Check for updates

OPEN ACCESS

EDITED BY Noha Mousaad Elemam, University of Sharjah, United Arab Emirates

REVIEWED BY

Pawel Swietach, University of Oxford, United Kingdom Jaromir Pastorek, Slovak Academy of Sciences, Slovakia

*CORRESPONDENCE Chanitra Thuwajit ⊠ chanitra.thu@mahidol.ac.th

SPECIALTY SECTION This article was submitted to Pathology, a section of the journal Frontiers in Medicine

RECEIVED 02 November 2022 ACCEPTED 17 February 2023 PUBLISHED 17 March 2023

CITATION

Numprasit W, Yangngam S, Prasopsiri J, Quinn JA, Edwards J and Thuwajit C (2023) Carbonic anhydrase IX-related tumoral hypoxia predicts worse prognosis in breast cancer: A systematic review and meta-analysis. *Front. Med.* 10:1087270. doi: 10.3389/fmed.2023.1087270

COPYRIGHT

© 2023 Numprasit, Yangngam, Prasopsiri, Quinn, Edwards and Thuwajit. This is an openaccess article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Carbonic anhydrase IX-related tumoral hypoxia predicts worse prognosis in breast cancer: A systematic review and meta-analysis

Warapan Numprasit^{1,2}, Supaporn Yangngam³, Jaturawitt Prasopsiri³, Jean A. Quinn², Joanne Edwards² and Chanitra Thuwajit³*

¹Division of Head Neck and Breast Surgery, Department of Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, ²School of Cancer Sciences, Wolfson Wohl Cancer Research Centre, University of Glasgow, Glasgow, United Kingdom, ³Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Background: Tumoral hypoxia is associated with aggressiveness in many cancers including breast cancer. However, measuring hypoxia is complicated. Carbonic anhydrase IX (CAIX) is a reliable endogenous marker of hypoxia under the control of the master regulator hypoxia-inducible factor- 1α (HIF- 1α). The expression of CAIX is associated with poor prognosis in many solid malignancies; however, its role in breast cancer remains controversial.

Methods: The present study performed a meta-analysis to evaluate the correlation between CAIX expression and disease-free survival (DFS) and overall survival (OS) in breast cancer.

Results: A total of 2,120 publications from EMBASE, PubMed, Cochrane, and Scopus were screened. Of these 2,120 publications, 272 full texts were reviewed, and 27 articles were included in the meta-analysis. High CAIX was significantly associated with poor DFS (HR=1.70, 95% CI=1.39–2.07, p<0.00001) and OS (HR=2.02, 95% CI 1.40–2.91, p=0.0002) in patients with breast cancer. When stratified by subtype, the high CAIX group was clearly associated with shorter DFS (HR=2.09, 95% CI =1.11–3.92, p=0.02) and OS (HR=2.50, 95% CI =1.53–4.07, p=0.0002) in TNBC and shorter DFS in ER⁺ breast cancer (HR=1.81 95% CI =1.38–2.36, p<0.0001).

Conclusion: High CAIX expression is a negative prognostic marker of breast cancer regardless of the subtypes.

KEYWORDS

breast cancer, carbonic anhydrase IX, meta-analysis, prognosis, survival

Introduction

The incidence of breast cancer has increased in recent decades, with an estimated 13% of women developing breast cancer in their lifetime and over 40,000 deaths per year (1, 2). The survival depends on clinicopathological factors, such as tumor size, nodal status, evidence of distant metastasis as well as biological markers, including estrogen receptor (ER), progesterone

receptor (PR), and human epidermal growth factor receptor 2 (HER2) status (3–5). The intrinsic breast cancer subtypes are currently significant prognostic and predictive markers. Five-year overall survival (OS) was the highest in the ER/PR-positive subtype (94%) as compared to the HER2-positive subtype (85%) and the triple-negative (TNBC) subtype (77%) (1). Breast cancer has distinct phenotypes as evidenced by patients who have a similar staging and molecular classification but have a different treatment response and prognosis (6–8). Thus, additional predictive and prognostic markers are warranted to improve the treatment and prognostic outcomes.

Tumoral hypoxia is a common characteristic of many solid tumors (9, 10). In breast cancer, median oxygen partial pressure is approximately 10 mmHg, which is less than that of the normal breast tissue (52–65 mmHg) (11, 12). Cancer cells adapt to survive under hypoxic conditions *via* hypoxia-inducible factor-1 α (HIF-1 α), leading to the transcription of targeted genes resulting in tumor progression and invasion (13). Subsequently, HIF-1 α can trigger the transcription of targeted genes, leading to tumor progression and invasion (14).

The expression of carbonic anhydrase IX (CAIX) is targeted by the HIF-1a transcriptional activity and controls the pH between intracellular and extracellular compartments (15). It is mainly dependent on HIF-1a regulation; therefore, it can also be a marker of tumor hypoxia (16, 17). However, hypoxia is not an obligated factor, and the inactivation of the von Hippel-Lindau (VHL) gene can stabilize HIF-1α under a non-hypoxic condition and subsequently activated the CAIX overexpression (15, 18). CAIX catalyzes extracellular hydrating CO2 into HCO3 and H+ and cooperates with other acid/base transporters to maintain extracellular acidosis and intracellular neutral/slight alkalosis (19). In contrast, CAIX-bound Cl⁻/HCO⁻₃ exchangers (AEs) can import or provide or export HCO-3 from intracellular compartment during cell migration (20). CAIX expression mediates cancer cell growth, migration, and invasion (18) by directly binding to β -catenin, resulting in the disruption of the E-cadherin/cytoskeleton/β-catenin complex; and an acidic extracellular pH also suppresses the function of cytotoxic T-cells (21).

Many studies have shown that high CAIX expression was associated with adverse survival outcomes. In breast cancer, some studies evaluated the importance of CAIX expression in relation to survival; however, those results were controversial and mostly included a small number of patients. Ong et al. reported that CAIX expression was the independent prognostic factor for disease-free survival (DFS) and OS in TNBC. Similarly, Brennan et al. reported that high CAIX was associated with shorter OS, breast cancer-specific survival (BCSS), and relapse-free survival (RFS) (22, 23). In contrast, Currie et al. found no association between the level of CAIX and DFS and OS (24), while Pinheiro et al. reported that only a high CAIX expression was related to DFS but not to OS (25).

To address this issue, a meta-analysis was conducted to evaluate the prognostic value of CAIX in breast cancer and to determine the correlation between CAIX and breast cancer subtypes. To date, this is the first meta-analysis to focus on the prognostic role of CAIX in breast cancer. The meta-analysis revealed that a high CAIX protein expression was associated with unfavorable survival outcomes and could discriminate the prognosis in the ER-positive and TNBC subtypes.

Materials and methods

Search strategy

This study used EMBASE, PubMed, Cochrane, and Scopus electronic databases to search for articles. The keywords including [(Prognos*) OR (surviv*) OR (hazard) OR (disease-free) OR ("disease free") OR (progression-free) OR ("progression-free") OR (Kaplan-Meier) OR ("Kaplan Meier") OR (predict*) OR (outcome) OR (efficacy) OR (effective*)] AND [(CAIX) OR (ca9) OR ("carbonic anhydrase IX") OR ("carbonic anhydrase 9") OR ("carbonic anhydrase-IX") OR ("carbonic anhydrase-9") OR (CA-IX) OR (ca-9) OR (G250)] AND [(breast cancer) OR (breast tumors*) OR (breast carcinoma)] were used.

Selection criteria

The inclusion criteria of the present study were as follows: (a) the patients in the study cohorts who were confirmed to have invasive breast cancer, regardless of the subtype, (b) CAIX expression which was detected by immunohistochemistry (IHC), (c) the studies that reported DFS or OS with hazards ratios (HRs) and 95% confidence intervals (CIs) or the Kaplan–Meier survival curves from which HRs and 95% CIs could be extracted, and (d) the studies that were published in English. The exclusion criteria for the present study were studies that failed to meet any of the inclusion criteria, were related to non-human studies, or contained duplicated and unavailable full texts.

Data extraction and quality assessment

The search with regard to data extraction and quality assessment was reviewed by three independent reviewers (WN, JP, and SY). The following information was extracted from each study: the first author's name, year of publication, the total number of patients, the scoring method and cut-off level for high or low CAIX expression, breast cancer subtypes, HRs, 95% CIs of DFS and OS, and whether univariate or multivariate analysis was performed.

Statistical methods

Pooled HRs and their 95% CIs were used to determine the association between CAIX expression and survival. Heterogeneity among studies was assessed using the chi-squared test and I². A *p*-values of <0.1 or an I² statistic of >50% was indicative of significant heterogeneity between studies; in these cases, a random-effects model was used. The meta-analysis was performed with Review Manager 5.4 (RevMan the Cochrane Collaboration; Oxford, England). The *p*-values of <0.05 were considered statistically significant.

Results

Study selection and characteristics

A PRISMA flow diagram for the process of study selection is summarized in Figure 1. Initially, 275 articles from EMBASE, 242

from PubMed, 19 from Cochrane, and 1,897 from Scopus were identified, and subsequently, 313 duplicated records were removed. A total of 2,120 papers were screened. A total of 1,848 studies were excluded based on the titles and abstracts resulting in 272 full texts being reviewed. Of these, 245 articles were excluded. Finally, 27 papers met the eligibility criteria (Figure 1; Table 1).

Study characteristics

The 27 included studies were published between 2001 and 2022. DFS was reported in 22 articles, 10 of which provided HRs and 95% CIs, while the OS analysis was included in 16 articles, 7 of which provided HRs and 95% CIs (Table 1). Most of the articles (20 out of 27, 74%) were reported on mixed breast cancer subtypes and provided data on ER, PR, and/or HER2 staining, with survival analysis on all cases, regardless of the subtype. Three studies focused on TNBC, two articles on ER-positive (ER⁺), one on ER-negative (ER⁻), and one on male breast cancer. The mean age of patients was between 46 and 62 years. Fifty percent of the studies used the primary antibody clone M75 to detect the CAIX expression. In most studies (80%), the level of CAIX expression was determined by estimating both staining intensity and the percentage of tumor cells stained. The remaining studies (20%) used only intensity or percentage. The low--high cutoff value varied across all studies. Overall, high CAIX expression in patients with breast cancer varied in each study, ranging from 8 to 91.1%. Most studies (45.5%, 12 out of 27 studies) did not report on the cellular location of CAIX expression. In 36% of studies, expression was reported in the cell membrane, in 9% of studies, CAIX expression was reported in the membrane and cytoplasm/nucleus, and in two studies, CAIX expression was reported in the exclusive cytoplasm or nuclear staining (9%).

High CAIX was associated with poor DFS in breast cancer

Twenty-two studies totaling 9,157 patients were analyzed for the effect of CAIX expression on DFS. Shamis et al. studied CAIX expression in two independent cohorts with specific HRs and 95% CIs and DFS in each cohort, and both cohorts were included in this meta-analysis (26). The study by Jubb et al. did not define the low/high cutoff for CAIX expression, but it provided the HR and 95% CI for each CAIX score of 1, 2, and 3 and compared each with that of the negative CAIX group (41). Hence, the HR and 95% CI for each CAIX score were included in the meta-analysis. High CAIX was significantly associated with poor DFS in patients with breast cancer (HR = 1.70, 95% CI = 1.39–2.07, p < 0.00001) with heterogeneity I² = 83% (Figure 2).

High CAIX was associated with poor OS in breast cancer

A total of 3,591 patients from the selected 17 studies were investigated for the association between CAIX expression and



TABLE 1 Characteristics of the eligible studies for meta-analysis in this study.

