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EXPRESSION DIFFERENCE BETWEEN SNAIL AND TWIST AS NEW-TYPE THERAPY TARGET IN PATIENTS WITH BREAST CANCER: A META-ANALYSIS

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Abstract

Background: With the application of targeted drugs, the prognosis of breast cancer has significantly ameliorated, and the survival time of patients is significantly prolonged. However, there are still numerous of breast cancer patients with poor therapeutic effect due to no specific treatment targets. Studies have demonstrated that block the function of epithelial-mesenchymal-transition (EMT) factors, Snail and Twist, can significantly improve the prognosis of breast cancer. Consequently, we may take the two factors into consideration as new therapeutic targets.

Objective: To investigate the expression difference between Snail and Twist as newly therapeutic target in breast cancer patients.

Methods: The databases including PubMed, Embase, Web of science, CNKI and WANFANG MED ONLINE were Retrieved. Revman software 5.0 was used to conduct fixed-effect model meta-analysis.

Results: 8 valid articles provided 2848 participants were analyzed. there was no statistical significance in the expression difference between Snail and Twist in breast cancer (Pooled OR=1.16; 95%CI: 0.97-1.37) and the following settings: ER-positive/negative (Pooled OR=1.16; 95%CI: 0.85-1.58), PR-positive/negative(Pooled OR=1.20; 95%CI: 0.87-1.65), HER2-overexpression/non-expression (Pooled OR=0.94; 95%CI: 0.62-1.43), lymph nodes positive/negative (Pooled OR=1.02; 95%CI: 0.80-1.31), patients with TNBC/non-TNBC (Pooled OR=0.93; 95%CI: 0.59-1.46), tumor stage3-4/1-2 (Pooled OR=1.00; 95%CI: 0.74-1.36).

Conclusion: The expression difference between Snail and Twist as new-type therapy target in breast cancer patients is not statistically significant, denoting that it is sensible to combine them together as novel targets for the treatment of breast cancer in clinical application.

Keywords: expression difference, Snail, Twist, breast cancer, therapy target, meta-analysis.

1. Introduction

The most common malignant tumor of women in the worldwide is breast cancer that gives rise to more than 400,000 deaths each year, and the primary reason of death is metastatic breast cancer[1]. Tumor cells metastasis is an intricate procedure divided into following steps, including separating tumor cells away from the primary tumor, invasion, intravascular perfusion, circulating memory activity, secondary site extravasation and colonization[2]. A large-scale cell movement called epithelial-mesenchymal-transition (EMT),

has found before the invasion of metastatic carcinoma. EMT refers to epithelial cells obtain mesenchymal properties by losing contact with their adjacent tissues and giving them the ability to penetrate through the basement membrane that isolates different tissues[3,4]. Increasing evidences indicate that EMT plays a crucial role in the process of tumor invasiveness and metastasis. The milestone of EMT occurs is the development of casting off E-cadherin and accelerating the N-cadherin expression. E-cadherin is considered to be an inhibitor of tumor invasion due to its ability to maintain the connectiveness among cells and epithelial phenotype[5]. Switches that regulate E-cadherin to N-cadherin transcription factors include Snail and Twist[6,7,8].

Snail family inhibitors Snail 1(Snail) and Snail 2 (Slug) are the best investigative regulatory factors for the core EMT[9,10], which possess the potential to regulate multiple genes of cell adhesion and cell junction[11−13]. Twist, which resembles to Snail and Slug, is a regulator for the core EMT, regulating cells destiny and cancer progression[14−20]. However, as the structurally different from Snail and Slug, Twist is a basic helix-loop-helix protein[14]. Snail-induced EMT accelerates tumor metastasis by giving rise to immunosuppression. Knockdown Snail enhances tumor infiltrating lymphocytes and systemic immune response, which significantly suppress lump growth and metastasis[21]. Slug has been thought to be related to the tumor distant metastasis ascribe to its great potency to block E-cadherin, as well as is a crucial intermediary for Twist-induced EMT[22,23]. Twist represses the epithelial branch of EMT by inducing Slug, and then two factors labor together to urge the process of EMT and tumor metastasis[23]. Based on the structural difference between Snail and Twist and their close association in process of EMT, was there expression difference of them in breast cancer patients?

