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SYSTEMATIC REVIEW

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Diagnostic accuracy of ESR1 mutation detection by cell-free DNA in breast cancer: a systematic review and meta-analysis of diagnostic test accuracy

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Abstract

Background Estrogen receptors express in nearly 70% of breast cancers (ER-positive). Estrogen receptor alpha plays a fundamental role as a significant factor in breast cancer progression for the early selection of therapeutic approaches. Accordingly, there has been a surge of attention to non-invasive techniques, including circulating Cell-free DNA (ccfDNA) or Cell-Free DNA (cfDNA), to detect and track ESR1 genotype. Therefore, this study aimed to examine the diagnosis accuracy of ESR1 mutation detection by cell-free DNA in breast cancer patientsthrough a systematic review and comprehensive meta-analysis.

Methods PubMed, Embase, and Web of Science databases were searched up to 6 April 2022. Diagnostic studies on ESR1 measurement by cfDNA, which was confirmed using the tumour tissue biopsy, have been included in the study. The sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR) and negative likelihood ratio (NLR) were considered to analyse the data.

Results Out of 649 papers, 13 papers with 15 cohorts, including 389 participants, entered the meta-analyses. The comprehensive meta-analysis indicated a high sensitivity (75.52, 95% CI 60.19–90.85), specificity (88.20, 95% CI 80.99–95.40), and high accuracy of 88.96 (95% CI 83.23–94.69) for plasma ESR1. We also found a moderate PPV of 56.94 (95% CI 41.70–72.18) but a high NPV of 88.53 (95% CI 82.61–94.44). We also found an NLR of 0.443 (95% CI 0.09–0.79) and PLR of 1.60 (95% CI 1.20–1.99).

Conclusion This systematic review and comprehensive meta-analysis reveal that plasma cfDNA testing exhibits high sensitivity and specificity in detecting ESR1 mutations in breast cancer patients. This suggests that the test could be a valuable diagnostic tool. It may serve as a dependable and non-invasive technique for identifying ESR1 mutations in breast cancer patients. However, more extensive research is needed to confirm its prognostic value.

Keywords ESR1, Breast cancer, Cell-free DNA, Diagnosis, Meta-analysis

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Introduction

Breast cancer (BC) is the most prevalent neoplasm in women globally and is the second leading cause of cancer-related mortality. It comprises different clinical, histopathological and molecular subgroups according to hormone receptor and human epidermal growth factor receptor-2 (HER-2/*neu*) status. Nearly 70% of breast cancers express estrogen receptor (ER-positive) [1–3]. Estrogen stimulates cell growth by binding to the ER's ligand-binding domain (LBD) [4]. Due to the importance of cell division in cancer development and estrogen's function in breast tumorigenesis and boosting mitotic activity in the mammary glands, ER significantly contributes to breast cancer progression [5, 6].

ER-positive patients have a lower risk of recurrence in the early stages of BC and are seven to eight times more likely to benefit from endocrine therapy than ER-negative patients [7, 8]. In metastatic breast cancer (MBC), ESR1 ligand-binding domain mutations lead to endocrine therapy resistance. Approximately 20% of ER-positive patients lose ER expression in metastases, worsening overall survival rates. Consequently, given that aromatase inhibitors and ovarian suppression therapies in premenopausal women act by depriving ligand, ER-positive breast cancer would be anticipated to be resistant to them. Accordingly, early diagnosis and determination of ESR1 are vital to preventing disease progression [9–11].

ESR1 genotyping relies on primary or metastatic lesion biopsy, which is the gold standard approach; however, due to some potential severe complications like pneumothorax and hemorrhagic shock, tissue biopsy is not an appropriate procedure for disease monitoring [12]. With such invasive procedures, processing tumour samples for quantity/quality tests can be complex. Monitoring tumour response and relapse during treatment using invasive approaches is also a significant challenge in tumour profiling [13]. Furthermore, if the tumour has spread and is constantly changing over time due to treatment, multiple biopsies may be required, as it is challenging to obtain a comprehensive picture of the tumour [13]. Moreover, the tissue biopsy provides limited spatial and temporal information, therefore failing to capture the complex heterogeneity of tumours [14].

Liquid biopsy is a new diagnostic approach to assess tumour biomarkers using body fluids, which can help to overcome many of the mentioned restrictions [9, 12, 15, 16]. Cell-free DNA (cfDNA) present a non-invasive way of cancer genotype profiling and monitoring the.

molecular changes during clinical follow-up [15, 17–19]. Genomic studies have demonstrated that cfDNA detected significantly more mutations and captured the majority of metastatic lesions compared to tumour biopsies [20, 21].

ESR1 mutations have been found in the cfDNA of MBC patients, and their potency as a biomarker for disease monitoring, prognosis prediction, and treatment decision-making has been elucidated by several studies [22–25]. Hence, through a non-invasive test and easily accessible material, cfDNA can fill that gap and deliver real-time personalized therapy [26].

We performed a comprehensive meta-analysis to systematise the most recent evidence on the diagnostic value of cfDNA for ESR1 genotyping in breast cancer patients to identify the accuracy of this approach for early diagnosis.

Methods

Search strategy

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines has been used to prepare the research design, search strategy, screening, and reporting. Three electronic databases of PubMed, Embase and Web of Science were searched using a comprehensive search strategy, including both MeSh indexed terms and free keywords up to 6 April 2022 as follows: ((Breast OR Mammary) AND (Cancer* OR Neoplasm* OR Tumour* OR Malignancy* OR Carcinoma*) AND (Cell-Free Nucleic Acid OR Circulating tumour DNA OR Estrogen receptor alpha) AND (Diagnos*)). We also utilised grey literature and selected papers' references to find other valuable data. We only considered papers published in English but did not apply date or location limitations.

Criteria study selection

Three researchers (M.R, K.H, M.T) independently performed the screening and assessed the papers. Studies that met the following criteria were included in the meta-analysis: (1) studies considered the accuracy of detecting ESR1 mutation by cfDNA in breast cancer patients: (2) studies with raw data could calculate the desired parameters mentioned in the data extraction section: (3) studies with ESR1 mutation analysis of tumour tissue samples. Papers were excluded if they were: (1) studies reported duplicate data; (2) review papers, case reports, comments, letters, in vitro studies and non-human subjects; (3) lacking adequate data for calculating desired parameters.

Data extraction

The data extraction checklist included the first author, publication year, country, study design, sample size, mean/median age, ESR1 mutation detection assay for cfDNA and tumour sample, tumour stage, true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN) as well as reported correlations.

Quality assessment & publication bias

We assessed the risk of bias with the Quality Assessment of Diagnostic Accuracy Studies criteria (QUADAS-2) [27], and disagreements were resolved by a third party independently. Begg's and Egger's tests were used for publication bias evaluation. RevMan version 5.4 (The Cochrane Collaboration Review Manager, Copenhagen) was used to generate the risk of bias plots.

