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ORIGINAL ARTICLE

Diagnostic Accuracy of Anti-Carbamylated Protein Antibodies in Rheumatoid Arthritis: a Systematic Review and Meta-Analysis

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SUMMARY

Background: **The purpose of this study was to estimate the diagnostic accuracy of anti-carbamylated protein (anti-CarP) antibodies in rheumatoid arthritis.**
Methods: **We searched the PubMed, EMBASE, Cochrane Library, Web of Science, and Scopus databases for studies published before January 1, 2019. Two investigators independently evaluated studies to determine their inclusion in the analysis, assess their quality, and extract the relevant data. The articles were assessed with the Quality Assessment of Diagnostic Accuracy Studies tool, and a bivariate mixed effects model was used to estimate the diagnostic indexes across studies.**
Results: **We included 16 published studies in this meta-analysis. The pooled sensitivity and specificity of anti-CarP were 43.1% and 94.4%, respectively. The area under the summary receiver operator characteristic curve was 0.55. The specificity estimates were highly heterogeneous, which could be partly explained by the higher specificity in the healthy control group (43.0%, 96.8%) than in the other disease group (43.4%, 89.8%).**
Conclusions: **Anti-CarP antibodies have a relatively low sensitivity and high specificity for rheumatoid arthritis. However, the specificity was lower in the other disease subgroups than in the healthy controls.**
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INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease that primarily affects joints, causing joint instability, acute pain and functional disability. In addition, it can also damage body systems, such as the cardiovascular or respiratory systems. In total, 0.5 - 1% of adults worldwide have RA. The damage is irreversible, and no drug or treatment can completely recover normal function once the damage has occurred. Effective detection biomarkers are important for the clinical management of RA patients [1].
Currently, anti-cyclic citrullinated peptide (anti-CCP) [2] and rheumatoid factor (RF) are the well-known serological markers that are used for the diagnosis of RA,

and both were included in the American College of Rheumatologists/European League Against Rheumatism (ACR/EULAR) criteria for the diagnosis of RA in 2010 [3]. A recent meta-analysis showed that the sensitivity and specificity of anti-CCP antibody were 67% and 95%, while those of RF were 69% and 85%, respectively [4]. Thus, a substantial proportion of the RA patients are not identified when anti-CCP antibody and/or RF are used. It would be beneficial to find new biomarkers to fill this gap.

In 2011, an anti-carbamylated protein (anti-CarP) antibody was detected that targets homocitrulline [5]. Carbamylation is a post-translational protein modification that converts lysine into homocitrulline by an irreversible chemical reaction that occurs in the presence of cyanate. Anti-CarP antibodies can be present in RA patients before disease onset [5-7]; they can predict the development of RA and are associated with increased joint destruction. Moreover, anti-CarP antibodies can be detected in anti-CCP antibody/RF-negative patients, which demonstrated that these antibodies have a cross-diagnostic effect that may be important in the diagnosis of RA [5]. In this meta-analysis, we summarize the sensitivity, specificity, and likelihood ratios of the anti-CarP antibody and assess its diagnostic accuracy using published data.

MATERIALS AND METHODS

Data sources and searches

We systematically searched PubMed, EMBASE, Cochrane Library, Web of Science, and Scopus for studies published before January 1, 2019, without any language restrictions. The index terms used were “antibody to carbamylated protein”, “antibody to CarP”, “autoantibody to carbamylated protein”, “autoantibody to CarP”, “anti-carbamylated protein antibody”, “anti-CarP antibody”, “rheumatoid arthritis”, and “RA”. The details of the search strategy are listed in the supplementary material 1 - Table1.

Study selection criteria

Studies were included in this meta-analysis if they (1) examined the diagnostic accuracy of anti-carbamylated protein antibody; (2) provided sufficient data to calculate the sensitivity and specificity; (3) included patients diagnosed with RA based on the international diagnostic criteria, healthy controls, and patients with other disease who were not going to develop RA [3,8]; and (4) enrolled at least 50 RA patients and 10 controls. The details of the reasons for the study exclusion are listed in the supplementary material 1 - Table 2. Two evaluators searched for and scanned the articles independently, and discrepancies between the reviewers were resolved by discussion.