In recent years, some studies had characterized that the expression of Snail and Twist was positively related to the grade, recrudesce, metastasis and poor prognosis in breast carcinoma[24,25] . Would Snail and Twist express differently as the tumor stage progresses and lymph nodes metastasize according to this characteristic? Breast cancer is classified into at least four categories of clinically correlative molecular subtypes: luminal A, luminal B, human epidermal growth factor receptor 2(HER2)-overexpression, and triple-negative breast cancer(TNBC) [26] due to its expression of different biomarkers, involving estrogen receptor (ER), progesterone receptor (PR), HER2 and Ki67[27]. Taking the deficiency of existing predictors and prognostic factors for breast cancer and amassing literatures demonstrate that interdicting the functions of Snail and Twist have a tremendous potential to restrain tumor invasion, metastasis and recurrence into account[28,29], provided Snail and Twist were appended as bran-new predictive and prognostic factors for breast cancer , Which one would possess more predictable significance? Therefore, we hypothesized that the expression between

SNAIL and TWIST was statistically significant in various biomarkers and categories of breast cancer, and commenced on implementing this meta-analysis for answering above suspicions.

2. Methods

2.1. Search strategy

FX Hao and WH Yin independently completed all the relevant citations retrieval with no restrictions, using the databases included: PubMed, Embase, Web of science, CNKI and WANFANG MED ONLINE, the search strategy comprised of three parts connected by "AND": disease (breast cancer), factor 1(SNAIL) and factor 2(TWIST). Two reviewers accomplished their search on 1 April 2018 and updated to 2 May 2018.

Inclusion criteria and study selection The articles that were satisfied with all the following inclusion criteria are included: Published

language in English or Chinese; shown the patients number of expressing Snail and Twist simultaneously; patients with breast cancer. Two reviews (FX Hao and WH Yin) sifted through all the citations in turn by titles, abstracts and full-texts, and only reserved available studies that met the inclusion criteria. If there were any di-

