Diagnostic value of BiP or anti-BiP antibodies for rheumatoid arthritis: a meta-analysis

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Abstract

Objective

To evaluate the diagnostic value of BiP or anti-BiP antibodies in patients with rheumatoid arthritis.

Methods

Relevant studies published on PubMed and CNKI from January 1995 to July 2016 were retrieved. Two reviewers independently evaluated studies and QUADAS tool was used to assess the quality of the included studies. A random-effects model was used to combine sensitivity, specificity, positive and negative likelihood ratios and diagnostic odds ratio. Stratified analysis was performed for exploring heterogeneity and funnel plot was examined for the possibility of publication bias.

Results

Nine studies met our inclusion criteria. The pooled sensitivity, specificity, LR+, LR-, DOR were 0.67 (95%CI, 0.64–0.70), 0.92 (95%CI, 0.90–0.93), 7.65(95%CI, 4.08–14.36), 0.36(95%CI, 0.33–0.39), 23.73(95%CI, 13.01–43.28), respectively.

Conclusion

This meta-analysis shows that BiP or anti-BiP antibodies have a moderate accuracy for the diagnosis of rheumatoid arthritis with a moderate sensitivity and high specificity. It can be an efficient supplement to the existing diagnostic method.

Key words

BiP, anti-BiP antibodies, rheumatoid arthritis, diagnosis, meta-analysis

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Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterised by chronic inflammation in the synovial membrane of affected joints that ultimately leads to loss of daily function due to chronic pain and fatigue (1). RA affects approximately 1% of the world's population (2) and complications of RA like cardiovascular diseases result in the increased mortality rate among RA patients (3, 4). Therefore, early and accurate diagnosis of RA could decrease the morbidity of functional disability and improve quality of life. So far, the recommended standard for RA is the 2010 revised criteria of the American College of Rheumatology (ACR) (5). In this criteria, rheumatoid factors (RF) and anti citrullinated peptide antibodies (ACPAs) are used as the serological biomarkers for the diagnosis of RA, both of which may play important roles in the pathogenic process (6-8). The sensitivity of RF is 60–80% while the specificity is relatively low because it can also be detected in patients suffering from other diseases and even in healthy individuals (9). The sensitivity and specificity of ACPAs are 67% and 95%, respectively (10). However, there are still misdiagnoses and missed diagnoses in clinical practice when RF and ACPAs are combined to use for diagnosing (11, 12).

BiP, also known as GRP78, is a ubiquitous endoplasmic reticulum (ER) resident protein vital for the folding of polypeptide chains and the protection of cells from apoptosis, when a cell is stressed (13, 14). Besides functioning as a molecular chaperone, it can also be secreted into extracellular environment (15). During the pathogenesis process of RA, BiP appears as a product of stress response and patients are likely to develop autoantibodies to BiP (16). Many studies have certified the increase of BiP and anti-BiP antibodies in RA patients. However, there are still controversies (17-19) about their diagnostic value in RA.

In our meta-analysis, we summarised published data on the sensitivity, specificity, likelihood ratios (LRs), diagnostic odds ratio (DOR) of BiP or anti-BiP antibodies for the diagnostic value of RA.

Methods

Data sources and searches

We developed a protocol for the review and followed standard reporting guidelines (20). PubMed and CNKI databases were searched for studies published in English or Chinese from January 1995 to July 2016. Our searches were based on combinations of the following index terms: rheumatoid arthritis; GRP78; glucose regulated protein 78; anti-GRP78 antibodies; anti-GRP78 antibody; BiP; immunoglobulin heavy chain binding protein; anti-BiP antibodies; anti-BiP antibody; p68; anti-p68 antibodies; anti-p68 antibody. We also reviewed the reference lists of retrieved studies and review articles.

Study selection

Two reviewers independently checked abstracts for the inclusion criteria. Discrepancies were resolved through discussion. Inclusion criteria are as follows: 1) Studies evaluated the diagnostic value of BiP antigen, anti-BiP antibodies in rheumatoid arthritis. 2) Patients met the 1987 (21) or 2010 (5) revised criteria of the American College of Rheumatology (ACR). 3) Enough data was provided to calculate the sensitivity and specificity for diagnosis of rheumatoid arthritis.

