Prognosis assessment of CD44+/CD24- in Breast Cancer Patients: a systematic review and meta-analysis

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Research Article

Keywords: CD44+/CD24-, Breast cancer, Meta-analysis, Prognosis

Posted Date: August 23rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-318997/v1>

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Version of Record: A version of this preprint was published at Archives of Gynecology and Obstetrics on April 18th, 2022. See the published version at <https://doi.org/10.1007/s00404-022-06402-w>.

Manuscript title: **Prognosis assessment of CD44⁺/CD24⁻ in Breast Cancer Patients: a systematic review and meta-analysis**

Running title: Meta-analysis of CD44⁺/CD24⁻ Prognosis Assessment in Breast Cancer Patients

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Abstract

Purpose: This meta-analysis investigated the relationships between the CD44⁺/CD24⁻ phenotype and tumor size, lymph node metastasis, distant metastasis, disease-free survival (DFS), and overall survival (OS) in 8036 postoperative breast cancer patients enrolled in 23 studies. **Methods:** A literature search of PubMed, Medline, Cochrane, Embase, and PMC was conducted to identify eligible studies. The combined odds ratios (ORs) and 95% confidence intervals (95% CIs) were analyzed to evaluate the relationships between the CD44⁺/CD24⁻ phenotype and the pathological and biological characteristics of breast cancer patients, and the combined hazard ratios (HRs) and 95% CIs were calculated to evaluate the relationships between CD44⁺/CD24⁻ and DFS and OS of breast cancer patients using Stata 12.0 software. **Results:** The CD44⁺/CD24⁻ phenotype were not related to the tumor size (tumor size > 2.0 cm vs ≤ 2.0 cm, combined OR = 0.98, 95% CI: 0.68–1.34, $p = 0.792$) and didn't promote lymph node metastasis (lymph node metastasis vs. no lymph node metastasis, combined OR = 0.94, 95% CI: 0.71–1.26, $p = 0.692$) and distant metastasis (distant metastasis vs no distant metastasis, combined OR = 3.88, 95% CI: 0.93–16.24, $p = 0.064$). The CD44⁺/CD24⁻ phenotype was negatively correlated with postoperative DFS (HR = 1.67, 95% CI: 1.35–2.07, $p < 0.00001$) and OS (combined HR = 1.52, 95% CI: 1.21–1.91, $p = 0.0004$). **Conclusion:** These results suggested expression of the CD44⁺/CD24⁻ phenotype can be used as a reliable indicator of clinical prognosis and a potential therapeutic targets in breast cancer patients.

Key words: CD44⁺/CD24⁻; Breast cancer; Meta-analysis; Prognosis.

Declarations**Funds**

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors

Conflicts of interest

We don't have a conflict of interest

Availability of data and material

I can always upload the data if needed.

Code availability

Stata12.0 and Revman5.3

Authors' contributions: Author Zhaoming Ma(Doctoral candidate)and author Guanhong Huang(Doctor)have given substantial contributions to the conception or the design of the manuscript, author Jingjing Gu and author Dandan Chen to acquisition, analysis and interpretation of the data,author Zhiqiang Li and Yongliang Yang to further review the data.All authors have participated to drafting the manuscript,author Zhaoming Ma and author Guanhong Huang revised it critically. All authors read and approved the final version of the manuscript. All authors contributed equally to the manuscript and read and approved the final version of the manuscript.

Ethics approval

Patients and the public were not involved in the design or conduct of the study.

Consent to participate

All the authors agreed to participate.

Consent for publication

All the authors agreed to publish.

Acknowledgements:

Thanks Jingjing Gu, Dandan Chen, Zhaoming Ma, Guanhong Huang, Zhiqiang Li and Yongliang Yang for their efforts on this article.

