after treatment,<sup>[29,31,33]</sup> and 5 studies (501 patients, 34.2%) collected their samples before or after treatment.<sup>[24,26–28,34]</sup> HRs

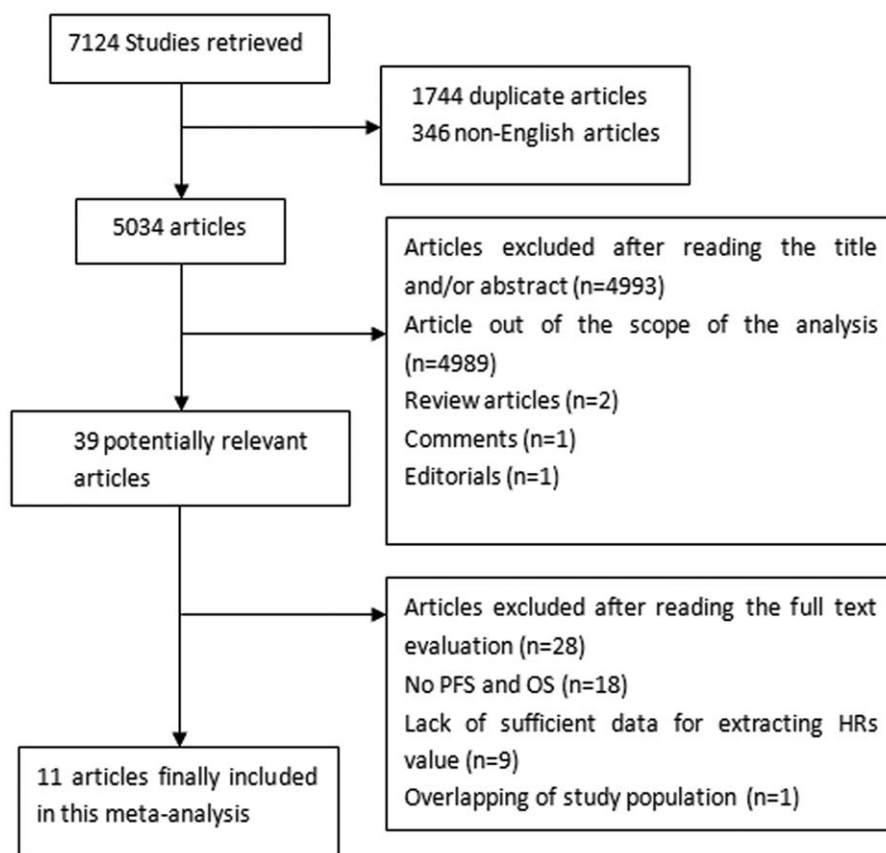


Figure 1. Literature search strategy and selection of study.

Table 1

Characteristics of the included studies.

Ref.	Country	No.	TNM stage	Method	Assay indicators	Sample origin	Sampling time	Endpoints	Research quality
Madhavan et al <sup>[29]</sup>	Germany	201	IV	qPCR	ALU LINE1 concentration and DNA Integrity	Plasma	After treatment	PFS/OS	High
Maxwell et al <sup>[30]</sup>	America	32	I-IV	Digital sequencing	TP53 mutation, panel-specific mutational load, PIK3CA mutation, and mutational heterogeneity	Plasma	Before treatment	PFS	High
Nakauchi et al <sup>[31]</sup>	Japan	14	IV	NGS	TP53 mutation, PIK3CA mutation	Plasma	After treatment	OS	High
Schwarzenbach et al <sup>[32]</sup>	Germany	102	I-IV	PCR-based microsatellite analysis	LOH	Serum	Before treatment	PFS/OS	High
Schwarzenbach et al <sup>[33]</sup>	Germany	76	I-III	Fluorescence-labeled PCR	LOH	Serum	After treatment	PFS	High
Takeshita et al <sup>[24]</sup>	Japan	119	I-IV	ddPCR	ESR1	Plasma	Before and after treatment	PFS/OS	High
Visvanathan et al <sup>[34]</sup>	America	141	IV	cMethDNA assay	Methylation	Serum	Before and after treatment	PFS/OS	High
Liang et al <sup>[28]</sup>	America	100	I-IV	Digital Sequencing	TP53 mutation, PIK3CA mutation	Plasma	Before and after treatment	PFS	High
Garcia-Murillas et al <sup>[26]</sup>	Britain	55	I-III	ddPCR	ctDNA	Plasma	Before and after treatment	PFS	High
Chandrarlapaty et al <sup>[25]</sup>	Randomized patients from 189 centers in 24 countries	541	IV	ddPCR	ESR1 mutation	Plasma	Before treatment	PFS/OS	High
Iqbal et al <sup>[27]</sup>	India	86	I-III	qPCR	ALU concentration and integrity	Serum	Before and after treatment	PFS/OS	High