Data extraction and study quality assessment

The meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (supplementary material 3) [9]. The data extracted from the reports included author, publication year, type of article, design, type of ELISA, reference standard for rheumatoid arthritis, method used define the cutoff value for the peptides, patient group region, number of participants, proportion of female participants, age, disease duration. In addition, the diagnostic indexes were summarized to fill the four cells of a diagnostic 2 x 2 table. We evaluated the quality of each study based on the tool from the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-1&2) [10,11]. Two reviewers, blinded to the published details, extracted the data and assessed the methodological quality of each report independently, and discrepancies were resolved by consensus.

Data analysis

We used a bivariate binomial mixed model to analyze the data from studies [12]. The following metrics were calculated: sensitivity, specificity, positive and negative likelihood ratios (LRs), and diagnostic odds ratio (DOR) [13,14]. Then, we constructed a summary receiver operating characteristic (SROC) curve and calculated the area under the curve (AUC) to evaluate the overall test performance [15,16]. If heterogeneity existed ($p < 0.05$ or $I^2 > 50\%$), we performed a subgroup analysis to examine the potential source of heterogeneity. Finally, a sensitivity analysis was used to evaluate the stability of the results of this meta-analysis. Publication bias was analyzed by Deek’s test, and if the p-value was less than 0.05, it indicated the presence of asymmetry. We used Stata (version 12.0) for this statistical analysis.

RESULTS

Search results

Figure 1 shows the process of study selection. We identified 1,851 published studies by electronic and manual searching. After removing duplicate studies and screening titles and abstracts, 60 records were selected for full-text review. Of these, 44 records were excluded for the listed reasons (supplementary material 1 - Table 2), and 16 records that met the inclusion criteria were included in our meta-analysis. Table 1 and Table 2 show the information about the included studies. There were 4,854 patients with RA and 5,843 controls without RA, and the characteristics of the control groups varied. Among the 16 studies, 8 used healthy persons as a control group, 6 used a mix of healthy persons and patients with other diseases, and 2 used a mix of healthy persons and healthy first-degree relatives (HFDRs) of RA patients.

The study quality was evaluated with QUADAS-1 and QUADAS-2. According to the 14 standard items of

Table 1. Characteristics of the 16 individual studies included in the meta-analysis.