Introduction

Breast cancer is the most common malignant tumor and the second leading cause of cancer death in women^[1]. According to the statistics of the World Health Organization (WHO), more than 4 million patients are diagnosed with breast cancer every year, and more than 1 million people die of breast cancer every year^[2]. According to multiple studies, breast cancer is a group of heterogeneous tumors with different proliferation rates, invasive capabilities, metastatic potential, and therapeutic effects^[3]. Although there are

many treatments, including surgery, radiotherapy, chemotherapy, endocrine therapy, targeted therapy, cytotoxic drug therapy, hormone therapy, and immunotherapy, a large proportion of breast cancer patients eventually die of breast cancer recurrence and metastasis. Therefore, early detection of sensitive prognostic markers is particularly important for the prognosis of breast cancer patients^[4]. There is evidence indicating that, although many malignant tumors are clonal in nature, they contain heterogeneous cell populations with different biological characteristics, and a small portion of these are cancer stem cells (CSCs)^[5]. Some experts believe that these self-renewing CSCs are the main reason for the failure of cancer treatment^[6]. In 2003, Al-Hajj and colleagues^[7] discovered that cells with the CD44⁺/CD24⁻ phenotype undergo a process similar to normal stem cell self-renewal and differentiation^[8]. Hence, the expression of CD44⁺/CD24⁻ cells has attracted the interest of experts^[9]. Hiroko Nogi found that cells with a expression of CD44⁺/CD24⁻ are associated with lymph node metastasis^[10], and there are reports suggesting that cells with high expression of this phenotype are associated with poor prognosis of breast cancer^[12]. Lee^[13] et al demonstrated that expression of CD44⁺/CD24⁻ phenotype cells is associated with breast cancer tumor progression, and emphasized the importance of CSC-targeted therapy in the treatment of breast cancer. Other reports have put forward different viewpoints from the above^[14] so that the role of CD44⁺/CD24⁻ tumor stem cells in breast cancer remains controversial^[15]. The purpose of this study was to establish a meta-analysis model for investigating the relationships between cells with expression of the CD44⁺/CD24⁻ phenotype and tumor size, lymph node metastasis, distant metastasis, disease-free survival (DFS), and overall survival (OS) in breast cancer patients.

Study selection

We searched the following combinations of medical subject words (MeSH) and text words in the PubMed, Medline, Cochrane, Embase, and PMC databases: (“breast cancer” or “breast cancer”) and (“CD44” and “CD24”) and (“prognosis” or “survival” or “outcome”). Then, in order to find other eligible papers avoiding the omissions of electronic retrieval methods, we further searched the references in the identified preliminary research papers and review articles. The date determining the inclusion of literature was December 2019.

Data extraction and quality evaluation

Two independent reviewers (Jingjing Gu and Dandan Chen) read the titles and abstracts of all candidate articles. If the nature of some articles could not be determined from the title or abstract, the full text was reviewed. Any differences in the two quality assessments and data collections were discussed with corresponding author until agreement was finally reached. A predefined form was used for data extraction. The extracted data included: the name of the first author, publication time, country, number of patients, patients with a expression of CD44⁺/CD24⁻ phenotype, the number of tumors > 2.0 cm, lymph node metastases, distant

metastases, follow-up time, detection method, HR, and 95% CI corresponding to OS and DFS. For articles that only included the Kaplan-Meier curve without providing HRs and 95% CIs, we used the methods in Tierney^[17] and Parmar et al^[18] to estimate HRs. And the prognosis-related survival rate was extracted from the Kaplan-Meier curve by GetData Graph digicater 2.24 software. Some papers, from which we still could not obtain the above information even after efforts to contact the author of the original material, were marked as “NA” (not available).