ddPCR = droplet digital polymerase chain reaction, LOH = loss of heterozygosity, NGS = next generation sequencing, OS = overall survival, PCR = quantitative polymerase chain reaction, PFS = progression-free survival, qPCR = quantitative polymerase chain reaction, TNM stage = tumor-node-metastasis stage.



were extracted directly in 5 studies<sup>[24,25,27,30,32]</sup> and could be calculated in 6 studies.<sup>[26,28,29,31,33,34]</sup> The quality score ranged from 5 to 7. Six studies were evaluated with score 5,<sup>[27-30,32,34]</sup> 4 studies were evaluated with score 6,<sup>[24,25,31,33]</sup> and 1 study was evaluated with score 7.<sup>[26]</sup> All the studies were regarded as high quality. The characteristics and quality of studies enrolled in this meta-analysis are summarized in Table 1. S1 Table, <http://links.lww.com/MD/C331> summarizes the NOS score of the enrolled studies.

**3.3. Prognostic value of cfDNA and heterogeneity**

The results of meta-analysis revealed a significant association between cfDNA and PFS in patients with breast cancer, with a pooled HR of 2.02 (95% CI 1.51–2.72, n=20). For OS, we found a pooled HR of 1.75 (95% CI 1.01–3.05, n=12), which indicated that significant association could also be observed for OS. However, high heterogeneities were presented in the statistical tests among study group of PFS ( $I^2=82\%$ ,  $P<.001$ ) and OS ( $I^2=92\%$ ,  $P<.001$ ). Therefore, we adopted random effects model (Fig. 2).

**3.4. Subgroup analysis**

Some factors are correlated with patient’s prognosis and may bring heterogeneity to the overall analysis. As high heterogeneities were observed in the analysis of all studies, we performed a subgroup analysis stratified according to the TNM stage (I-III, IV, I-IV), region, method of cfDNA assessment, origin of cfDNA, cfDNA analysis (quantification or mutation), and number of patients. In the subgroup analysis stratified by TNM staging, the pooled HRs of stage I-III patients for PFS and OS were 7.62 (95% CI, 2.53-22.97;  $P=.002$ ;  $I^2=74\%$ ) and 1.86 (95% CI, 0.87–3.98;  $P=.19$ ;  $I^2=41\%$ ), respectively; the pooled HRs of stage IV patients for PFS and OS were 1.22 (95% CI, 0.84–1.76;  $P<.001$ ;  $I^2=88\%$ ) and 1.59 (95% CI, 0.80–3.18;  $P<.001$ ;  $I^2=94\%$ ), respectively; the pooled HRs of stage I-IV patients for PFS and OS were 2.14 (95% CI, 1.67–2.73;  $P=.38$ ;  $I^2=6\%$ ) and 2.10 (95% CI, 1.03–4.29;  $P=.26$ ;  $I^2=20\%$ ), respectively. The presence of ESR1 mutation and high levels of ALU concentration were associated with a worse OS (HR 1.35, 95% CI 1.07–1.69,  $P=.60$ ,  $I^2=0$ ; HR 3.97, 95% CI 2.69–5.87,  $P=.87$ ,  $I^2=0$ , respectively). In addition, our results indicated that PFS was poorer for patients with TP-53 mutation, and