Statistical analysis

Positive predictive values (PPV), negative predictive values (NPV), sensitivity (SE), specificity (SP), accuracy, positive likelihood ratio (PLR) and negative likelihood ratio (NLR), as well as subgroup analyses, all with a 95% confidence interval, were analysed using Comprehensive Meta-Analysis (CMA) v. 2.2.064 software. Following the Cochrane Handbook for Systematic Reviews of Interventions [28], we used the I-square (I2) test to assess statistical heterogeneity. In the case of extreme heterogeneities, the random-effects model was used, and the fixed-effect model with inverse variance model was utilised in the other cases. *P*-values less than 0.05 were considered statistically significant.

Result

Study selection process

Our initial database search resulted in 649 papers. After removing duplicate records, we evaluated 427 remaining papers by title and/or abstract and 85 papers by full text. Ultimately, 13 papers with 15 cohorts entered into the meta-analysis. PRISMA flow diagram for the study selection process is presented in Fig. 1.

Study characteristics

Our study includes 13 studies with 15 cohorts with a sample size ranging from 6 to 77, including 389 participants. Characteristics of studies entered into meta-analysis are presented in Table 1.

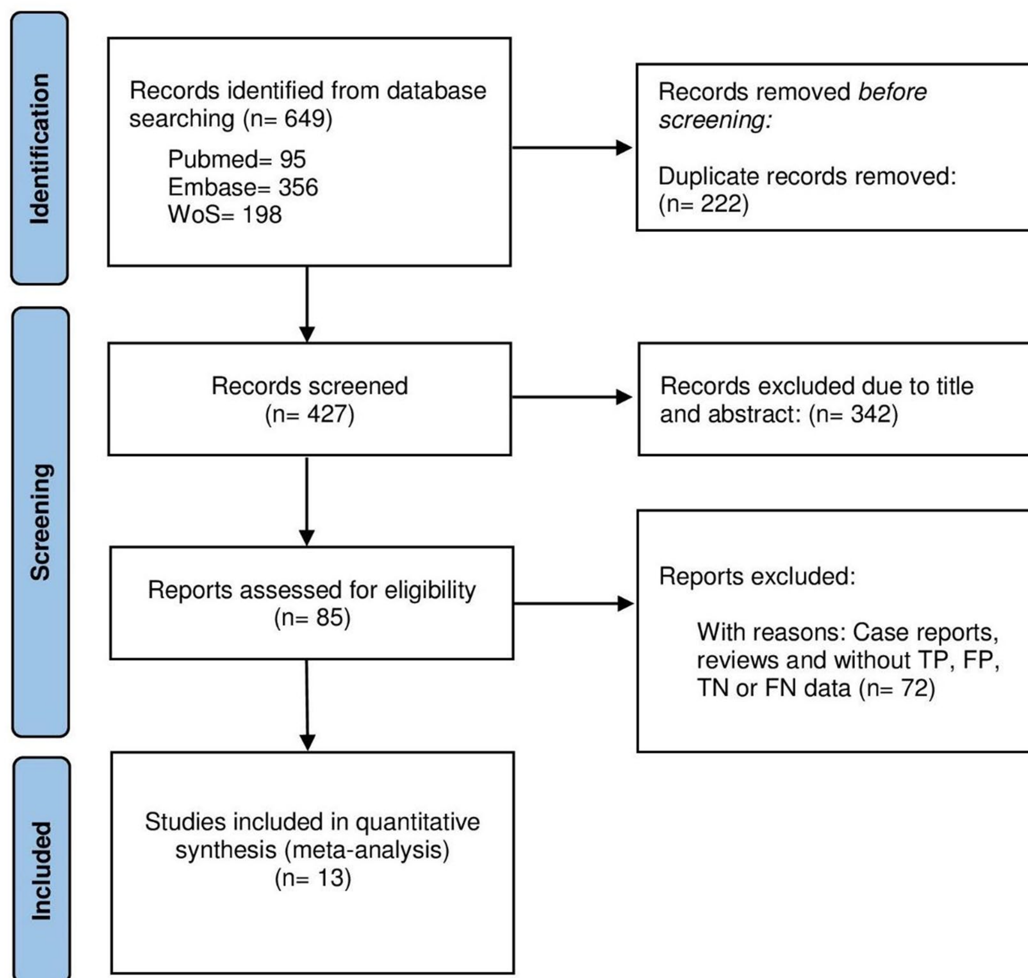


Fig. 1 PRISMA flow diagram of the study selection process

Table 1 Characteristics of studies included in the meta-analysis of the diagnostic accuracy of ESR1 mutation detection in cfDNA

Study	Country	*N*	Age	stage	cfDNA volume	Plasma volume	Tumour sample's mutation detection assay	cfDNA's mutation detection assay	TP	FP	FN	TN
G. Schiavon (2015) [9]	US	31	NA	MBC	NA	2 ml	PCR	PCR	3	0	1	27
D. Sefrioui (2015) [29]	France	7	NA	MBC	1–2 ng	0.4-2 ml	dPCR	dPCR	4	0	0	3
J. H. Chung (2017) [30]	US	14	58 (32–85)	MBC	≥ 20 ng	NA	NGS (Hybrid capture method)	NGS (Hybrid capture method)	4	1	0	9
J. H. Chung (2017) [30]	US	14	58 (32–85)	MBC	≥ 20 ng	NA	NGS (Hybrid capture method)	NGS (Hybrid capture method)	2	2	1	9
M.P. Goetz (2017) [31]	US	14	NA	MBC or locally recurrent breast cancer	≥ 15 ng	1–5 ml	NGS (Amplicon-based method)	NGS (Amplicon-based method)	0	2	0	12
T. Takeshita (2017) [32]	Japan	35	56.4 (31–84)	MBC	NA	2 ml	dPCR	dPCR	1	4	5	25
A. Moretti (2017) [33]	Italy	7	NA	MBC	NA	NA	NGS-dPCR	NGS	1	2	1	3
L. Lupini (2018) [34]	Italy	6	NA	MBC	1–20 ng	1 ml	NGS (Amplicon-based method)-PCR	NGS (Amplicon-based method)-PCR	1	2	1	2
Y. N. Kuang (2018) [35]	US	23	NA	MBC	5 µl	1–5 ml	dPCR	dPCR	7	2	0	14
J. S. Winn (2020) [36]	US	19	47.6 (32–69)	IBC	10–100 ng	3–6 ml	NGS	NGS	0	0	1	18
N. C. Turner (2020) [37]	UK	77	NA	ABC	NA	NA	dPCR	dPCR	31	27	1	18
D. Stergiopoulou (2021) [38]	US	32	NA	MBC	8 µl	2 ml	dPCR	dPCR	5	0	3	24
R. A. Aboelwafa (2021) [39]	Egypt	45	NA	MBC or locally recurrent breast cancer	2.5 µl	1–5 ml	dPCR	dPCR	2	0	0	43
R. A. Aboelwafa (2021) [39]	Egypt	45	NA	MBC or locally recurrent breast cancer	2.5 µl	1–5 ml	dPCR	dPCR	9	1	0	35
H. Shim (2021) [40]	Korea	20	NA	MBC	NA	2 ml	dPCR	dPCR	0	3	0	17