Author [reference]	Year	Type of article	Design	Method (antigen, type)	Plate and antibody brands of ELISA	Reference standard	Cutoff	Patient groups	RA-female median	RA-age (yrs)	Disease duration	Healthy control-female median	Healthy control-age (yrs)
Shi et al. [5]	2011	Journal article	case-control	ELISA (FCS, IgG)	Plate: Thermo Scientific; antibody: Dako	the 1987 ACR criteria	Mean + 2SD	Leiden	NR	NR	< 2 year	NR	NR
Montes et al. [17]	2014	Meeting abstract	case-control	ELISA (FCS, IgG)	NR	the 1987 ACR criteria	Mean + 2SD	Spanish	76.90%	63	NR	50%	63
Shi et al. [18]	2015	Journal article	case-control	ELISA (FCS, IgG)	NR	the 2010 ACR criteria	Mean + 2SD	Leiden	NR	NR	< 2 year	NR	NR
Pecani et al. [19]	2015	Meeting abstract	case-control	ELISA (FCS, IgG)	NR	the 2010 ACR criteria	NR	Italian	NR	NR	NR	NR	NR
Alessandri et al. [20]	2015	Journal article	case-control	ELISA (FCS, IgG)	Plate: Thermo Scientific; antibody: Sigma	the 2010 ACR/EULAR criteria	Mean + 3SD	NR	41%	57.1	NR	HFDRs: 42% NHS: 45%	HFDRs: 54.6% NHS: 44.6%
Brink et al. [21]	2015	Journal article	case-control	ELISA (FCS, IgG)	Plate: Nunc; antibody: DAKO	the 1987 ACR criteria	a specificity of 97% of ROC curves	Vasterbotten northern Sweden	75%	57.5	median 7 months	73.10%	57.5
Janssen et al. [22]	2015	Journal article	case-control	ELISA (FCS, IgG)	Plate: Thermo Scientific; antibody: DAKO	established	Mean + 2SD	Caucasian	56%	57	NR	60%	26
Verheul et al. [23]	2015	Letter to the editor	case-control	ELISA (FCS, IgG)	Plate: Thermo Scientific; antibody: DAKO	the 1987 ACR criteria	a specificity of 97% of ROC curves	Japanese	NR	NR	mean 3.6 years	NR	NR
Pecani et al. [24]	2016	Journal article	case-control	ELISA (FCS, IgG)	Plate: Thermo Scientific; antibody: Sigma	the 2010 ACR/EULAR criteria	Mean + 3SD	Rome	77%	55.4	mean 107 months	73	53.1
Koppejan et al. [25]	2016	Journal article	case-control	ELISA (FCS, IgG)	Plate: Nunc; antibody: Sigma	the 1987 ACR criteria	Mean + 2SD	Manitoba	82%	45	NR	79	36
Challener et al. [26]	2016	Journal article	case-control	ELISA (FCS, IgG)	Plate: R&D Systems; antibody: Kirkegaard & Perry Laboratories Inc	the 1987 ACR criteria	mean+2SD	North American	70.30%	57	> 20 year	NR	NR
Verheul et al. [27]	2016	Letter to the editor	case-control	ELISA (FCS, IgG)	NR	the 1987 ACR criteria	a specificity of 97% of ROC curves	Leiden	67%	57	NR	51	44
Nakabo et al. [28]	2017	Journal article	case-control	ELISA (FCS, IgG)	Plate: Thermo Scientific; antibody: Promega	established	Mean + 2SD	Japanese	NR	NR	NR	NR	NR
Van Delf et al. [29]	2017	Journal article	case-control	ELISA (FCS, IgG)	antibody: DAKO	the 1987 ACR criteria	Mean + 2SD	Leiden	68.10%	57.2	NR	50%	44.1
Spinelli et al. [30]	2017	Journal article	case-control	ELISA (FCS, IgG)	Plate: Thermo Scientific; antibody: Sigma	the 2010 ACR/EULAR criteria	Mean + 3SD	Rome	68%	58.4	127 months	66.60%	56.7
Verheul et al. [31]	2017	Journal article	case-control	ELISA (FCS, IgG)	Plate: Thermo Scientific; antibody: DAKO	the 1987 ACR criteria	Mean + 2SD	Leiden	66.40%	54,9	NR	51.30%	41,8

Table 2. Characteristics of the 16 individual studies included in the meta-analysis.

Author	Year	Case number (RA)	Control participants (number)	TP	FP	FN	TN	SEN	SPE
Shi et al.	2011	557	HC: 305	250	9	307	296	44.90%	97.05%
Montes et al.	2014	520	HC: 305	188	10	332	198	36.20%	95.20%
Shi et al.	2015	969	T: 1,422 HC: 305 OD: 1,117	426	156	543	1,266	44.00%	89.00%
Pecani et al.	2015	178	HC: 68	82	1	96	67	45.93%	98.53%
Alessandri et al.	2015	63	T: 197 HC: 56, HFDR: 141	29	14	34	183	46.00%	92.90%
Brink et al.	2015	192	HC: 197	81	7	111	190	42.20%	96.40%
Janssen et al.	2015	86	T: 271 HC: 36 OD: 235	41	10	45	261	47.70%	96.30%
Verheul et al.	2015	268	HC: 324	121	10	147	314	45.10%	96.90%
Pecani et al.	2016	309	T: 298 HC: 98 OD: 200	117	35	192	263	34.40%	88.26%
Koppejan et al.	2016	95	T: 232 HC: 123 HFDR: 109	42	27	53	205	44.30%	88.36%
Challener et al.	2016	212	HC: 65	81	3	131	62	38.20%	95.40%
Verheul et al.	2016	557	T: 1,101 HC: 209 OD: 518 Smokers: 374	250	45	307	1,001	44.90%	95.70%
Nakabo et al.	2017	266	T: 696 HC: 80 OD: 616	124	149	141	548	46.80%	78.60%
Van Delf et al.	2017	373	HC: 196	184	6	189	190	49.20%	97.05%
Spinelli et al.	2017	50	HC: 30	19	0	31	30	38.00%	100.00%
Verheul et al.	2017	160	T: 287 HC: 80 OD: 207	60	26	100	261	37.50%	90.80%