References	Country	Mean age	BC subtypes (n)	Stage	Treatment (n)	IHC score method	CAIX cut- off level	CAIX high (%)	Ab clones	HR (95% CI) for DFS	<i>p</i> -value	HR (95% CI) for OS	<i>p</i> -value
		NA	ED (272)	I III	CMT (110)			0		UV = 1.81 (1.12-2.92)	0.018		
Shamia at al. (20)	United	NA	EK+ (3/3)	1-111	CM1 (110)	Weight H	Log-rank	9	1/75	MV = 1.04 (0.46-2.35)	0.926	NIA	NA
Snamis et al. (20)	Kingdom	NIA	ED (295)	1 111	CMT (71)	score	Studio	28	M1/5	UV = 1.64 (1.14-2.37)	0.008		INA
		INA	ER+ (285)	1-111	CM1 (71)			28		MV = 1.74 (1.08-2.82)	0.023		
Ong et al. (22)	Singapore	55	TNBC (306)	NA	NA	I and P	≥1	39.3	NA	MV 2.77 (1.78–4.31)	<0.001	MV 2.48 (1.50- 4.09)	<0.001
Li et al. (27)	China	49	ER+ (55)	Recurrence	NA	I and P	NA	34.5	ab108351	UV* 2.64 (1.28-5.44)	0.0086	NA	NA
Alves et al. (28)	Brazil	49.6	Mixed BC (196)	IIb or III	CMT (196)	I and P	≥3	7.4	ab15086	UV* 0.32 (0.19–0.55)	<0.00001	UV* 0.33 (0.15- 0.66)	<0.00001
Ozretic et al. (29)	Croatia	60	TNBC (64)	NA	NA	I and P	>60	77	ab15086	NA	NA	UV 2.85 (0.36– 22.25)	0.32
Jin et al. (30)	South Korea	NA	TNBC (270)	I–II	NA	NA	≥10%	21.9	NA	UV* 1.45 (0.77-2.67)	0.25	NA	NA
Chu et al. (31)	China	55.34	Mixed (149)	I–IV	СМТ	I and P	Strong intensity in ≥10% cells	15	NA	MV 5.758 (2.28-14.50)	<0.001	NA	NA
Samaka et al. (32)	Egypt	48	Mixed (56)	I-IV	NA	I and P	>1%	91.1	ab107257	NA	NA	UV* 2.09 (1.05- 4.19)	0.0358
Aomatsu et al. (33)	Japan	NA	Mixed (102)	IIA–IIIA	CMT (102)	I and P	Moderate to strong staining in >10% cells	46	M75	UV* 4.52 (2.05–9.97)	0.0002	UV* 3.31 (1.56– 7.05)	0.0018
Deb et al. (34)	Australia	NA	Male (276)	I–IV	NA	I and P	Strong intensity in ≥10% cells	8	NA	UV 2.2 (0.8–5.7)	0.11	NA	NA

10.3389/fmed.2023.1087270

(Continued)

References	Country	Mean age	BC subtypes (n)	Stage	Treatment (n)	IHC score method	CAIX cut- off level	CAIX high (%)	Ab clones	HR (95% Cl) for DFS	p-value	HR (95% CI) for OS	p-value
Kim et al. (35)	South Korea	52	Mixed metastasis (162)	IV	NA	I and P	≥2	19.8	NA	NA	NA	MV 1.69 (0.77- 3.69)	0.189
Noh et al. (36)	South Korea	NA	ER-AR+ (127)	I–III	NA	I and P	≥2	28.7	NA	MV 2.231 (0.670– 7.426)	0.191	MV 15.89 (1.82- 131.6)	0.01
Betof et al. (37)	United States	48	Mixed (209)	I–III	CMT (209)	I and P	≥50	88	M75	UV* 1.75 (0.92-3.31)	0.088	UV* 2.73 (1.2-6.21)	0.0166
Kaya et al. (38)	Turkey	46	Mixed (111)	I–III	NA	I	Any staining	55.8	H-120	UV* 0.86 (0.54–1.36)	0.5253	UV* 2.77 (1.58- 4.85)	0.0004
Beketic-Oreskovic										UV 6.74 (2.27-20.03)	<0.001	UV 5.68 (2.11- 15.31)	<0.001
et al. (39)	Croatia	61.5	Mixed (40)	1-111	NA	I and P	52.5	60	NA	MV 4.14 (1.28-13.35)	0.018	MV 3.99 (1.38- 11.59)	0.011
Lou et al. (40)	Canada	NA	Mixed (3,630)	I–III	NA	I and P	Any staining	15.6	M75	UV* 2.30 (1.91–277)	<0.00001	NA	NA
Pinheiro et al. (25)	Portugal	NA	Mixed (122)	T1-3anyN	NA	I and P	≥3	18	ab15086	UV* 2.24 (0.79–6.35)	0.1294	NA	NA
Jubb et al. (41)	United Kingdom	57 (27–80)	Mixed (151)	I-III	CMT (63)	I and P	>10%	32	M75	CAIX score 1; UV 0.63 (0.29–1.41)	0.26	NA	NA
										CAIX score 2; UV 1.24 (0.49–3.13)	0.65		
										CAIX score 3; UV 1.83 (0.86–3.89)	0.12	-	

(Continued)

frontiersin.org

05

References	Country	Mean age	BC subtypes (n)	Stage	Treatment (n)	IHC score method	CAIX cut- off level	CAIX high (%)	Ab clones	HR (95% Cl) for DFS	p-value	HR (95% CI) for OS	p-value
Tan et al. (42)	United Kingdom	55	Mixed (407)	I–III	NA	I and P	≥10%	14	M75	UV* 1.81 (1.14-2.86)	0.0119	UV* 4.29 (2.61- 7.04)	<0.00001
Crabb et al. (43)	Canada	NA	Mixed (602)	II–III	NA	NA	NA	16.7	M75	MV 1.58 (1.12-2.22)	0.008	NA	NA
Kyndi et al. (44)	Denmark	NA	Mixed (945)	II–III	NA	I and P	≥10%	16	M75	UV 1.29 (1.02–1.62)	<0.05	UV 1.3 (1.06- 1.60)	<0.05
Hussain et al. (45)	United Kingdom	62	Mixed (144)	I–II	NA	I and P	Weak or strong staining and focal or	26	M75	NA	NA	UV 2.63 (1.21- 5.70)	0.01
							diffuse distribution					MV 2.43 (1.07– 5.53)	0.035
Trastour et al. (46)	France	62	Mixed (132)	I–III	CMT/ET	I and P	>1%	29	M75	MV 2.0 (1.0-4.2)	0.05	NA	0.2
Brennan et al. (23)	Ireland	NA	Mixed (400)	Π	ET (199)	I	Any staining	11	M75	UV* 1.62 (1.02-2.72)	0.041	UV* 1.92 (1.09– 3.38)	0.0239
Generali et al. (47)	United Kingdom	NA	Mixed (166)	T2-4N0-1	CMT/ET (187)	I and P	Any staining	24.7	M75	UV* 1.79 (0.84–3.89)	0.1315	UV* 1.99 (0.79– 5.02)	0.1443
Tomes et al. (48)	Canada	NA	Mixed (53)	any T,N	NA	Р	NA	NA	M75	NA	NA	UV* 0.50 (0.30- 0.85)	<0.0001
Chia et al. (49)	Canada	59	Mixed (103)	I–III	CMT (27)/ET (80)	I and P	≥1	48	M75	UV* 2.38 (1.34-4.22)	0.0031	UV 2.61 (1.01– 6.75)	0.05



OS. High CAIX expression was statistically significantly associated with shorter OS (HR = 2.05, 95% CI 1.44–2.91, p < 0.0001) with heterogeneity I² = 80% (Figure 3).

High CAIX was associated with poor OS and DFS in ER⁺ and TNBC subtypes

Three articles focused on the CAIX expression in 640 TNBC cases. One study reported both DFS and OS, while the other two reported either DFS or OS, resulting in 576 TNBC cases included in the DFS analysis and 370 TNBC cases included in the OS analysis. Two articles focused on CAIX expression and DFS in ER⁺ breast cancer from 731 ER⁺ breast cancer cases. The results revealed that, when compared to patients with a low CAIX expression, patients with a high CAIX expression were clearly associated with shorter DFS in TNBC (HR=2.09, 95% CI =1.11–3.92, p=0.02) with heterogeneity I² = 63% and OS (HR=2.50, 95% CI =1.53–4.07, p=0.0002) without heterogeneity I² = 0%; and shorter DFS in ER⁺ breast cancer (HR=1.81 95% CI =1.38–2.36, p<0.0001) without heterogeneity I² = 0% (Figure 4).

The antibody does not affect CAIX survival

The studies used a variety of CAIX antibodies for IHC. Twelve studies used an M75 antibody clone: 1 from BioScience, 1 from Novus Biologicals, and 1 from Bayer, but the other 9 could not be identified. The HR for DFS was 1.66 (95% CI: 1.35–2.0, p < 0.00001). Clones used in other studies were as follows: 6 studies used Abcam, 1 from Cell Marque, 1 from Novus Biologicals, and 2 from Santa Cruz Biotechnology (Table 1), which also demonstrated the effect of CAIX with HR for DFS 1.94 (95% CI: 1.06–3.57; p < 0.0001; Figure 5). There was no significant difference between the M75 antibody and other antibodies (p = 0.63; Figure 5). The HR for OS in the group stained with the M75 antibody was 2.01 (95% CI: 1.19–3.38; p = 0.009), and it was 2.10 (95% CI: 1.26–3.52; p = 0.002) for the other antibody group (Figure 6). There was no significant difference between the M75 antibody and the other antibodies in terms of OS (p = 0.90; Figure 6).

Discussion

This meta-analysis focused on the prognostic role of CAIX expression in breast cancer. Hypoxia, as determined by the CAIX

Hazard Ratio

IV, Random, 95% CI

5 10

ż

0.5

Improve outcome Poor outcome

0.1 0.2

				Hazard Rado		Hazaro Rauo
Study or Subgroup	log[Hazard Ratio]	SE	Weight	IV, Random, 95% Cl	Year	IV, Random, 95% Cl
Ong et al. 2022	0.9083	0.2565	7.2%	2.48 [1.50, 4.10]	2022	_ -
Alves et al. 2019	-1.1087	0.4023	6.0%	0.33 [0.15, 0.73]	2019	_
Ozretic et al. 2017	1.0473	1.0486	2.2%	2.85 [0.36, 22.25]	2017	
Samaka et al. 2015	0.7372	0.3512	6.4%	2.09 [1.05, 4.16]	2015	
Noh et al. 2014	2.7657	1.1056	2.0%	15.89 [1.82, 138.74]	2014	│ ———→
Aomatsu et al. 2014	1.1969	0.3838	6.1%	3.31 [1.56, 7.02]	2014	
Kim et al. 2014	0.5247	0.3991	6.0%	1.69 [0.77, 3.69]	2014	
Betof et al. 2012	1.0043	0.4194	5.8%	2.73 [1.20, 6.21]	2012	
Kaya et al. 2012	1.0188	0.2864	7.0%	2.77 [1.58, 4.86]	2012	
Beketic-Oreskovic et al. 2011	1.737	0.5052	5.1%	5.68 [2.11, 15.29]	2011	
Tan et al. 2009	1.4563	0.2535	7.3%	4.29 [2.61, 7.05]	2009	
Hussain et al. 2008	0.967	0.3961	6.0%	2.63 [1.21, 5.72]	2008	
Kyndi et al. 2008	0.2624	0.1041	8.3%	1.30 [1.06, 1.59]	2008	-
Brennan et al. 2006	0.6523	0.2889	7.0%	1.92 [1.09, 3.38]	2006	
Generali et al. 2006	0.6881	0.4714	5.4%	1.99 [0.79, 5.01]	2006	+
Tomes et al. 2003	-0.6931	0.2606	7.2%	0.50 [0.30, 0.83]	2003	
Chia et al. 2001	0.9594	0.4844	5.2%	2.61 [1.01, 6.75]	2001	
Total (95% CI)			100.0%	2.05 [1.44, 2.91]		◆
Heterogeneity: Tau ^z = 0.38; Chi ^z = Test for overall effect: Z = 3.99 (P	= 80.58, df = 16 (P < 0.0001)	< 0.0000	1); I² = 80	%		0.01 0.1 1 10 100 Improve outcome Poor outcome

