Review Article

Immunohistochemical Changes After Neoadjuvant Chemotherapy and Their Impact on Breast Cancer Survival: A Systematic Review and Meta-analysis

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Abstract

Changes in immunohistochemical (IHC) profiles following neoadjuvant chemotherapy (NAC) may impact therapeutic decisions and prognosis in breast cancer patients. However, the clinical significance of these biomarker conversions remains uncertain. To evaluate the frequency of IHC marker conversion (estrogen receptor [ER], progesterone receptor [PR], and HER2) after NAC and its association with pathological complete response (pCR), overall survival (OS), and disease-free survival (DFS). We conducted a systematic review and meta-analysis of cohort studies reporting pre- and post-NAC IHC profiles in breast cancer. A comprehensive search was performed in PubMed, Embase, Scopus, and Web of Science. The ROBINS-I tool was used to assess risk of bias. Random-effects models were applied to calculate pooled conversion rates and assess the prognostic impact of IHC changes. Twenty-four studies (n = 5891 patients) were included. The pooled conversion rates were 9.2% for ER, 15.1% for PR, 8.6% for HER2. Loss of hormone receptor positivity was associated with a lower pCR rate and worse DFS (HR 1.42; 95% CI, 1.11-1.81). HER2 gain correlated with improved pCR. High heterogeneity was observed, and sensitivity analyses confirmed the robustness of the results. IHC profile changes after NAC are frequent and clinically relevant. Loss of hormone receptor expression may indicate poorer

Abbreviations: BC, Breast cancer; DFS, Disease-free survival; ER, Estrogen receptor; HER2, Human epidermal growth factor receptor 2;HR, Hormone receptor; IHC, Immunohistochemical; NAC, Neoadjuvant chemotherapy; OS, Overall survival; pCR, Pathological complete response; PR, Progesterone receptor; R ROBINS-I, Risk of bias in non-randomized studies of interventions; T-DM1, Trastuzumab emtansine; TNBC, Triple-negative breast cancer.

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prognosis, while HER2 gain suggests improved treatment sensitivity. Reassessment of IHC markers post-NAC should be considered to optimize adjuvant therapy decisions.

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Keywords: Biomarker conversion, Immunohistochemistry, Breast cacner, Neoadjuvant chemotherapy, Survival outcomes

Introduction

Breast cancer (BC) remains the most frequently diagnosed cancer in women worldwide, accounting for approximately 2.3 million new cases annually and nearly one-quarter of all female malignancies.[1] Despite advances in early detection and systemic therapy, BC continues to be a leading cause of cancer-related death.[2] Over recent decades, BC management has shifted from uniform treatment approaches to precision medicine paradigms, fundamentally transforming patient outcomes and survival rates.

Neoadjuvant chemotherapy (NAC) has become a cornerstone in the management of both locally advanced and early-stage breast cancer, fulfilling several key therapeutic objectives beyond tumor downstaging.[3] Initially developed for inoperable cases, NAC is now routinely used to enable breast-conserving surgery, assess tumor biology and chemosensitivity in vivo, eradicate micrometastatic disease, and provide important prognostic information through pathological response evaluation.[4,5] The ability to monitor tumor response to systemic therapy in real time has deepened our understanding of tumor biology and established pathological complete response (pCR) as a validated surrogate endpoint for survival across multiple BC subtypes.[6]

Immunohistochemistry (IHC) plays a central role in BC characterization and treatment planning, particularly through the evaluation of key biomarkers such as estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki-67.[7] These markers guide clinical decisions regarding endocrine therapy, HER2-targeted treatment, and chemotherapy, and they form the basis of current breast cancer subtype classification.[6]

NAC can substantially modify biomarker expression, challenging the assumption of tumor biological stability. Reported conversion rates range from 8% to 55%, with the most frequent changes affecting hormone receptor and HER2 status. These dynamic shifts have direct implications for treatment eligibility, including endocrine therapy and anti-HER2 agents.[8] In one recent cohort of 203 breast cancer patients, post-NAC conversion rates ranged from 11.8% for ER to 24.6% for PR, with HER2 conversion observed in 12.5% of cases—underscoring the dynamic nature of tumor biology in response to systemic therapy.[9]

However, the assumption of biomarker stability between diagnostic biopsy and surgical resection has been increasingly challenged. NAC can induce significant changes in IHC marker expression, with reported conversion rates ranging from 8% to 55%, most commonly involving hormone receptors and HER2. Such alterations may influence treatment eligibility and prognosis.[8,9] For example, tumors that lose ER or PR expression may no longer benefit from endocrine therapy, while those that acquire HER2 or hormone receptor positivity may become candidates for targeted therapy not previously indicated.[9-12]

These dynamic changes are believed to result from both intrinsic tumor heterogeneity and selective pressure exerted by chemotherapy, which may eliminate chemosensitive clones while allowing resistant subclones to persist and dominate. In this context, biomarker conversion may reflect the unmasking of pre-existing cellular subpopulations rather than true phenotypic evolution.

Although the phenomenon of biomarker conversion has been increasingly recognized, its clinical significance remains poorly defined. There is currently no consensus on the prognostic value of specific conversion patterns, nor are there standardized guidelines on whether and how to modify treatment based on changes in receptor status. Furthermore, the impact of conversion may vary across molecular subtypes and individual biomarkers. The recent recognition of HER2-low tumors as a distinct therapeutic category adds further complexity, particularly in patients whose tumors convert from HER2-positive to HER2-low status after NAC.

To address these uncertainties, we conducted a systematic review and meta-analysis to assess the prevalence, patterns, and prognostic significance of IHC biomarker conversion following NAC in breast cancer patients. By synthesizing data from diverse cohorts, we aim to clarify whether reassessment of ER, PR, and HER2 status in residual disease should be integrated into routine clinical practice to optimize adjuvant therapy decisions and improve patient outcomes.

Methods

Study Design and Protocol Registration

This systematic review and meta-analysis were conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement to ensure methodological rigor and transparent reporting.[13] The review protocol was developed prospectively and followed established guidelines for systematic reviews of prognostic studies. It was formally registered in PROSPERO (CRD420250655833) due to the focus on prognostic rather than intervention studies, the methodology was predetermined and documented to minimize bias and enhance reproducibility. The completed PRISMA checklist is provided as Supplementary Material.

Search Strategy and Information Sources

A comprehensive systematic literature search was conducted across multiple electronic databases including PubMed/MEDLINE, EMBASE, Web of Science, Cochrane Central Register of Controlled Trials (CENTRAL) and the Google Scholar. The search strategy encompassed publications from database inception through June

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2025, with no language restrictions initially applied to maximize sensitivity.

The search strategy was developed in collaboration with a medical librarian and utilized a combination of Medical Subject Headings (MeSH) terms and free-text keywords to optimize retrieval of relevant studies. The core search strategy for PubMed included the following terms

Primary Search Terms: ("breast neoplasms"[MeSH] OR "breast cancer" OR "breast carcinoma" OR "mammary carcinoma")

AND ("neoadjuvant therapy"[MeSH] OR "neoadjuvant chemotherapy" OR "preoperative chemotherapy" OR "primary systemic therapy" OR "preoperative treatment")

AND ("immunohistochemistry"[MeSH] OR "biomarkers"[MeSH] OR "receptor conversion" OR "biomarker change" OR "subtype conversion")

AND ("estrogen receptor" OR "progesterone receptor" OR "HER2" OR "hormone receptor")

AND ("survival"[MeSH] OR "prognosis"[MeSH] OR "mortality" OR "recurrence" OR "outcome")

Similar search strategies were adapted for other databases with appropriate syntax modifications and database-specific controlled vocabulary terms. The EMBASE search utilized Emtree terms, while Web of Science employed topic searches with relevant truncation and wildcard operators.

Eligibility Criteria

Studies were considered eligible for inclusion based on predefined criteria established using the Population, Intervention, Comparator, Outcomes, and Study design (PICOS) framework:

Population: Adult women (≥18 years) with histologically confirmed invasive breast cancer of any stage, who received neoadjuvant chemotherapy followed by surgical resection, both early-stage and locally advanced disease were considered eligible.

Intervention/Exposure: Assessment of immunohistochemical biomarker expression both before (pre-treatment core biopsy) and after (posttreatment surgical specimen) neoadjuvant chemotherapy, documentation of biomarker conversion status (defined as a change in receptor status from positive to negative or vice versa), evaluation of at least one of the following biomarkers: ER, PR, HER2.

Comparator: Comparison of survival outcomes between patients with and without biomarker conversion, analysis of conversion patterns and their association with clinical outcomes.

Outcomes: Primary outcomes: Overall survival (OS) and disease-free survival (DFS)

Secondary outcomes: Pathological complete response (pCR) rates, biomarker conversion frequencies, time to recurrence, and patterns of biomarker change.

Study Design: Randomized controlled trials (RCTs), prospective cohort studies, and high-quality retrospective cohort studies with adequate follow-up duration (defined as a minimum median follow-up of 24 months).

Inclusion Criteria

Studies were eligible for inclusion if they involved breast cancer patients who received neoadjuvant chemotherapy with documented assessment of biomarker expression both before and after treatment. Additionally, included studies were required to report survival outcomes or provide sufficient data for their calculation, maintain a minimum sample size of ≥50 evaluable patients, and demonstrate adequate methodological quality based on standardized quality assessment tools. Full-text articles available in English were prioritized, although abstracts in other languages were considered when sufficient data were provided to allow for robust analysis.

Exclusion Criteria

Studies were excluded if they involved only metastatic breast cancer patients or lacked paired pre- and post-treatment biomarker assessments. Additionally, exclusion criteria encompassed studies without survival outcome data or with insufficient follow-up, case reports and case series with fewer than 50 patients, editorials, letters, or reviews without original data, and studies with inadequate methodological quality or a high risk of bias. Studies focusing exclusively on neoadjuvant endocrine therapy without chemotherapy, those involving only male breast cancer patients, and studies with substantial missing data that could not be imputed or obtained from authors were also excluded from the analysis.

Study Selection and Data Extraction Process

The study selection process was conducted independently by two reviewers using a standardized approach. Initial screening involved the review of titles and abstracts using the predefined eligibility criteria.

Data extraction was performed using a comprehensive, standardized form designed specifically for this review. The extraction form was developed based on established guidelines for prognostic systematic reviews and was piloted on five included studies to ensure completeness and reproducibility.

Quality Assessment

The risk of bias in the included studies was evaluated using the Risk Of Bias In Non-randomized Studies – of Interventions (ROBINS-I) tool, which is recommended for cohort studies assessing the impact of interventions. The tool evaluates seven bias domains: (1) confounding, (2) selection of participants, (3) classification of interventions, (4) deviations from intended interventions, (5) missing data, (6) measurement of outcomes, and (7) selection of the reported result. Each domain was independently assessed by two reviewers and classified as low, moderate, serious, or critical risk of bias. Disagreements were resolved by consensus or third-party adjudication.

A visual summary of domain-level risk of bias for all included studies is presented in Figure. The overall risk of bias was determined for each study based on the highest level of risk identified across domains. Studies with critical risk of bias in any domain were excluded from sensitivity analyses.

Statistical Analysis

All statistical analyses were performed using R software (version 4.3.0) with the meta, metafor, and survival packages. Given the anticipated heterogeneity between studies due to differences in patient populations, treatment protocols, and biomarker assessment methods, random-effects models were employed for all analyses

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using the DerSimonian-Laird method. Prevalence estimates: Pooled proportions with 95% confidence intervals for biomarker conversion rates, Primary analysis were Risk Ratios (RR) with 95% confidence intervals comparing survival outcomes between patients with and without biomarker conversion

For studies reporting risk ratios or hazard ratios with confidence intervals, these were directly incorporated into the meta-analysis. When effect estimates were not provided, they were calculated from available survival data using established methods. For prevalence estimates, the Freeman-Tukey double arcsine transformation was used to stabilize variances.