Data extraction and quality assessment

Data were extracted using a standard form that included the author, publication year, demographic characteristics of the RA patients, true-positive results, false-positive results, false-negative results, true-negative results, constitution of the control group, samples and the diagnostic test used. Study quality was assessed by two independent reviewers using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) (22) tool. All disagreements were resolved through discussion.

Data analysis

A random-effects model was used to combine sensitivity, specificity, positive and negative likelihood ratios and diagnostic odds ratio. Summary receiver operator characteristic (SROC) curve was constructed and Q* values

were calculated from the SROC curve. Stratified analysis was conducted by the sample and diagnostic test used to further assess the heterogeneity. Finally, funnel plot was examined for diagnostic odds ratios to explore the possibility of publication bias. For this statistical analysis, the software Meta-DiSc (v. 1.4) was performed.

Results

Study selection

Our initial data search yielded a total of 95 articles. Among them, 75 articles were excluded by reviewing the titles and abstracts, whereas the remaining 20 were considered as potentially eligible for our analysis. After careful reading of the entire full text, 9 articles (23-31) met the inclusion criteria and were included in the systematic review. A flow diagram (Fig. 1) shows the flow chart of the literature search.

Study characteristics

Table I summed up the characteristics of the included 9 studies. Three studies (27, 30, 31) used anti-p68 antibody for diagnosing, which was proved to be identical to anti-BiP antibody (24). The sensitivities of BiP or anti-BiP antibodies for the diagnosis of RA in the involved studies ranged from 63% to 83% and the specificities from 62% to 99%, respectively. Control groups consist of healthy persons and other rheumatic diseases such as systemic lupus erythematosus (SLE), osteoarthritis (OA), scleroderma, psoriatic arthritis (PsA) and mixed connective tissue disease (MCTD). Seven studies (24, 25, 27-31) detected anti-BiP antibodies by ELISA or western blot, whereas two studies (23, 26) detected BiP by western blot. Of the nine studies, two (26, 27) collected synovium for diagnosing, one (23) collected salivary and the others collected sera.

Study quality

The quality of the studies was summarised in Fig. 2. Most of the studies had high quality with a median score of 12 using the QUADAS tool. Two studies (23, 25) satisfy all the criteria of the quality check list. One study (29) satisfies 13 items of the 14 standard items, two studies (30, 31) satisfy 12 items, three studies (24, 27, 28) satisfy 11 items and one study (26) only satisfies 8 items.