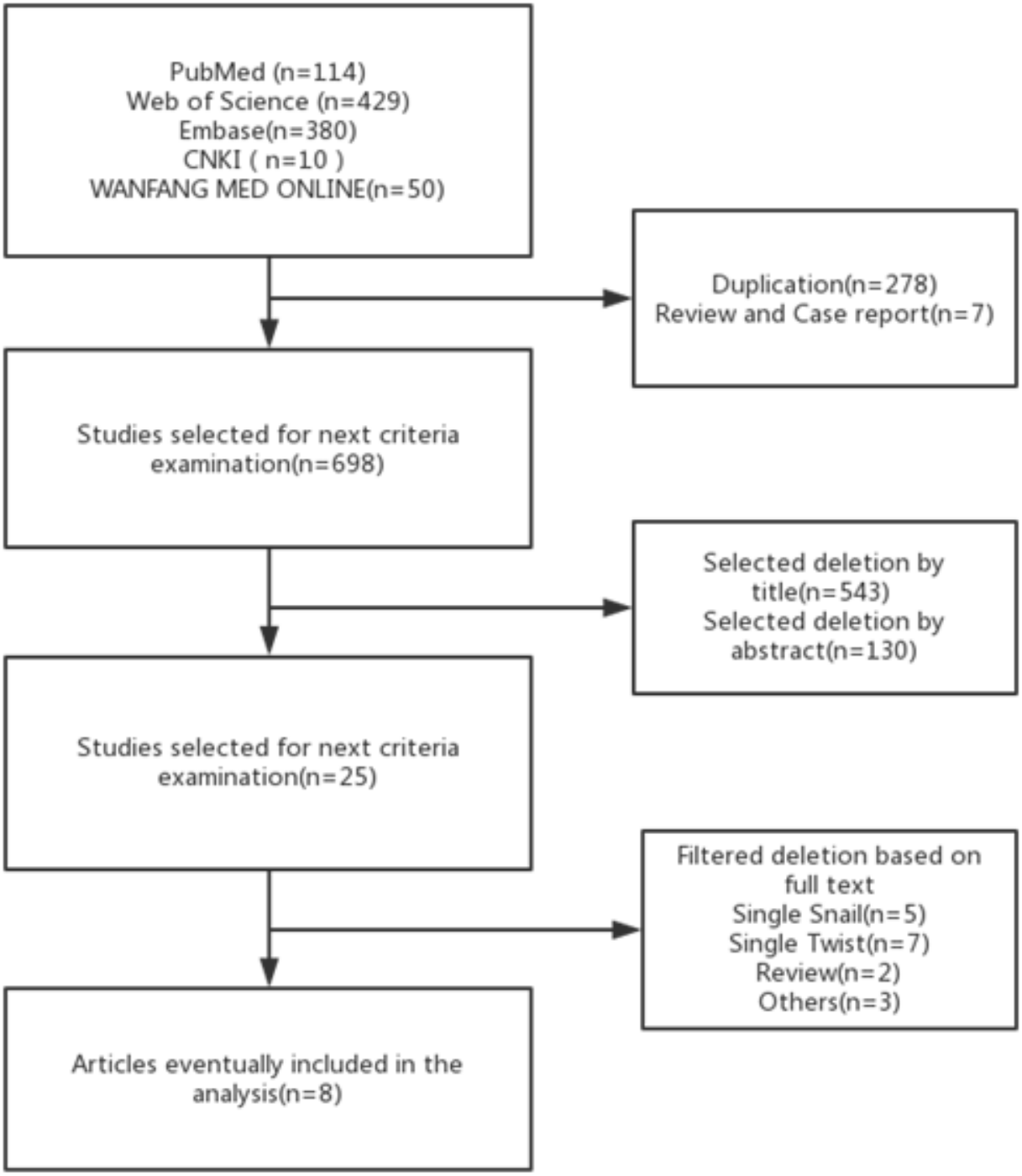

Figure 1: The procedure of study selection

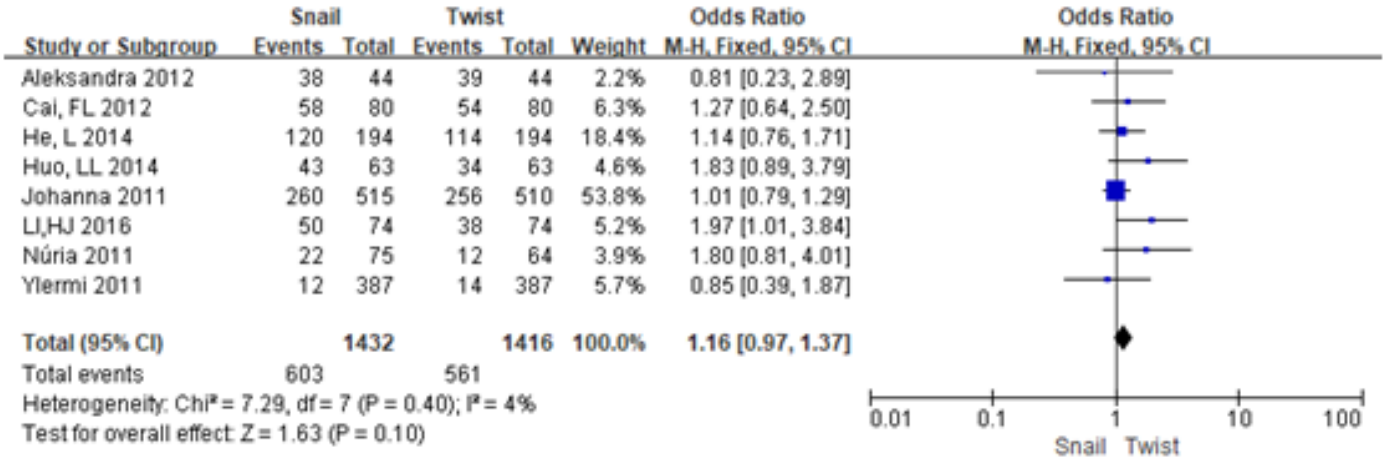

Figure 2: The expression difference between Snail and Twist in patients withbreast cancer.

vergences that could be resolved by the third reviewer (CQ Li).