US = United States; Pro. = Prospective; PCR = Polymerase chain reaction; NGS = Next-Generation Sequencing, NA = Not available; TP = True positive; FP = False positive; FN = False negative; TN = True negative; CI = Confidence interval; MBC = Metastatic breast cancer; IBC = Inflammatory breast cancer

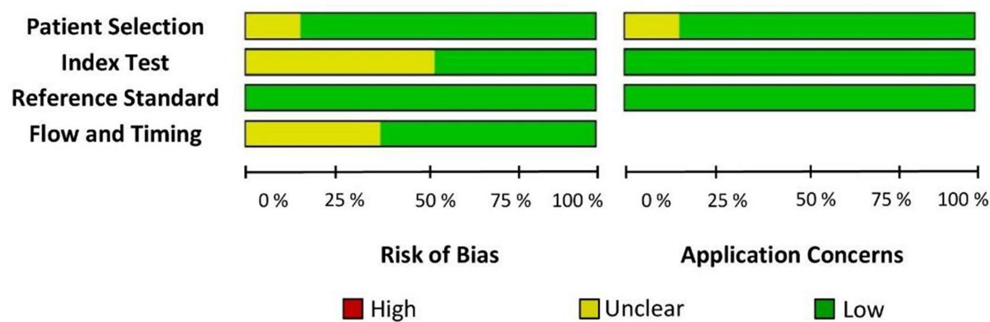


Fig. 2 Summary of risk of bias for studies entered into the meta-analysis

Quality assessment & publication bias

Quality assessment findings for studies entered into meta-analysis were fair (Fig. 2). Begg's and Egger's tests findings were as follows for publication bias: Sensitivity (*P*B=00.6, *P*E=0.02), Specificity (*P*B=0.01, *P*E=0.01), PPV (*P*B=0.36, *P*E=0.87), NPV (*P*B=0.54, *P*E=0.91), PLR (*P*B=0.06, *P*E=0.19), NLR (*P*B=0.36, *P*E=0.56) and Accuracy (*P*B=0.07, *P*E=0.03).

Main outcomes

Sensitivity (Fig. 3)

According to the meta-analysis of 13 cohorts, compared to histopathological examination, cfDNA assessment indicated a sensitivity of 75.52 (95% CI 60.19–90.85). Significant heterogeneity was observed (I^2=75.47%, $P<0.001$).

Specificity (Fig. 3)

Meta-analysis showed the specificity of 88.20 (95% CI 80.99–95.40) for ESR1 mutation detection through cfDNA in breast cancer. High heterogeneity was observed (I^2=81.36%, $P<0.001$).

PPV & NPV (Fig. 4)

The meta-analysis demonstrated the PPV of 56.94 (95% CI 41.70–72.18) and the NPV of 88.53 (95% CI 82.61–94.44) for ESR1 detection by cfDNA. The heterogeneity was moderate for NPV (I^2=43.36%, $P>0.05$) but notable heterogeneity was observed for PPV (I^2=73.36%, $P<0.001$).

Accuracy (Fig. 5)

We identified an accuracy of 88.96 (95% CI 83.23–94.69) for this approach by examining sensitivity and specificity. Significant heterogeneity was observed (I^2=78.91%, $P<0.001$).

PLR & NLR (Fig. 5)

Meta-analysis found the PLR of 1.60 (95% CI 1.20–1.99) and NLR 0.44 (95% CI 0.09–0.79) for ESR1 detection via cfDNA. No heterogeneity for PLR (I^2=00.0%, $P>0.05$) but a substantial heterogeneity for NLR (I^2=58.38%, $P<0.05$, respectively) was observed.

Subgroup analysis (Tables 2 and 3)

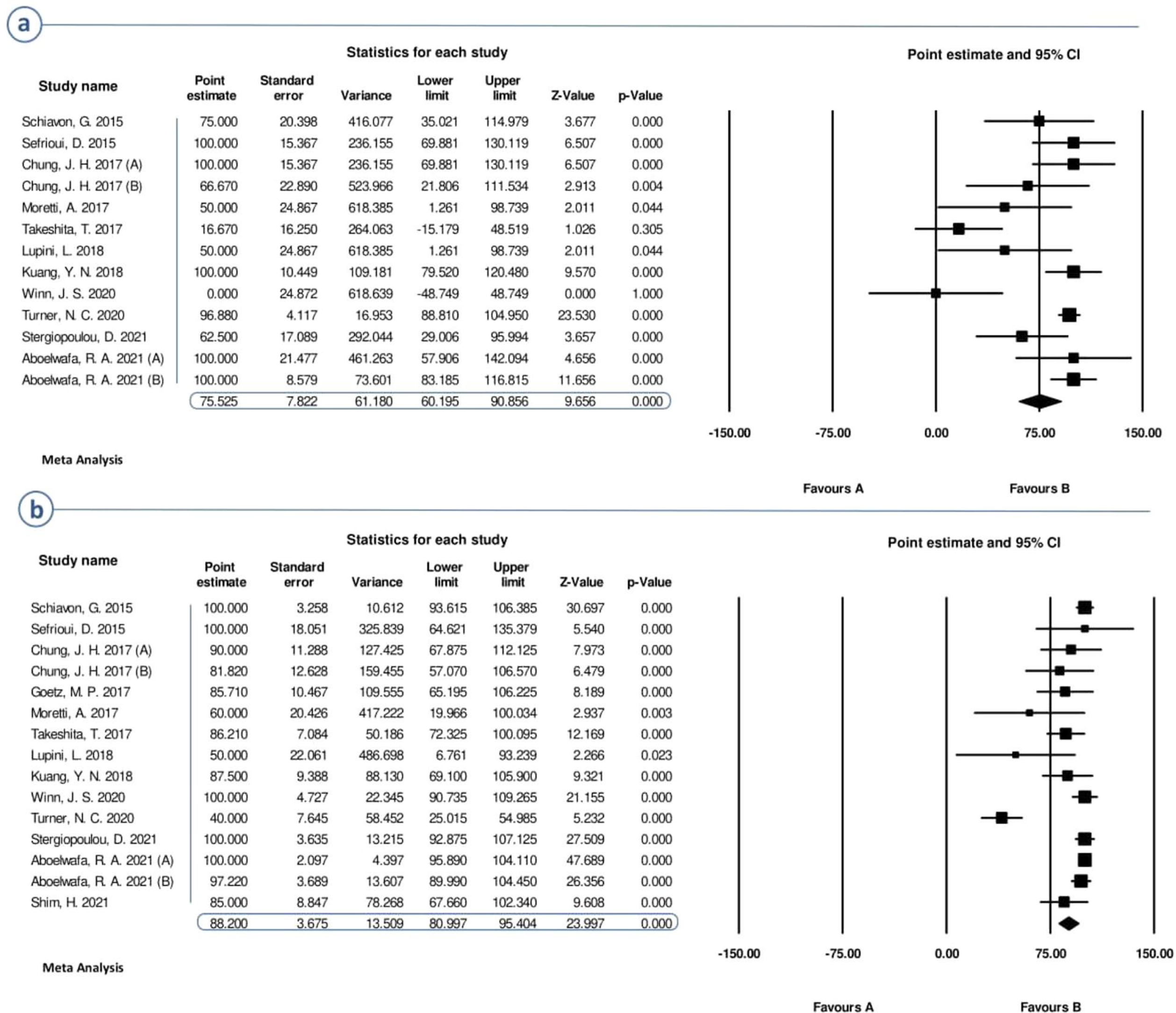
In our meta-analysis, subgroup analysis based on type of ESR1 detection assay (dPCR or NGS) (Table 2), source of malignancy (Primary or Metastatic) as well as type of tissue biopsy (archival tissue samples or fresh tumors) were both indicated in the Table 3.