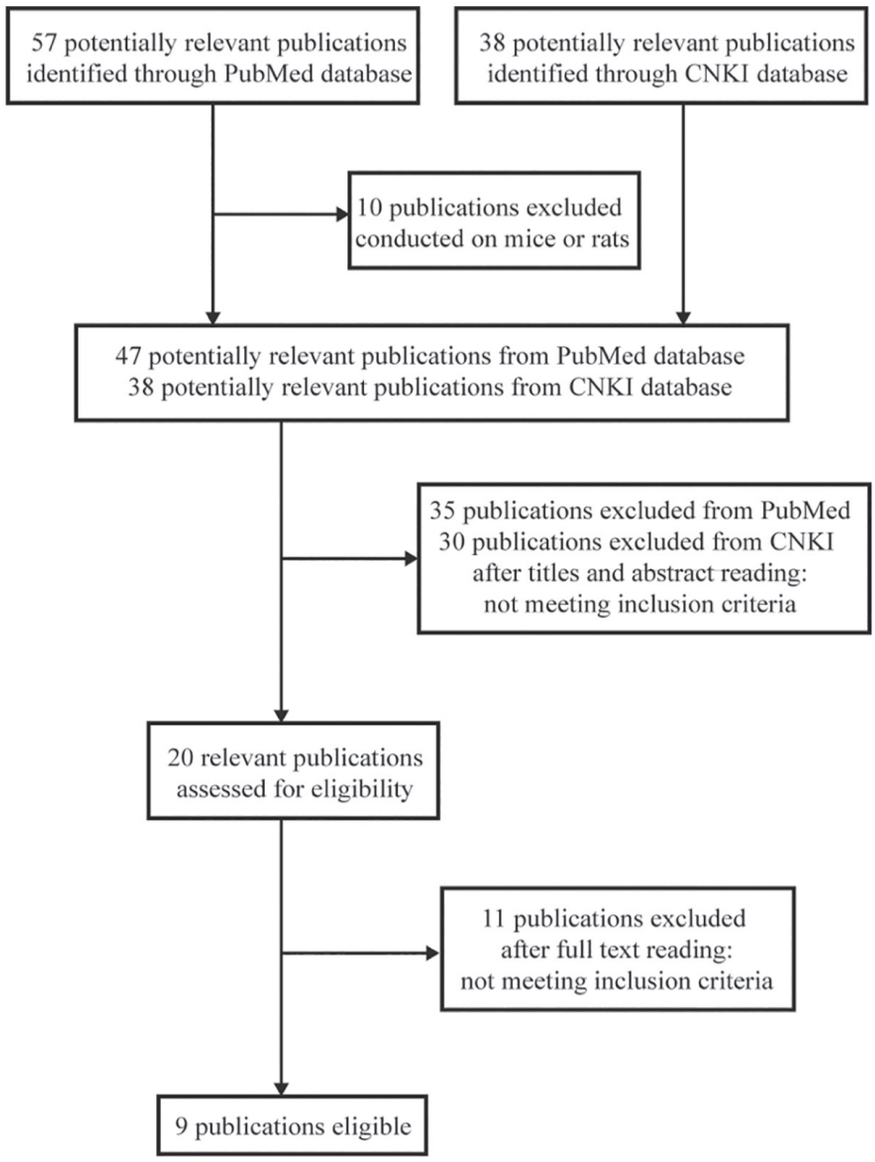

Fig. 1 Flowchart depicting literature search and selection.

Diagnostic value of BiP

As shown in Fig. 3, the pooled sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-) in our meta-analysis were 0.67 (95%CI, 0.64–0.70), 0.92 (95%CI, 0.90–0.93), 7.65(95%CI, 4.08–14.36) and 0.36(95%CI, 0.33–0.39), respectively. The diagnostic odds ratio (DOR) was 23.73(95%CI, 13.01–43.28) (Fig. 4A). The SROC curve was asymmetric and there was no clear trade-off between sensitivity and specificity (Fig. 4B).

Stratified analyses

For the stratified analyses, Cochran Q statistic method was used to assess the heterogeneity among the estimates of diagnostic OR in subgroups divided by various control groups, races, and diagnostic tests. As is shown in Table II for stratified analyses, the DOR estimate of the healthy persons subgroup, was 17 times of the one of mix subgroup and was 9.7 times of the one of persons with other diseases subgroup. Also, the DOR estimate of the western blot subgroup was 2 times of the one of ELISA subgroup. As for races, there were no significant differences in the DOR estimate between the European and the Chinese.

Table I. Characteristics of studies included in the meta-analysis.

Author	Year	Country	RA Patients	Mean Age (yrs)	Disease duration (yrs)	Controls	TP	FP	FN	TN	Sample	Diagnostic test used
Giusti *et al*.	2010	Italy	20	57.62±11.09	9.50±6.15	HC (n=20) [a]	17	1	3	19	Salivary	BiP by WB
Blass *et al*	2001	Germany	400	45	NA	HC (n=150) ORD (n=200)	252	15	148	335	Sera	Anti-BiP by WB
Bodman-Smith *et al*.	2004	UK	96	58.2	NA	HC (n=45) ORD (n=51)	70	28	26	68	Sera	Anti-BiP by ELISA
Corrigall *et al*.	2004	UK	18	NA	NA	ORD (n=13)	13	5	5	8	Synovium	BiP by WB
Blass *et al*.	1995	Germany	167	NA	NA	HC (n=55) ORD (n=98)	107	1	60	152	Synovium	Anti-p68 by WB
Chen *et al*.	2007	China	65	NA	NA	HC (n=71) ORD (n=103)	49	11	16	163	Sera	Anti-BiP by ELISA
Zou *et al*.	2009	China	79	NA	NA	HC (n=173) ORD (n=100)	53	19	26	254	Sera	Anti-BiP by ELISA
Sun *et al*.	2004	China	183	52±6	8±8	HC (n=81) ORD (n=114)	124	17	59	178	Sera	Anti-p68 by ELISA
Yang *et al*.	2009	China	71	52.43±13.68	7.98±5.93	HC (n=30) ORD (n=42)	53	11	18	61	Sera	Anti-p68 by ELISA

[a]HC: healthy control; ORD: other rheumatic disease; WB: western blot.