в

Α

Jin et al. 2016

Ong et al. 2022

Total (95% CI)

Study or Subgroup log[Hazard Ratio]

Test for overall effect: Z = 2.29 (P = 0.02)

Heterogeneity: Tau² = 0.13; Chi² = 2.70, df = 1 (P = 0.10); l^2 = 63%

5							
					Hazard Ratio	Hazard Ratio	
_	Study or Subgroup	log[Hazard Ratio]	SE	Weight	IV, Fixed, 95% Cl	IV, Fixed, 95% Cl	
	Ong et al. 2022	0.9083	0.2565	94.4%	2.48 [1.50, 4.10]		
	Ozretic et al. 2017	1.0473	1.0486	5.6%	2.85 [0.36, 22.25]		
	Total (95% CI)			100.0%	2.50 [1.53, 4.07]	•	
	Heterogeneity: Chi ² = Test for overall effect:	0.02, df = 1 (P = 0.90 Z = 3.68 (P = 0.0002)); I² = 0%)	6		0.05 0.2 1 5	20

Hazard Ratio

1.45 [0.77, 2.73] 2016

2.77 [1.78, 4.31] 2022

2.09 [1.11, 3.92]

SE Weight IV, Random, 95% Cl Year

0.3716 0.3229 43.6%

1.0188 0.2256 56.4%

100.0%

С

				Hazard Ratio		Hazard Ratio
Study or Subgroup	log[Hazard Ratio]	SE	Weight	IV, Fixed, 95% CI	Year	IV, Fixed, 95% Cl
Shamis et al. 2022	0.5933	0.2449	31.4%	1.81 [1.12, 2.93]	2022	
Shamis et al. 2022	0.4947	0.1855	54.8%	1.64 [1.14, 2.36]	2022	
Li et al. 2020	0.9708	0.3694	13.8%	2.64 [1.28, 5.45]	2020	
Total (95% CI)			100.0%	1.81 [1.38, 2.36]		•
Heterogeneity: Chi ² =	1.33, df = 2 (P = 0.5)	2); I ² = 0%	6			
Test for overall effect:	Z = 4.31 (P ≤ 0.0001)				Improve outcome Poor outcome

FIGURE 4

A Forest plot of HR and 95% CI for the association of CAIX with (A) DFS, (B) OS of patients with TNBC, and (C) DFS of patients with ER⁺ BC.