Heterogeneity Assessment and Risk of Bias

Statistical heterogeneity was quantified using, I^2 statistic: Values of 25%, 50%, and 75% were interpreted as low, moderate, and high heterogeneity, respectively; Cochran's Q test: Statistical significance was set at $P < .10$; Tau² (τ^2): Measure of between-study variance 95% prediction intervals: To estimate the range of true effects in similar future studies

Publication bias was evaluated using multiple approaches such as visual inspection of Funnel plots of effect size versus standard error and Egger's regression test and

Statistical significance was set at $P < .05$ for all analyses except heterogeneity testing ($P < .10$). All tests were two-sided, and multiple comparison adjustments were applied when appropriate using the Bonferroni correction.

Ethical Considerations

As this study involved analysis of previously published data, institutional review board approval was not required. All included studies were required to have appropriate ethical approval as reported in their original publications. Patient confidentiality was maintained throughout the review process, and no individual patient data were extracted or analyzed. The study was formally registered in PROSPERO (CRD420250655833).

Results

The systematic literature search identified 295 records across five databases. After removing 113 duplicate and automatically generated records, 182 unique records underwent initial screening. Following title and abstract review, 142 reports were sought for full-text retrieval, of which 100 were successfully obtained and assessed for eligibility. After comprehensive full-text review, 76 studies were excluded due to inadequate survival outcome reporting, resulting in 24 studies included in the final systematic review and meta-analysis.[8,10,14-35] The study selection process is summarized in Figure 1 PRISMA Flowchart diagram.[13,36]

Study Characteristics

The 24 included studies comprised entirely of observational cohort studies published between 2007 and 2025, with the majority published in the last decade (11 studies from 2020 to 2025 and 12 studies from 2010 to 2019) .[8,10,14-35] The studies originated from diverse geographic regions, with Asia representing 58.3% (14/24 studies), Europe 16.7% (4/24 studies), South America 16.7% (4/24 studies), and North America 4.2% (1/24 studies). Among individual countries, China contributed the highest number of studies (33.3%, 8/24 studies), followed by Turkey (12.5%, 3/24 studies) and Brazil (8.3%, 2/24 studies).

The studies included a total of 5263 patients with residual disease following NAC who underwent biomarker re-evaluation. Sample sizes ranged from 30 to 578 patients (median: 179 patients), with total study populations ranging from 52 to 710 patients when reported. The mean age across studies ranged from 46 to 55 years, reflecting typical breast cancer populations undergoing neoadjuvant treatment.

All included studies evaluated biomarker expression changes between pretreatment needle biopsies and postneoadjuvant surgical specimens. Studies assessed various combinations of immunohistochemical markers including ER, PR, and HER2, with immunohistochemical subtype conversion being the primary endpoint of interest. Follow-up duration, when reported, ranged from 36 to 120 months, ensuring adequate time for survival outcome assessment. Detailed characteristics of all included studies are presented in Table 1 .

Quality Assessment

Risk of bias was assessed across 24 cohort studies using the ROBINS-I tool. The overall risk of bias was classified as low in 10 studies (41.6%), moderate in 8 studies (33.3%), serious in 5 studies (20.8%), and critical in 1 study (4.1%).

The most frequent concerns were observed in bias due to confounding (Domain 1) and selection of participants (Domain 2), largely attributable to retrospective designs and limited baseline comparability. In contrast, most studies had low risk in outcome measurement (Domain 6) and reporting (Domain 7), reflecting acceptable endpoint definitions and survival analysis methods.

Studies by Al-Saleh et al,[21] Coiro et al,[21] and Rey-Vargas et al[21] were judged to have elevated risks across multiple domains, whereas Antonini et al,[21] He et al,[21] La Cruz et al.,[21] and Peng et al[21] were rated consistently as low risk across all domains. The detailed summary is presented in Supplementary Figure, which provides a visual summary of domain-specific judgments for all included studies using the ROBINS-I tool Supplemental Table 1

Heterogeneity Assessment and Risk of Bias

Statistical heterogeneity was systematically evaluated across all meta-analyses using multiple statistical measures including the I^2 statistic, Cochran's Q test, and Tau² values. Substantial to high heterogeneity was observed in most analyses, with I^2 values ranging from 0% to 96.3% depending on the specific outcome and subgroup analyzed. The highest heterogeneity was observed in the prevalence of subtype conversion ($I^2 = 89.76\%$), reflecting significant variation in institutional practices and patient populations across studies. Moderate heterogeneity was present in OS analyses ($I^2 = 62.0\%$), while some subgroup analyses, particularly for HER2-positive tumors, demonstrated excellent consistency ($I^2 = 0\%$).

Publication bias was systematically assessed using visual inspection of funnel plots and Egger's regression test for all meta-analyses with sufficient studies. The majority of analyses showed no evidence of significant publication bias, with Egger's test P-values $> .05$ for most outcomes. However, potential publication bias was identified

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Table 1 Study Characteristics; NR: Not Reported. AC adriblastin and ciclofosfamide, T taxane, H trastuzumab, P pertuzumab, Carbo carboplatin

Authors	Year	Country	Design	NAC Regimes	Total Patients	Age(years)	Subtypes no-pCR		Patients NAC				IHC Change		Follow-up (months)
									pCR		no-pCR				
					n				n	%	n	%	n	%	
Antonini et al.[37]	2025	Brazil	Retrospective cohort	AC-T	540	55 ± 2.3	Luminal	227	171	31.7	369	68.3	154	41.7	60
				AC-TH			HER-2	75							
				AC-TCarbo			TNBC	42							
							HR/HER2	25							
Uzun et al.[8]	2024	Turkey	Retrospective cohort	AC-T	476	51.5 ± 11.4	Luminal	155	272	57.1	204	42.9	56	27.5	60
				AC-TH			HER-2	12							
				AC-TCarbo			TNBC	42							
							HR/HER2	4							
Pons et. al.[29]	2023	Spain	Retrospective cohort	THP / AC-THP	150	53.4 ± 11.4	Luminal	32	76	50.7	74	49.3	26	35.1	NR
				ACdd – TCarbo			HER-2	8							
				TPembro-AC			TNBC	32							
							HR/HER2	2							
He et al.[24]	2023	China	Retrospective cohort	AC -T	374	-	Luminal	205	80	21.4	294	78.6	27	9.2	60
							HER-2	52							
							TNBC	14							
							HR/HER2	23							
Santos et al.[25]	2023	Brazil	Retrospective cohort	AC-T	-	49.65±10.9	Luminal	48	-	-	86	26	30.2	60	
				TC			HER-2	5							
							TNBC	21							
							HR/HER2	2							
Özdemir et al.[27]	2022	Turkey	Retrospective cohort	NR	142	48	Luminal	77	30	21.1	112	78.9	18	16.1	NR
							HER-2	17							
							TNBC	14							
							HR/HER2	4							
Al-Saleh et al.[14]	2021	Saudi Arabi	Retrospective cohort	AC-T	250	47,0 ± 14.0	Luminal	47	159	63.6	91	36.4	20	22.0	120
				FAC-T			HER-2	38							
				TAC			TNBC	12							
							HR/HER2	4							
Coiro et al.[20]	2021	Italy	Retrospective cohort	NR	394	53.8 ± 13.2	Luminal	102	129	32.7	265	67.3	72	27,2	NR
							HER-2	85							
							TNBC	58							
							HR/HER2	20							

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Table 1 (*continued*)

Authors	Year	Country	Design	NAC Regimes	Total Patients	Age(years)	Subtypes no-pCR		Patients NAC				IHC Change		Follow-up (months)
									pCR		no-pCR				
					n				n	%	n	%	n	%	
Candás et al.[17]	2021	Argentina	Retrospective cohort	AC-T	172	51 ± 11.7	Luminal	82	49	28.5	123	71.5	21	17.1	NR
				AC-THP			HER-2	15							
				TCarb			TNBC	18							
							HR/HER2	8							
Rey-Vargas et. al[10]	2020	Colombia	Retrospective cohort	AC-T	127	-	Luminal	60	49	38.6	78	61.4	12	15.4	NR
				AC-TH			HER-2	6							
				AC-TCarb			TNBC	8							
							HR/HER2	2							
Ding et al.[22]	2020	China	Retrospective cohort	AC-T	614	50 ± 9.2	Luminal	224	132	21.5	482	78.5	82	17.0	60
							HER-2	100							
							TNBC	83							
							HR/HER2	56							
Peng et al.[28]	2019	China	Retrospective cohort	NR	157	48.6 ± 8.2	Luminal	48	45	28.7	112	71.3	30	26.8	NR
							HER-2	38							
							TNBC	19							
							HR/HER2	7							
La Cruz et al[21]	2018	EUA	Retrospective cohort	AC-T	52	52 ± 11.7	Luminal	17	22	42.3	30	57.7	5	16.7	NR
							HER-2	6							
							TNBC	8							
							HR/HER2	-							
Wu et al.[32]	2017	China	Retrospective cohort	NR	-	-	Luminal	342	-	-	572	140	24.5	NR	
							HER-2	-							
							TNBC	-							
							HR/HER2	-							
Yoshida et al.[34]	2017	Japan	Retrospective cohort	AC-T	697	51.7	Luminal	326	119	17.1	578	82.9	44	7.6	60
				AC-TH			HER-2	78							
							TNBC	153							
							HR/HER2	21							
Lim et al.[26]	2016	Korea	Retrospective cohort	AC-T	322	46 ± 8.2	Luminal	156	32	9.9	290	90.1	69	23.8	60
				AC-TH			HER-2	62							
							TNBC	28							
							HR/HER2	20							

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Table 1 (*continued*)

Authors	Year	Country	Design	NAC Regimes	Total Patients	Age(years)	Subtypes no-pCR		Patients NAC				IHC Change		Follow-up (months)
									pCR		no-pCR				
					n				n	%	n	%	n	%	
Gahlaut et al.[23]	2016	United Kingdom	Retrospective cohort	NR	246	47.8 ± 10.3	Luminal	133	67	27.2	179	72.8	20	11.2	NR
							HER-2	17							
							TNBC	-							
							HR/HER2	-							
Zhou et al.[35]	2015	China	Retrospective cohort	AC-T	231	NR	Luminal	52	124	53.7	107	46.3	26	24.3	NR
				TAC			HER-2	32							
							TNBC	13							
							HR/HER2	20							
Chatterjee et al.[18]	2015	India	Retrospective cohort	NR	156	48.0 ± 14.0	Luminal	78	50	32.1	106	67.9	13	12.3	NR
							HER-2	7							
							TNBC	14							
							HR/HER2	7							
Avci et al.[16]	2015	Turkey	Retrospective cohort	AC-T	100	47.8±11.4	Luminal	75	13	13.0	87	87.0	23	26.4	NR
							HER-2	21							
							TNBC	6							
							HR/HER2	-							
Tan et al.[31]	2014	China	Retrospective cohort	FAC	296	46	Luminal	163	29	9.8	267	90.2	48	18.0	36
				AC-T			HER-2	96							
							TNBC	-							
							HR/HER2	33							
Yang et al.[33]	2013	China	Retrospective cohort	NR	122	48.0 ± 14.0	Luminal	79	9	7.4	113	92.6	14	12.4	NR
							HER-2	13							
							TNBC	21							
							HR/HER2	-							
Chen et al.[19]	2012	China	Retrospective cohort	FEC	259	48.0 ± 14.0	Luminal	224	35	13.5	224	86.5	34	15.2	NR
				TCarbo			HER-2	-							
							TNBC	-							
							HR/HER2	-							
Tacca et al.[30]	2007	France	Retrospective cohort	FEC	710	49.5 ± 11.7	Luminal	275	290	40.8	420	59.2	98	23.3	120
				T			HER-2	-							
							TNBC	-							
							HR/HER2	-							