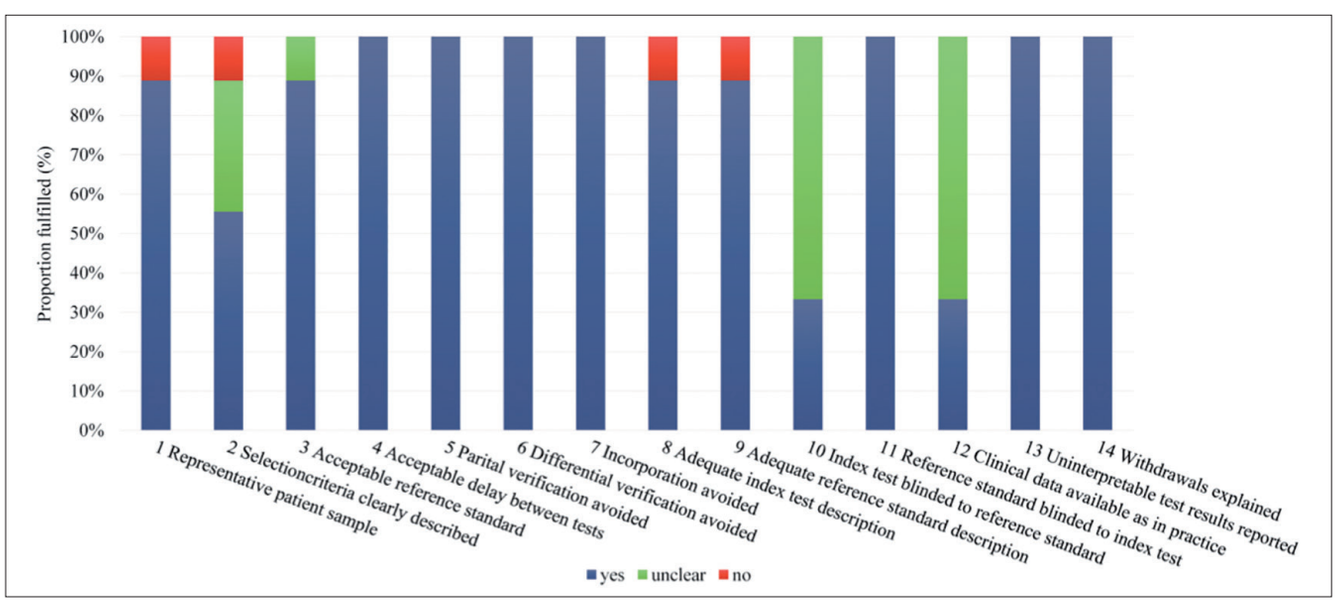

Fig. 2. Bar graph of study assessment with QUADAS checklist.

Publication bias

To assess the publication bias of the studies, the Deeks' funnel plot was conducted by Stata 12.0 (shown in Fig. 5). With a *p*-value of 0.85, no significant publication bias in our meta-analysis was found.

Discussion

RA is a ubiquitous and disabling chronic inflammatory disease which is difficult to cure. Early diagnosis and treatment can prevent major damage of joint tissue and improve prognosis, thus enhancing the patients' life quality. However, the diagnostic value of existing serological biomarkers, such as RF, ACPAs, anti-SA or anti-RA33 antibodies are not satisfactory enough (9, 32, 33). Thus, new biomarkers need to be developed.

In RA patients, BiP or anti-BiP antibodies were found to be increased. Furthermore, upregulation of BiP appeared to be distinctive of RA and even drugs treatment independent (23), and antibodies to BiP are found in the sera antedating the onset of RA (25). These findings suggested that BiP or anti-BiP antibodies could be explored as a potential biomarker for improving the diagnostic algorithms of RA. Therefore, in this article, we assess the diagnostic value of BiP or anti-BiP antibodies in RA. Our results showed that BiP or anti-BiP antibodies had a high specificity 0.92 (95%CI, 0.90–0.93) and moderate sensitivity 0.67 (95%CI, 0.64–0.70). The pooled positive likelihood ratio (LR+) was 8, indicating that patients with RA had an 8-fold higher chance of being BiP or anti-BiP antibodies