Sindy of Subgroup big (Hazard Ratio) SE Weight IV, Random, 95% Cl Vear IV, Ran					Hazard Ratio		Hazard Ratio
b.1.1.Children M 3 Shamis et al. 2022 (1) 0.14947 0.1855 5.3% 1.64 [1.14, 2.36] 2022 Shamis et al. 2022 (2) 0.1655 0.286 6.5% 1.18 [1.12, 1.24] 2022 Shamis et al. 2014 1.5080 0.324 3.2% 4.52 [2.05, 9.37] 2014 Jubb et al. 2010 (3) -0.462 0.3968 3.3% 0.63 [0.29, 1.37] 2010 Jubb et al. 2010 (4) 0.2151 0.473 7.7% 1.24 (0.48, 3.14) 210 Jubb et al. 2010 (5) 0.6043 0.3263 3.4% 1.83 [0.66, 3.89] 2010 Jubb et al. 2008 0.4547 0.3264 6.4% 1.58 [1.12, 2.23] 2008 Kyndi et al. 2007 0.6931 0.3267 3.7% 2.001 [10.0, 4.00] 2007 Brennan et al. 2006 0.4224 0.236 4.8% 1.62 [1.02, 2.57] 2006 Chai et al. 2001 0.8677 0.2391 4.2% 2.38 [1.34, 4.23] 2001 Stabtotal (96% C1) 6.68% 1.66 [1.35, 2.05] 1.66 [1.35, 2.05] 1.66 [1.35, 2.05] 1.66 [1.35, 2.05] I et al. 2016 0.79706 <	Study of Subgroup	log[Hazard Ratio]	SE.	weight	IV, Random, 95% CI	rear	IV, Random, 95% CI
Sharns is al. 2022 (2) 0.1655 0.0266 6.5% 1.18 (1.14, 2.30 2022 Armatsu et al. 2014 1.5065 0.0266 6.5% 1.18 (1.14, 2.124) 2022 Armatsu et al. 2014 1.5065 0.0266 6.5% 1.18 (1.14, 2.124) 2022 Armatsu et al. 2012 0.5596 0.3281 3.2% 4.52 (2.05, 9.37) 2014 Betof et al. 2010 (3) -0.462 0.3698 3.3% 0.68 (0.29, 1.37) 2010 Jubb et al. 2010 (3) -0.462 0.3698 3.3% 0.68 (0.29, 1.37) 2010 Jubb et al. 2010 (4) 0.2161 0.4737 2.7% 1.24 (0.49, 3.14) 2010 Jubb et al. 2010 (5) 0.6043 0.3653 3.4% 1.83 (0.68, 3.89) 2010 Jubb et al. 2008 0.4574 0.1756 5.4% 1.58 (1.12, 2.123) 2008 Trastour et al. 2008 0.4574 0.1756 5.4% 1.58 (1.12, 2.123) 2008 Trastour et al. 2008 0.4574 0.1756 5.4% 1.58 (1.12, 2.13) 2008 Bernan et al. 2006 0.46924 0.236 4.6% 1.62 (1.02, 5.7) 2006 Bernani et al. 2006 0.46924 0.236 4.6% 1.62 (1.02, 5.7) 2006 Bernani et al. 2006 0.46924 0.236 4.6% 1.62 (1.02, 5.7) 2006 Bernani et al. 2006 0.46922 0.386 3.4% 1.79 (0.84, 3.81) 2006 Chai et al. 2001 0.8671 0.2931 4.23 2001 Bernani et al. 2002 0.9708 0.3684 3.4% 2.77 [1.78, 4.31] 2022 te stor overall effect Z = 4.77 (P < 0.00001); P = 81% Test for overall effect Z = 4.77 (P < 0.00001); P = 81% Test for overall effect Z = 4.77 (P < 0.00001); P = 81% Test for overall effect Z = 4.77 (P < 0.00001); P = 81% Test for overall effect Z = 2.14 (P = 0.3) Total (95% C) 3.322 3.9% 1.45 [0.77, 2.73] 2016 Jun et al. 2014 0.7086 0.5161 2.5% 2.20 (1.13, 2.07] Heterogeneity; Tau*= 0.79; Chi*= 65.11, df = 9 (P < 0.00001); P = 83% Test for overall effect Z = 2.14 (P = 0.03) Total (95% C) 3.32% 1.94 (1.06, 3.57] Heterogeneity; Tau*= 0.79; Chi*= 65.11, df = 9 (P < 0.00001); P = 83% Test for overall effect Z = 2.14 (P = 0.03) Total (95% C) 3.32% 5.74 2.7% 5.76 (2.27, 2.001] Job 6.2 1 5.16 (P < 0.00001); P = 83% Test for overall effect Z = 2.14 (P = 0.03) Total (95% C) 3.32% 5.74 2.7% 5.76 (2.27, 2.001] Job 6.2 1 5.20 Improve outcome Poor outcome Poor outcome Poor outcome Feotro outcome Poor outcome Heterogeneity; Tau*= 0.16; Chi*= 14 (P < 0.00001); P =	Chamic at al. 2022 (1)	0 40 47	0 1055	ຮ່ວທ	4 6 4 14 4 4 3 361	2022	
Sharth stat. 2022 (2) 0.1633 0.0280 0.3% 1.16 [1.2, 1.4] 2022 Avanatsu et al. 2014 1.5080 0.4334 3.2% 4.52 (25, 9.37) 2014 Edet of et al. 2012 0.6596 0.3281 3.3% 0.63 [0.29, 1.37] 2010 Jubb et al. 2010 (3) -0.462 0.3968 3.3% 0.63 [0.29, 1.37] 2010 Jubb et al. 2010 (4) 0.2151 0.4737 2.7% 1.24 (0.49, 3.14] 2010 Jubb et al. 2010 (5) 0.6043 0.3863 3.4% 1.83 [0.66, 3.89] 2010 Jubb et al. 2008 0.4544 0.1766 5.4% 1.58 [1.12, 2.23] 2008 Kyndi et al. 2008 0.42544 0.1718 5.6% 5.4% 1.58 [1.12, 2.23] 2008 Grane at al. 2008 0.42544 0.1718 5.6% 1.29 [1.02, 1.63] 2008 Grane at al. 2008 0.4254 0.236 4.9% 1.29 [1.02, 1.63] 2008 Grane at al. 2006 0.5922 0.386 3.4% 1.82 [1.02, 2.57] 2068 Grane at al. 2006 0.5922 0.386 3.4% 1.82 [1.02, 2.57] 2068 Grane at al. 2006 0.5922 0.386 3.4% 1.29 [1.02, 1.63] 2007 Grane at al. 2006 0.5922 0.386 3.4% 1.29 [1.02, 1.63] 2006 Grane at al. 2006 0.5922 0.386 3.4% 1.29 [1.02, 1.63] 2006 Grane at al. 2006 0.5922 0.386 3.4% 1.29 [1.02, 4.30] 2007 Grane at al. 2006 0.5922 0.386 4.4% 2.277 [1.78, 4.31] 2022 Let at. 2000 0.9708 0.3894 3.5% 2.64 [1.28, 5.46] 2006 Chu et al. 2016 1.37606 0.4713 2.7% 5.76 [2.29, 1.450] 2016 Chu et al. 2016 1.7696 0.4713 2.7% 5.76 [2.29, 1.450] 2016 Chu et al. 2016 1.7696 0.4713 2.7% 5.76 [2.29, 1.450] 2016 Chu et al. 2011 1.988 0.5563 2.2% 6.77 (4.27, 2011) 2012 Bektet: Oreskowic et al. 2011 1.989 0.5574 2.3% 6.74 [2.7, 2.001] 2014 Wes et al. 2011 0.892 0.5754 2.3% 6.74 [2.7, 2.001] 2014 Grane at al. 2012 -0.1508 0.2274 4.48% 0.66 [0.54, 1.37] 2012 Bektet: Oreskowic et al. 2011 1.989 0.5574 2.7% 6.77 [2.7, 2.001] 2014 Thehrein et al. 2011 0.982 0.5754 2.3% 6.74 [2.7, 2.001] 2014 Thehrein et al. 2011 0.982 0.5754 2.3% 6.74 [2.7, 2.001] 2014 Thehrein et al. 2011 0.982 0.5754 2.3% 6.74 [2.7, 2.001] 2014 Thehrein et al. 2011 0.982 0.5754 2.7% 6.74 [2.7, 2.001] 2014 Thehrein et al. 2014 0.93 (FP = 0.63), F= 0% Test for subgroup differences: ChP = 0.23, df = 1 (P = 0.63), F = 0% Solutiont [effect Z = 2, 16 (P = 0.030)] Total (95% C) -0.2 i 5.2 i	Shamis et al. 2022 (1) Chamie et al. 2022 (2)	0.4947	0.1800	0.3% C 50/	1.04 [1.14, 2.30]	2022	-
Holinsberg 1, 2014 Holinsberg 1, 2014 Loue tai, 2011 Loue tai, 2010 Loue tai, 2010 Crabb tai, 2008 Loss 2, 2038 Loss 2, 2008 Loss 2, 200	Snamis et al. 2022 (2) Association of al. 2014	0.1000	0.0200	0.0%	1.10[1.12, 1.24]	2022	
Debi et al. 2012 0.3380 0.3281 3.338 1.75 (0.32, 3.33 2012 Lub et al. 2011 0.3280 0.0346 6.1% 2.30 (1.91, 2.77 2011 Lub et al. 2010 (3) 0.462 0.3956 3.3% 0.63 (0.29, 1.37) 2010 Tan et al. 2009 0.5933 0.2359 4.8% 1.81 (1.4, 2.87) 2009 Tan et al. 2009 0.5933 0.2359 4.8% 1.81 (1.4, 2.87) 2009 Crabb et al. 2010 (6) 0.5623 0.2546 0.1198 6.0% 1.29 (1.02, 1.63) 2009 Errenan et al. 2008 0.2546 0.1198 6.0% 1.29 (1.02, 2.57) 2006 General et al. 2006 0.5857 3.7% 2.00 (1.00, 4.00) 2007 Errenan et al. 2006 0.5827 0.386 3.4% 1.79 (0.84, 3.81] 2006 Cha et al. 2001 0.8671 0.2931 4.2% 2.38 (1.34, 4.23) 2001 Subtotal (95% CI) - 0.8671 0.2931 4.2% 2.38 (1.34, 4.23) 2001 Subtotal (95% CI) - 0.9708 0.3694 3.5% 2.64 (1.28, 5.45) 2020 Aves et al. 2019 0.10, Chi ^a = 75.53, df = 14 (P < 0.00001); P = 81% Test for verall effect Z = 4.77 (P < 0.00001) St.12 Other clones Ong et al. 2020 0.9708 0.3694 3.5% 2.64 (1.28, 5.45) 2020 Aves et al. 2019 - 1.1384 0.266 4.5% 0.32 (0.19, 0.54) 2019 Aves et al. 2014 0.7686 0.571 1.9% 2.23 (0.80, 6.05) 2.014 No h et al. 2014 0.7805 0.5161 2.5% 2.20 (0.80, 6.05) 2.014 No h et al. 2011 0.882 0.5754 2.1% 2.44 (0.78, 7.54) 2011 Prinetro et al. 2011 0.882 0.5754 2.1% 2.44 (0.78, 7.54) 2011 Prinetro et al. 2011 0.882 0.5754 2.1% 2.44 (0.78, 7.54) 2011 Prinetro et al. 2011 0.882 0.5754 2.1% 2.44 (0.78, 7.54) 2011 Prinetro et al. 2011 0.882 0.5754 2.1% 2.44 (0.78, 7.54) 2011 Prinetro et al. 2011 0.882 0.5754 2.1% 2.44 (0.78, 7.54) 2011 Prinetro et al. 2011 0.882 0.5754 2.1% 2.44 (0.78, 7.54) 2011 Prinetro et al. 2011 0.882 0.5754 2.1% 2.44 (0.78, 7.54) 2011 Prinetro et al. 2016 0.0374 1.9% 2.00 (0.80, 6.57] Test for subproput differences: Chi ^p = 0.23, df = 1 (P = 0.63), P = 0% <u>Sobtotal (65% CI) 0.0001</u> Fe as % Test for overall effect Z = 5.16 (P < 0.00001) Fe as % Test for overall effect Z = 5.16 (P = 0.23), df = 1 (P = 0.63), P = 0% <u>Sobtotal (65% CI) 1.0007</u> Sobtotal (65% CI) 1.0007 (Chi Chi Chi Chi Chi Chi Chi Chi Chi Chi	Romaisu et al. 2014 Detefictioli 2012	1.0000	0.4034	3.270	4.02 [2.00, 9.97]	2014	
Lobe et al. 2011 (3) $-0.482 + 0.394 + 0.13 + 2.50 + 1.31 + 2.71 + 2.01$	Beturetal. 2012	0.0000	0.3281	3.970 G 104	1.70 [0.92, 3.33]	2012	
Jubb et al. 2010 (a) 0.0402 (b) (c) (c) (c) (c) (c) (c) (c) (c) (c) (c	Louiet al. 2011 Jubbiot al. 2010 (2)	0.0328	0.0940	0.170	2.30[1.81, 2.77]	2011	
Judb et al. 2010 (4) 0.2131 0.473 2.7.9 1.24 (9.49, 1.11) 2010 Tan et al. 2009 0.5933 0.2359 4.8% 1.81 (11.4, 2.87) 2009 Crabb et al. 2008 0.4574 0.756 5.4% 1.58 (11.2, 2.23) 2008 Kyndi et al. 2008 0.2566 6.1198 6.0% 1.29 (10.2, 16.3) 2008 Frenson et al. 2006 0.4824 0.236 4.8% 1.62 (10.2, 2.77) 2006 Generali et al. 2006 0.4824 0.236 4.8% 1.62 (10.2, 2.77) 2006 Generali et al. 2001 0.8671 0.2931 4.2% 2.38 (1.3.4, 4.2) 2001 Subtotal (95% CI) 0.8671 0.2931 4.2% 2.38 (1.3.4, 4.2) 2001 Stat 2019 .1394 0.266 4.9% 2.77 [1.76, 4.31] 2022 Li et al. 2020 0.9708 0.3694 3.5% 2.64 [1.28, 5.45] 2020 Li et al. 2016 0.3716 0.3229 3.9% 1.45 [0.77, 2.73] 2016 Chu et al. 2014 0.8226 0.6181 2.75% 2.20 [0.80, 6.52] 2014 Kaya et al. 2014	Jubb et al. 2010 (3)	-0.402	0.3800	3.370 2.704	0.03 [0.29, 1.37]	2010	
Date at 2010 (0.) 0.0034 0.0037 1.03 (0.00, 0.03 200) Crabe tal. 2009 0.0333 0.2354 4.8% 1.81 [1.14, 2.23] 2009 Crabe tal. 2008 0.2456 0.1189 6.0% 1.89 [1.14, 2.23] 2009 Crabe tal. 2008 0.2456 0.1189 6.0% 1.29 [1.02, 1.63] 2008 Trastour et al. 2007 0.6893 0.3537 3.7% 2.00 [1.00, 4.00] 2007 Brennan et al. 2006 0.4824 0.236 4.8% 1.62 [1.02, 2.57] 2006 Generali et al. 2001 0.8671 0.2931 4.2% 2.38 [1.34, 4.23] 2001 Chia et al. 2001 0.8671 0.2931 4.2% 2.38 [1.34, 4.23] 2001 Heterogeneity: Tau ² = 0.10; Chi ^p = 75.53, df = 14 (P < 0.00001); P = 81%	Jubb et al. 2010 (4) Jubb et al. 2010 (5)	0.2131	0.4737	2.770	1.24 [0.45, 3.14]	2010	
Carbib et al. 2003 0.3633 0.3634 1.03 1.03 1.15 11.12, 2.23 2008 Kyndi et al. 2008 0.2546 0.1186 6.0% 1.29 1.02 1.63 2008 Kyndi et al. 2006 0.4524 0.236 4.8% 1.29 1.00 1.00 2007 Brennan et al. 2006 0.4624 0.236 4.8% 1.62 1.20 2006 Generali et al. 2006 0.6627 0.2361 4.2% 2.38 1.34, 2.31 2006 Chia et al. 2001 0.6677 0.231 4.2% 2.38 1.34, 2.31 2006 Subtotal (95% C1) 66.8% 1.66 1.35, 2.05] 1.66 1.35, 2.05] State al. 2012 1.0188 0.2256 4.9% 2.77 [1.76, 4.31] 2022 Let al. 2020 0.3716 0.3684 3.5% 2.64 [1.26, 5.05] 2019 Jin et al. 2016 1.7506 0.471 2.7% 2.20 1.018 0.2563 2.2% 2.016, 0.55 2.04 Let al. 2014 0.8024 0.653 2.2% 2.20 <	Tan et al. 2010 (3)	0.0043	0.3033	J.470 1 2 96	1.03 [0.00, 3.03]	2010	
0.130.0 tail. 2000 0.174.0 1.129 [1.02, 1.23] 2003 Trastour et al. 2007 0.6931 0.2537 3.7% 2.00 [1.00, 4.00] 2007 Brennan et al. 2006 0.4824 0.2284 4.8% 1.62 [1.02, 2.57] 2006 Generali et al. 2006 0.4824 0.2331 4.2% 2.38 [1.34, 4.23] 2001 Chia et al. 2001 0.8671 0.2931 4.2% 2.38 [1.34, 4.23] 2001 Chia et al. 2001 0.8671 0.2931 4.2% 2.38 [1.34, 4.23] 2001 Subtotal (95% C1) 0.668.% 1.66 [1.35, 2.05] 0.667.43 2.01 0.67.43 2.01 Heterogeneity: Tau"= 0.10; Chi"= 75.53, df = 14 (P < 0.00001); P= 81%	Crabbatal 2009	0.0500	0.2339	4.070	1.61 [1.14, 2.07]	2009	
Characterization of the second	Kvn diotol 2000	0.4574	0.1730	0, 4.C 2013	1.30 [1.12, 2.23]	2000	
Indicidual 2001 0.4824 0.236 4.8% 1.82 [102, 2.57] 2006 Generalitet al. 2006 0.5822 0.386 3.4% 1.79 [0.84, 3.81] 2006 Chia et al. 2001 0.8671 0.2931 4.2% 2.38 [1.34, 4.23] 2001 Subtotal (95% CI) 66.8% 1.66 [1.35, 2.05] 1.66 [1.35, 2.05] 1.67 [1.02] Heterogeneity: Tau ² = 0.10; Chi ² = 75.53, df = 14 (P < 0.00001); P = 81%	Tractour et al. 2000	0.2340	0.1130	37%	2 00 [1 00 / 00]	2000	
$ \begin{array}{c} \text{Chine He is 1200} \\ Comparison of the image o$	Brennan et al. 2007	0.0331	0.0007	4.8%	1 62 [1 02, 2 57]	2007	_
$\begin{array}{c} \text{Order that of a 1 2001} \\ \text{Output of the 1 2001} \\ \text{Subtotal (95\% CI)} \\ \text{Heterogeneity: Tau" = 0.10; Chi" = 75.53, df = 14 (P < 0.00001); P = 81% \\ \text{Test for overall effect } Z = 4.77 (P < 0.00001) \\ \text{S.1.2 Other clones} \\ \text{Ong et al. 2022} \\ \text{Li et al. 2020} \\ \text{Output of 1 2000} \\ \text{Output of 1 20000} \\ \text{Output of 1 2000} \\ Outp$	Generali et al. 2000 Generali et al. 2006	0.4024	0.200	3196	1 70 [0.02, 2.37]	2000	
$\begin{array}{c} \text{Loc} (11, \text{Col}) & \text{Col} (13, 14, 12) \\ \text{Heterogeneity: Tau" = 0.10; Chi" = 75.53, df = 14 (P < 0.00001); P = 81\% \\ \text{Test for overall effect: } Z = 4.77 (P < 0.00001) \\ \text{S1.2 Other clones} \\ \text{Ong et al. 2022} & 1.0188 0.2256 4.9\% 2.77 [1.78, 4.31] 2022 \\ \text{Lit cal. 2020} & 0.9708 0.3694 3.5\% 2.64 [1.28, 545] 2020 \\ \text{Aves et al. 2019} & -1.1394 0.266 4.5\% 0.32 [0.19, 0.54] 2019 \\ \text{Jin et al. 2016} & 0.3716 0.3229 3.9\% 1.45 [0.77, 2.73] 2016 \\ \text{Chu et al. 2016} & 1.7506 0.4713 2.7\% 5.76 [2.29, 14.50] 2014 \\ \text{Obe et al. 2014} & 0.8024 0.6137 1.9\% 2.23 [0.67, 7.43] 2014 \\ \text{Chu et al. 2014} & 0.8024 0.6137 1.9\% 2.23 [0.67, 7.43] 2014 \\ \text{Kaya et al. 2012} & -0.1508 0.2374 4.8\% 0.86 [0.54, 1.37] 2011 \\ \text{Pinheiro et al. 2011} & 0.892 0.5754 2.1\% 2.44 [0.79, 7.54] 2011 \\ \text{Subtotal (95\% Cl)} & 100.0\% 1.70 [1.39, 2.07] \\ \text{Heterogeneity: Tau" = 0.79; Chi" = 66.11, df = 9 (P < 0.00001); I" = 88\% \\ \text{Test for overall effect: } Z = 2.14 (P = 0.03) \\ \text{Test for subgroup differences: Chi" = 0.23, df = 1 (P = 0.63), I" = 0\% \\ \hline 30 \text{Colorelis} \\ \text{(1) cohort II} \\ \text{(2) cohort II} \\ \text{(2) cohort II} \\ \text{(2) cohort II} \\ \text{(3) CAX score 1} \\ \text{(4) CAX score 2} \\ \text{(5) CAX score 3} \\ \end{array}$	Chia et al. 2000	0.3622	0.000	4 7%	7 38 [1 34 4 23]	2000	