To ensure the quality of each document, the NOS scale^[16] bias assessment tool (Table 1) was used to evaluate the quality of the literature, and was independently completed and checked by two researchers (Jingjing Gu and Dandan Chen). Studies with a total score of 6–9 were regarded as high-quality research, and all papers of included in this meta-analysis were rated 6–9. And the following criteria had to be met: (1) all articles included in this study were cohort studies; (2) research subjects were postoperative patients diagnosed with breast cancer by pathology; (3) expression of CD44⁺/CD24⁻ in breast cancer was detected by immunohistochemistry (IHC); (4) sufficient data were presented in the paper, including the number of patients with tumor size > 2.0 cm, lymph node metastasis, and distant metastasis, in order to estimate the odds ratio (OR), hazard ratio (HR), and 95% confidence interval (95% CI) that corresponded to the overall survival rate (OS) or disease-free survival rate (DFS), or so that we could calculate these measures based on the information in the article; and (5) the study was published in English.

Exclusion criteria

The following publications were excluded: (1) summaries, comments, letters to editors, and articles published in one or more unpublished books; (2) articles related to CD44⁺/CD24⁻, or articles that detected CD44⁺/CD24⁻ in the blood; (3) articles that only reported animal experiments.

Statistical Analysis

ORs and 95% CIs were used to estimate the relationships between tumor stem cells CD44⁺/CD24⁻ and breast cancer pathological parameters (tumor size, lymph node status, distant metastasis). We evaluated the correlations between cells with expression of the CD44⁺/CD24⁻ phenotype and patients' OS and DFS by HRs and 95% CIs. The extracted data were analyzed using Stata12.0 and RevMan 5.3 analysis software (Cochrane Collaboration). In this study, OR > 1 meant that cells with expression of the CD44⁺/CD24⁻ phenotype were closely related to tumors > 2 cm, lymph node metastasis, and distant metastasis. HR > 1 indicated that cells with expression of the CD44⁺/CD24⁻ phenotype were negatively correlated with OS and DFS. The Q test was used to evaluate the heterogeneity of the study, and the I² value indicated the degree of heterogeneity. A *p* value > 0.05 and I² < 50% meant no heterogeneity, and a fixed-effect model was needed; otherwise, we selected a random-effect model^[19]. In addition, we evaluated the publication bias of the studies using Begg's funnel

plot, Begg's test and Egger's test. If the the graph appeared like a symmetrical inverted funnel and the p values of Begg's test and Egger's test were both > 0.05 , this indicated that publication bias was not detected. We verified the reliability of the results by sensitivity analysis. All statistical tests in this meta-analysis were two-tailed and p -values < 0.05 were considered statistically significant.

Results

Characteristics of Included Studies

According to the above search strategy, a total of 332 articles were initially retrieved. By screening the topics, eight repeated articles and 38 reviews were excluded. In total, 197 non-human experiments or non-breast cancer or non-CD44⁺/CD24⁻-related studies were excluded by reviewing the abstracts, and we excluded 66 articles that detected the expression of CD44⁺/CD24⁻ in the blood. Finally, we included 23 studies in the meta-analysis, and the flowchart of the search strategy is shown in Figure 1. The total number of patients included in this meta-analysis was 8,036, from 5 studies on tumor size, 16 studies on lymph node status, and 5 studies on distant metastasis. We conducted 11 studies on OS and 9 studies on DFS.

Table 2 summarizes the main characteristics of the data related to the cancer stem cells CD44⁺/CD24⁻ in this meta-analysis.

Correlations between the CD44⁺/CD24⁻ Markers and the Pathological and Biological Characteristics of Breast Cancer Patients

The correlation between expression of the CD44⁺/CD24⁻ phenotype and the tumor size in breast cancer patients is shown in Figure 2. Overall analysis showed that cells with the expression of CD44⁺/CD24⁻ phenotype were not related to the tumor size (tumor size > 2.0 cm vs ≤ 2.0 cm, combined OR = 0.98, 95% CI: 0.68–1.34, $p = 0.792$, fixed-effect model), lymph node metastasis in breast cancer patients (Figure 4, lymph node metastasis vs no lymph node metastasis, combined OR = 0.94, 95% CI: 0.71–1.26, $p = 0.692$, random-effect model), and distant metastasis of breast cancer patients (Figure 6, distant metastasis vs no distant metastasis, combined OR = 3.88, 95% CI: 0.93–16.24, $p = 0.064$, random-effect model).