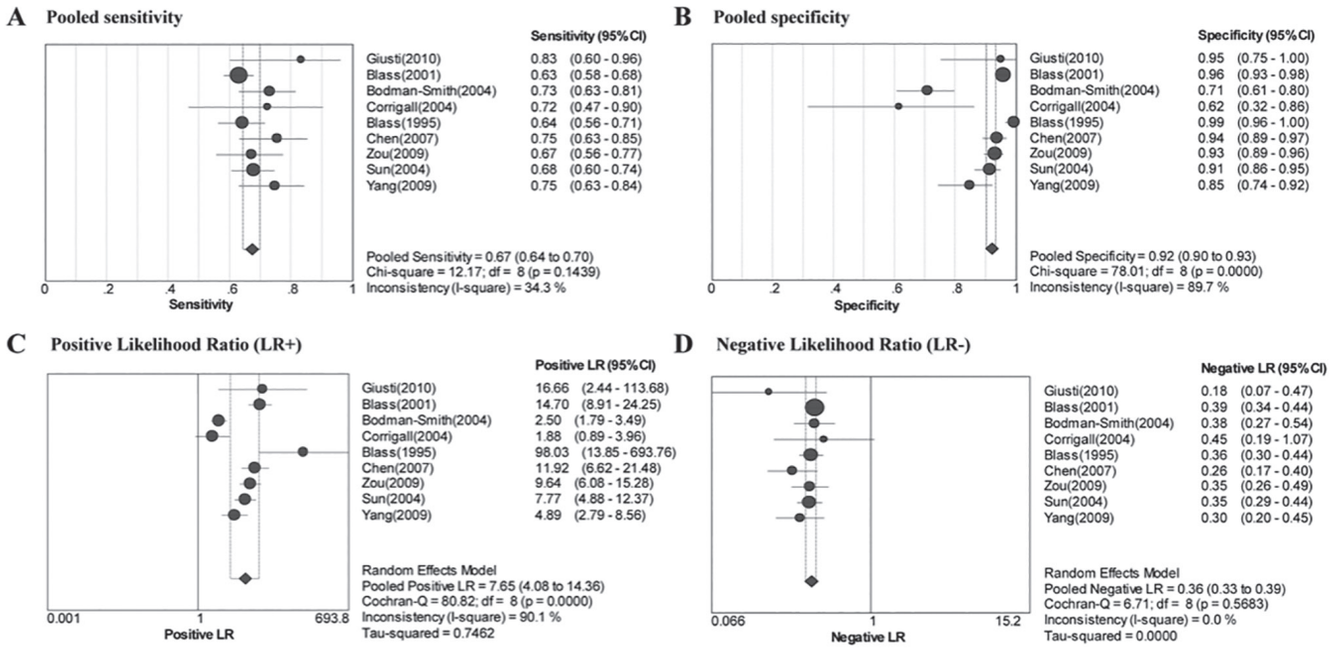

Fig. 3. Pooled sensitivity, specificity, positive and negative likelihood ratio from the meta-analysis of BiP or anti-BiP antibodies studies. **A**: Pooled sensitivity. **B**: Pooled specificity. **C**: Positive likelihood ratio (LR+). **D**: Negative likelihood ratio (LR-). Each solid circle represents each study and the size of each study is indicated by the size of the solid circle.