Discussion

This systematic review and comprehensive meta-analysis is carried out to determine diagnostic values for evaluating ESR1 mutation *via* cfDNA in breast cancer. We systematically reviewed studies to pool the available data initially, the specificity of ESR1 identification by cfDNA was found to be 88.20 (95% CI 80.99–95.40) in our meta-analysis; hence, the ability of plasma cfDNA to identify breast cancer patients who have a negative result is high due to the existence or absence of an ESR1 mutation. In detail, a test's specificity refers to its ability to correctly classify a person as disease-free or identify all true negative cases based on results relative to a gold standard [41].

We primarily require accurate diagnostic tests with minor errors to apply suitable therapies for diseases. These approaches should be high-tech, cost-effective, rapid, and safe, leading to the precise diagnosis of the specific disease [42].

Focusing on our study, mutations in ER-positive MBC patients in ER gene's ligand-binding domain have recently been identified as endocrine resistance mechanisms that are mostly determined using tissue biopsy [43]. Traditional tissue biopsy is highly accurate, but tissue processing is challenging, lengthy, and invasive. Frequent invasive procedures could harm the patient, and due to the small sample volume, tumour tissue is not always accessible for genetic characterisation. While the liquid biopsy is a non-invasive and rapid approach, its ability to detect resistance mechanisms during therapy could assist in the early detection of disease progression, followed by better treatment strategies. ESR1 mutations



a

Statistics for each study

Study name	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Schiavon, G. 2015	75.000	20.398	416.077	35.021	114.979	3.677	0.000
Sefrioui, D. 2015	100.000	15.367	236.155	69.881	130.119	6.507	0.000
Chung, J. H. 2017 (A)	100.000	15.367	236.155	69.881	130.119	6.507	0.000
Chung, J. H. 2017 (B)	66.670	22.890	523.966	21.806	111.534	2.913	0.004
Moretti, A. 2017	50.000	24.867	618.385	1.261	98.739	2.011	0.044
Takeshita, T. 2017	16.670	16.250	264.063	-15.179	48.519	1.026	0.305
Lupini, L. 2018	50.000	24.867	618.385	1.261	98.739	2.011	0.044
Kuang, Y. N. 2018	100.000	10.449	109.181	79.520	120.480	9.570	0.000
Winn, J. S. 2020	0.000	24.872	618.639	-48.749	48.749	0.000	1.000
Turner, N. C. 2020	96.880	4.117	16.953	88.810	104.950	23.530	0.000
Stergiopoulou, D. 2021	62.500	17.089	292.044	29.006	95.994	3.657	0.000
Aboelwafa, R. A. 2021 (A)	100.000	21.477	461.263	57.906	142.094	4.656	0.000
Aboelwafa, R. A. 2021 (B)	100.000	8.579	73.601	83.185	116.815	11.656	0.000
Meta Analysis	75.525	7.822	61.180	60.195	90.856	9.656	0.000