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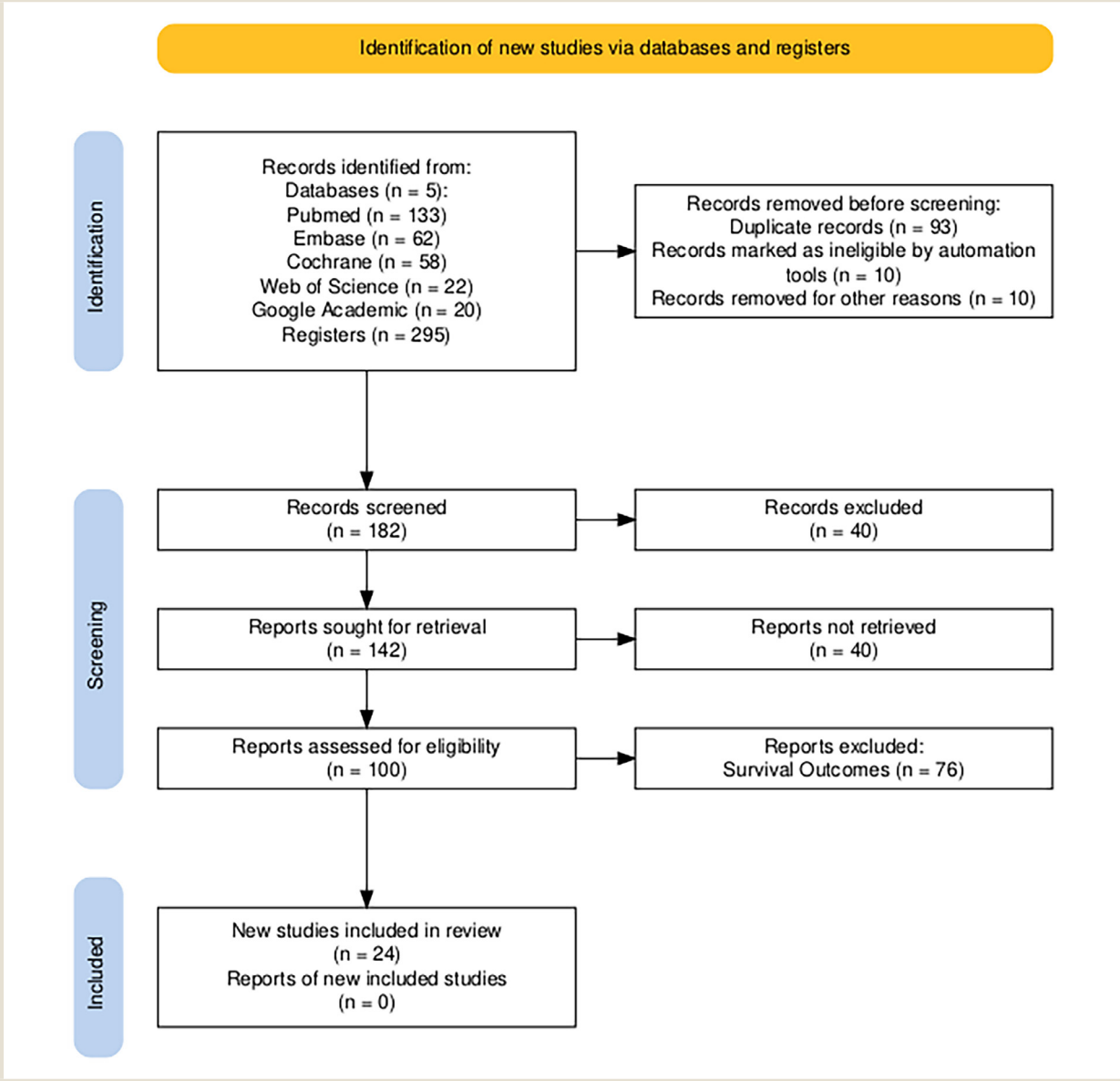
Figure 1 PRISMA 2020 flow diagram illustrating the study selection process. PRISMA checklist available as Supplementary.



in the luminal breast cancer disease-free survival analysis (Egger's test: $P = .044$), suggesting possible overestimation of treatment effects in smaller or less precise studies. Comprehensive funnel plots for all analyses and detailed Egger's test results are presented in and Supplemental Table 1, respectively.

Prevalence of Immunohistochemical Subtype Conversion

The prevalence of molecular subtype conversion among patients with residual disease varied considerably across studies, ranging from 8% to 42%. The pooled analysis revealed an overall prevalence of 0.20 (95% CI, 0.16-0.24), indicating that approximately 20% of patients with residual disease experienced molecular subtype conversion following neoadjuvant chemotherapy. Substantial heterogeneity was observed across studies ($I^2 = 90.18\%$, $P < .0001$), likely reflecting differences in patient populations, treatment protocols, and biomarker assessment methodologies. Individual study results and forest plot analysis are presented in Figure 2.

Prevalence of Subtype Conversion

Luminal Breast Cancer Subtype Conversion. The meta-analysis of luminal BC subtype conversion included 18 studies with 2847 patients with residual disease following NAC. The pooled prevalence of molecular subtype conversion in luminal breast cancer was 0.19 (95% CI, 0.14-0.25), indicating that approximately 19% of luminal breast cancer patients experienced molecular subtype conversion following neoadjuvant chemotherapy. Substan-

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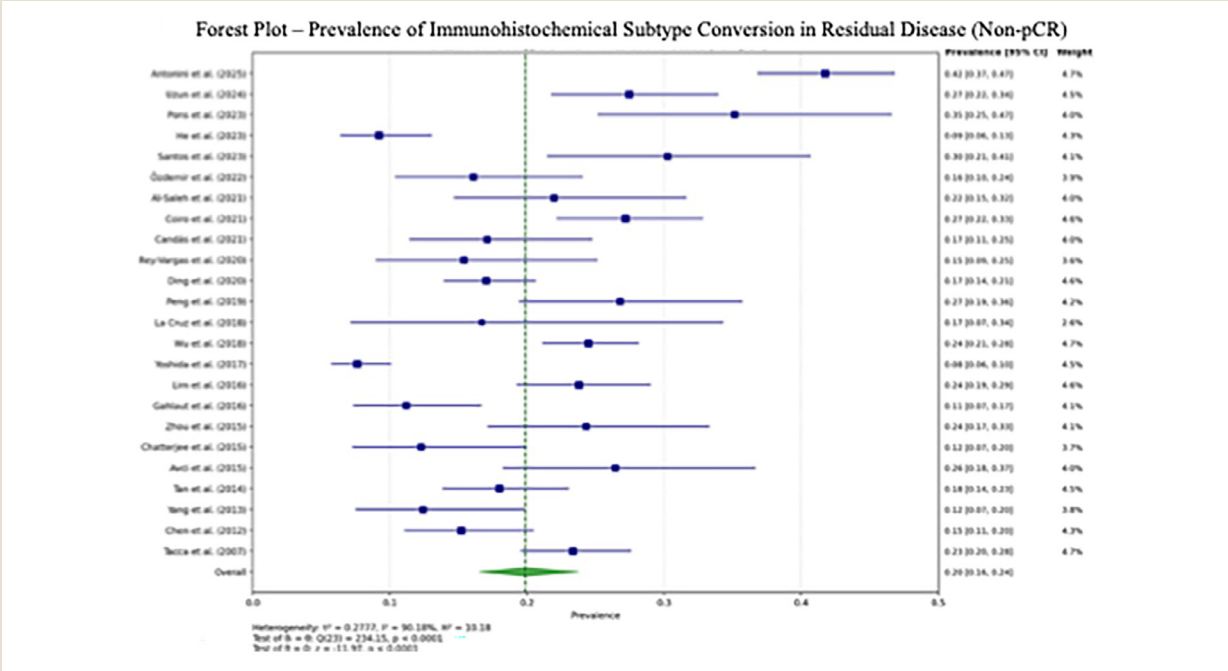
Figure 2 Forest plot of immunohistochemical subtype conversion prevalence in breast cancer patients with residual disease after neoadjuvant chemotherapy.



tial heterogeneity was observed among studies (I^2 = 88.2%, P < .0001), with individual study conversion rates ranging from 7.6% to 35.1%, primarily reflecting the predominant pattern of hormone receptor loss rather than gain, which has significant implications for endocrine therapy eligibility (Figure 3A).

HER2-Positive Breast Cancer Subtype Conversion. For HER2-positive breast cancer, the meta-analysis encompassed 14 studies with 1156 patients with residual disease after NAC. The pooled prevalence of molecular subtype conversion in HER2-positive breast cancer was 0.18 (95% CI, 0.12-0.26), indicating that approximately 18% of HER2-positive patients experienced molecular subtype conversion following neoadjuvant treatment. Moderate heterogeneity was observed across studies (I^2 = 76.8%, P = .0003), with conversion rates ranging from 4.4% to 26.8%, reflecting the complex receptor crosstalk patterns between HER2 and hormone receptors that influence both treatment response and conversion patterns, particularly given that 60%−70% of HER2+ breast cancers co-express hormone receptors (Figure 3B).

Triple-Negative Breast Cancer (TNBC) Subtype Conversion. The analysis of TNBC subtype conversion included 12 studies with 789 patients with residual disease following NAC. The pooled prevalence of molecular subtype conversion in TNBC was 0.15 (95% CI, 0.09-0.23), indicating that approximately 15% of TNBC patients experienced molecular subtype conversion following neoadjuvant chemotherapy. High heterogeneity was observed among TNBC studies (I^2 = 85.4%, P < .0001), with individual study conversion rates ranging from 8.1% to 30.2%, reflecting the inherent biological diversity within the triple-negative classification and the potential for acquisition of hormone receptor expression or HER2 positivity, which creates new therapeutic opportunities for targeted treatment (Figure 3C).

Comparative Analysis of Subtype-Specific Conversion Rates. When comparing conversion rates across molecular subtypes, luminal breast cancers demonstrated the highest prevalence of molecular subtype conversion (19%), followed by HER2-positive tumors (18%), and TNBC (15%), though the confidence intervals overlapped and statistical comparison revealed no significant difference between subtypes (P = .42) (Figure 3). The biological mechanisms underlying these conversion patterns are primarily attributed to tumor heterogeneity and treatment-induced selective pressure, where neoadjuvant chemotherapy preferentially eliminates chemosensitive tumor cell clones while sparing resistant subclones with altered biomarker profiles, resulting in apparent conversion that reflects "unmasking" of pre-existing minor subclones rather than true biological transformation. These findings support routine biomarker re-evaluation in residual disease following neoadjuvant chemotherapy, regardless of molecular subtype, as conversion patterns directly impact adjuvant treatment decision-making and eligibility for targeted therapies, ultimately affecting treatment efficacy and long-term survival outcomes.

Survival Outcomes Meta-Analysis

Overall Population Analysis. The meta-analysis included 6 studies comparing OS between patients with and without biomarker

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Figure 3 Forest plots of immunohistochemical subtype conversion prevalence by molecular subtype in breast cancer patients with residual disease after neoadjuvant chemotherapy. (A) Luminal subtype conversion, (B) HER2-positive subtype conversion, (C) Triple-negative breast cancer (TNBC) Subtype conversion.