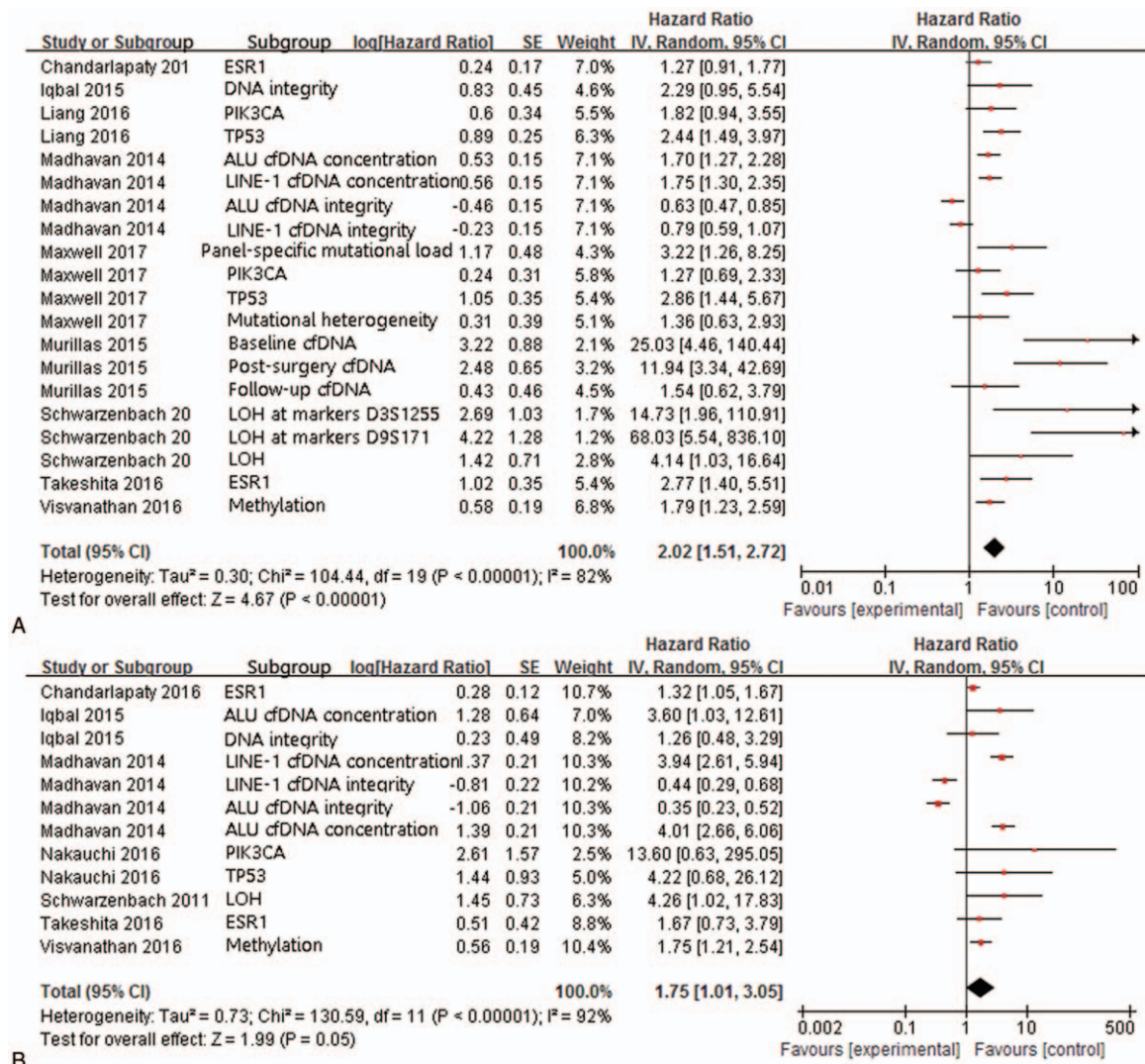


Figure 2. Forest plot of pooled hazard ratio (HR) for the impact of cfDNA on PFS (A) and OS (B) in breast cancer patients.