b

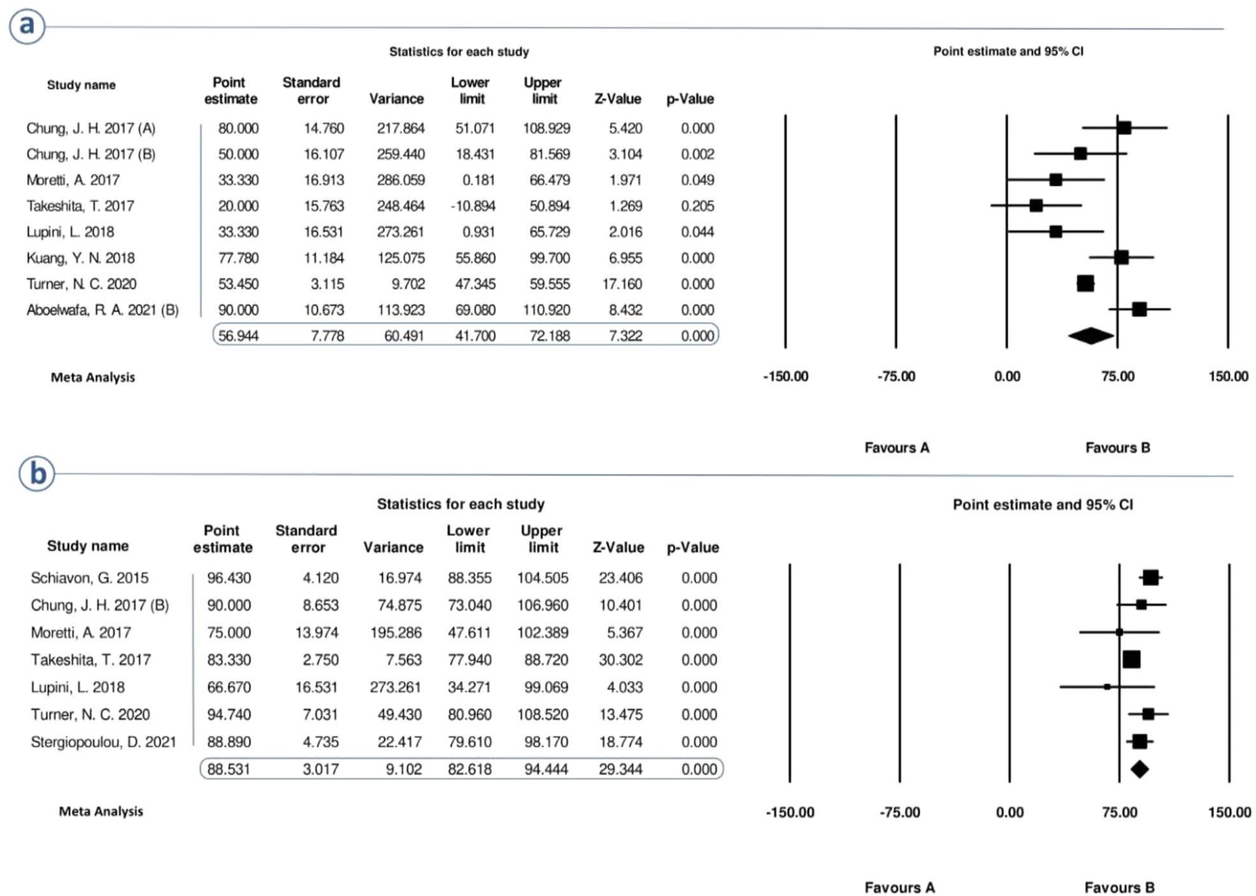
Statistics for each study

Study name	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Schiavon, G. 2015	100.000	3.258	10.612	93.615	106.385	30.697	0.000
Sefrioui, D. 2015	100.000	18.051	325.839	64.621	135.379	5.540	0.000
Chung, J. H. 2017 (A)	90.000	11.288	127.425	67.875	112.125	7.973	0.000
Chung, J. H. 2017 (B)	81.820	12.628	159.455	57.070	106.570	6.479	0.000
Goetz, M. P. 2017	85.710	10.467	109.555	65.195	106.225	8.189	0.000
Moretti, A. 2017	60.000	20.426	417.222	19.966	100.034	2.937	0.003
Takeshita, T. 2017	86.210	7.084	50.186	72.325	100.095	12.169	0.000
Lupini, L. 2018	50.000	22.061	486.698	6.761	93.239	2.266	0.023
Kuang, Y. N. 2018	87.500	9.388	88.130	69.100	105.900	9.321	0.000
Winn, J. S. 2020	100.000	4.727	22.345	90.735	109.265	21.155	0.000
Turner, N. C. 2020	40.000	7.645	58.452	25.015	54.985	5.232	0.000
Stergiopoulou, D. 2021	100.000	3.635	13.215	92.875	107.125	27.509	0.000
Aboelwafa, R. A. 2021 (A)	100.000	2.097	4.397	95.890	104.110	47.689	0.000
Aboelwafa, R. A. 2021 (B)	97.220	3.689	13.607	89.990	104.450	26.356	0.000
Shim, H. 2021	85.000	8.847	78.268	67.660	102.340	9.608	0.000
Meta Analysis	88.200	3.675	13.509	80.997	95.404	23.997	0.000

Fig. 3 Forest plot of cfDNA-ESR1 sensitivity **(a)** and specificity **(b)**

confer ligand-independent function, tolerance to estrogen deprivation, and relative resistance to tamoxifen and fulvestrant, according to liquid biopsy studies [44–47]. However, plasma cfDNA has several limitations that affect its utility as a definite marker for ESR1 detection in breast cancer [48]. Some investigations have reported that low concentrations of plasma cfDNA constitute a significant obstacle to its isolation (average 30 ng/mL, range 1.8–44 ng/mL) [49, 50]. In addition, due to the short half-life of cfDNA, the purification process must be operating immediately [48].

Effective extraction procedures for cell-free DNA (cfDNA) should ideally be rapid, sturdy, straightforward, and capable of automation. These methods should yield cfDNA of acceptable purity and quantity, which is vital for the dependability of subsequent applications like Next-Generation Sequencing (NGS) or Polymerase Chain Reaction (PCR). Nevertheless, the selection of an appropriate method is also influenced by the quantity and volume of the samples under consideration [51]. At present, the market offers over 40 distinct methods for the extraction of cell-free DNA (cfDNA), encompassing both manual and automated isolation kits. These techniques vary fundamentally, typically relying on the binding of DNA molecules to silica gel membranes, magnetic particles or organic chemicals [52]. A research study conducted in Germany sought to evaluate six commercially available cfDNA kits with the objective of extracting high-purity cfDNA from human plasma samples. The compared methods were two spin-column based, three magnetic bead-based and two automatic magnetic bead-based methods. The yield of DNA, as quantified by the Qubit Fluorometer and Bioanalyzer, have exhibited notable variations among the different extraction kits,



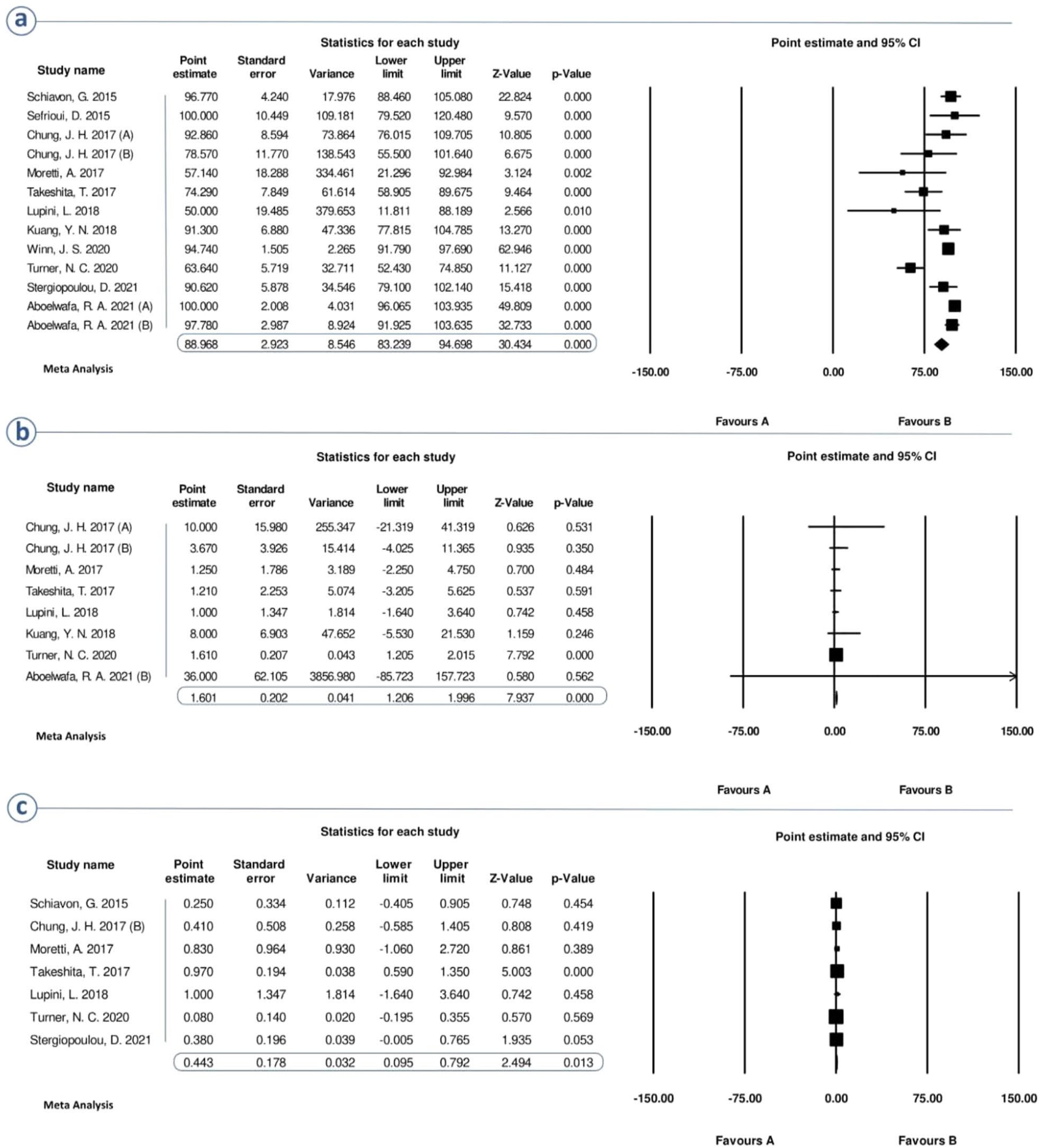
Study name	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Chung, J. H. 2017 (A)	80.000	14.760	217.864	51.071	108.929	5.420	0.000
Chung, J. H. 2017 (B)	50.000	16.107	259.440	18.431	81.569	3.104	0.002
Moretti, A. 2017	33.330	16.913	286.059	0.181	66.479	1.971	0.049
Takeshita, T. 2017	20.000	15.763	248.464	-10.894	50.894	1.269	0.205
Lupini, L. 2018	33.330	16.531	273.261	0.931	65.729	2.016	0.044
Kuang, Y. N. 2018	77.780	11.184	125.075	55.860	99.700	6.955	0.000
Turner, N. C. 2020	53.450	3.115	9.702	47.345	59.555	17.160	0.000
Aboelwafa, R. A. 2021 (B)	90.000	10.673	113.923	69.080	110.920	8.432	0.000
Meta Analysis	56.944	7.778	60.491	41.700	72.188	7.322	0.000