Heterogeneity: Tau ² = 0.10; Ch ² = 75.53, df = 14 (P < 0.00001); P = 81% Test for overall effect: Z = 4.77 (P < 0.00001) 5.1.2 Other clones Orig et al. 2022 1.0188 0.2256 4.9% 2.77 [1.78, 4.31] 2022 Li et al. 2020 0.9708 0.3694 3.5% 2.64 [1.29, 5.45] 2020 Alves et al. 2019 1.1394 0.266 4.5% 0.32 [0.19, 0.54] 2019 Jin et al. 2016 1.7506 0.4713 2.7% 5.76 [2.29, 14.50] 2016 Chu et al. 2016 1.7506 0.4713 2.7% 5.76 [2.29, 14.50] 2016 Deb et al. 2014 0.7885 0.5161 2.5% 2.20 [0.80, 6.05] 2014 Noh et al. 2014 0.8024 0.6137 1.9% 2.23 [0.67, 7.43] 2014 Kaya et al. 2011 0.9892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.6754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.6754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.6754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.6754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.6754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.6754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.6754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2014 0.63.57] Heterogeneity: Tau ² = 0.16; Chi ² = 143.46, df = 24 (P < 0.00001); P = 88% Test for overall effect: Z = 5.16 (P < 0.00001) Test for subgroup differences: Chi ² = 0.23, df = 1 (P = 0.63), P = 0% <u>Coohotes</u> (1) cohort II 2) cohort II 3) CAX score 1 4) CAX score 2 5) CAX score 3	Subtotal (95% CI)	0.0011	0.2001	66.8%	1.66 [1.35, 2.05]	2001	•
5.1.2 Other clones Ong et al. 2022 1.0188 0.2256 4.9% 2.77 [1.78, 4.31] 2022 Li et al. 2020 0.9708 0.3694 3.5% 2.64 [1.85, 5.45] 2020 Alves et al. 2019 -1.1394 0.266 4.5% 0.32 [0.19, 0.54] 2019 Jin et al. 2016 0.3716 0.3229 3.9% 1.45 [0.77, 2.73] 2016 Chu et al. 2016 1.7506 0.4713 2.7% 5.76 [2.29, 14.50] 2016 Deb et al. 2014 0.7885 0.5161 2.5% 2.20 [0.80, 6.05] 2014 Noh et al. 2014 0.8024 0.6137 1.9% 2.23 [0.67, 7.43] 2012 Beketic-Oreskowic et al. 2011 1.9081 0.5553 2.2% 6.74 [2.27, 20.01] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Subtotal (95% CI) 33.2% 1.94 [1.06, 3.57] Heterogeneity: Tau ² = 0.79; Chi ² = 66.11, df = 9 (P < 0.00001); I ² = 88% Test for overall effect: $Z = 5.16$ (P < 0.00001) Test for subgroup differences: Chi ² = 0.23, df = 1 (P = 0.63), I ² = 0% <u>50 cohort II</u> 2) cohort II 2) cohort II 2) cohort II 2) cohort II 2) cohort II 2) CAX score 3	Heterogeneity: Tau² = 0.10; Chi Test for overall effect: Z = 4.77 (i² = 75.53, df = 14 (P (P ≤ 0.00001)	< 0.0000	1); I² = 81	%		
Ong et al. 2022 1.0188 0.2256 4.9% 2.77 [1.78, 4.31] 2022 Li et al. 2020 0.9708 0.3894 3.5% 2.64 [1.28, 5.45] 2020 Alves et al. 2019 -1.1394 0.266 4.5% 0.32 [0.19, 0.54] 2019 Jin et al. 2016 0.3716 0.3229 3.9% 1.45 [0.77, 2.73] 2016 Chu et al. 2016 0.7766 0.4713 2.7% 5.76 [2.29, 14.50] 2016 Deb et al. 2014 0.7885 0.5161 2.5% 2.20 [0.80, 6.05] 2014 Noh et al. 2014 0.8024 0.6137 1.9% 2.23 [0.67, 7.43] 2012 Beketic-Oreskovic et al. 2011 1.9081 0.5553 2.2% 6.74 [2.27, 20.01] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.78, 7.54] 2011 Subtoal (95% CI) 33.2% 1.94 [1.06, 3.57] Heterogeneity: Tau ² = 0.79; Chi ² = 66.11, df = 9 (P < 0.00001); I ² = 88% Test for overall effect: $Z = 2.14$ (P = 0.03) Total (95% CI) 100.0% 1.70 [1.39, 2.07] Heterogeneity: Tau ² = 0.16; Chi ² = 143.46, df = 24 (P < 0.00001); I ² = 88% Test for subgroup differences: Chi ² = 0.23, df = 1 (P = 0.63), I ² = 0% <u>Southots</u> (1) cohort II (2) cohort I (3) CAIX score 1 (4) CAX score 2 (5) CAIX score 3	5.1.2 Other clones						
Li et al. 2020 0.9708 0.3694 3.5% 2.64 [1.28, 5.45] 2020 Alves et al. 2019 -1.1394 0.266 4.5% 0.32 [0.19, 0.54] 2019 Jin et al. 2016 0.3716 0.3229 3.9% 1.45 [0.77, 2.73] 2016 Chu et al. 2016 1.7506 0.4713 2.7% 5.76 [2.29, 14.50] 2016 Deb et al. 2014 0.8024 0.6137 1.9% 2.23 [0.67, 7.43] 2014 Kaya et al. 2012 -0.1508 0.2374 4.8% 0.86 [0.54, 1.37] 2012 Beketic-Oreskovic et al. 2011 1.9081 0.5553 2.2% 6.74 [2.27, 2011 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2016 0.50 0.2 1 5 20 Improve outcome Poor	Ong et al. 2022	1.0188	0.2256	4.9%	2.77 [1.78, 4.31]	2022	
Alves et al. 2019 -1.1394 0.266 4.5% 0.32 [0.19, 0.54] 2019 Jin et al. 2016 0.3716 0.3229 3.9% 1.45 [0.77, 2.73] 2016 Chu et al. 2016 1.7506 0.4713 2.7% 5.76 [2.29, 14.50] 2016 De be tal. 2014 0.8024 0.6137 1.9% 2.23 [0.67, 7.43] 2014 Kaya et al. 2012 -0.1508 0.2374 4.8% 0.86 [0.54, 1.37] 2012 Beketic-Oreskovic et al. 2011 1.9081 0.5553 2.2% 6.74 [2.27, 20.01] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Subtotal (95% Cl) 33.2% 1.94 [1.06, 3.57] Heterogeneity: Tau ² = 0.79; Chi ² = 66.11, df = 9 (P < 0.00001); I ² = 86% Test for overall effect: $Z = 2.14$ (P = 0.03) Total (95% Cl) 100.0% 1.70 [1.39, 2.07] Heterogeneity: Tau ² = 0.16; Chi ² = 143.46, df = 24 (P < 0.00001); I ² = 83% Test for subgroup differences: Chi ² = 0.23, df = 1 (P = 0.63), I ² = 0% <u>Footnotes</u> (1) cohort II (2) cohort II (2) cohort II (2) CAIX score 1 (4) CAX score 2 (5) CAIX score 3	Li et al. 2020	0.9708	0.3694	3.5%	2.64 [1.28, 5.45]	2020	
Jin et al. 2016 0.3716 0.3229 3.9% 1.45 [0.77, 2.73] 2016 Chu et al. 2016 1.7606 0.4713 2.7% 5.76 [2.29, 14.50] 2016 Deb et al. 2014 0.7885 0.5161 2.5% 2.20 [0.80, 6.05] 2014 Noh et al. 2014 0.8024 0.6137 1.9% 2.23 [0.67, 7.43] 2014 Kaya et al. 2012 -0.1508 0.2374 4.8% 0.86 [0.54, 1.37] 2012 Beketic-Oreskovic et al. 2011 1.9081 0.5553 2.2% 6.74 [2.27, 20.01] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Subtotal (95% CI) 33.2% 1.94 [1.06, 3.57] Heterogeneity: Tau ² = 0.79; Chi ² = 66.11, df = 9 (P < 0.00001); I ² = 88% Test for overall effect: Z = 2.14 (P = 0.03) Total (95% CI) 100.0% 1.70 [1.39, 2.07] Heterogeneity: Tau ² = 0.16; Chi ² = 143.46, df = 24 (P < 0.00001); I ² = 83% Test for overall effect: Z = 5.16 (P < 0.00001) Test for subgroup differences: Chi ² = 0.23, df = 1 (P = 0.63), I ² = 0% <u>Footnotes</u> [1) cohort II [2) cohort II [2) cohort II [3) CAIX score 1 [4) CAIX score 2 [5) CAIX score 3	Alves et al. 2019	-1.1394	0.266	4.5%	0.32 [0.19, 0.54]	2019	_
Chu et al. 2016 1.7506 0.4713 2.7% 5.76 [2.29, 14.50] 2016 Deb et al. 2014 0.7885 0.5161 2.5% 2.20 [0.80, 6.05] 2014 Noh et al. 2014 0.8024 0.6137 1.9% 2.23 [0.67, 7.43] 2014 Kaya et al. 2012 -0.1508 0.2374 4.8% 0.86 [0.54, 1.37] 2012 Beketic-Oreskovic et al. 2011 1.9081 0.5553 2.2% 6.74 [2.27, 20.01] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Subtotal (95% Cl) 33.2% 1.94 [1.06, 3.57] Heterogeneity: Tau ² = 0.79; Chi ² = 66.11, df = 9 (P < 0.00001); I ² = 86% Test for overall effect: Z = 2.14 (P = 0.03) Total (95% Cl) 1.70 [1.39, 2.07] Heterogeneity: Tau ² = 0.16; Chi ² = 143.46, df = 24 (P < 0.00001); I ² = 83% Test for overall effect: Z = 5.16 (P < 0.00001) Test for subgroup differences: Chi ² = 0.23, df = 1 (P = 0.63), I ² = 0% <u>Footnotes</u> [10] cohort II [2] cohort II [2] cohort II [3] CAIX score 1 [4] CAIX score 3	Jin et al. 2016	0.3716	0.3229	3.9%	1.45 [0.77, 2.73]	2016	
Deb et al. 2014 0.7885 0.5161 2.5% 2.20 [0.80, 6.05] 2014 Noh et al. 2014 0.8024 0.6137 1.9% 2.23 [0.67, 7.43] 2014 Kaya et al. 2012 -0.1508 0.2374 4.8% 0.86 [0.54, 1.37] 2012 Beketic-Oreskovic et al. 2011 1.9081 0.5553 2.2% 6.74 [2.27, 20.01] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Subtotal (95% Cl) 33.2% 1.94 [1.06, 3.57] Heterogeneity: Tau ² = 0.79; Chi ² = 66.11, df = 9 (P < 0.00001); I ² = 86% Test for overall effect: Z = 2.14 (P = 0.03) Total (95% Cl) 100.0% 1.70 [1.39, 2.07] Heterogeneity: Tau ² = 0.16; Chi ² = 143.46, df = 24 (P < 0.00001); I ² = 83% Test for overall effect: Z = 5.16 (P < 0.00001) Test for subgroup differences: Chi ² = 0.23, df = 1 (P = 0.63), I ² = 0% <u>Footnotes</u> (1) cohort II (2) cohort I (3) CAIX score 1 (4) CAIX score 2 (5) CAIX score 3	Chu et al. 2016	1.7506	0.4713	2.7%	5.76 [2.29, 14.50]	2016	
Noh et al. 2014 0.8024 0.6137 1.9% 2.23 [0.67, 7.43] 2014 Kaya et al. 2012 -0.1508 0.2374 4.8% 0.86 [0.54, 1.37] 2012 Beketic-Oreskovic et al. 2011 1.9081 0.5553 2.2% 6.74 [2.27, 20.01] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Subtotal (95% Cl) 33.2% 1.94 [1.06, 3.57] Heterogeneity: Tau ² = 0.79; Chi ² = 66.11, df = 9 (P < 0.00001); I ² = 86% Test for overall effect: $Z = 2.14$ (P = 0.03) Total (95% Cl) 100.0% 1.70 [1.39, 2.07] Heterogeneity: Tau ² = 0.16; Chi ² = 143.46, df = 24 (P < 0.00001); I ² = 83% Test for overall effect: $Z = 5.16$ (P < 0.00001) Test for subgroup differences: Chi ² = 0.23, df = 1 (P = 0.63), I ² = 0% Tootnotes (1) cohort II (2) cohort I (3) CAIX score 1 (4) CAIX score 2 (5) CAIX score 3	Deb et al. 2014	0.7885	0.5161	2.5%	2.20 [0.80, 6.05]	2014	
Kaya et al. 2012 -0.1508 0.2374 4.8% 0.86 [0.54, 1.37] 2012 Beketic-Oreskovic et al. 2011 1.9081 0.5553 2.2% 6.74 [2.27, 20.01] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Subtotal (95% CI) 33.2% 1.94 [1.06, 3.57] Heterogeneity: Tau ² = 0.79; Chi ² = 66.11, df = 9 ($P < 0.00001$); $P = 86\%$ Test for overall effect: $Z = 2.14$ ($P = 0.03$) Total (95% CI) 1.70 [1.39, 2.07] Heterogeneity: Tau ² = 0.16; Chi ² = 143.46, df = 24 ($P < 0.00001$); $P = 83\%$ Test for overall effect: $Z = 5.16$ ($P < 0.00001$) Test for subgroup differences: Chi ² = 0.23, df = 1 ($P = 0.63$), $P = 0\%$ Tootnotes (1) cohort II (2) cohort I (3) CAIX score 1 (4) CAIX score 2 (5) CAIX score 3	Noh et al. 2014	0.8024	0.6137	1.9%	2.23 [0.67, 7.43]	2014	
Beketic-Oreskovic et al. 2011 1.9081 0.5553 2.2% 6.74 [2.27, 20.01] 2011 Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Subtotal (95% Cl) 33.2% 1.94 [1.06, 3.57] Heterogeneity: Tau ² = 0.79; Chi ² = 66.11, df = 9 (P < 0.00001); I ² = 86% Test for overall effect: Z = 2.14 (P = 0.03) Total (95% Cl) 100.0% 1.70 [1.39, 2.07] Heterogeneity: Tau ² = 0.16; Chi ² = 143.46, df = 24 (P < 0.00001); I ² = 83% Test for overall effect: Z = 5.16 (P < 0.00001) Test for subgroup differences: Chi ² = 0.23, df = 1 (P = 0.63), I ² = 0% Eootnotes (1) cohort II (2) cohort I (3) CAIX score 1 (4) CAIX score 2 (5) CAIX score 3	Kaya et al. 2012	-0.1508	0.2374	4.8%	0.86 [0.54, 1.37]	2012	
Pinheiro et al. 2011 0.892 0.5754 2.1% 2.44 [0.79, 7.54] 2011 Subtotal (95% Cl) 2.1% 33.2% 1.94 [1.06, 3.57] Heterogeneity: Tau ² = 0.79; Chi ² = 66.11, df = 9 (P < 0.00001); I ² = 86% Test for overall effect: $Z = 2.14$ (P = 0.03) Total (95% Cl) 100.0% 1.70 [1.39, 2.07] Heterogeneity: Tau ² = 0.16; Chi ² = 143.46, df = 24 (P < 0.00001); I ² = 83% Test for overall effect: $Z = 5.16$ (P < 0.00001) Test for subgroup differences: Chi ² = 0.23, df = 1 (P = 0.63), I ² = 0% Eootnotes (1) cohort II (2) cohort II (2) cohort I (3) CAIX score 1 (4) CAIX score 3	Beketic-Oreskovic et al. 2011	1.9081	0.5553	2.2%	6.74 [2.27, 20.01]	2011	
Subtotal (95% CI) 33.2% 1.94 [1.06, 3.57] Heterogeneity: Tau ² = 0.79; Chi ² = 66.11, df = 9 (P < 0.00001); I ² = 86% Test for overall effect: $Z = 2.14$ (P = 0.03) Total (95% CI) 100.0% 1.70 [1.39, 2.07] Heterogeneity: Tau ² = 0.16; Chi ² = 143.46, df = 24 (P < 0.00001); I ² = 83% 0.05 0.2 1 5 20 Test for overall effect: $Z = 5.16$ (P < 0.00001)	Pinheiro et al. 2011	0.892	0.5754	2.1%	2.44 [0.79, 7.54]	2011	
Heterogeneity: Tau ² = 0.79; Chi ² = 66.11, df = 9 (P < 0.00001); I ² = 86% Test for overall effect: Z = 2.14 (P = 0.03) Total (95% Cl) 100.0% 1.70 [1.39, 2.07] Heterogeneity: Tau ² = 0.16; Chi ² = 143.46, df = 24 (P < 0.00001); I ² = 83% Test for overall effect: Z = 5.16 (P < 0.00001) Test for subgroup differences: Chi ² = 0.23, df = 1 (P = 0.63), I ² = 0% <u>Footnotes</u> (1) cohort II (2) cohort I (3) CAIX score 1 (4) CAIX score 3	Subtotal (95% CI)			33.2%	1.94 [1.06, 3.57]		◆
Total (95% CI) 100.0% 1.70 [1.39, 2.07] Heterogeneity: Tau ² = 0.16; Chi ² = 143.46, df = 24 (P < 0.00001); I ² = 83% 0.05 0.2 5 20 Itest for overall effect: Z = 5.16 (P < 0.00001)	Heterogeneity: Tau² = 0.79; Chi Test for overall effect: Z = 2.14 (i² = 66.11, df = 9 (P ≺ (P = 0.03)	0.00001); I² = 86%)		
Heterogeneity: Tau ² = 0.16; Chi ² = 143.46, df = 24 (P < 0.00001); I ² = 83% Test for overall effect: Z = 5.16 (P < 0.00001) Test for subgroup differences: Chi ² = 0.23, df = 1 (P = 0.63), I ² = 0% <u>Footnotes</u> (1) cohort II (2) cohort I (3) CAIX score 1 (4) CAIX score 2 (5) CAIX score 3	Total (95% CI)			100.0%	1.70 [1.39, 2.07]		•
Test for overall effect: Z = 5.16 (P < 0.00001)	Heterogeneity: Tau ² = 0.16 [°] Chi	i ² = 143,46. df = 24 (F	P < 0.000	01); I ² = 8	3%		
(1) cohort II (2) cohort I (3) CAIX score 1 (4) CAIX score 2 (5) CAIX score 3	Test for overall effect: Z = 5.16 (Test for subgroup differences:	(P < 0.00001) Cbi²=0.23 df=1 (P	= 0.63)	P=0%			0.05 0.2 1 5 20 Improve outcome Poor outcome
(1) cohort II (2) cohort I (3) CAIX score 1 (4) CAIX score 2 (5) CAIX score 3	Footnotes		0.00/1	/0			
(2) cohort I (3) CAIX score 1 (4) CAIX score 2 (5) CAIX score 3	(1) cohort II						
(3) CAIX score 1 (4) CAIX score 2 (5) CAIX score 3	(2) cohort I						
(4) CAIX score 2 (5) CAIX score 3	(3) CAIX score 1						
(5) CAIX score 3	(4) CAIX score 2						
	(5) CAIX score 3						
	(0) 0/1// 00010 0						