2.2. Data abstraction

The following information was extracted by two authors using Excel version 2016: the first author, publication year, sample size, the patients number of Snail and Twist expression, as well as the two agents expressed in the below settings, including ER-positive/negative, PR-positive/negative, HER2-overexpression/non-expression, lymph nodes positive/negative, tumor stage3-4/1-2, patients with TNBC/non-TNBC. If there were some inconsistences, they were solved by discussion.

2.3. Data analysis

The crude data were analyzed by using Revman software version 5.30. The pooled results were presented as odd ratio (OR) and its 95% confidence interval(CI), and heterogeneity between studies was evaluated by a heterogeneity Chi squared test with significant level of $P<0.1$. When heterogeneity between studies was not significant ($P>0.1$), a fixed-effect model was used to pool the data; otherwise, the random-effect model was applied. The publication bias was assessed by creating funnel plot.

3. Results

Search results

After the procedure of comprehensive retrieval, we obtained 983 citations to be gradually omitted by repetition, reviews and case reports, as well as screened basing on titles and abstracts, and then 25 citations remained. Further, we read through the full-texts meticulous and found that there were 5 papers only reported Snail, 7 papers covered Twist homogeneously, 2 reviews and 3 other articles. Eventually, we included 8 articles for this meta-analysis (Figure 1).

Characteristics of included studies

The included articles came from China(n=4), Netherlands(n=1), Poland(n=1), Spain(n=1), Finland(n=1). The sample size of it for the meta-analysis ranged from 44 to 667 (median=107). There were some studies showed the sample size about Snail expression and Twist expression under the following conditions: ER-positive/negative(n=3), PR- positive/negative(n=2), HER2

overexpression/non-overexpression(n=4), patients with TNBC/non-TNBC(n=3), lymph node positive/negative(n=5), tumor stage3-4/1-2(n=4). The specific details were shown in Supplementary Table 1 and Supplementary Table 2.

3.1. Meta-analysis results

The expression difference between Snail and Twist

8 valid articles provided 2848 participants for analyzing the expression difference between Snail