Effects of the CD44⁺/CD24⁻ Phenotype on Postoperative Disease-free Survival and Overall Survival

The above method was used to conduct 11 studies on the OS and nine studies on the DFS of breast cancer patients. The correlations between the expression of the CD44⁺/CD24⁻ phenotype and DFS and OS of breast cancer patients are shown in Figures 5 and 6. The results indicated that expression of the cancer stem cell marker CD44⁺/CD24⁻ and DFS (Figure 8, HR = 1.67, 95% CI: 1.35–2.07, $p < 0.00001$, fixed-effect model) and OS (Figure 10, combined HR = 1.52, 95% CI: 1.21–1.91, $p = 0.0004$, fixed-effect model) were negatively correlated, and their differences were statistically significant.

Publication Bias

Begg's funnel plot, Begg's test and Egger's test were used to evaluate publication bias. The graphs of Begg's funnel plot appeared like a symmetrical inverted, and the correlations between expression of the CD44⁺/CD24⁻ phenotype and a patient's tumor size (Begg's test: $p = 0.221$, Egger's test: $p = 0.204$), lymph node metastasis (Begg's test: $p = 0.344$, Egger's test: $p = 0.171$), distant metastasis (Begg's test: $p = 0.462$, Egger's test: $p = 0.066$), DFS (Begg's test: $p = 0.602$, Egger's test: $p = 0.78$), and OS (Begg's test: $p = 1$, Egger's test: $p = 0.665$) showed there was no obvious publication bias.

Sensitivity Analysis

The sensitivity analysis results (Figures 12, 13, 14, 15 and 16) showed that the correlations between the cells that expressed the CD44⁺/CD24⁻ phenotype and tumor size, lymph node metastasis, DFS, and OS of breast cancer patients were stable. However, as shown in Figure 14, the results of the correlation between expression of the CD44⁺/CD24⁻ phenotype and the distant metastasis of breast cancer patients were not stable.

Discussion

Since Hamburger and other experts explicitly proposed the “cancer stem cell” (CSC) hypothesis for the first time in 1997, research on cancer stem cells has attracted much attention from researchers. The hypothesis proposes that tumors originate from a small subset of rare cells that have the ability to self-renew and differentiate into other cancer cells, thereby promoting tumor cell proliferation, tumor generation, and growth^[37]. There is increasing evidence to support the CSC hypothesis^[38]. Such research is mainly based on xenotransplantation experiments, in which human breast cancer cells are transplanted into immunocompromised mice. The results have shown that only CSCs (usually <1% of malignant tumors) can produce tumors^[39], and also that CSCs are the only cells participating in tumor recurrence and metastasis^[40]. Some researchers also believe that CSCs promote tumor growth, invasion, and metastasis by stimulating blood vessels to obtain sufficient blood and nutrition^[41]. Some experts have suggested that CSCs in human breast cancer are related to recurrence and metastasis in breast cancer patients^[42], and that breast cancer stem cells induce local lymph node metastasis in breast cancer patients^[43]. Al-Hajj and colleagues proposed for the first time that cells with expression of the CD44⁺/CD24⁻ phenotype in breast cancer show the characteristics of tumor stem cells^[44]. Many subsequent studies have shown that cells with expression of the CD44⁺/CD24⁻ phenotype have tumorigenic ability and invasive characteristics^[45]. Some experts suggested that breast cancer phenotype CD44⁺/CD24⁻ cells have greater invasive ability *in vitro* and greater metastatic ability *in vivo* than other cells^[46]. Studies have shown that expression of the CD44⁺/CD24⁻ phenotype is associated with DFS and OS in breast cancer patients^[47]. However, the role of the CSC phenotype CD44⁺/CD24⁻ in breast cancer remains controversial. The research results of Mylona et al showed that there was no obvious correlation between cells with expression of the CD44⁺/CD24⁻ phenotype and DFS and OS