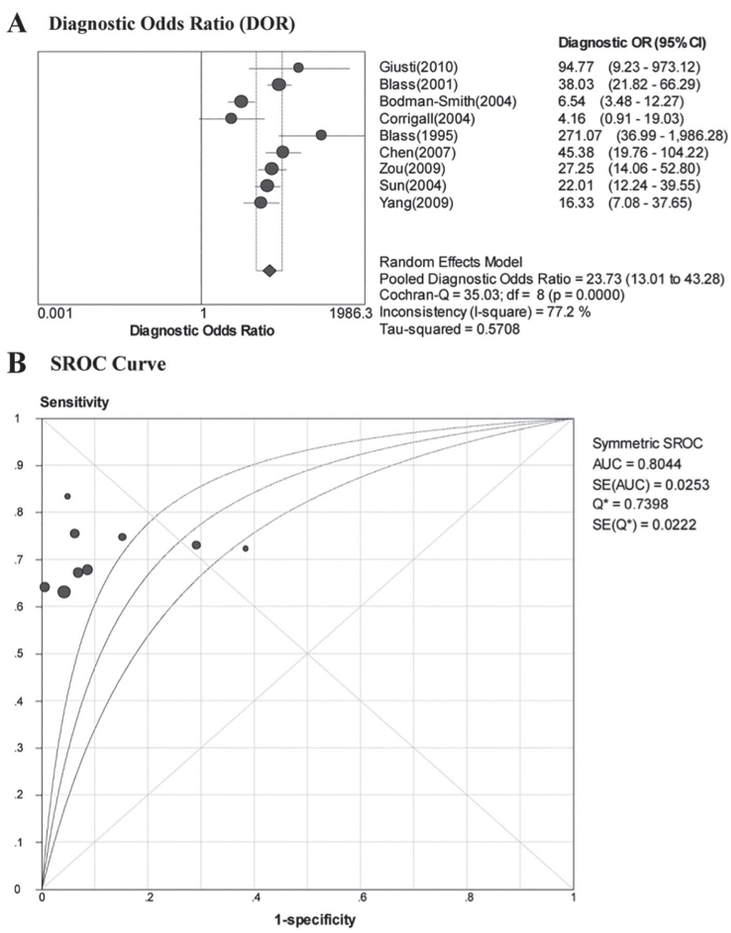

Fig. 4. Diagnostic odds ratio and SROC curve. **A**: Diagnostic odds ratio (DOR). **B**: SROC curve of the BiP or anti-BiP antibodies in the meta-analysis. SROC: summary receiver operative curves; AUC: area under the curve; SE: standard error; Q*: Cochran Q.

test positive compared with patients without RA. The diagnostic odds ratio (DOR) is a measure of test performance which combines the strengths of sensitivity and specificity with the advantage of accuracy as a single indicator (34). In our meta-analysis, the pooled DOR was 23.73 (13.1–43.28), suggesting that BiP or anti-BiP antibody test could be useful in the diagnosis of RA. Moreover, the SROC curve with AUC value of 0.80 and Q* value of 0.74 indicated that BiP or anti-BiP antibodies had a moderate accuracy for the diagnosis of RA.

Our results indicated that BiP or anti-BiP antibodies have a remarkably higher specificity compared to Rheumatoid Factor. Meanwhile, the difference of sensitivity is not obvious. RF, autoantibody against the Fc portion of IgG, formulates the immune complexes that exceed the process of RA as well as other rheumatoid diseases, which may contribute to the low specificity. On the other hand, BiP or anti-BiP antibodies is close to ACPAs in sensitivity (67% *vs*. 67%) and had a slightly lower specificity than ACPAs (92% *vs*. 95%). This similarity may derive from their similar role in RA pathogenesis. Citrullinated proteins released from necrotic inflammatory cells bind to HLA-DRB1

Table II. Stratified analyses of the included studies about the diagnostic value of BiP in RA.

Subtype	Pooled DOR		Pooled sensitivity		Pooled specificity		LR+	LR-
	DOR	95%CI	Sen	95%CI	Spe	95%CI		
Control Group								
Healthy	247.33	94.04-650.46	0.66	0.63-0.69	1	0.99-1.00	64.92	0.33
ORD	14.52	8.32-25.34	0.66	0.63-0.69	0.88	0.86-0.91	4.9	0.38
MIX[a]	25.61	13.83-47.42	0.67	0.64-0.70	0.92	0.91-0.94	8.75	0.36
Diagnostic test								
Western blot	39.3	8.52-181.32	0.64	0.60-0.68	0.96	0.94-0.97	12.65	0.38
ELISA	19.25	10.17-36.42	0.71	0.66-0.75	0.89	0.87-0.91	6.32	0.34
Race								
European	25.33	6.63-96.88	0.65	0.62-0.69	0.92	0.90-0.94	8.6	0.38
Chinese	25.29	17.52-36.50	0.7	0.65-0.75	0.92	0.90-0.94	8.14	0.33

[a]Include persons with other rheumatic disease and healthy persons.