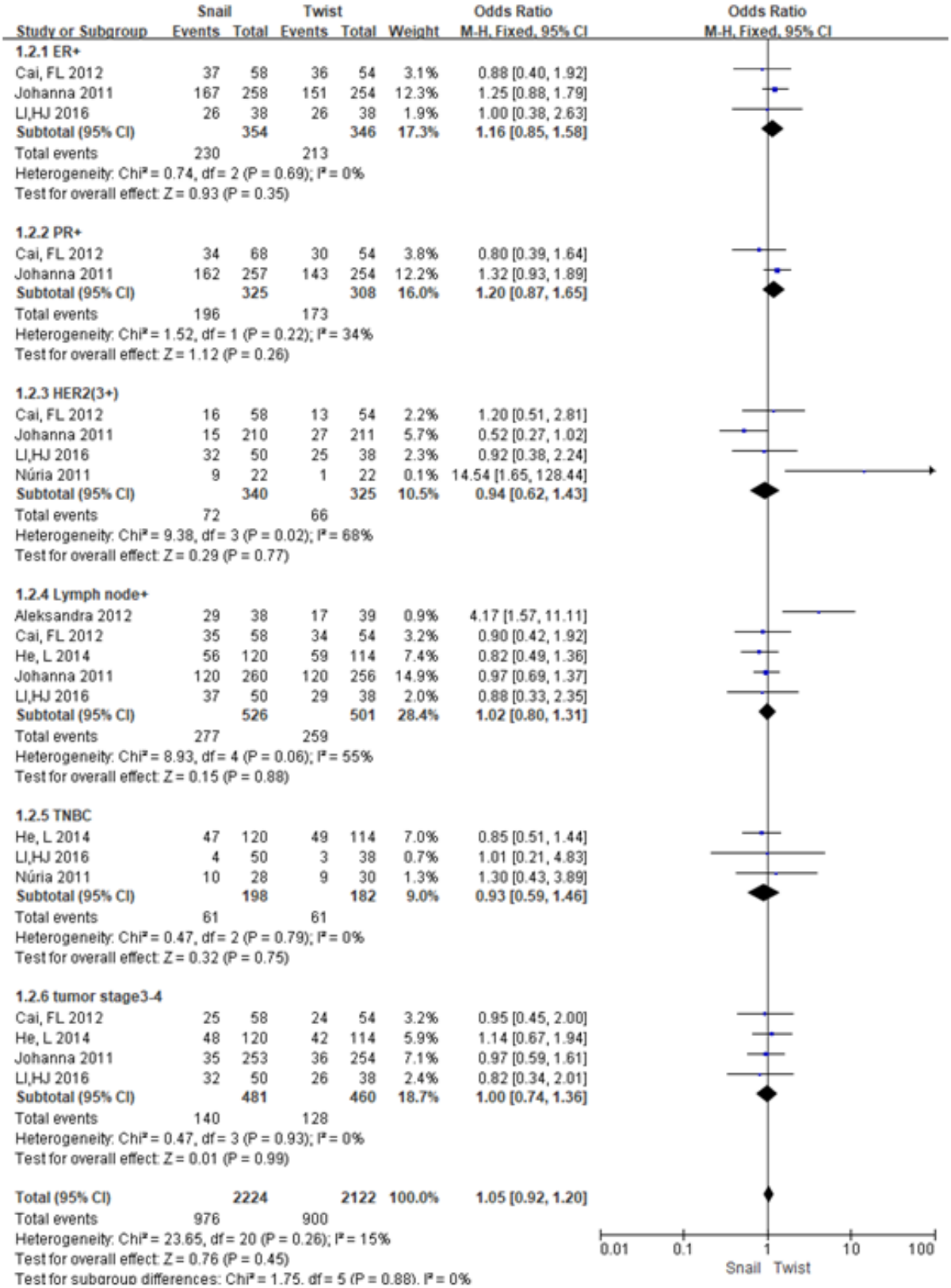

Figure 3: The expressiondifference between Snail and Twist in subgroup analysis.