of postoperative patients^[15]. Here, we investigated the correlations between the CSC CD44⁺/CD24⁻ phenotype and the pathological and biological characteristics of breast cancer patients, and the effects of this phenotype on the prognosis of breast cancer patients. We found that cells with the expression of CD44⁺/CD24⁻ phenotype were not related to the tumor size (tumor size > 2.0 cm vs ≤ 2.0 cm, combined OR = 0.98, 95% CI: 0.68–1.34, $p = 0.792$, fixed-effect model) and didn't promot local lymph node metastasis (lymph node metastasis vs. no lymph node metastasis, combined OR = 0.94, 95% CI: 0.71–1.26, $p = 0.692$, random-effect model) and distant metastasis (distant metastasis vs no distant metastasis, combined OR = 3.88, 95% CI: 0.93–16.24, $p = 0.064$, random-effect model). Expression of the CD44⁺/CD24⁻ marker was negatively correlated with postoperative DFS (HR = 1.67, 95% CI: 1.35–2.07, $p < 0.00001$, fixed-effect model) and OS (combined HR = 1.52, 95% CI: 1.21–1.91, $p = 0.0004$, fixed-effect model), and their differences were statistically significant.

The CSC CD44⁺/CD24⁻ phenotype has always been a research hot spot. Many investigators^[48,49] have a strong interest in the mechanism of the CD44⁺/CD24⁻ phenotype in breast cancer since there has recently been renewed interest in CSCs. Hence, we felt that the CD44⁺/CD24⁻ phenotype still has a certain value and significance in predicting the prognosis of breast cancer patients. Some meta-analyses have previously studied the correlations between the CD44⁺/CD24⁻ phenotype of cancer stem cells and the pathological and biological characteristics of breast cancer patients, and there is also a correlation between the CD44⁺/CD24⁻ phenotype and prognosis of breast cancer patients. Zhou^[50] suggested that expression of the CD44⁺/CD24⁻ phenotype is not correlated with tumor size and lymphatic metastasis in breast cancer patients, but is negatively correlated with the OS of breast cancer patients. However, Wang's study found that expression of the CD44⁺/CD24⁻ phenotype was not significantly associated with the OS of breast cancer patients^[51]. The two meta-analysis results are different, which highlights and emphasizes the necessity of our meta-analysis. Compared with the studies of Zhou and Wang, our meta-analysis proved that expression of the CD44⁺/CD24⁻ phenotype is not closely related to tumor size, lymph node metastasis, and distant metastasis in breast cancer patients, but negatively correlated with DFS and OS. In support of our results, we included more research samples found through more search channels. Our meta-analysis involved a total of 23 studies, including 8,036 breast cancer patients. More importantly, we did not find heterogeneity or publication bias, and the sensitivity analysis also suggested that the results are stable on the correlation between the expression of the CD44⁺/CD24⁻ phenotype and the tumor size, lymph node metastasis, DFS and OS in breast cancer patients. Therefore, we conclude that breast cancer cells with expression of the CD44⁺/CD24⁻ phenotype are not related to the pathological and biological characteristics of postoperative patients with breast cancer, and the phenotype can be used as a tumor marker to predict the prognosis of breast cancer patients. Moreover, it can be a clinical predictor of potential therapeutic targets for breast cancer patients.