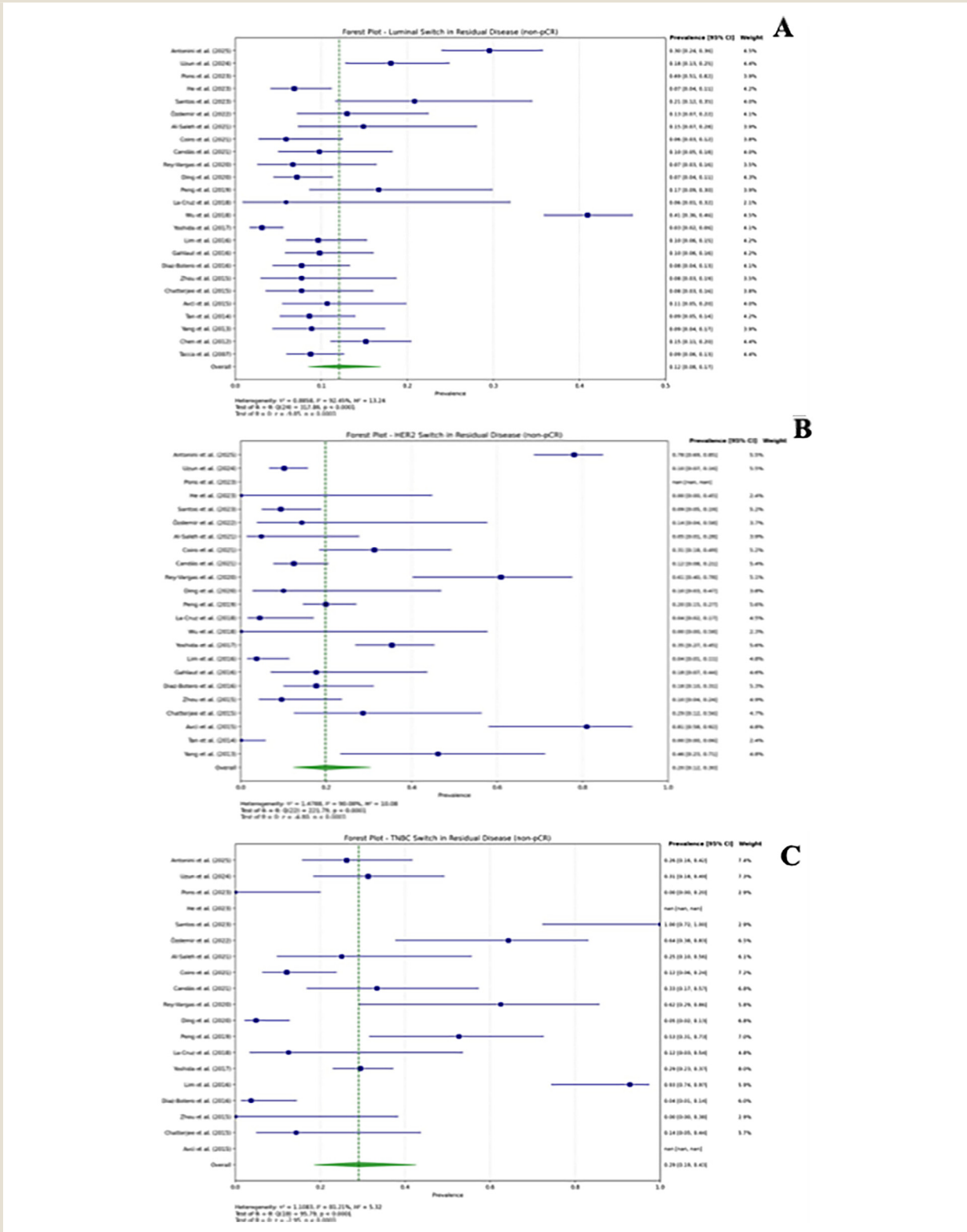

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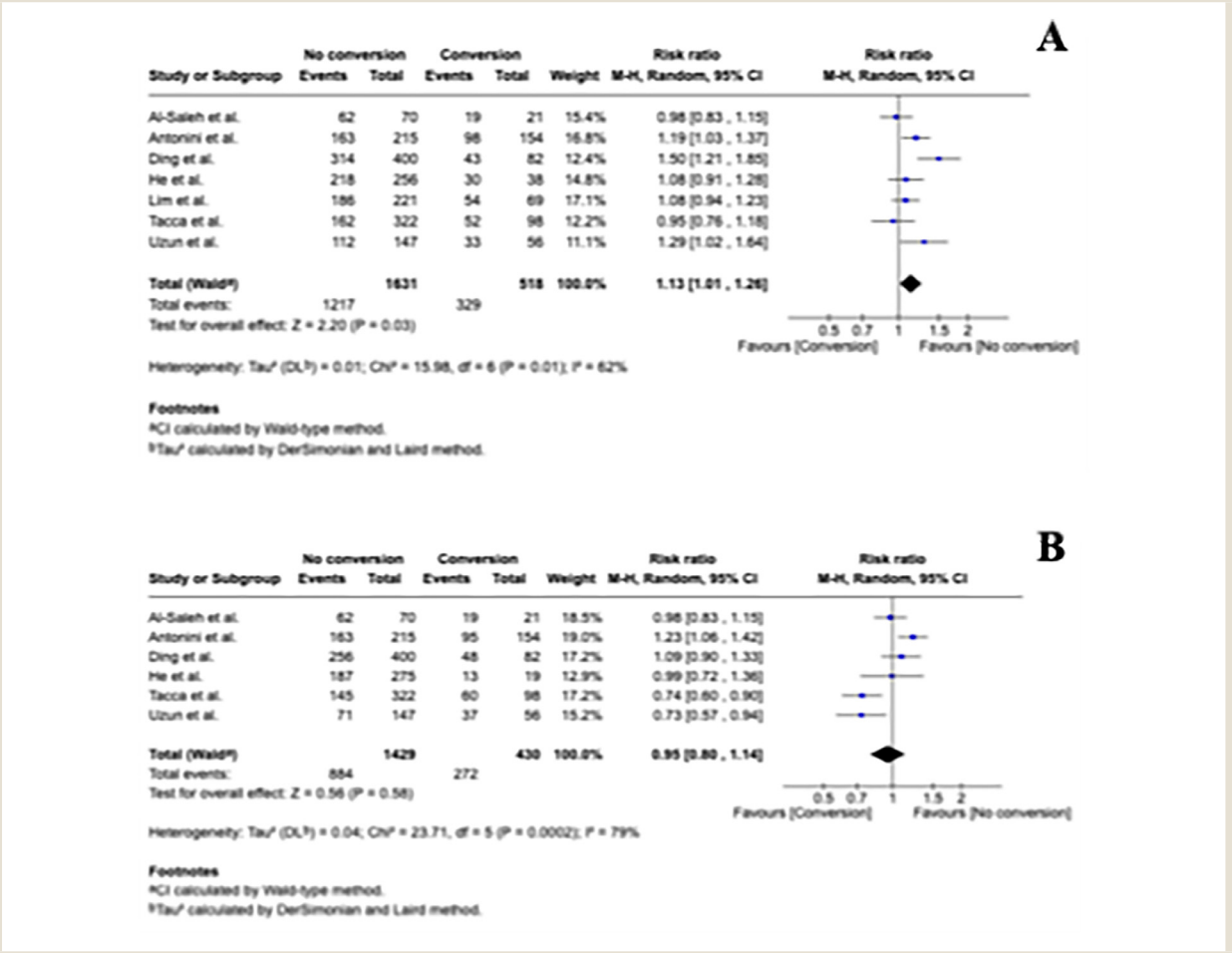
Figure 4 Forest plots of survival outcomes all breast cancer conversion. (A) Overall survival; (B) Disease-free survival (DFS).



conversion. Patients without molecular subtype conversion demonstrated a statistically significant survival advantage with a risk ratio of 1.13 (95% CI, 1.01-1.26, $P = .03$), indicating a 13% higher chance of survival compared to those experiencing biomarker conversion. Moderate heterogeneity was observed ($I^2 = 62.0\%$, $Tau^2 = 0.01$, $P = .01$), and Egger's test showed no evidence of publication bias (intercept = 0.35, $P = .33$).

The analysis of DFS encompassed 6 studies and revealed no statistically significant difference between groups (RR: 0.95, 95% CI, 0.80-1.14, $P = .58$). Substantial heterogeneity was observed ($I^2 = 79.0\%$, $Tau^2 = 0.04$, $P = .0002$), and Egger's test indicated no publication bias (intercept = −0.46, $P = .25$), though the negative intercept suggested a slight tendency toward smaller effects in less precise studies. Figure 4 presents the forest plots of survival outcomes associated with biomarker conversion in breast cancer.

When evaluating the prognostic implications of biomarker conversion, the majority of studies reported survival outcomes specifically for loss of receptor expression (positive to negative), which we therefore adopted as the primary event in our pooled analysis. Loss of ER or PR after NAC was consistently associated with decreased DFS and OS, especially in luminal tumors. HER2 loss, although less frequent, was also linked to poorer survival in several cohorts. Conversely, biomarker gain (negative to positive) was rarely reported with sufficient survival data and could not be included in the quantitative synthesis. As a result, our survival analyses should be interpreted predominantly as reflecting the adverse prognostic impact of biomarker loss.

Molecular Subtype-Specific Analysis.

Luminal Breast Cancer. Among luminal breast cancer patients, those without biomarker conversion demonstrated a 19% higher chance of OS compared to those with conversion (RR: 1.19, 95% CI, 1.01-1.41, $P = .04$). This represents the most pronounced survival benefit among all molecular subtypes. Moderate heterogeneity was observed ($I^2 = 42\%$, $P = .10$), and Egger's test approached statistical significance (intercept = 0.86, $P = .068$), suggesting possible publication bias. For DFS, a non-significant trend favored patients without conversion (RR: 1.15, 95% CI, 0.98-1.35, $P = .09$) with no observed heterogeneity between studies

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($I^2 = 0\%$, $P = .32$). However, Egger's test revealed a statistically significant publication bias (intercept = 0.97, $P = .044$).

HER2-positive Breast Cancer. Analysis of HER2-positive tumors showed no statistically significant difference in OS between patients with and without biomarker conversion (RR: 1.11, 95% CI, 0.95-1.31, $P = .19$). Notably, there was no heterogeneity was observed ($I^2 = 0\%$, $P = .66$), indicating highly consistent results, and Egger's test showed no publication bias (intercept = 0.052, $P = .77$). For DFS, a non-significant trend favored patients with biomarker conversion (RR: 0.79, 95% CI, 0.53-1.18, $P = .25$), though substantial heterogeneity was present ($I^2 = 72\%$, $P = .01$). Egger's test was non-significant (intercept = −0.99, $P = .065$).

Triple Negative Breast Cancer. Among TNBC patients, no significant difference in overall survival was observed between those with and without biomarker conversion (RR: 0.98, 95% CI, 0.84-1.16, $P = .85$), with the risk ratio close to 1.0 indicating essentially equivalent outcomes. Minimal heterogeneity was observed ($I^2 = 12\%$, $P = .33$), and Egger's test showed no publication bias (intercept = −0.23, $P = .43$). Disease-free survival analysis for triple-negative breast cancer patients lacked sufficient data for robust meta-analysis, with limited studies providing adequate information for this specific subgroup outcome. The available data suggested no clear difference between groups, and no publication bias was detected, as illustrated in . Figure 5 presents forest plots illustrating survival outcomes stratified by molecular subtype conversion.

Discussion

To our knowledge, this is the first systematic review and meta-analysis to evaluate the prevalence, patterns, and prognostic implications of IHC biomarker conversion following NAC in BC. Our findings confirm that approximately 20% of patients with residual disease experience IHC subtype conversion after NAC, and that biomarker stability is associated with superior OS, particularly in luminal tumors.