Study name	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Schiavon, G. 2015	96.430	4.120	16.974	88.355	104.505	23.406	0.000
Chung, J. H. 2017 (B)	90.000	8.653	74.875	73.040	106.960	10.401	0.000
Moretti, A. 2017	75.000	13.974	195.286	47.611	102.389	5.367	0.000
Takeshita, T. 2017	83.330	2.750	7.563	77.940	88.720	30.302	0.000
Lupini, L. 2018	66.670	16.531	273.261	34.271	99.069	4.033	0.000
Turner, N. C. 2020	94.740	7.031	49.430	80.960	108.520	13.475	0.000
Stergiopoulou, D. 2021	88.890	4.735	22.417	79.610	98.170	18.774	0.000
Meta Analysis	88.531	3.017	9.102	82.618	94.444	29.344	0.000

Fig. 4 Forest plot of cfDNA-ESR1 Positive Predictive Value (PPV) **(a)**, and Negative Predictive Value (NPV) **(b)**

with differences reaching up to 4.3-fold. Predominantly, all kits isolated small fragments that corresponded to mono-nucleosomal sizes. The manual QIAamp Circulating Nucleic Acid Kit (spin column) and the automated MagNA Pure Total NA Isolation Kit (automatic magnetic bead) demonstrated the highest yield and reproducibility. These findings underscore the necessity of standardizing preanalytical conditions, which should be tailored to the specific needs of the downstream applications [51].

Furthermore, the microfluidic platform supplies molecular perception [53] with advanced nucleic acid extraction [48] and micrometre-scale liquid utilisation [54]. This approach reduces the need for a high sample volume and, by reducing the processing time, makes it possible to examine many samples in a fast-paced clinical environment [55, 56]. In a Korean study that aimed to isolate cfDNA for liquid biopsy by microfluidic platform compared to the conventional gold standard QIAGEN method, microfluidic technology has shown a high performance. The platform could detect a point mutation in phosphatidylinositol-4,5-bisphosphate 3-kinase (PIK3CA) in metastatic HER-2 breast cancer patients [48]. Microfluidic systems have been developed for the analysis and detection of circulating tumor cells (CTCs), which involve cell capture methods based on affinity binding and label-free methods, and detection based on electrical, chemical, and optical sensors [57]. Moreover, microfluidic lab-on-a-chip technology has been spotlighted for exosome isolation and molecular analysis, offering high sensitivity, quick analysis time, and a sample-to-answer format [58]. While other cell-free DNA (cfDNA) monitoring strategies have demonstrated clinical validity across various solid tumor types, including breast cancer, microfluidic approaches offer several advantages. They provide high sensitivity and flexibility, low sample usage, cost-effectiveness, and the ability for automation [59]. Furthermore, cfDNA-based approaches can effectively compensate for the shortcomings of traditional screening and monitoring methods, which fail to provide real-time information and prospective guidance for breast cancer diagnosis and treatment [60]. In terms of cost, cfDNA monitoring has been found to be the lowest-cost strategy, even if the cost of cfDNA were doubled [61]. In this regard, the utilisation of the microfluidic platform in the clinic, especially in monitoring patients



Study name	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Schiavon, G. 2015	96.770	4.240	17.976	88.460	105.080	22.824	0.000
Sefrioui, D. 2015	100.000	10.449	109.181	79.520	120.480	9.570	0.000
Chung, J. H. 2017 (A)	92.860	8.594	73.864	76.015	109.705	10.805	0.000
Chung, J. H. 2017 (B)	78.570	11.770	138.543	55.500	101.640	6.675	0.000
Moretti, A. 2017	57.140	18.288	334.461	21.296	92.984	3.124	0.002
Takeshita, T. 2017	74.290	7.849	61.614	58.905	89.675	9.464	0.000
Lupini, L. 2018	50.000	19.485	379.653	11.811	88.189	2.566	0.010
Kuang, Y. N. 2018	91.300	6.880	47.336	77.815	104.785	13.270	0.000
Winn, J. S. 2020	94.740	1.505	2.265	91.790	97.690	62.946	0.000
Turner, N. C. 2020	63.640	5.719	32.711	52.430	74.850	11.127	0.000
Stergiopoulou, D. 2021	90.620	5.878	34.546	79.100	102.140	15.418	0.000
Aboelwafa, R. A. 2021 (A)	100.000	2.008	4.031	96.065	103.935	49.809	0.000
Aboelwafa, R. A. 2021 (B)	97.780	2.987	8.924	91.925	103.635	32.733	0.000
Meta Analysis	88.968	2.923	8.546	83.239	94.698	30.434	0.000