HC - healthy control, T - the total of all the control participants, OD - other disease, HFDRs - healthy first-degree relatives of RA patients.

QUADAS-1, none of the studies satisfied all criteria; the median quality score was 11, and 11 studies had a QUADAS score > 10. The details of the QUADAS-1 results and the risk of bias and applicability concerns graph from QUADAS-2 are reported in the supplementary material 2.

Diagnostic accuracy of anti-CarP antibody

A forest plot of the sensitivity and specificity of the anti-CarP antibody in detecting RA is shown in Figure 2. The sensitivity and specificity ranged from 36.1% to 49.2% and from 78.6% to 100%, respectively, and the pooled sensitivity and specificity were 43.1% (95% CI, 41% to 45.2%) and 94.4% (95% CI, 91.1% to 96.1%), respectively. The positive LR, negative LR, and DOR were 7.69 (95% CI, 5.268 to 11.224), 0.603 (95% CI, 0.576 to 0.631), and 12.752 (95% CI, 8.459 to 19.223), respectively. The AUC was 0.55 (95% CI, 0.50 to 0.59) according to the SROC curve (Figure 3). Table 3 lists the details of the summary diagnostic indexes.

Heterogeneity and subgroup analysis

The meta-regression analysis showed that the study quality was not the source of heterogeneity, and the proportion of heterogeneity indicated there was no threshold effect. However, the I^2 for specificity was comparatively high, which meant there was statistically significant heterogeneity among the studies ($I^2 \geq 50$). We tried

Table 3. The details of the summary diagnostic indexes of the anti-CarP antibody.

Group	Number of studies	No. RA	No. control	Sensitivity (95% CI)	Heterogeneity of I^2	Heterogeneity of p-value	Specificity (95% CI)	Heterogeneity of I^2	Heterogeneity of p-value	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)	AUC (95% CI)
Anti-Carp	16	4,854	5,843	0.431 (0.410, 0.452)	49.31	0.01	0.944 (0.919, 0.961)	94.25	< 0.001	7.690 (5.268, 11.224)	0.603 (0.576, 0.631)	12.752 (8.459, 19.223)	0.55 (0.50, 0.59)
In the healthy control	16	4,854	2,422	0.430 (0.409, 0.452)	49.39	0.01	0.968 (0.960, 0.975)	0	0.72	13.560 (10.486, 17.536)	0.588 (0.565, 0.612)	23.052 (17.369, 30.593)	0.84 (0.81, 0.87)
In the disease control	6	2,346	3,268	0.434 (0.413, 0.455)	45.31	0.1	0.898 (0.834, 0.939)	96.65	< 0.001	4.263 (2.527, 7.189)	0.630 (0.583, 0.681)	6.762 (3.728, 12.263)	0.46 (0.42, 0.51)