Patients without biomarker conversion had a 13% higher chance of OS (RR: 1.13; 95% CI, 1.01-1.26; $P = .03$), with the effect being more pronounced in luminal breast cancer (RR: 1.19; 95% CI, 1.01-1.41; $P = .04$). This supports the clinical relevance of reassessing ER, PR, and HER2 expression on surgical specimens after NAC, as changes can directly influence adjuvant therapy decisions. Prior studies have similarly shown that biomarker reassessment modifies treatment strategies in up to 30% of patients.[38]

Among conversion patterns, hormone receptor loss emerged as the most clinically significant. Jin et al identified HR loss post-NAC as an independent predictor of worse disease-free and overall survival.[39] Conversely, gain of ER or PR expression may enable endocrine therapy that was previously contraindicated, highlighting the need for systematic retesting. Regarding HER2, although our meta-analysis did not identify a significant OS difference, HER2 conversion, particularly from HER2-positive to HER2-low, has therapeutic implications in the era of antibody-drug conjugates.[40] This reinforces the need for accurate HER2 reassessment after NAC.

Beyond reporting pooled conversion rates, our findings highlight a critical and unresolved clinical dilemma: how should treatment strategies be adapted when biomarker profiles change after NAC? The current literature offers no consensus on the best approach for patients who lose hormone receptor expression, lose HER2 positivity, or gain HER2 expression in residual disease. These scenarios carry immediate therapeutic consequences—potential withdrawal or initiation of endocrine or HER2-targeted therapies—yet are managed heterogeneously in daily practice. Importantly, the survival disadvantage associated with receptor loss underscores the urgency of clarifying whether continuing standard adjuvant therapy remains beneficial in these patients. Conversely, HER2 gain represents a therapeutic opportunity that might not be systematically exploited.

By consolidating evidence across nearly 6000 patients, our study provides the strongest signal to date that biomarker conversion is not only frequent but also clinically meaningful. More importantly, it exposes the absence of high-level evidence to guide adjuvant treatment adaptation in this setting. In this sense, our meta-analysis should not be interpreted as redundant but rather as a stimulus for designing prospective studies specifically aimed at evaluating treatment strategies in patients with biomarker conversion, ultimately moving toward evidence-based recommendations for this complex group.

Biomarker conversion likely reflects tumor heterogeneity and the selective pressure exerted by chemotherapy. NAC can eliminate chemosensitive clones while sparing resistant subpopulations with different biomarker profiles, resulting in an "unmasking" of pre-existing subclones rather than true phenotypic transformation.[3,31] Reductions in PR and Ki-67 after NAC, as reported by Peng et al, may indicate a shift toward more resistant tumor phenotypes, affecting prognosis and treatment planning[28] Supplemental Figures 1 and 2

Our results align with previous cohorts, such as He et al and Özdemir et al, which reported the prognostic significance of receptor changes.[24,27] However, studies like Yoshida et al[34] found no association between HER2 conversion and survival, suggesting subtype-specific differences. By pooling data across 24 studies, we provide stronger evidence that biomarker stability is a favorable prognostic factor, overcoming the limitations of individual, underpowered studies Supplemental Figures 3 and 4

Technical variables such as preanalytical tissue handling, IHC scoring variability, and inter-observer interpretation may contribute to apparent biomarker discrepancies. Nevertheless, prior studies indicate that these artifacts are less frequent than true biological changes.[41] Our meta-analysis was limited to studies with paired pre- and post-NAC specimens to minimize bias Supplemental Figures 5 and 6

Several limitations should be considered. Most included studies were retrospective, with inherent risks of selection bias and heterogeneous treatment protocols. High statistical heterogeneity was observed in conversion prevalence ($I^2 = 90.2\%$), likely due to variability in IHC thresholds, NAC regimens, and geographic differences (eg, 58% of studies originated from Asia). Although random-effects models were applied, residual confounding cannot be excluded. Furthermore, data from modern therapeutic contexts—such as immunotherapy[42-44]—were limited, and none of the included studies specifically assessed the HER2-low classification Supplemental Figures 7 and 8

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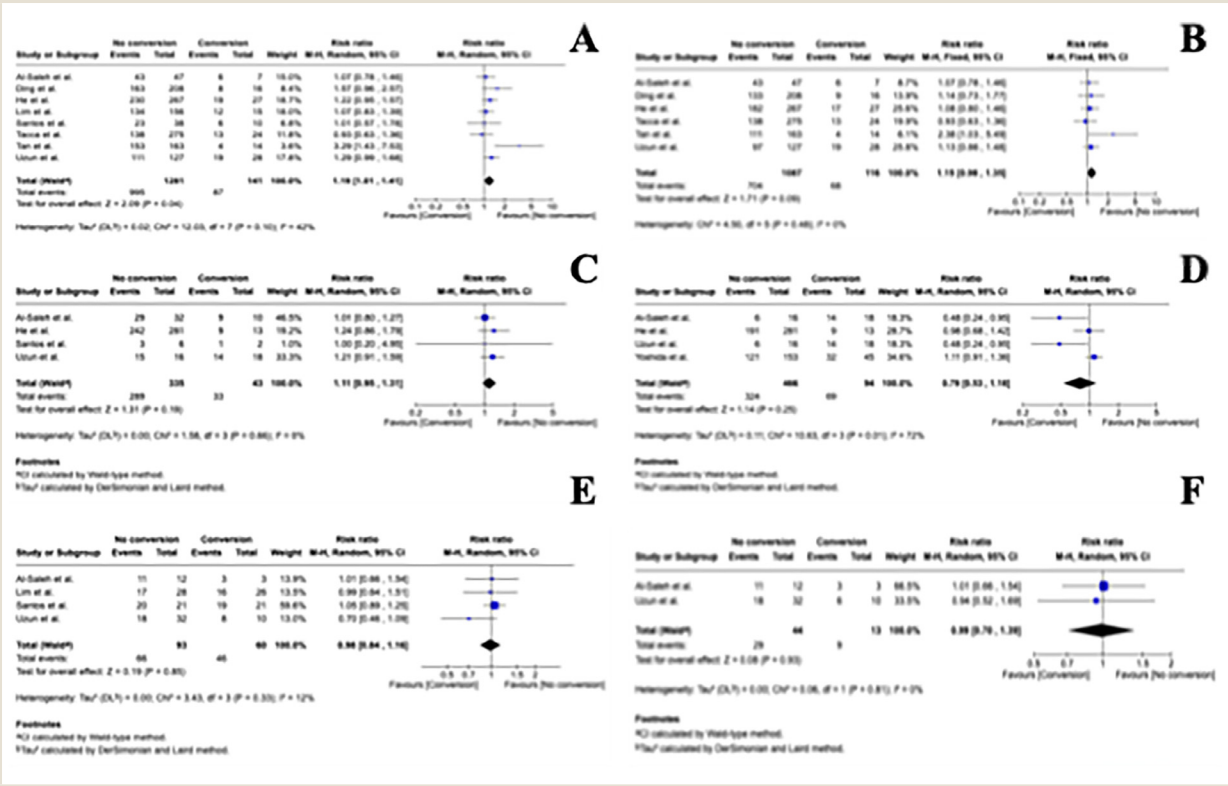
Figure 5 Forest plots of survival outcomes by subtype conversion, A. Overall survival (OS) – Luminal subtype, B. Disease-free survival (DFS) – Luminal subtype, C. Overall survival (OS) – HER2-positive subtype, D. Disease-free survival (DFS) – HER2-positive subtype, E. Overall survival (OS) – Triple negative breast cancer (TNBC) subtype, Disease-free survival (DFS) – Triple negative breast cancer (TNBC) subtype.



An additional limitation of our study is the geographic distribution of the included cohorts. More than half of the studies (58%) originated from Asian institutions, particularly China, Korea, and Japan. While this reflects regions with high research productivity in neoadjuvant settings, it may restrict the external validity of our pooled estimates. Differences in genetic background, tumor biology, screening practices, and access to systemic therapies between Asian and Western populations could influence both the likelihood of biomarker conversion and its prognostic implications. Therefore, the generalizability of our findings to non-Asian populations should be interpreted with caution, and validation in large prospective cohorts from diverse geographic regions remains essential Supplemental Figure 9.

Another critical limitation is the lack of information on how adjuvant treatment decisions were adapted following biomarker conversion. For luminal tumors, ER loss after NAC raises uncertainty regarding the continuation of endocrine therapy: while some guidelines support its maintenance, many clinicians choose to discontinue it, assuming loss of endocrine sensitivity. This highlights the unresolved question of whether micrometastatic disease remains hormone-responsive despite receptor loss in the residual tumor. A similar dilemma applies to HER2-positive tumors that become HER2-negative, where practice varies regarding continuation of HER2-targeted therapy. Importantly, none of the included studies provided detailed data on HER2-low tumors, a category that has gained therapeutic relevance with agents such as trastuzumab-deruxtecan. In ER+/HER2+ tumors, ER loss seems to have a less detrimental impact compared to luminal ER+/HER2– tumors that convert to triple negative, underscoring subtype-specific differences. Conversely, the clinical significance of biomarker gain (ER, PR, or HER2) remains unclear, as it is not established whether adjuvant endocrine or HER2-targeted therapies act effectively on the residual positive clone or on heterogeneous micrometastatic disease.

These results highlight the need to integrate biomarker reassessment into clinical workflows. Current ASCO guidelines recommend pathological complete response as a surrogate endpoint but do not mandate IHC retesting post-NAC.[45] Our findings support routine reassessment of ER, PR, and HER2 in residual disease to identify patients who may benefit from therapy adjustments, particularly those converting to triple-negative phenotype, which is associated with worse prognosis.[39]

Future studies should prioritize large prospective cohorts with standardized IHC protocols to validate the prognostic implications of biomarker conversion. It is also essential to investigate whether modifying adjuvant treatments based on post-NAC receptor changes can improve survival outcomes. Additionally, integrat-

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ing complementary biomarkers such as tumor-infiltrating lymphocytes (TILs)[46] and other molecular predictors may enhance risk stratification and allow for more personalized treatment strategies.

Conclusion

This systematic review and meta-analysis demonstrate that immunohistochemical biomarker conversion occurs in approximately 20% of breast cancer patients with residual disease following neoadjuvant chemotherapy. Notably, biomarker stability—particularly in luminal subtypes—is associated with significantly improved overall survival, reinforcing its prognostic relevance. These findings highlight the clinical importance of reassessing ER, PR, and HER2 status in surgical specimens after NAC. Changes in biomarker expression can alter treatment eligibility, guide adjuvant therapy decisions, and ultimately influence patient outcomes. In the era of personalized oncology, post-NAC IHC profiling should be integrated into routine clinical workflows to ensure accurate risk stratification and optimized therapeutic planning.

Further prospective studies are needed to confirm whether adapting adjuvant treatment based on post-NAC biomarker changes can improve survival. Nonetheless, our results support immediate implementation of biomarker reassessment as a standard component of care in patients undergoing neoadjuvant chemotherapy for breast cancer.

Clinical Practice Points

- Immunohistochemical (IHC) biomarker conversion occurs in approximately 20% of breast cancer patients with residual disease after neoadjuvant chemotherapy (NAC).
- Loss of hormone receptor expression post-NAC is associated with poorer prognosis, particularly in luminal tumors.
- HER2 gain after NAC is linked to higher rates of pathological complete response and may open new therapeutic opportunities.
- Routine reassessment of ER, PR, and HER2 in surgical specimens after NAC can influence adjuvant therapy decisions and improve individualized treatment planning.

Ethics Approval and Consent to Participate

This study was registered in the PROSPERO database (registration number CRD42023456585) and conducted in accordance with the ethical principles of the Declaration of Helsinki. As this was a systematic review and meta-analysis of previously published data, approval from an ethics committee and informed consent were not required.