Study name	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Chung, J. H. 2017 (A)	10.000	15.980	255.347	-21.319	41.319	0.626	0.531
Chung, J. H. 2017 (B)	3.670	3.926	15.414	-4.025	11.365	0.935	0.350
Moretti, A. 2017	1.250	1.786	3.189	-2.250	4.750	0.700	0.484
Takeshita, T. 2017	1.210	2.253	5.074	-3.205	5.625	0.537	0.591
Lupini, L. 2018	1.000	1.347	1.814	-1.640	3.640	0.742	0.458
Kuang, Y. N. 2018	8.000	6.903	47.652	-5.530	21.530	1.159	0.246
Turner, N. C. 2020	1.610	0.207	0.043	1.205	2.015	7.792	0.000
Aboelwafa, R. A. 2021 (B)	36.000	62.105	3856.980	-85.723	157.723	0.580	0.562
Meta Analysis	1.601	0.202	0.041	1.206	1.996	7.937	0.000

Study name	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Schiavon, G. 2015	0.250	0.334	0.112	-0.405	0.905	0.748	0.454
Chung, J. H. 2017 (B)	0.410	0.508	0.258	-0.585	1.405	0.808	0.419
Moretti, A. 2017	0.830	0.964	0.930	-1.060	2.720	0.861	0.389
Takeshita, T. 2017	0.970	0.194	0.038	0.590	1.350	5.003	0.000
Lupini, L. 2018	1.000	1.347	1.814	-1.640	3.640	0.742	0.458
Turner, N. C. 2020	0.080	0.140	0.020	-0.195	0.355	0.570	0.569
Stergiopoulou, D. 2021	0.380	0.196	0.039	-0.005	0.765	1.935	0.053
Meta Analysis	0.443	0.178	0.032	0.095	0.792	2.494	0.013

Fig. 5 Forest plot of cfDNA-ESR1 Accuracy **(a)**, Positive Likelihood Ratio (PLR) **(b)** and ESR1 Negative Likelihood Ratio (NLR) **(c)**

with breast cancer can be a helpful tool to improve the screening of these patients.

However, the major limitation of using cfDNA as a tissue substitute is the high degree of heterogeneity in the concordance rate between gene alterations in tumour tissues and those in their corresponding plasma [32]. In Schiavon *et al.'s* study, tumour tissue DNA and cfDNA had a 97% (30/31) agreement on the existence of ESR1 mutations [23]. A screening test's sensitivity can be described as the test's ability to recognise true positive cases or, in other words, recognise all cases with the considered condition based on results relative to a gold standard [62]. As we found in our meta-analysis, the sensitivity for ESR1 detection using cfDNA is 75.52 (95% CI

Table 2 Results of subgroup analysis

	NGS			dPCR		
	n	**ES (LL to UL)**	**I^2**	**N**	**ES (LL to UL)**	**I^2**
SE	4	56.78 (13.89–99.67)	75.66	8	81.01 (64.04–97.99)	75.74
SP	5	90.14 (79.17–101.10)	33.98	9	90.44 (82.55–98.33)	87.72
PPV	3	55.39 (28.17–82.61)	56.27	4	61.76 (38.40-85.11)	84.84
NPV	3	94.74 (94.73–94.74)	12.87	3	90.99 (21.78–81.84)	77.67
NLR	4	1.008 (0.94–1.07)	4.95	4	0.42 (-0.004-0.85)	78.54
PLR	3	1.75 (-1.41-4.92)	0.00	4	1.61 (1.21–2.01)	0.00
ACC	4	88.35 (76.87–99.82)	50.18	8	89.81 (81.70-97.93)	84.53

ES: Effect size; N: Number of studies; SE= sensitivity; SP= Specificity; PPV= Positive predictive value; NPV = Negative predictive value, PLR = Positive likelihood ratio; NLR = Negative likelihood ratio; ACC = Accuracy; LL= Lower limit; UL: Upper limit

60.19–90.85), which indicates that the ability of the test using cfDNA to identify breast cancer patients who have a positive result due to the existence or absence of ESR1 mutations is high and reliable.

The PPV measure shows how many people with positive test results have the condition based on a screening test [62]. Our meta-analysis revealed that the PPV for ESR1 detection by cfDNA is sustainable in sensitivity and indicates an acceptable outcome compared to the gold standard.

The NPV indicates the proportion of people with a negative test result that truly does not have a disease [63]. Our meta-analysis revealed that the NPV for ESR1 detection via cfDNA is high and suggests effective performance compared to the gold standard.

Also, our study found an reasonable accuracy for ESR1 detection via cfDNA, which indicates acceptable efficiency. The prevalence of target conditions influences tests' diagnostic accuracy, and the ability of a test to distinguish between the target condition and health is referred to as diagnostic accuracy [64].

As an alternative statistic, the positive and negative likelihood ratio relates the probability of test results in cases with the condition to the probability of a test result in situations without the condition [65]. According to studies, PLR of more than ten and NLR of less than 0.1 gave considerable evidence for diagnosis [66]. Regarding this, PLR 1.60 (95% CI 1.20–1.99) and NLR 0.44 (95% CI 0.09–0.79) indicate an association with the presence and absence of the condition, respectively.

Temporal tumour heterogeneity particularly influences concordance between tissue-based testing and blood and can be pronounced in breast cancer with different discordance in studies between primary and metastatic tumour biopsies [67]. According to the biopsy strategy, tissue archive samples taken long before mutational profiling are most commonly used in clinical practice. However, blood and tissue-based concordance studies where archival tissue tests are utilized regularly report significantly lower concordance (<80%) than those using simultaneous biopsies [68, 69]. In addition, ESR1 mutations are far more common in metastatic (up to 40%) than primary (less than 1%) breast tumours. Also, the impact of temporal and spatial heterogeneity cannot be ignored when assessing the accuracy of cfDNA profiling [70].

In this regard, due to the high heterogeneity in our overall results, subgroup analysis was performed according to different covariates, such as the source of malignancy (primary or metastatic) as well as the type of tissue biopsy (archival tissue samples or fresh tumours). According to the limited number of studies included in the subgroup analysis of the type of tissue biopsy in contrast to the source of malignancy subgroup analysis, the type of tissue biopsy shows no statistically significant subgroup effect. However, the diagnostic accuracy in primary tissues was slightly improved (SE from 75.52 to 87.4, ACC from 88.96 to 98.42, and SP from 88.20 to 99.56). In this regard, these results indicate that detecting ESR1 in cfDNA was likely more accurate in this group.