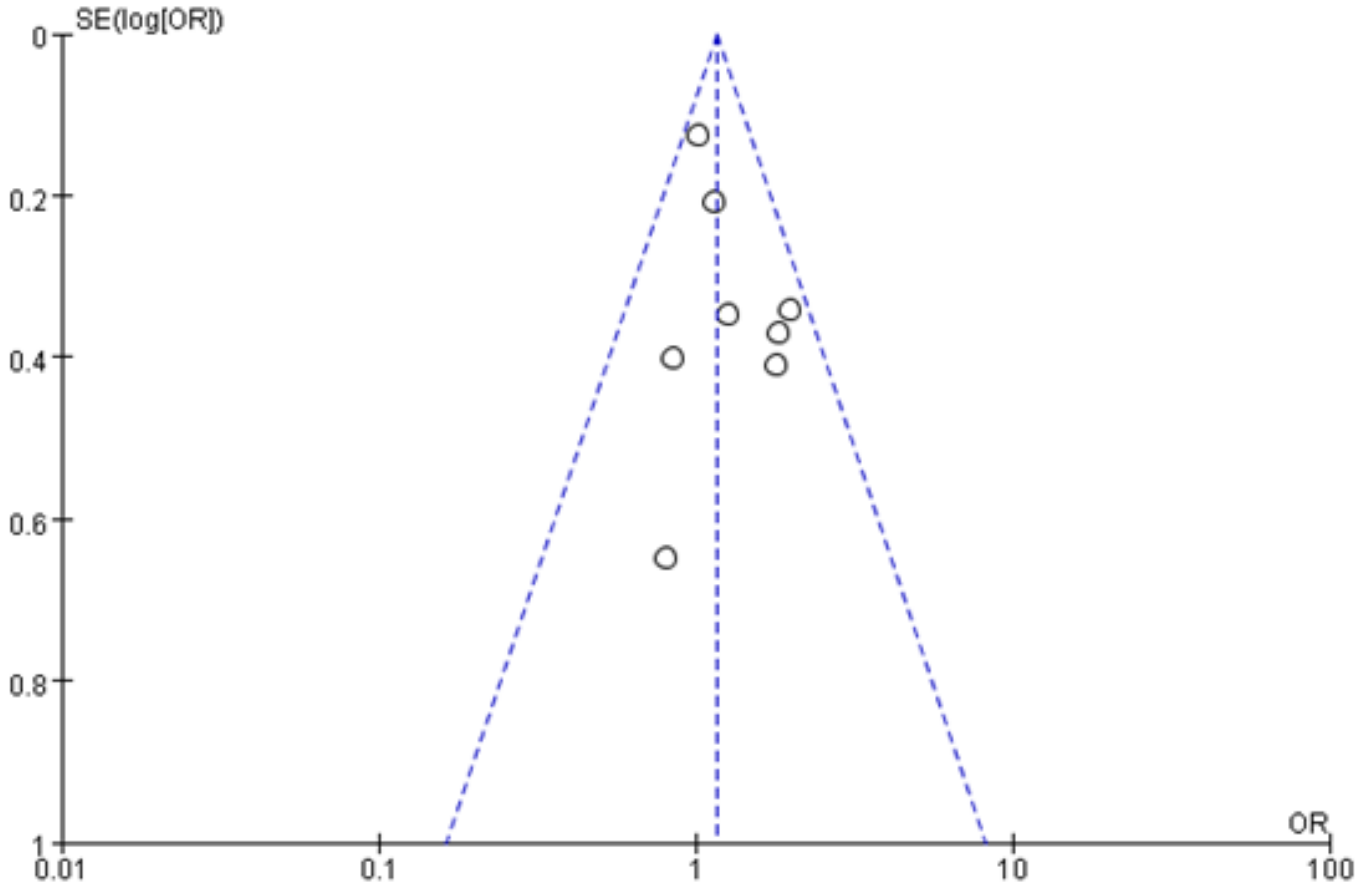

Figure 4: The funnel plot with its 95 % confidence interval wasedto evaluate the presence of publication bias.

and Twist in patients with breast cancer. When the data from all the articles were pooled together, no heterogeneity was found(P=0.40). On this account, a fixed-effect model was utilized by analyzing it. The data revealed that there was no statistical significance of expression difference between Snail and Twist in breast tumor patients (Pooled OR=1.16; 95%CI: 0.97-1.37) (Figure 2).

The expression difference between Snail and Twist in subgroup analysis

And then we arranged all the different conditions together for subgroup analysis. The fixed-effect model was adopted due to the heterogeneity test was not statistically significant(P=0.26) (Figure 3).

There were 3 related articles providing 700 patients for analyzing the differential expression of

Snail and Twist in ER-positive/negative breast cancer patients. The pooled result showed that there was no statistical significance (Pooled OR=1.16; 95%CI: 0.85-1.58) (Figure 3).

We analyzed two relevant studies which supplied 633 participants for the PR-positive/negative breast cancer patients. The pooled data was no significant difference (Pooled OR=1.20; 95%CI: 0.87-1.65) (Figure 3).

For the HER2 over-expression/non-overexpression, the pooled data from 4 effective papers which comprised of 665 patients suggested that there was no statistical significance (Pooled OR=0.94; 95%CI: 0.62-1.43) (Figure 3).

All the data consisted of 1027 participants in correlative 5 studies for lymph nodes-positive/negative was calculated. The pooled result manifested that there was no statistical significance (Pooled OR=1.02; 95%CI: 0.80-1.31) (Figure 3).

While we analyzed 3 valid articles providing 380 TNBC/non-TNBC patients, the pooled data of it was not statistically significant (Pooled OR=0.93;

95%CI: 0.59-1.46) (Figure 3).

4 papers provided 941 participants for the tumor stage3-4/1-2 analysis, the pooled result revealed that there was no significant difference (Pooled OR=1.00; 95%CI: 0.74-1.36) (Figure 3).

3.2. Public bias

We elaborated a funnel plot to evaluate the publication bias in this meta-analysis. The data was evenly distributed on both sides of the plot, suggesting that the heterogeneities in partial branches of the subgroup analysis were unlikely due to publication bias (Figure 4).