Consent for Publication

Not applicable.

Key Findings

- Approximately one in five breast cancer patients with residual disease after neoadjuvant chemotherapy (NAC) experience immunohistochemical (IHC) biomarker conversion.
- Hormone receptor loss after NAC is the most frequent and clinically relevant change, correlating with worse overall survival, especially in luminal tumors.
- HER2 gain post-NAC is associated with higher pathological complete response rates, suggesting increased treatment sensitivity.
- Routine reassessment of ER, PR, and HER2 in surgical specimens after NAC can modify adjuvant treatment strategies in up to 30% of patients.
- Incorporating post-NAC IHC re-evaluation into clinical workflows may improve patient selection for targeted and endocrine therapies, ultimately impacting long-term outcomes.

Disclosure

AM received honoraria from Roche, AstraZeneca, Novartis, Exact Siences, and Eli Lilly. The other authors have no conflicts of interest to declare.

CRediT authorship contribution statement

Marcelo Antonini: Writing – original draft, Methodology, Data curation, Conceptualization. **André Mattar:** Writing – review & editing, Data curation, Conceptualization. **Gil Facina:** Writing – review & editing, Investigation. **Francisco Pimentel Cavalcante:** Writing – review & editing, Formal analysis. **Felipe Zerwes:** Writing – review & editing, Methodology. **Fabricio Palermo Brenelli:** Writing – review & editing. **Antônio Luis Frasson:** Writing – review & editing, Supervision. **Eduardo Camargo Millen:** Writing – review & editing, Formal analysis. **Rodrigo Caires Campos:** Writing – review & editing. **Letícia Xavier Félix:** Writing – review & editing, Formal analysis. **Juliana Calado Vieira:** Writing – review & editing. **Marina Diógenes Teixeira:** Writing – review & editing, Investigation. **Marcelo Madeira:** Writing – review & editing, Methodology. **Rogério Fenile:** Writing – review & editing, Investigation. **Henrique Lima Couto:** Writing – review & editing, Data curation. **Leonardo Ribeiro Soares:** Writing – review & editing, Data curation. **Ruffo de Freitas Junior:** Writing – review & editing, Data curation. **Renata Arakelian:** Writing – review & editing, Writing – original draft. **Renata Montarroyos Leite:** Writing – review & editing. **Vitoria Rassi Mahamed Rocha:** Writing – review & editing, Writing – original draft. **Luiz Henrique Gebrim:** Writing – review & editing, Supervision, Conceptualization.

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Supplementary Material

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Table

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Page 1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Page 3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Page 7
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Page 8
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Page 9
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Page 10
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Page 10
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Page 11
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Page 11
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (eg for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Page 11
	10b	List and define all other variables for which data were sought (eg participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Page 11
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Page 11
Effect measures	12	Specify for each outcome the effect measure(s) (eg risk ratio, mean difference) used in the synthesis or presentation of results.	Page 11
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (eg tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Page 10
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Page 11
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Page 11
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Page 11
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (eg subgroup analysis, meta-regression).	Page 11

(*continued on next page*)

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Table (*continued*)

Section and Topic	Item #	Checklist item	Location where item is reported
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Page 11
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Page 12
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Page 12
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Page 13
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Page 14
Study characteristics	17	Cite each included study and present its characteristics.	Page 15
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Page 17
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (eg confidence/credible interval), ideally using structured tables or plots.	Page 19
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Page 19
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (eg confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Page 18-19
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Page 19
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Page 20
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Page 27-28
	23b	Discuss any limitations of the evidence included in the review.	Page 27-28
	23c	Discuss any limitations of the review processes used.	Page 27-28
	23d	Discuss implications of the results for practice, policy, and future research.	Page 27-28
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Page 11
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Page 11
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Page 29-30
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Page 29-30
Competing interests	26	Declare any competing interests of review authors.	Page 29-30
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Page 29-30

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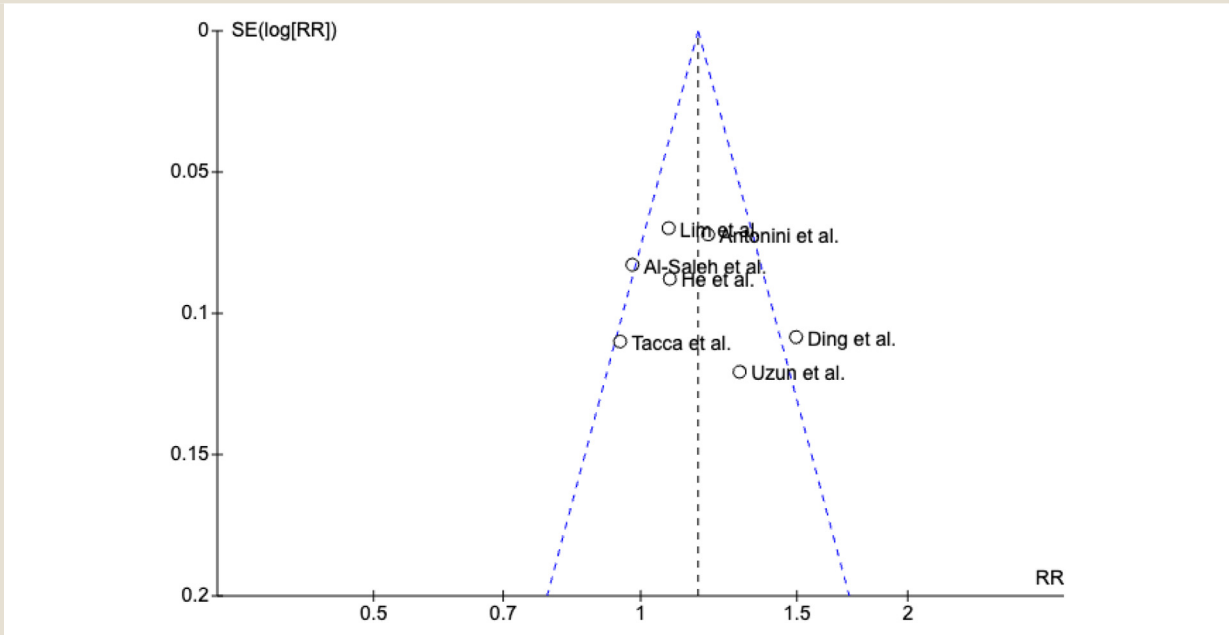
Supplemental Table 1 Egger's Test Results for Publication Bias Assessment Across All Meta-Analyses

Population Analysis	Outcome	Number of Studies	Intercept	*P*-value	Interpretation
All	OS	6	0.35	0.33	No evidence of publication bias
	DFS	6	−0.46	0.25	No evidence of publication bias
Luminal	OS	6	0.86	0.068	Possible asymmetry, approaching significance
	DFS	6	0.97	0.044*	Statistically significant publication bias
HER2-positive	OS	4	0.052	0.77	No evidence of publication bias
	DFS	4	−0.99	0.065	No significant asymmetry
TNBC	OS	4	−0.23	0.43	No evidence of publication bias
	DFS	2	−0.25	NC†	Insufficient studies for reliable assessment

Legend: Abbreviations: TNBC, Triple-Negative Breast Cancer; NC, Not Calculable.
Notes: *Statistically significant at $P < .05$, †Egger's test could not be calculated due to insufficient number of studies ($n = 2$), Intercept values represent the bias coefficient from Egger's linear regression test, Positive intercepts suggest overestimation of effects in smaller studies, Negative intercepts suggest underestimation of effects in smaller studies, *P*-values < 0.05 indicate statistically significant evidence of publication bias.
Interpretation: The majority of meta-analyses showed no evidence of publication bias based on Egger's test. Only the disease-free survival analysis for the luminal subtype demonstrated statistically significant publication bias ($P = .044$), suggesting potential overestimation of treatment effects in smaller or less precise studies. The overall survival analysis for the luminal subtype approached significance ($P = .068$), indicating possible but not definitive evidence of asymmetry. All remaining analyses, including the overall population, HER2-positive and TNBC subtypes, demonstrated no evidence of publication bias, reinforcing the reliability of the meta-analysis.

Supplemental Figure 1 **Funnel plot for publication bias assessment in Overall survival (OS) Meta-analysis comparing patients with and without biomarker conversion after neoadjuvant chemotherapy. Funnel plot displaying the relationship between study precision (standard error of the log risk ratio, SE(log[RR])) on the y-axis and effect size (risk ratio, RR) on the x-axis for overall survival outcomes. Each circle represents an individual study included in the meta-analysis. The vertical dashed line represents the pooled effect estimate (RR = 1.13). The diagonal dashed lines represent the 95% confidence interval boundaries for the expected distribution of studies in the absence of publication bias. Studies are expected to be symmetrically distributed around the pooled estimate if publication bias is absent. Egger's regression test: intercept = 0.35, $P = .33$, indicating no statistically significant evidence of publication bias.**



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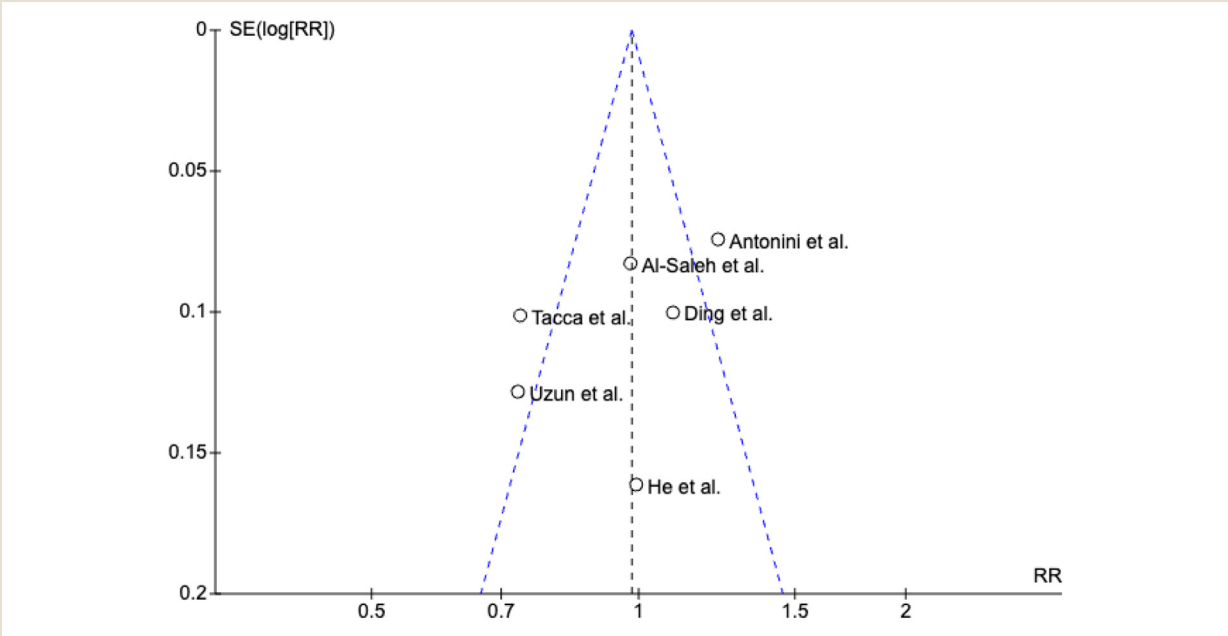
Supplemental Figure 2 Funnel plot for publication bias assessment in Disease-free survival (DFS) Meta-analysis comparing patients with and without biomarker conversion after neoadjuvant chemotherapy. Funnel plot displaying the relationship between study precision (standard error of the log risk ratio, SE(log[RR])) on the y-axis and effect size (risk ratio, RR) on the x-axis for disease-free survival outcomes. Each circle represents an individual study included in the meta-analysis. The vertical dashed line represents the pooled effect estimate (RR = 0.95). The diagonal dashed lines represent the 95% confidence interval boundaries for the expected distribution of studies in the absence of publication bias. Studies are expected to be symmetrically distributed around the pooled estimate if no publication bias is present. Egger's regression test: intercept = −0.46, $P = .25$, indicating no statistically significant evidence of publication bias, though the negative intercept suggests a slight tendency toward smaller effects in less precise studies.