expression, has been associated with poor survival outcomes, independent of other clinicopathological factors in many solid malignancies, including breast cancer (50). The current meta-analysis included a greater number of studies and confirmed a negative survival outcome in patients with breast cancer who had a high CAIX expression. To our knowledge, this is the first meta-analysis that has examined the CAIX expression exclusively in breast cancer. The results of this meta-analysis may lead to the use of CAIX expression as a prognostic marker, resulting in better treatment options for patients with breast cancer.

High CAIX was significantly associated with poor DFS (HR = 1.70, 95% CI = 1.39–2.07, p < 0.00001) and OS (HR = 2.02, 95% CI 1.40–2.91, p = 0.0002), despite the high heterogeneity of DFS, I² = 83%, and OS, I² = 81%. This heterogeneity could be explained by the bias in the scoring method and cutoff level as most of the studies determined the CAIX protein expression by the intensity and percentage of tumor cell staining and with individual cutoff levels. However, this meta-analysis did support the use of CAIX as a

prognostic marker; therefore, the evaluation of CAIX expression should be considered in breast cancer.

Tumoral hypoxia has long been established as a factor in the progression and metastasis of cancer cells (51). CAIX protein expression is a reliable endogenous hypoxic marker as its expression is dependent on the HIF-1 α activity (16). CAIX is a zinc metalloproteinase that is located at the transmembrane and acts to convert CO₂ to HCO⁻₃ and H⁺ (52). This process occurs extracellularly and results in an extracellular acidic pH. The cancer cells exploit the extracellular acidity to invade the stroma by promoting epithelial-mesenchymal transition (EMT) and cell motility as well as suppressing anti-tumor immunity by, for example, dysregulating cytotoxic T-cell functions while enhancing the function of M2 macrophages and myeloid-derived suppressor cells (MDSCs) (53, 54). These effects may explain the correlation between the increased expression of CAIX and poor survival outcomes.