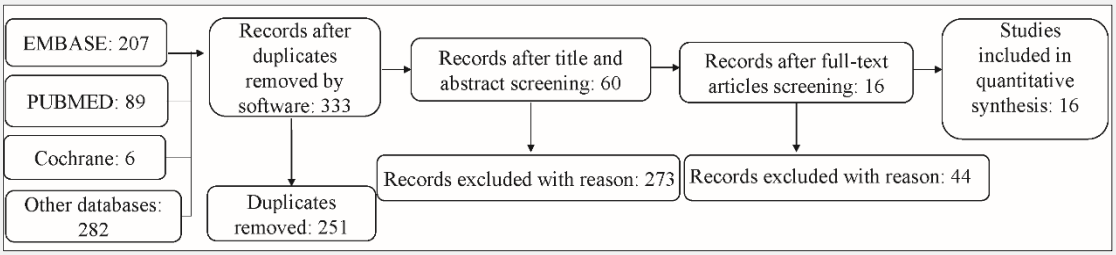

Figure 1. Study selection process.

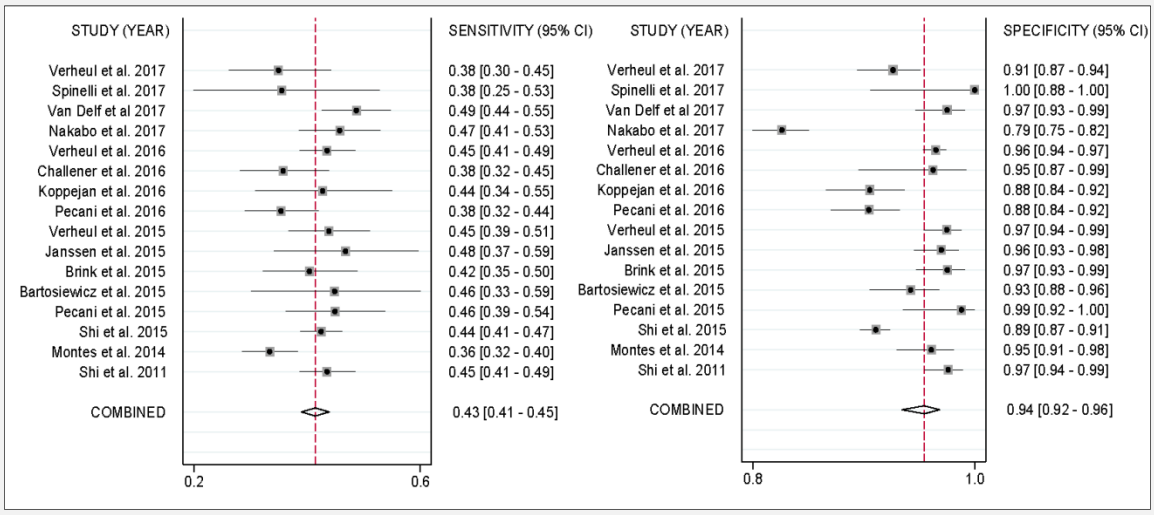

Figure 2. Forest plot of the estimates of sensitivity and specificity for the anti-CarP antibody in RA patients.

Circles and lines represent point estimates and 95% confidence intervals, respectively.

to determine the cause via subgroup analysis.

In this meta-analysis, two studies compared results using RA patients with healthy first-degree relatives, which might result in overestimation, and the number of studies was not enough to be considered representative. Some included studies used healthy persons as the controls, but some used a mixture of people with other diseases and healthy individuals as controls. Thus, we divided the included studies into two subgroups as follows: the healthy control group and the other diseases group. There were 16 reports in the healthy control group and 8 reports in the other diseases group. The summary estimates in the healthy control group were as follows: sensitivity 43% (95% CI, 40.9% to 45.2%), specificity 96.8% (95% CI, 96% to 97.5%), PLR 13.56 (95% CI, 10.486 to 17.536), NLR 0.588 (95% CI, 0.565 to 0.612), DOR 23.052 (95% CI, 17.369 to 30.593), and AUC 0.84 (95% CI, 0.81 to 0.87). The summary estimates in the other diseases group were as follows: sensitivity 43.4% (95% CI, 41.3% to 45.5%), specificity 89.8% (95% CI, 83.4% to 93.9%), PLR 4.263 (95% CI, 2.527 to 7.189), NLR 0.63 (95% CI, 0.583 to 0.681), DOR 6.762 (95% CI, 3.728 to 12.263), and AUC 0.46 (95% CI, 0.42 to 0.51). The details of the summary diagnostic indexes are listed in Table 3, and Figures 4, 5, and 6 show the forest plot and the SROC curve.