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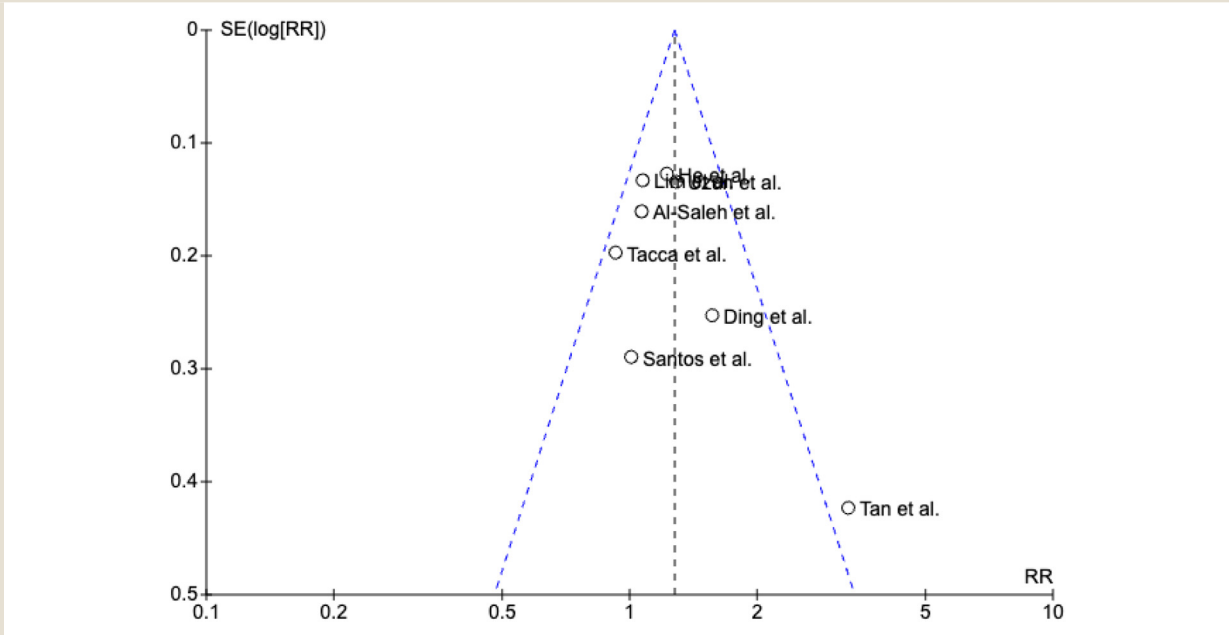
Supplemental Figure 3 Funnel plot for publication bias assessment in Overall survival (OS) Meta-analysis for Luminal breast cancer patients comparing those with and without biomarker conversion after neoadjuvant chemotherapy. Funnel plot displaying the relationship between study precision (standard error of the log risk ratio, SE(log[RR])) on the y-axis and effect size (risk ratio, RR) on the x-axis for overall survival outcomes specifically in the luminal breast cancer subtype. Each circle represents an individual study included in the meta-analysis. The vertical dashed line represents the pooled effect estimate (RR = 1.19). The diagonal dashed lines represent the 95% confidence interval boundaries for the expected distribution of studies in the absence of publication bias. Studies are expected to be symmetrically distributed around the pooled estimate if no publication bias is present. Egger's regression test: intercept = 0.86, $P = .068$, approaching statistical significance and suggesting possible asymmetry. The positive intercept indicates that studies with lower precision tend to report larger effects, suggesting potential publication bias in the luminal subtype analysis.



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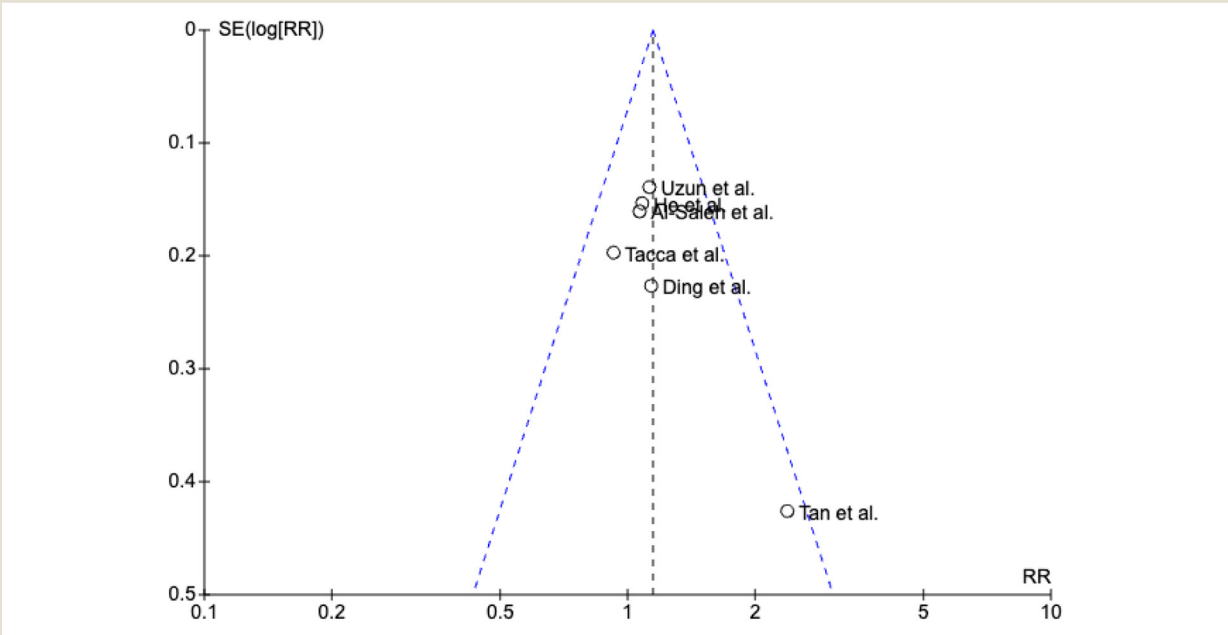
Supplemental Figure 4 Funnel plot for publication bias assessment in Disease-free survival (DFS) Meta-analysis for Luminal breast cancer patients comparing those with and without biomarker conversion after neoadjuvant chemotherapy. Funnel plot displaying the relationship between study precision (standard error of the log risk ratio, SE(log[RR])) on the y-axis and effect size (risk ratio, RR) on the x-axis for disease-free survival outcomes specifically in the luminal breast cancer subtype. Each circle represents an individual study included in the meta-analysis. The vertical dashed line represents the pooled effect estimate (RR = 1.15). The diagonal dashed lines represent the 95% confidence interval boundaries for the expected distribution of studies in the absence of publication bias. Studies are expected to be symmetrically distributed around the pooled estimate if no publication bias is present. Egger's regression test: intercept = 0.97, $P = .044$, indicating statistically significant asymmetry and evidence of publication bias. The positive intercept suggests that studies with lower precision tend to report larger effects, indicating potential overestimation of treatment effects in smaller or less precise studies for luminal DFS outcomes.



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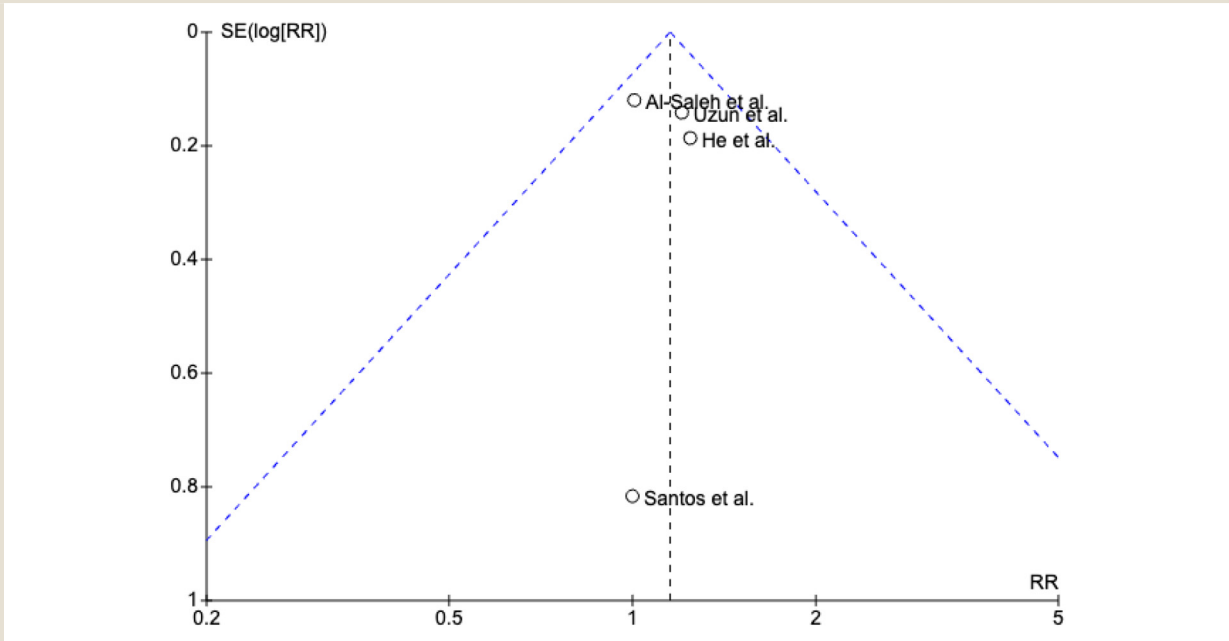
Supplemental Figure 5 Funnel plot for publication bias assessment in Overall survival (OS) Meta-analysis for HER2-positive breast cancer patients comparing those with and without biomarker conversion after neoadjuvant chemotherapy. Funnel plot displaying the relationship between study precision (standard error of log risk ratio, SE(log[RR])) on the y-axis and effect size (risk ratio, RR) on the x-axis for overall survival outcomes specifically in the HER2-positive breast cancer subtype. Each circle represents an individual study included in the meta-analysis. The vertical dashed line represents the pooled effect estimate (RR = 1.11). The diagonal dashed lines represent the 95% confidence interval boundaries for the expected distribution of studies in the absence of publication bias. Studies are expected to be symmetrically distributed around the pooled estimate if no publication bias is present. Egger's regression test: intercept = 0.052, $P = .77$, indicating no evidence of publication bias. The intercept is very close to zero and non-significant, suggesting excellent symmetry in the distribution of studies and highly reliable results for HER2-positive overall survival outcomes.