Carbonic anhydrase IX is highly induced in a HIF-1-dependent manner and is constitutively expressed in VHL-defective cells. While

tudy or Subaroup	log[Hazard Ratio]	SE	Weight	IV. Random, 95% CI	Year	IV. Random, 95% CI
.1.1 Clone M75	log[nazara nado]	95	reight	Try Randolly 55% Cr	Tour	
omatsulet al 2014	1 1 9 6 9	0 3838	61%	3 31 [1 56 7 02]	2014	
etofet al 2012	1.1303	0.0000	5.8%	2 73 [1 20 6 21]	2014	
an et al 2009	1.0043	0.4104	73%	4 29 [2 61 7 05]	2012	
vndietal 2008	0.2624	0.1041	8.3%	1 30 [1 06 1 59]	2008	+
lussain et al. 2008	0.2021	0.3961	6.0%	2 63 [1 21 5 72]	2008	
ienerali et al. 2006	0.6881	0.0001	5.4%		2006	
omes et al. 2003	-0.6931	0.2606	7.2%	0.50 (0.30, 0.83)	2003	_ _
nia et al. 2001	0.9594	0.4844	5.2%	2 61 [1 01 6 75]	2001	
ubtotal (95% CI)			51.2%	2.01 [1.19, 3.38]		•
leterogeneity: Tau² = 0.44; Cr est for overall effect: Z = 2.61	ni² = 46.75, df = 7 (P < (P = 0.009)	0.00001)); I² = 85%)		
.1.2 Other clones						
ing et al. 2022	0.9083	0.2565	7.2%	2.48 [1.50, 4.10]	2022	
lves et al. 2019	-1.1087	0.4023	6.0%	0.33 [0.15, 0.73]	2019	- _
zretic et al. 2017	1.0473	1.0486	2.2%	2.85 [0.36, 22.25]	2017	
amaka et al. 2015	0.7372	0.3512	6.4%	2.09 [1.05, 4.16]	2015	— •—
ïm et al. 2014	0.5247	0.3991	6.0%	1.69 [0.77, 3.69]	2014	+
loh et al. 2014	2.7657	1.1056	2.0%	15.89 [1.82, 138.74]	2014	
aya et al. 2012	1.0188	0.2864	7.0%	2.77 [1.58, 4.86]	2012	
eketic-Oreskovic et al. 2011	1.737	0.5052	5.1%	5.68 [2.11, 15.29]	2011	
rennan et al. 2006	0.6523	0.2889	7.0%	1.92 [1.09, 3.38]	2006	
ubtotal (95% Cl)			48.8%	2.10 [1.26, 3.52]		◆
leterogeneity: Tau ² = 0.40; Ch est for overall effect: Z = 2.84	ni² = 30.15, df = 8 (P = (P = 0.005)	0.0002);	I² = 73%			
otal (95% CI)			100.0%	2.05 [1.44, 2.91]		•
leterogeneity: Tau ² = 0.38; Ct	ni² = 80.58 df = 16 (P	< N NNNN	1) [,] I ² = 80 [,]	%	⊢	
est for overall effect: Z = 3.99	(P < 0.0001) (Chi≅= 0.02, df= 1./D	- 0.000	.,,. = 00 ≆= ∩∞	~	Ö.I	01 0.1 1 10 100 Improve outcome Poor outcome
estion subgroup amerences.	. Cm = 0.02, ui = 1 (P	- 0.90), 1	- 0%			

CAXII is upregulated in VHL-defective renal tumors and induced hypoxia in tumor cells, its dependence on HIF is not well established (15). Additionally, it is well known that the tumor expression of HIF-1 α and CAIX was correlated with poor patient survival, CAXII, which lacks the extracellular proteoglycan domain of CAIX implicated in cell adhesion, had a less obvious survival effect (17). CAXII expression is related to better survival statistics for patients (55–57). In breast cancer, there is a strong association between luminal cancers and CAXII expression. Moreover, CAXII is also a biomarker of favorable prognosis in lung (58) and brain (59) tumors but is associated with a poor prognosis in colorectal cancer (60).

Additionally, this meta-analysis clarified the importance of CAIX expression associated with survival outcomes in both ER^+ and TNBC. Li et al. reported increased tamoxifen resistance in ER^+ breast cancer with a high CAIX expression (27). Similarly, a study by Tan et al. demonstrated the adverse effect of CAIX expression on basal-like breast cancer subtypes by escalating the chemotherapy resistance (42). This may imply that CAIX overexpression is a hostile factor mediating treatment resistance. Thus, a combination of chemotherapy and CAIX inhibitors may be helpful in the prevention of chemoresistance. This meta-analysis had several limitations. The high degree of heterogeneity of the study indicated that we were unable to accurately define a CAIX expression scoring method and optimal threshold values. Further studies to standardize the IHC protocol for CAIX are needed. The publication bias might overestimate the survival outcome as articles reporting positive findings were selected.

Conclusion

Our results highlight the importance of a high CAIX expression being associated with poor DFS and OS in patients with breast cancer. This information may be useful for future studies, leading to the incorporation of CAIX inhibitors in treatment regimens for patients with breast cancer. High-quality studies with larger homogeneous samples are required to determine the prognostic role of CAIX in different breast cancer subtypes.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

WN and CT contributed to the framework and the overall perspective of the study design. The literature search was carried out by WN, SY, and JP. SY and JP extracted the data and assisted with quality control. JP carried out the statistical analysis. WN wrote the manuscript and created the tables and figures. The statistical analysis was supervised and verified by JE and CT. WN, JE, and CT contributed to the study's quality assessment and manuscript revision. JQ checked and edited the English grammar. All authors contributed to the article and approved the submitted version.

Funding

This study received funding from the National Research Council of Thailand (NRCT) and Mahidol University (grant no. N42A650343).

References

1. Giaquinto AN, Sung H, Miller KD, Kramer JL, Newman LA, Minihan A, et al. Breast cancer statistics, 2022. *CA Cancer J Clin.* (2022) 72:524–41. doi: 10.3322/ caac.21754

 Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin. (2022) 72:7–33. doi: 10.3322/caac.21708

3. Phung MT, Tin Tin S, Elwood JM. Prognostic models for breast cancer: a systematic review. *BMC Cancer*. (2019) 19:230. doi: 10.1186/s12885-019-5442-6

4. Yersal O, Barutca S. Biological subtypes of breast cancer: prognostic and therapeutic implications. *World J Clin Oncol.* (2014) 5:412–24. doi: 10.5306/wjco.v5.i3.412

5. Soerjomataram I, Louwman MWJ, Ribot JG, Roukema JA, Coebergh JWW. An overview of prognostic factors for long-term survivors of breast cancer. *Breast Cancer Res Treat.* (2008) 107:309–30. doi: 10.1007/s10549-007-9556-1

6. Schaafsma E, Zhang B, Schaafsma M, Tong C-Y, Zhang L, Cheng C. Impact of Oncotype DX testing on ER+ breast cancer treatment and survival in the first decade of use. *Breast Cancer Res.* (2021) 23:74. doi: 10.1186/s13058-021-01453-4

7. Almstedt K, Heimes AS, Kappenberg F, Battista MJ, Lehr HA, Krajnak S, et al. Long-term prognostic significance of HER2-low and HER2-zero in node-negative breast cancer. *Eur J Cancer.* (2022) 173:10–9. doi: 10.1016/j.ejca.2022.06.012

8. Wang C. A meta-analysis of efficacy and safety of PD-1/PD-L1 inhibitors in triplenegative breast cancer. *J Oncol.* (2022) 2022:1–7. doi: 10.1155/2022/2407211

9. Semenza GL. Hypoxia and cancer. Cancer Metastasis Rev. (2007) 26:223-4. doi: 10.1007/s10555-007-9058-y

10. Vaupel P, Mayer A. Hypoxia in cancer: significance and impact on clinical outcome. *Cancer Metastasis Rev.* (2007) 26:225-39. doi: 10.1007/s10555-007-9055-1

11. McKeown SR. Defining normoxia, physoxia and hypoxia in tumours-implications for treatment response. *Br J Radiol*. (2014) 87:20130676. doi: 10.1259/bjr.20130676

12. Godet I, Doctorman S, Wu F, Gilkes DM. Detection of hypoxia in cancer models: significance, challenges, and advances. *Cells.* (2022) 11:686. doi: 10.3390/cells11040686

13. Ziello JE, Jovin IS, Huang Y. Hypoxia-inducible factor (HIF)-1 regulatory pathway and its potential for therapeutic intervention in malignancy and ischemia. *Yale J Biol Med.* (2007) 80:51–60. PMID: 18160990

14. Semenza GL. Targeting HIF-1 for cancer therapy. *Nat Rev Cancer*. (2003) 3:721–32. doi: 10.1038/nrc1187

15. Wykoff CC, Beasley NJ, Watson PH, Turner KJ, Pastorek J, Sibtain A, et al. Hypoxia-inducible expression of tumor-associated carbonic anhydrases. *Cancer Res.* (2000) 60:7075–83. PMID: 11156414

16. Kaluz S, Kaluzova M, Liao SY, Lerman M, Stanbridge EJ. Transcriptional control of the tumor- and hypoxia-marker carbonic anhydrase 9: a one transcription factor (HIF-1) show? *Biochim Biophys Acta.* (2009) 1795:162–72. doi: 10.1016/j. bbcan.2009.01.001

17. Pastorekova S, Zatovicova M, Pastorek J. Cancer-associated carbonic anhydrases and their inhibition. *Curr Pharm Des.* (2008) 14:685–98. doi: 10.2174/138161208783877893

18. Robertson N, Potter C, Harris AL. Role of carbonic anhydrase IX in human tumor cell growth, survival, and invasion. *Cancer Res.* (2004) 64:6160–5. doi: 10.1158/0008-5472.CAN-03-2224

19. Queen A, Bhutto HN, Yousuf M, Syed MA, Hassan MI. Carbonic anhydrase IX: a tumor acidification switch in heterogeneity and chemokine regulation. *Semin Cancer Biol.* (2022) 86:899–913. doi: 10.1016/j.semcancer.2022.01.001

20. Becker HM. Carbonic anhydrase IX and acid transport in cancer. *Br J Cancer*. (2020) 122:157–67. doi: 10.1038/s41416-019-0642-z

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

21. Svastová E, Zilka N, Zaťovicová M, Gibadulinová A, Ciampor F, Pastorek J, et al. Carbonic anhydrase IX reduces E-cadherin-mediated adhesion of MDCK cells via interaction with beta-catenin. *Exp Cell Res.* (2003) 290:332–45. doi: 10.1016/S0014-4827(03)00351-3

22. Ong CHC, Lee DY, Lee B, Li H, Lim JCT, Lim JX, et al. Hypoxia-regulated carbonic anhydrase IX (CAIX) protein is an independent prognostic indicator in triple negative breast cancer. *Breast Cancer Res.* (2022) 24:38. doi: 10.1186/s13058-022-01532-0

23. Brennan DJ, Jirstrom K, Kronblad A, Millikan RC, Landberg G, Duffy MJ, et al. CA IX is an independent prognostic marker in premenopausal breast cancer patients with one to three positive lymph nodes and a putative marker of radiation resistance. *Clin Cancer Res.* (2006) 12:6421–31. doi: 10.1158/1078-0432.CCR-06-0480