4. Discussion

Increasing voices appeal to prevent Snail and Twist function as new groundbreaking targets in breast cancer treatment due to the existing prognostic and prognostic factors not meet the needs. Considering the oncologists have been trekking on the way to contrive some new targeted therapies, so it is particularly important to choose a more meaningful factor of them. Our result certified that there was no statistical significance of the expression difference between Snail and Twist in breast cancer patients and in the subdivided situations, including ER-positive/negative, PR-positive/negative, HER2 overexpression/non-overexpression, TNBC/non-TNBC, lymph node positive/negative as well as tumor stage3-4/1-2.

EMT not only donates the metastatic and invasive ability to tumor cells , but also confers them with stem cell-like properties, which is mainly responsible for causing immunosuppression and tumor recurrence[30−33]. In breast cancer, the process of EMT and enhancive mammo-sphere-forming capability are caused by the co-expression of Snail and Twist [31]. Twist works on Snail upstream and induces Snail expression, and their synergistic effect can downregulate the E- cadherin expression and boost EMT[34,35]. In order to explore which factor expression with more representative status in breast cancer, Johanna et al. found that no significant difference was observed[36], which was testified by our results, indicating that it is highly undesirable to choose only one of them as a prognostic and predictive maker in patients with breast cancer.

In our study, we had demonstrated that Snail expression was not discriminated in patients with ER-positive/negative breast tumor compared with Twist expression. Nevertheless, the co-expression of Snail and Twist is positively correlated with recurrence in patients with ER-positive tumors, but is not statistically significant as a prognostic factor, either single or united, for patients with ER-negative tumors[36]. Therefore, the above descriptions indicate that we should combine the two markers expression together to judge the prognosis only in ER-positive breast tumor and cannot divide them from each other in clinical application.

A preselected invasive subpopulations of tumor cells caused by EMT occurs more frequently in lymph node metastasis than in primary tumors, where these invasive cells organize only an infrequency of cells, contributing to the levels of expressing Snail and Twist are significantly greater in lymph node metastasis than in primary tumors[37,38]. Some clinical studies had shown that the co-expression of Snail and Twist was closely associated with the tumor stage and lymph node metastasis, but not with the aged, tumor size, PR- status, TNBC and HER2 overexpression[39−41]. Again, our results had stated that there were no significant differences of Snail expression compared to Twist expression in the following categories, including PR-positive/negative, HER2 overexpression/non-overexpression, patients with TNBC/non-TNBC, lymph node positive/negative as well as tumor stage3-4/1-2. These aforementioned fruits indicate that it is practicable to monitor co-expression of two markers as the newly-added predictors and prognostic factors for lymph node metastasis and tumor stage3-4 breast cancer patients, but not for PR-positive, HER2 overexpression and TNBC patients.

This novel meta-analysis was the first article to compare the expression difference between the two EMT factors, Snail and Twist, in breast cancer patients according to our knowledge. Indeed, this analysis presents with some limitations.

First, we didn't analyze the race and age of patients, tumor size, as well as luminal A and luminal B breast cancer attribute to we found that the included papers did not offer these precise classifications. Second, only English and Chinese literatures were included, which might lead to selective bias, although no publication bias was found. Third, with using a fixed-effect model, no heterogeneity was tested in the integrated-group and subgroup analysis, however we found moderate heterogeneity in some branches of subgroup analysis, which might be due to the clinical heterogeneity existing in the included studies. Finally, considering the limited number of partial branches of subgroup analysis, it did not provide sufficient information for the meta-analysis, which might contain result bias.

Despite with these imperfections, this article provided a novel standpoint that co-expression of Snail and Twist could be used as newly predictive and prognostic markers for patients with breast cancer at present, particularly in ER-positive, lymph node positive and tumor stage3-4 situations. In the future, it is considered that apply these conclusions to give assistance for diagnosis and detecting prognosis in breast cancer in clinical implications. In later study, we will collect more clinical data to analyze the expression difference between Snail and Twist of the conditions of age, tumor grade, luminal A and luminal B.

5. Conclusion

Our results show that expression difference between Snail and Twist in breast cancer patients is not statistically significant, suggesting that it is recommendable to bond the two factors together for prognosticating the outcome of patients with breast cancer.

Competing interests The authors declare no competing interests.

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