Various assays have been used to detect ESR1 mutations in cfDNA detection. The benefit of real-time PCR is that it is easy to use and is more tolerant of variable DNA content, but it only supports limited multiplex capability. On the other hand, NGS may be unable to detect the existence of the ESR1 mutation according to the small amount of cfDNA present in the blood, resulting in false-negative results, and it is more technically challenging and costly to employ. However, NGS allows for the simultaneous analysis of multiple genomic locations while disclosing the precise sequence changes [71, 72].

Table 3 Results of subgroup analysis

	Primary			Metastatic			Primary and metastatic			Archived			Fresh tissue			Mixed		
	N	ES (LL to UL)	I^2	*N*	ES (LL to UL)	I^2	*N*	ES (LL to UL)	I^2	*N*	ES (LL to UL)	I^2	*N*	ES (LL to UL)	I^2	*N*	ES (LL to UL)	I^2
SE	4	87.41 (68.26-106.55)	35.77	7	64.65 (34.32–94.98)	82.39	2	79.85 (35.66-124.04)	71.09	11	78.68 (63.60-93.77)	71.65	1	100.00 (69.88-130.11)	0.00	1	0.00 (-48.74-48.74)	0.0
SP	4	99.56 (96.71-102.42)	0.00	7	91.37 (83.92–98.82)	14.97	3	59.19 (25.02–93.36)	86.64	12	86.31 (77.81–94.82)	84.89	1	100.00 (64.62-135.37)	0.00	2	94.36 (80.12–108.60)	52.28
PPV	1	90.00 (69.08-110.92)	0.00	5	53.58 (29.81–77.34)	70.43	2	49.94 (34.97–64.90)	30.10	8	56.94 (41.70-72.18)	73.36	-	-	-	-	-	-
NPV	2	93.02 (85.66-100.37)	30.71	3	83.63 (78.58–88.68)	0.00	2	84.69 (58.31-111.06)	59.04	7	88.53 (82.61–94.44)	43.36	-	-	-	-	-	-
NLR	2	0.34 (0.01–0.67)	0.00	3	0.89 (0.54–1.24)	0.00	2	0.09 (-0.18-0.36)	0.00	7	0.44 (0.095–0.79)	58.38	-	-	-	-	-	-
PLR	1	36.00 (-85.72-157.72)	0.00	5	1.79 (-0.73-4.321)	0.00	2	1.59 (1.19–1.99)	0.00	8	1.60 (1.20–1.99)	0.00	-	-	-	-	-	-
ACC	4	98.42 (95.48-101.36)	0.00	7	88.34 (80.26–96.39)	52.84	2	62.55 (51.80-73.31)	73.31	11	86.07 (78.14–94.01)	82.34	1	100.00 (79.52-120.48)	0.00	1	94.74 (91.79–97.69)	0.0

ES: Effect size; N: Number of studies; SE = sensitivity; SP = Specificity; PPV = Positive predictive value; NPV = Negative predictive value, PLR = Positive likelihood ratio; NLR = Negative likelihood ratio; ACC = Accuracy; LL = Lower limit; UL: Upper limit

In our meta-analysis, we observed variability in assays for ESR1 mutation detection in cfDNA and tumor samples across different studies. Each of these techniques has its own strengths and limitations, which could impact the detection of ESR1 mutations. For instance, PCR is a cost-effective and rapid method but may lack the sensitivity to detect low-frequency mutations [73]. In contrast, NGS can provide high-throughput data and detect multiple mutations simultaneously, but it requires a more complex workflow and data analysis [74]. Therefore, the choice of assay technique could significantly influence the outcomes of ESR1 mutation detection in cfDNA. Future studies should aim to standardize these techniques to reduce variability and improve the reliability of results. Despite these challenges, our study underscores the potential of cfDNA as a non-invasive diagnostic tool for tracking ESR1 mutations in breast cancer patients.

In this regard, subgroup analysis was performed according to ESR1 mutation detection assay, we observed distinct performance characteristics between Next-Generation Sequencing (NGS) and digital PCR (dPCR) in detecting ESR1 mutations in cell-free DNA for breast cancer. While both methods demonstrated high specificity (NGS: 90.14%, dPCR: 90.44%), dPCR outperformed NGS in terms of sensitivity (dPCR: 81.01% vs. NGS: 56.78%). This suggests that dPCR may offer a more reliable approach for detecting ESR1 mutations. However, the interpretation of these results should consider the substantial heterogeneity among the included studies, as indicated by the high I-square values. Further research is warranted to validate these findings and determine the optimal method for ESR1 mutation detection in breast cancer.

According to the study limitation concerning the type of detection assay, studies particularly indicate that tumour tissue achieve high concordance with cfDNA assays while variant allele fraction (VAF) detection limit was close to 0.1% [75, 76]. In addition, due to spatial heterogeneity, another relevant drawback of this study, a pivotal study by Parikh and colleagues, illustrates this matter by confirming the ability of blood-based profiling to identify a pool of mutations from various metastatic sites rather than just from one biopsied [77]. However, the lack of data on these matters may have affected the results. Additionally, the small sample sizes in the included studies and incomplete subgroup analyses (due to insufficient data on tumor stage, treatment history, and molecular subtype) are notable limitations of this meta-analysis that could impact diagnostic performance. This challenge is common in research and underscores the need for further studies with larger sample sizes to validate and extend our findings.

Although this study is a study to evaluate the diagnostic accuracy of ESR1 mutation detection, however, the lack

of clinical data, especially the lack of survival data, may be an insurmountable limitation in evaluating the usefulness of the ESR1 test. Therefore, future studies with a more clinical approach to evaluate the usefulness of the ESR1 test can be helpful.

Eventually, according to the above, our meta-analysis supports the idea that detecting ESR1 gene mutation in cfDNA has been accepted as a gold verification test for initial screening results. Based on the evidence, this test can be reliable in determining ESR1 mutation in breast cancer patients, particularly in low-income settings, because of its time-saving and ease of implementation concerns. However, further studies with a larger sample size are needed to robust the findings of this study.

Conclusion

This systematic review and comprehensive meta-analysis demonstrated that plasma cfDNA has high specificity and sensitivity for identifying ESR1 mutation in breast cancer patients. Hence, our findings proposed that the test has high diagnostic value in this setting. It is probably consider as a reliable non-invasive method for detecting ESR1 mutation and therapeutic decision-making in this breast cancer patients. Nevertheless, due to the limited number of papers included in this meta-analysis, comprehensive prospective studies are needed to robust the evidence on the prognostic power of this approach in breast cancer.

Author contributions
M.R. and A.H, designed the study; M.T and M.R carried out the data extraction; K.H, R.A-N, F.E and M.T analyzed the data; M.R. wrote the manuscript in consultation with A.R, D.SH., F.M and H.N.; and all of the authors read and revised the final version of the manuscript.

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Data availability
The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This is a systematic review and comprehensive meta-analysis study. The Mazandaran university of medical science Research Ethics Committee has confirmed that no ethical approval is required.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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