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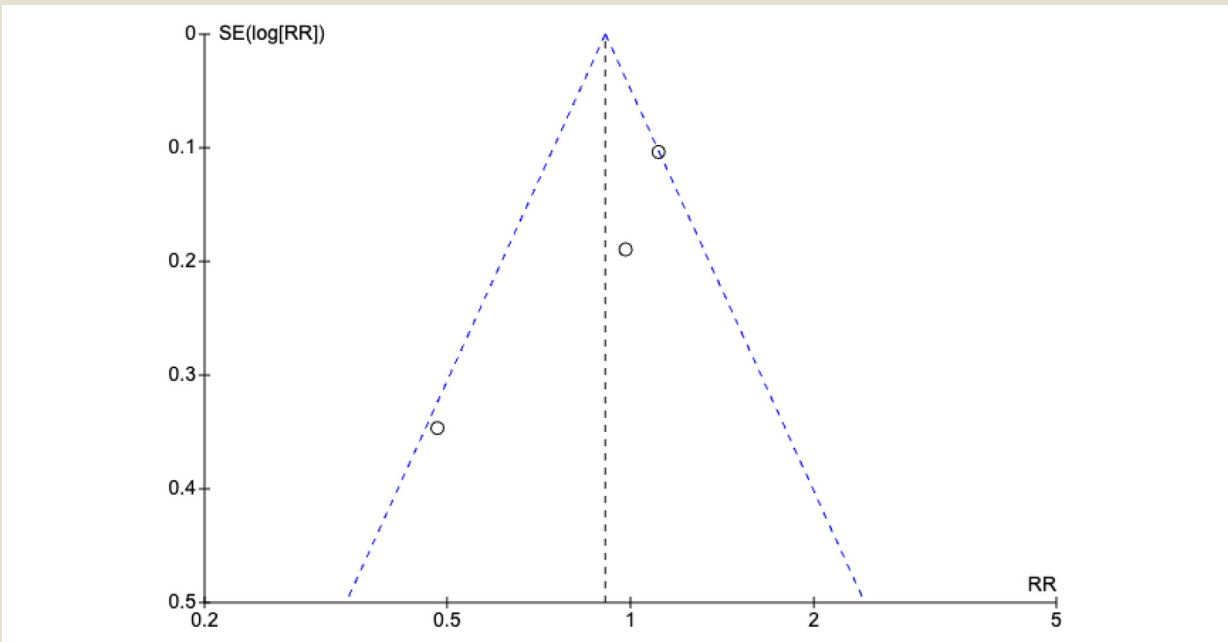
Supplemental Figure 6 Funnel plot for publication bias assessment in Disease-free survival (DFS) Meta-analysis for HER2-positive breast cancer patients comparing those with and without biomarker conversion after neoadjuvant chemotherapy. Funnel plot displaying the relationship between study precision (standard error of log risk ratio, SE(log[RR])) on the y-axis and effect size (risk ratio, RR) on the x-axis for disease-free survival outcomes specifically in the HER2-positive breast cancer subtype. Each circle represents an individual study included in the meta-analysis. The vertical dashed line represents the pooled effect estimate (RR = 0.79). The diagonal dashed lines represent the 95% confidence interval boundaries for the expected distribution of studies in the absence of publication bias. Studies are expected to be symmetrically distributed around the pooled estimate if no publication bias is present. Egger's regression test: intercept = −0.99, $P = .065$, approaching but not reaching statistical significance. The negative intercept suggests a tendency toward smaller effects in less precise studies, but this asymmetry is not statistically significant, indicating no clear evidence of publication bias for HER2-positive DFS outcomes.



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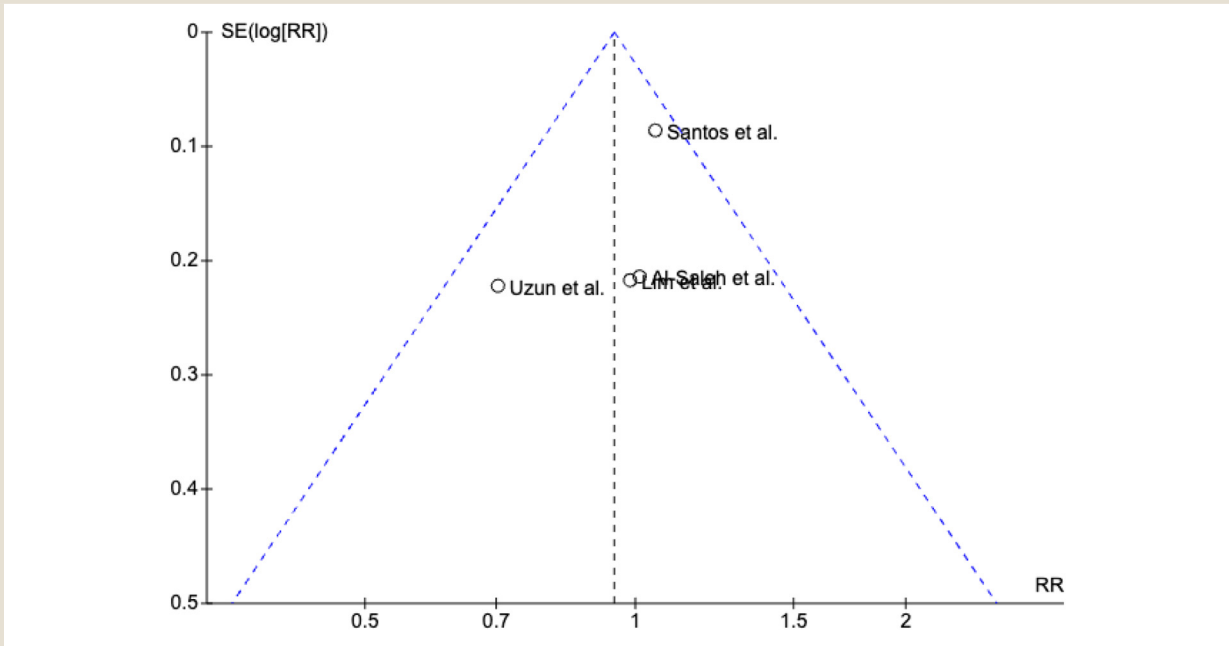
Supplemental Figure 7 Funnel plot for publication bias assessment in Overall survival (OS) Meta-analysis for Triple-negative breast cancer (TNBC) patients comparing those with and without biomarker conversion after neoadjuvant chemotherapy. Funnel plot displaying the relationship between study precision (standard error of log risk ratio, SE(log[RR])) on the y-axis and effect size (risk ratio, RR) on the x-axis for overall survival outcomes specifically in the triple-negative breast cancer (TNBC) subtype. Each circle represents an individual study included in the meta-analysis. The vertical dashed line represents the pooled effect estimate (RR = 0.98). The diagonal dashed lines represent the 95% confidence interval boundaries for the expected distribution of studies in the absence of publication bias. Studies are expected to be symmetrically distributed around the pooled estimate if no publication bias is present. Egger's regression test: intercept = −0.23, $P = .43$, indicating no evidence of publication bias. The intercept is close to zero and non-significant, suggesting good symmetry in the distribution of studies and reliable results for TNBC overall survival outcomes.



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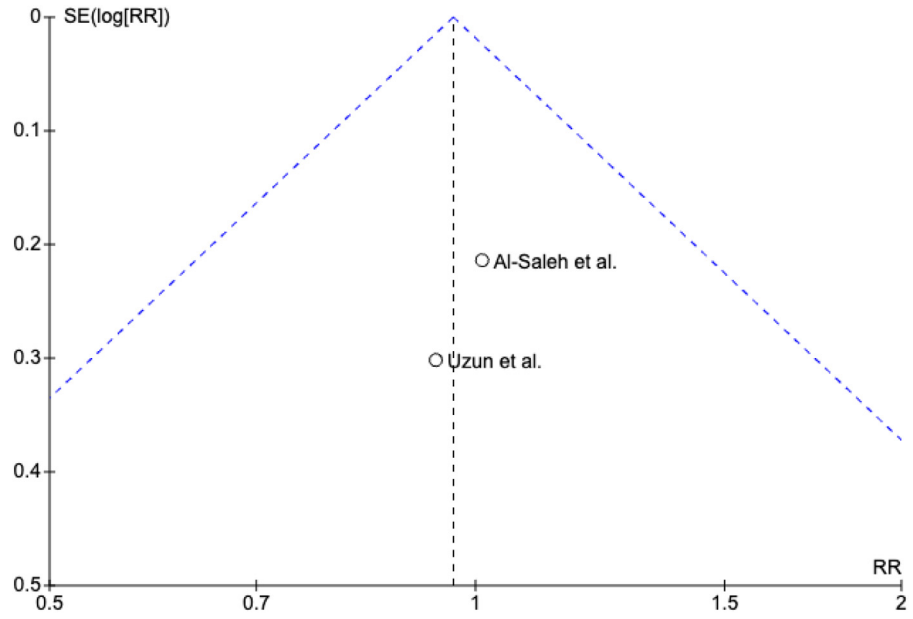
Supplemental Figure 8 Funnel Plot for Publication Bias Assessment in Disease-Free Survival (DFS) Meta-Analysis for Triple-Negative Breast Cancer (TNBC) Patients Comparing Those With and Without Biomarker Conversion After Neoadjuvant Chemotherapy. Funnel plot displaying the relationship between study precision (standard error of log risk ratio, SE(log[RR])) on the y-axis and effect size (risk ratio, RR) on the x-axis for disease-free survival outcomes specifically in the triple-negative breast cancer (TNBC) subtype. Each circle represents an individual study included in the meta-analysis. The vertical dashed line represents the pooled effect estimate (RR = 0.99). The diagonal dashed lines represent the 95% confidence interval boundaries for the expected distribution of studies in the absence of publication bias. Studies are expected to be symmetrically distributed around the pooled estimate if no publication bias exists. Egger's regression test: intercept = −0.25, *P* = not calculable, as the regression generated a statistical error due to the limited number of studies (only two studies available for this analysis). The small number of studies limits the ability to assess publication bias reliably for TNBC disease-free survival outcomes.



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Supplemental Figure 9

Risk of bias assessment across included studies using the ROBINS-I tool. The figure summarizes the risk of bias judgments across seven domains for each included retrospective cohort study: D1 (bias due to confounding), D2 (bias due to selection of participants), D3 (bias in classification of interventions), D4 (bias due to deviations from intended interventions), D5 (bias due to missing data), D6 (bias in measurement of outcomes), and D7 (bias in selection of the reported result). Judgments are color-coded as follows: green = low risk, yellow = moderate risk, red = serious risk, and dark red = critical risk. The overall risk of bias is presented in the last column. The bar chart below displays the percentage distribution of risk levels across all domains. To further evaluate methodological rigor beyond the Newcastle-Ottawa Scale, we conducted a detailed domain-specific risk of bias analysis for the included retrospective cohort studies using the ROBINS-I (Risk Of Bias In Non-randomized Studies - of Interventions) tool. The visual summary of domain-specific judgments is presented in Figure X. Overall, 10 of the 24 studies (41.6%) were judged as having a low overall risk of bias, 8 studies (33.3%) as moderate, 5 studies (20.8%) as serious, and 1 study (4.1%) as critical. The most frequent sources of bias were related to confounding (Domain 1), selection of participants (Domain 2), and deviations from intended interventions (Domain 4). Notably, bias due to confounding was classified as moderate or worse in 8 studies (33.3%), reflecting limitations in baseline adjustment and control of prognostic variables. In addition, 6 studies showed serious or critical concerns in selection bias, likely reflecting retrospective designs and unclear inclusion criteria. Domains related to outcome measurement (D6) and reporting (D7) had predominantly low risk assessments, indicating general reliability in survival data collection and endpoint definitions. The studies by Al-Saleh et al., Coiro et al., and Rey-Vargas et al. were highlighted as having elevated risks across multiple domains, whereas studies such as Antonini et al., He et al., Peng et al., and La Cruz et al. demonstrated consistently low risk profiles across all evaluated domains. These results support the inclusion of a wide range of real-world evidence studies while acknowledging their inherent limitations. The summary of domain-specific risks reinforces the need for cautious interpretation of effect estimates, particularly in studies with serious or critical bias classifications.

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