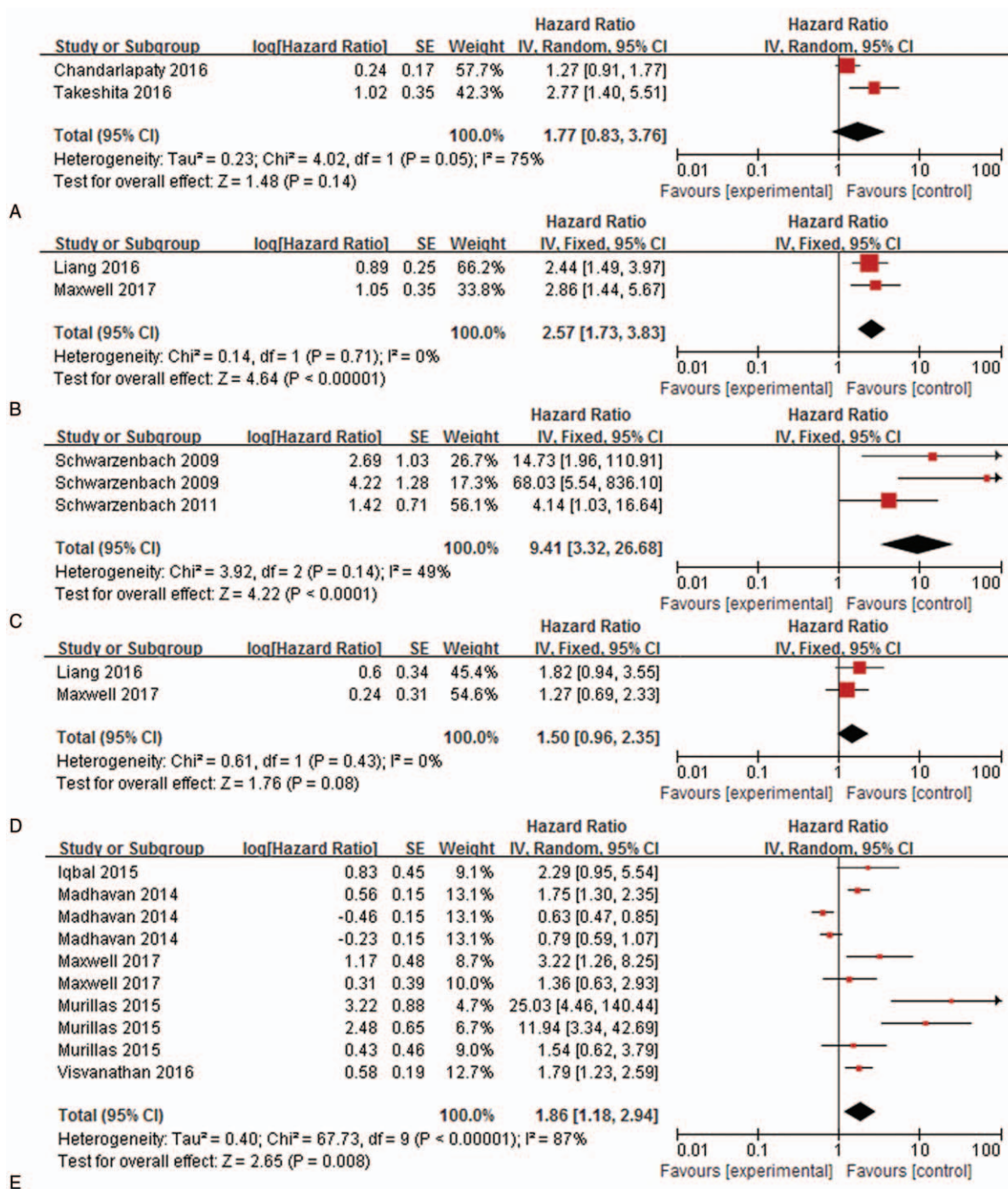


Figure 3. Subgroup analysis of PFS studies stratified according to the assay indicators. (A) ESR1, (B) TP-53, (C) LOH, (D) PIK3CA, (E) Other assay indicators.

LOH (HR 2.57, 95% CI 1.73–3.83,  $P = .71$ ,  $I^2 = 0$ ; HR 9.41, 95% CI 3.32–26.68,  $P = .14$ ,  $I^2 = 49\%$ , respectively) than those without TP-53 mutation and LOH. The pooled HRs of patients with other mutations or high level of cfDNA for PFS and OS were 1.86 (95% CI, 1.18–2.94;  $P < .001$ ;  $I^2 = 81\%$ ) and 1.06 (95% CI, 0.40–2.79;  $P < .001$ ;  $I^2 = 96\%$ ), respectively. With respect to the sample, the majority of studies tested cfDNA in the plasma rather than in the serum of patients. The combined HRs of both groups were  $>1$  (Fig. 3, S1–S9 Fig, <http://links.lww.com/MD/C331>). Table 2 summarizes the results of subgroup analyses.