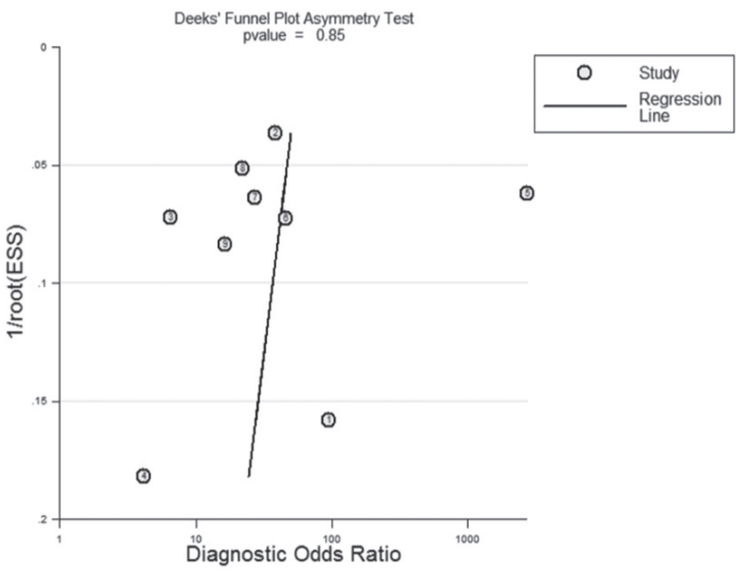

Fig. 5. Funnel plot for the assessment of potential publication bias between studies. ESS, effective sample size.

and can lead to the activation of $CD4^+$T cells. Therefore, a B-cell response to citrullinated antigens will be generated, resulting in the formation of immune complexes to stimulate the inflammatory process by upregulation of pro-inflammatory cytokines (35, 36). Meanwhile, intensive inflammatory reactions lead to rapid consumption of oxygen and glucose, causing micro-environmental stresses (16) to increase the secretion of BiP into the intercellular matrix. These extracellular BiPs act as a ligand binding to a cell-surface receptor, eventually decelerate the inflammatory process (13). Meanwhile, RA patients are likely to develop auto-antibodies against extracellular BiP. The formed immune complex stimulates the inflammatory process (17) to exacerbate the RA pathogenic process. Furthermore, through binding with BiP, these antibodies could attenuate the immune regulation function of extracellular BiP. It suggests that anti-BiP antibodies are likely to have a closer relationship with RA activity compared to ACPAs and would shed new light on RA diagnosis. In our meta-analysis, we also concern about the heterogeneity between studies. With a Spearman correlation coefficient of 0.317 and *p*-value of 0.406, the shape of the SROC curve indicated that variability in the thresholds used in studies had no significant influences on heterogeneity. We further investigated heterogeneity through stratified analysis. It seems that different control groups and different diagnostic tests used could lead to heterogeneity. In detail, the healthy control had a 9.7-fold higher DOR compared with other rheumatoid diseases control. Also, western blot showed a higher DOR than ELISA in diagnostic tests. Besides, BiP or anti-BiP antibodies showed no distinct differences between European and Chinese. The quality of the included studies may also cause extra heterogeneity. In order to have a complete understanding of the quality of the included articles, we used the QUADAS tool to assess them. Six studies (24, 26-28, 30, 31) did not clarify whether the results of the index test were judged objectively. So the results may be affected by the interpreter's subjective intention. For example, the reference standard results or the clinical data may have an influence on the index test results. For this reason, we gave those articles an "unclear" on item 10 and 12. One study (26) did not give an explicit reference standard, nor did it describe the index test in detail. Therefore, it only met 8 of the 14 items. These aspects would increase the studies' heterogeneity and influence the diagnostic accuracy of BiP or anti-BiP antibodies.

Some limitations of our meta-analysis should be acknowledged. First, due to the insufficiency of relevant studies, we did not perform stratified analysis on certain subgroups, for instance, the synovium subgroup and the sera subgroup. As we all know, immune response in the synovium is important in the pathogenic process in RA, which indicates

that synovium may be a valuable sample for diagnosing. We did not compare the BiP subgroup with anti-BiP antibodies subgroup, either. Second, only studies published in Chinese and English were included in this meta-analysis due to the linguistic limitations of the reviewers, which may lead to a language bias. Third, many factors such as age, sex, disease duration that possibly contribute to the heterogeneity could not be assessed because they were only reported in a few studies. Fourth, only the available published articles were included, which might result in the missing of some important ongoing or unpublished research data.

In conclusion, BiP or anti-BiP antibodies as a biomarker showed a moderate accuracy for the diagnosis of RA. With a moderate sensitivity and high specificity, BiP or anti-BiP antibodies can be an efficient supplement to the existing diagnostic method. Due to their different roles in RA pathogenesis, we believe a combined use of BiP and anti-BiP antibodies with RF or ACPAs would be helpful for the clinical diagnosis of RA.

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