Although our research has many advantages, our study still had certain limitations. For example, on the correlation between cells with expression of the CD44⁺/CD24⁻ phenotype and distant metastasis, although obvious publication bias was not found in the results, the sensitivity analysis indicated that the results were not stable. There might be several causes for this. First, although we have checked English websites such as PubMed, Medline, Cochrane, Embase, and PMC, there were some unpublished or non-English studies that we did not collect. Second, the breast cancer patients included in the study may have been treated with different treatment methods, which would have a certain impact on the outcome of the study. Third, some studies we included only had Kaplan-Meier survival curves, so that there may have been some deviations in extracting survival data from the survival curves. Fourth, in the studies we included, the authors' follow-up times for patients were different, which may have had a certain impact on the survival data of our study. Fifthly, although the cancer stem cell CD44⁺/CD24⁻ phenotype was extracted by IHC in the studies we included, the cut-off values were different. All of the above factors may cause deviations in the research results.

In summary, the results of this study indicated that, in breast cancer patients, tumor cells with expression of the CD44⁺/CD24⁻ phenotype didn't promote tumor tissue growth, lymph node metastasis, and distant metastasis. However, they were closely related to DFS and OS. Therefore, we concluded that the cancer stem cell CD44⁺/CD24⁻ phenotype can be used as a reliable indicator for the clinical prognosis of breast cancer patients and a potential therapeutic target.

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Table I (The quality of the included studies assessed by NOS)

Study	Selection				Comparability	Exposure			Scores	
					Same method					
	Adequate	Selection			Control for		to ascertain			
	definition	Representativeness of	of	Definition	important	Ascertainment	for cases	Non-response		
	of cases	of cases	controls	Of controls	factor	of exposure	and controls	rate		
Gabriella ^[20]	☆	☆	☆	☆	☆	☆	-	☆	7	
Alexandra ^[21]	☆	☆	☆	☆	☆	☆	☆	☆	8	

Hiroko ^[10]	☆	☆	☆	☆	☆	☆	☆	-	7
James ^[22]	☆	☆	☆	☆	-	☆	☆	☆	7
Mohamed ^[23]	☆	☆	☆	☆	☆	☆	☆	☆	8
Gulnur ^[24]	☆	☆	☆	☆	-	☆	☆	☆	7
Julia ^[25]	☆	☆	☆	☆	☆	☆	☆	-	7
Giordano ^[26]	☆	☆	☆	☆	☆	☆	☆	☆	8
Agnieszka ^[27]	☆	☆	☆	☆	☆	☆	☆	☆	8
Diego ^[28]	☆	☆	☆	☆	-	☆	☆	☆	7
Nilgün ^[14]	☆	☆	☆	-	☆	☆	☆	-	6
Chen ^[4]	☆	☆	☆	-	☆	☆	-	☆	6
Zhao ^[29]	☆	☆	☆	☆	☆	☆	☆	-	7
Bane ^[30]	☆	☆	☆	☆	-	☆	☆	-	6
Seo ^[31]	☆	☆	☆	☆	☆	☆	☆	-	7
Rabinovich ^[32]	☆	☆	☆	☆	-	☆	☆	-	6
Eleni ^[33]	☆	☆	☆	☆	☆☆	☆	☆	☆	9
Lin ^[34]	☆	☆	☆	☆	-	☆	☆	-	6
Ali ^[35]	☆	☆	☆	☆	☆☆	☆	☆	☆	9
Abraham ^[36]	☆	☆	☆	☆	☆	☆	☆	☆	8
Aulmann ^[37]	☆	☆	☆	☆	-	☆	☆	-	6
Hashimoto ^[9]	☆	☆	☆	☆	☆	☆	☆	-	6

Table 2— Main characteristics of the included studies on CD44⁺/CD24⁻.Abbreviations:NA,not available.