### 3.5. Publication bias

Begg test and Egger test were performed to assess the publication bias in our meta-analysis. The results revealed no evidence of significant publication bias in eligible studies of OS (Begg test,  $P = .447$ ) and PFS (Egger test,  $P = .106$ ). The  $P$  values for publication bias of each subgroup are summarized in Table 2.

### 4. Discussion

As a noninvasive approach, detection of cfDNA has been reported to be a potential method to predict survival in patients



**Table 2**

**Subgroup analysis of included studies.**

Variables	PFS					OS				
	HR (95%CI)	n	P	I <sup>2</sup>	Publication bias P	HR (95%CI)	n	P	I <sup>2</sup>	Publication bias P
Overall	2.02 (1.51–2.72)	20	<.001	82%	.005	1.75 (1.01–3.05)	12	<.001	92%	.447
TNM stage										
I-III	7.62 (2.53–22.97)	6	.002	74%	.091	1.86 (0.87–3.98)	2	.19	41%	.317
IV	1.22 (0.84–1.76)	6	<.001	88%	.822	1.59 (0.80–3.18)	8	<.001	94%	.702
I-IV	2.14 (1.67–2.73)	8	.38	6%	.105	2.10 (1.03–4.29)	2	.26	20%	.317
Assay indicators										
ESR1	1.77 (0.83–3.76)	2	.05	75%	.317	1.35 (1.07–1.69)	2	.60	0	.317
ALU concentration	1.70 (1.27–2.28)	1	–	–	–	3.97 (2.69–5.87)	2	.87	0	.317
TP-53	2.57 (1.73–3.83)	2	.71	0	.317	4.22 (0.68–26.12)	1	–	–	–
LOH	9.41 (3.32–26.68)	3	.14	49%	.117	4.26 (1.02–17.83)	1	–	–	–
PIK3CA	1.50 (0.96–2.35)	2	.43	0	.317	13.60 (0.63–295.05)	1	–	–	–
Others	1.86 (1.18–2.94)	10	<.001	87%	.069	1.06 (0.40–2.79)	5	<.001	96%	.801
Sample										
Serum	4.11 (1.67–10.13)	5	.01	68%	.014	1.85 (1.34–2.57)	4	.38	3%	.174
Plasma	1.80 (1.31–2.48)	15	<.001	83%	.028	1.60 (0.76–3.39)	8	<.001	94%	.64
Sampling time										
Before treatment	1.56 (1.23–1.97)	7	.16	35%	.099	1.90 (0.66–5.50)	2	.11	60%	.317
After treatment	2.50 (1.36–4.57)	8	<.001	91%	.288	1.79 (0.56–5.71)	6	<.001	96%	.655
Before and after treatment	2.08 (1.63–2.65)	5	.75	0	.327	1.76 (1.29–2.39)	4	.63	0	1.000
Method										
ddPCR	3.44 (1.43–8.24)	5	<.001	83%	.086	1.35 (1.07–1.69)	2	.60	0	.317
qPCR	1.22 (0.75–1.96)	5	<.001	90%	1.000	1.46 (0.51–4.14)	6	<.001	96%	1.000
Others	2.28 (1.61–3.22)	10	.03	51%	.012	1.96 (1.38–2.79)	4	.30	19%	.174

ddPCR=droplet digital polymerase chain reaction, LOH=loss of heterozygosity, qPCR=quantitative polymerase chain reaction, TNM stage=tumor-node-metastasis stage.

with breast cancer.<sup>[35]</sup> Furthermore, the development of more effective methods such as quantitative polymerase chain reaction (qPCR) and droplet digital polymerase chain reaction (ddPCR) has allowed both screening and validation of genomic alteration in cfDNA, thus ensuring the availability of cfDNA detection.<sup>[36]</sup> To the best of our knowledge, this is the first meta-analysis to systematically estimate the association between cfDNA and prognosis of breast cancer patients. This meta-analysis enrolled 11 publications including 1336 patients with breast cancer. The results revealed that concentration, mutation of cfDNA, and cfDNA integrity were significantly associated with outcome of breast cancer patients. Compared with patients with mutation or high levels of cfDNA, patients without mutation or with low levels of cfDNA tend to have a favorable PFS.