24. Currie MJ, Beardsley BE, Harris GC, Gunningham SP, Dachs GU, Dijkstra B, et al. Immunohistochemical analysis of cancer stem cell markers in invasive breast carcinoma and associated ductal carcinoma in situ: relationships with markers of tumor hypoxia and microvascularity. *Hum Pathol.* (2013) 44:402–11. doi: 10.1016/j. humpath.2012.06.004

25. Pinheiro C, Sousa B, Albergaria A, Paredes J, Dufloth R, Vieira D, et al. GLUT1 and CAIX expression profiles in breast cancer correlate with adverse prognostic factors and MCT1 overexpression. *Histol Histopathol.* (2011) 26:1279–86. doi: 10.14670/HH-26.1279

26. Shamis SAK, Quinn J, Mallon EEA, Edwards J, McMillan DC. The relationship between the tumor cell expression of hypoxic markers and survival in patients with ER-positive invasive ductal breast cancer. *J Histochem Cytochem*. (2022) 70:479–94. doi: 10.1369/00221554221110280

27. Li Y, Chen X, Zhou Z, Li Q, Westover KD, Wang M, et al. Dynamic surveillance of tamoxifen-resistance in ER-positive breast cancer by CAIX-targeted ultrasound imaging. *Cancer Med.* (2020) 9:2414–26. doi: 10.1002/cam4.2878

28. Alves W, Bonatelli M, Dufloth R, Kerr LM, Carrara GFA, da Costa RFA, et al. CAIX is a predictor of pathological complete response and is associated with higher survival in locally advanced breast cancer submitted to neoadjuvant chemotherapy. *BMC Cancer*. (2019) 19:1173. doi: 10.1186/s12885-019-6353-2

29. Ozretic P, Alvir I, Sarcevic B, Vujaskovic Z, Rendic-Miocevic Z, Roguljic A, et al. Apoptosis regulator Bcl-2 is an independent prognostic marker for worse overall survival in triple-negative breast cancer patients. *Int J Biol Markers*. (2018) 33:109–15. doi: 10.5301/iibm.5000291

30. Jin MS, Lee H, Park IA, Chung YR, Im SA, Lee KH, et al. Overexpression of HIF1 α and CAXI predicts poor outcome in early-stage triple negative breast cancer. *Virchows Arch.* (2016) 469:183–90. doi: 10.1007/s00428-016-1953-6

31. Chu CY, Jin YT, Zhang W, Yu J, Yang HP, Wang HY, et al. CA IX is upregulated in CoCl2-induced hypoxia and associated with cell invasive potential and a poor prognosis of breast cancer. *Int J Oncol.* (2016) 48:271–80. doi: 10.3892/ijo.2015.3253

32. Samaka RM, Abd El-Wahed MM, Al Sharaky DR, Shehata MA, Hegazy SE, Aleskandarany MA. Overexpression of carbonic anhydrase IX is a dismal prognostic marker in breast carcinoma in Egyptian patients. *Appl Immunohistochem Mol Morphol.* (2016) 24:405–13. doi: 10.1097/PAI.000000000000208

33. Aomatsu N, Yashiro M, Kashiwagi S, Kawajiri H, Takashima T, Ohsawa M, et al. Carbonic anhydrase 9 is associated with chemosensitivity and prognosis in breast cancer patients treated with taxane and anthracycline. *BMC Cancer*. (2014) 14:400. doi: 10.1186/1471-2407-14-400

34. Deb S, Johansson I, Byrne D, Nilsson C, Constable L, Fjällskog ML, et al. Nuclear HIF1A expression is strongly prognostic in sporadic but not familial male breast cancer. *Mod Pathol.* (2014) 27:1223–30. doi: 10.1038/modpathol.2013.231

35. Kim HM, Jung WH, Koo JS. Site-specific metabolic phenotypes in metastatic breast cancer. J Transl Med. (2014) 12:354. doi: 10.1186/s12967-014-0354-3

36. Noh S, Kim JY, Koo JS. Metabolic differences in estrogen receptor-negative breast cancer based on androgen receptor status. *Tumour Biol.* (2014) 35:8179–92. doi: 10.1007/s13277-014-2103-x

37. Betof AS, Rabbani ZN, Hardee ME, Kim SJ, Broadwater G, Bentley RC, et al. Carbonic anhydrase IX is a predictive marker of doxorubicin resistance in early-stage breast cancer independent of HER2 and TOP2A amplification. *Br J Cancer*. (2012) 106:916–22. doi: 10.1038/bjc.2012.32

38. Kaya AO, Gunel N, Benekli M, Akyurek N, Buyukberber S, Tatli H, et al. Hypoxia inducible factor-1 alpha and carbonic anhydrase IX overexpression are associated with poor survival in breast cancer patients. *J BUON*. (2012) 17:663–8. PMID: 23335522

39. Beketic-Oreskovic L, Ozretic P, Rabbani ZN, Jackson IL, Sarcevic B, Levanat S, et al. Prognostic significance of carbonic anhydrase IX (CA-IX), endoglin (CD105) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) in breast cancer patients. *Pathol Oncol Res.* (2011) 17:593–603. doi: 10.1007/s12253-010-9355-6

40. Lou Y, McDonald PC, Oloumi A, Chia S, Ostlund C, Ahmadi A, et al. Targeting tumor hypoxia: suppression of breast tumor growth and metastasis by novel carbonic anhydrase IX inhibitors. *Cancer Res.* (2011) 71:3364–76. doi: 10.1158/0008-5472. CAN-10-4261

41. Jubb AM, Soilleux EJ, Turley H, Steers G, Parker A, Low I, et al. Expression of vascular notch ligand delta-like 4 and inflammatory markers in breast cancer. *Am J Pathol.* (2010) 176:2019–28. doi: 10.2353/ajpath.2010.090908

42. Tan EY, Yan M, Campo L, Han C, Takano E, Turley H, et al. The key hypoxia regulated gene CAIX is upregulated in basal-like breast tumours and is associated with resistance to chemotherapy. *Br J Cancer*. (2009) 100:405–11. doi: 10.1038/sj.bjc.6604844

43. Crabb SJ, Bajdik CD, Leung S, Speers CH, Kennecke H, Huntsman DG, et al. Can clinically relevant prognostic subsets of breast cancer patients with four or more involved axillary lymph nodes be identified through immunohistochemical biomarkers? A tissue microarray feasibility study. *Breast Cancer Res.* (2008) 10:R6. doi: 10.1186/bcr1847

44. Kyndi M, Sørensen FB, Knudsen H, Alsner J, Overgaard M, Nielsen HM, et al. Carbonic anhydrase IX and response to postmastectomy radiotherapy in high-risk breast cancer: a subgroup analysis of the DBCG82 b and c trials. *Breast Cancer Res.* (2008) 10:R24. doi: 10.1186/bcr1981

45. Hussain SA, Ganesan R, Reynolds G, Gross L, Stevens A, Pastorek J, et al. Hypoxiaregulated carbonic anhydrase IX expression is associated with poor survival in patients with invasive breast cancer. *Br J Cancer*. (2007) 96:104–9. doi: 10.1038/sj.bjc.6603530

46. Trastour C, Benizri E, Ettore F, Ramaioli A, Chamorey E, Pouysségur J, et al. HIF-1alpha and CA IX staining in invasive breast carcinomas: prognosis and treatment outcome. *Int J Cancer*. (2007) 120:1451–8. doi: 10.1002/ijc.22436

47. Generali D, Berruti A, Brizzi MP, Campo L, Bonardi S, Wigfield S, et al. Hypoxiainducible factor-1alpha expression predicts a poor response to primary chemoendocrine therapy and disease-free survival in primary human breast cancer. *Clin Cancer Res.* (2006) 12:4562–8. doi: 10.1158/1078-0432.CCR-05-2690 48. Tomes L, Emberley E, Niu Y, Troup S, Pastorek J, Strange K, et al. Necrosis and hypoxia in invasive breast carcinoma. *Breast Cancer Res Treat.* (2003) 81:61–9. doi: 10.1023/A:1025476722493

49. Chia SK, Wykoff CC, Watson PH, Han C, Leek RD, Pastorek J, et al. Prognostic significance of a novel hypoxia-regulated marker, carbonic anhydrase IX, in invasive breast carcinoma. *J Clin Oncol.* (2001) 19:3660–8. doi: 10.1200/JCO.2001.19.16.3660

50. van Kuijk SJ, Yaromina A, Houben R, Niemans R, Lambin P, Dubois LJ. Prognostic significance of carbonic anhydrase IX expression in cancer patients: a meta-analysis. *Front Oncol.* (2016) 6:69. doi: 10.3389/fonc.2016.00069

51. Muz B, de la Puente P, Azab F, Azab AK. The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. *Hypoxia*. (2015) 3:83–92. doi: 10.2147/HP.S93413

52. Sedlakova O, Svastova E, Takacova M, Kopacek J, Pastorek J, Pastorekova S. Carbonic anhydrase IX, a hypoxia-induced catalytic component of the pH regulating machinery in tumors. *Front Physiol.* (2014) 4:400. doi: 10.3389/fphys.2013.00400

53. Huber V, Camisaschi C, Berzi A, Ferro S, Lugini L, Triulzi T, et al. Cancer acidity: an ultimate frontier of tumor immune escape and a novel target of immunomodulation. *Semin Cancer Biol.* (2017) 43:74–89. doi: 10.1016/j.semcancer.2017.03.001

54. Boedtkjer E, Pedersen SF. The acidic tumor microenvironment as a driver of cancer. *Annu Rev Physiol.* (2020) 82:103–26. doi: 10.1146/annurev-physiol-021119-034627

55. Barnett DH, Sheng S, Charn TH, Waheed A, Sly WS, Lin CY, et al. Estrogen receptor regulation of carbonic anhydrase XII through a distal enhancer in breast cancer. *Cancer Res.* (2008) 68:3505–15. doi: 10.1158/0008-5472.CAN-07-6151

56. Wykoff CC, Beasley N, Watson PH, Campo L, Chia SK, English R, et al. Expression of the hypoxia-inducible and tumor-associated carbonic anhydrases in ductal carcinoma in situ of the breast. *Am J Pathol.* (2001) 158:1011–9. doi: 10.1016/S0002-9440(10)64048-5

57. Watson PH, Chia SK, Wykoff CC, Han C, Leek RD, Sly WS, et al. Carbonic anhydrase XII is a marker of good prognosis in invasive breast carcinoma. *Br J Cancer*. (2003) 88:1065–70. doi: 10.1038/sj.bjc.6600796

58. Ilie MI, Hofman V, Ortholan C, Ammadi RE, Bonnetaud C, Havet K, et al. Overexpression of carbonic anhydrase XII in tissues from resectable non-small cell lung cancers is a biomarker of good prognosis. *Int J Cancer*. (2011) 128:1614–23. doi: 10.1002/ ijc.25491

59. Nordfors K, Haapasalo J, Korja M, Niemelä A, Laine J, Parkkila AK, et al. The tumour-associated carbonic anhydrases CA II, CA IX and CA XII in a group of medulloblastomas and supratentorial primitive neuroectodermal tumours: an association of CA IX with poor prognosis. *BMC Cancer.* (2010) 10:148. doi: 10.1186/1471-2407-10-148

60. Kivelä A, Parkkila S, Saarnio J, Karttunen TJ, Kivelä J, Parkkila AK, et al. Expression of a novel transmembrane carbonic anhydrase isozyme XII in normal human gut and colorectal tumors. *Am J Pathol.* (2000) 156:577–84. doi: 10.1016/S0002-9440(10)64762-1