Name	Year	Country	Marker	Techn- ology	Cohort	CD44 ⁺ /CD24 ⁻ (+) Tumor size>2cm	CD44 ⁺ / CD24 ⁻ (+) LN (+)	CD44 ⁺ /C D24 ⁻ (+) M (+)	Follow - up time	Cut-off value	OS DFS HR95%CI(Minimu m-Maximum value)	
Gabriella	2008	Sweden	CD44 ⁺ /CD24 ⁻	IHC	240	40	45	23	NA	NA	NA	NA
Alexandra	2011	Greece	CD44 ⁺ /CD24 ⁻	IHC	139	NA	8	NA	NA	10%	NA	NA
Hiroko	2011	Japan	CD44 ⁺ /CD24 ⁻	IHC	271	NA	21	NA	NA	10%	NA	NA

JamesM	2012	USA	CD44 ⁺ /CD24 ⁺	IHC	66	NA	18	NA	NA	3%	NA	NA
Mohamed	2011	UK	CD44 ⁺ /CD24 ⁺	IHC	306	22	15	11	NA	NA	NA	NA
Gulnur	2012	USA	CD44 ⁺ /CD24 ⁺	IHC	338	NA	8	7	NA	NA	NA	NA
Julia	2012	HongKong	CD44 ⁺ /CD24 ⁺	IHC	540	NA	109	NA	NA	NA	NA	NA
Giordano	2012	USA	CD44 ⁺ /CD24 ⁺	IHC	103	NA	32	NA	60	NA	NA	(1.10-68.99)
Agnieszka	2014	Poland	CD44 ⁺ /CD24 ⁺	IHC	156	NA	15	NA	NA	NA	NA	NA
Diego	2014	Brazil	CD44 ⁺ /CD24 ⁺	IHC	28	12	6	NA	NA	NA	NA	NA
Nilgün	2015	Turkey	CD44 ⁺ /CD24 ⁺	IHC	57	NA	31	NA	NA	Scores>3	NA	NA
Chen	2015	China	CD44 ⁺ /CD24 ⁺	IHC	120	NA	42	23	160	NA	2.12(1.25-3.57)	2.05(1.22-3.46)
Zhao	2016	China	CD44 ⁺ /CD24 ⁺	IHC	242	NA	NA	NA	80	NA	1.98(0.83-4.72)	1.43(0.99-2.07)

Bane	2013	Canada	CD44 ⁺ /CD24 ⁻	IHC	262	17	18	NA	148	Scores>3	1.18(0.62-2.27)	NA
Seo	2016	UK	CD44 ⁺ /CD24 ⁻	IHC	241	NA	41	NA	118.1	10%	2.02(1.14-3.57)	2.29(1.28-4.09)
Rabinovich	2018	Italy	CD44 ⁺ /CD24 ⁻	IHC	140	NA	9	NA	180	5%	1.63(0.68-3.89)	2.86(0.68-14.06)
Eleni	2008	Greece	CD44 ⁺ /CD24 ⁻	IHC	147	NA	NA	NA	135	10%	0.7 (0.22-2.21)	0.76(0.21-2.15)
Yanlin	2007	China	CD44 ⁺ /CD24 ⁻	IHC	147	NA	NA	NA	84	NA	2.24(1.35-3.72)	1.96(1.16-3.31)
Ali	2011	UK	CD44 ⁺ /CD24 ⁻	IHC	4152	NA	NA	NA	102	NA	0.73(0.4-1.33)	NA
Abraham	2005	India	CD44 ⁺ /CD24 ⁻	IHC	122	20	16	3	85.4	NA	1.46(0.67-3.18)	1.22(0.67-2.22)
Aulmann	2010	Germany	CD44 ⁺ /CD24 ⁻	IHC	50	NA	NA	NA	41	5%	2.61(0.49-14.07)	NA
Hashimoto	2012	Japan	CD44 ⁺ /CD24 ⁻	IHC	77	NA	NA	NA	26.4	10%	1.06(0.26-4.29)	NA
Lee	2011	Korea	CD44 ⁺ /CD24 ⁻	IHC	92	NA	NA	NA	36	5%	NA	1.78(0.51-6.15)

Table2:(CD44⁺/CD24⁺(+)LN (+) means the number of lymph node metastases in patients with high expression of CD44⁺/CD24⁺ phenotype cells,CD44⁺/CD24⁺(+)M means the number of distant metastases in patients with high expression of phenotype cells.)