Previous studies have reported that cfDNA could be observed in plasma of healthy individuals but was increased in patients with cancer.<sup>[37]</sup> The main source of circulating cfDNA is apoptosis of cells in healthy controls, whereas both apoptosis and necrosis contribute to elevated levels of cfDNA in cancer patients.<sup>[38]</sup> The result of our subgroup analysis stratified by assay indicators suggested that elevated ALU and presence of LOH were significantly correlated with patients' poor PFS and OS; TP-53 mutation was shown to be significantly associated with worse PFS; ESR1 mutation was suggested to be significantly associated with poor OS. In addition, our results showed a tendency of poor outcome among breast cancer patients with PIK3CA and some other mutations, although without statistical significance. As a predictive HR value of more than 2.0 was considered to be statistically strong,<sup>[39]</sup> ALU concentration, TP-53 mutation, and presence of LOH were proved to be good predictors for survival. Moreover, LOH was shown to be the most effective factor that was associated with shorter PFS and OS. It might be because loss of tumor suppress gene

and cyclin-dependent kinase inhibitor could promote cell proliferation.<sup>[33]</sup>

Subgroup analysis based on patients' TNM staging demonstrated that cfDNA was a good prognostic maker for PFS in stage I-III and stage I-IV breast cancer patients, while the prognostic value of cfDNA for PFS among stage IV patients remained to be validated. However, cfDNA was shown to be a reliable prognostic marker in patients with stage I-IV. As to the subgroups stratified by sampling time, the results indicated that cfDNA could be a good predictor for PFS among patients before treatment and after treatment, but its prognostic value for OS among this patient population should be further interpreted. Furthermore, in the population mixed with patients before and after treatment, cfDNA was proved to be a good predictor. Regarding origin of sample, both serum and plasma were having good origin in the detection of cfDNA.

As subgroup analysis classified by analytical methods, our results revealed that ddPCR and some other methods were more effective than qPCR in detection of cfDNA. The HR value of ddPCR group in prediction of PFS was 3.44. As reported in previous studies, a prognostic parameter with HR>2 is considered to be useful, which indicated that ddPCR was a good method to detect cfDNA.<sup>[39]</sup> This might be the reason that ddPCR technology has an excellent precision in quantification of sample.<sup>[40]</sup> In this way, ddPCR should consider to be adopted in the detection of cfDNA.

There were several limitations in our meta-analysis. First, the number of included studies is relatively small, which is partially due to the fact that articles in other languages were not included in our meta-analysis or that we excluded several studies for that HRs cannot be extracted. Second, heterogeneity was observed in this meta-analysis, so we performed subgroup analyses. Third, only published studies were included in this meta-analysis.

Despite these limitations, our meta-analysis is very valuable and crucial. First of all, we searched the relevant studies by using searching words; meanwhile, related articles and reference lists of these articles were also reviewed to identify all available studies. Second, 2 investigators independently examined the eligibility of studies and performed the quality assessment; all the eligible studies were demonstrated as having high quality. Third, patients of different ethnicities were included in our meta-analysis, which increased the generalizability of our results. Last but not least, we performed subgroup analyses to explore whether our results were influenced by other confounding factors, which demonstrated that our findings were reliable.

## 5. Conclusion

Our meta-analysis revealed that cfDNA could serve as a good prognostic factor for patients with breast cancer, which may help clinicians identify patients at a high risk of relapse or progression. Elevated circulating cfDNA, TP-53 mutation, and ESR1 mutation were significantly associated with worse survival of patients. However, the results of our meta-analysis need to be validated by further research in this field.

## Author contributions

**Conceptualization:** Jing Yang, Li Cheng, Jing Zhang, Xuelei Ma.

**Data curation:** Jing Yang, Jing Zhang, Linyan Chen.

**Formal analysis:** Jing Yang.

**Methodology:** Jing Yang, Li Cheng, Jing Zhang, Denian Wang, xinli Guo, Xuelei Ma.

**Software:** Jing Yang, Li Cheng, Linyan Chen, Denian Wang, xinli Guo.

**Supervision:** Xuelei Ma.

**Validation:** Jing Yang, Jing Zhang.

**Writing – original draft:** Jing Yang, Li Cheng, Denian Wang.

**Writing – review & editing:** Jing Yang, Li Cheng, Jing Zhang, Linyan Chen, Denian Wang, xinli Guo, Xuelei Ma.

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