Figure 1-The search strategy flow chart of this meta-ananlysis.

Figure 2-Forest plot was assessed for the correlation of CD44⁺/CD24⁺ with tumor size>2cm in breast cancer. Heterogeneity chi-squared = 1.88 (d.f. = 4), $p = 0.758$,I-squared (variation in OR attributable to heterogeneity) =0.0%,Test of OR=1 : $z=0.26$, $p= 0.792$.

Figure 3-Begg's funnel plot was used to detect publication bias on the correlation of CD44⁺/CD24⁺ with tumor size in breast cancer.Begg's test $p= 0.221$;Egger's test $p=0.204$.

Figure 4-Forest plot was assessed for the correlation of CD44⁺/CD24⁺ with Lymph node metastasis in breast cancer. Heterogeneity chi-squared=31.35 (d.f. = 15) $p = 0.008$, I-squared (variation in OR attributable to heterogeneity) =52.2%, Estimate of between-study variance Tau-squared =0.1599, Test of OR=1 : $z= 0.40$, $p = 0.692$.

Figure 5-Begg's funnel plot was assessed for publication bias on the correlation of CD44⁺/CD24⁺ with Lymph node metastasis in breast cancer.Begg's test $p= 0.344$; Egger's test $p=0.171$.

Figure 6-Forest plot was assessed for the correlation of CD44⁺/CD24⁺ with distant metastasis in breast cancer.Heterogeneity chi-squared = 36.20 (d.f. = 4) $p= 0.0001$,I-squared (variation in OR attributable to heterogeneity)=89.0%,Estimate of between-study variance Tau-squared =2.1575,Test of OR=1 : $z=1.85$, $p= 0.064$.

Figure 7-Begg's funnel plot was assessed for publication bias on the correlation of CD44⁺/CD24⁻ with distant metastasis in breast cancer. Begg's test $p=0.462$; Egger's test $p=0.066$.

Figure 8-Forest plot was assessed for the correlation of CD44⁺/CD24⁻ with DFS in breast cancer. Heterogeneity chi-squared = 8.65 (d.f. = 8); $p=0.373$; I-squared (variation in ES attributable to heterogeneity) = 7.5%; Test of ES=1 : $z=4.77$; $p<0.00001$.

Figure 9-Begg's funnel plot was used to detect publication bias on the correlation of CD44⁺/CD24⁻ with DFS in breast cancer. Begg's test $p=0.602$; Egger's test $p=0.78$.

Figure 10-Forest plot was assessed for the correlation of CD44⁺/CD24⁻ with OS in breast cancer. Heterogeneity chi-squared = 12.94 (d.f. = 10) $p=0.227$; I-squared (variation in ES attributable to heterogeneity) = 22.7%; Test of ES=1 : $z=3.58$, $p=0.0004$.

Figure 11-Begg's funnel plot was assessed for publication bias on the correlation of CD44⁺/CD24⁻ with OS in breast cancer. Begg's test $p=1$; Egger's test $p=0.665$.

Figure 12-Sensitivity analysis was assessed for the stability of results about the correlation of CD44⁺/CD24⁻ with tumor size >2.0cm in breast cancer.

Figure 13-Sensitivity analysis was used to detect the stability of results about the correlation of CD44⁺/CD24⁻ with Lymph node metastasis in breast cancer.

Figure 14-Sensitivity analysis was used to detect the stability of results about the correlation of CD44⁺/CD24⁻ with distant metastasis in breast cancer.

Figure 15-Sensitivity analysis was used to detect the stability of results about the correlation of CD44⁺/CD24⁻ with DFS in breast cancer.

Figure 16 Sensitivity analysis was used to detect the stability of results about the correlation of CD44⁺/CD24⁻ with OS in breast cancer.

Figures

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The search strategy flow chart of this meta-ananlysis.

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Figure 15

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