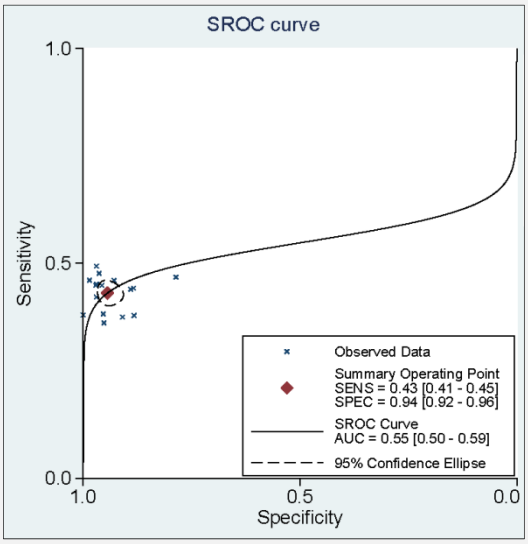

Figure 3. Summary receiver operating characteristic curves for the anti-CarP antibody for the diagnosis of RA.

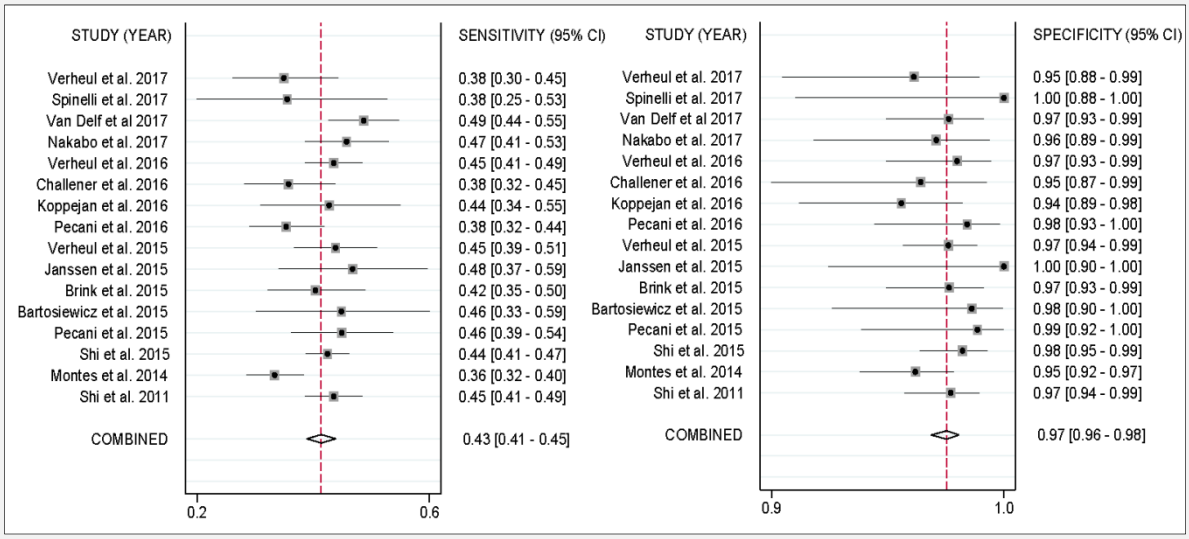

Figure 4. Forest plot of the estimates of sensitivity and specificity for the anti-CarP antibody in the healthy control group.

Publication bias

The sensitivity analysis indicated that the results of this meta-analysis were stable (supplementary material 2 - Table 3). The Deek's test revealed little publication bias in the studies (the mixed control $p = 0.216$, the healthy control group $p = 0.584$, the disease control group $p = 0.752$, $p > 0.05$).

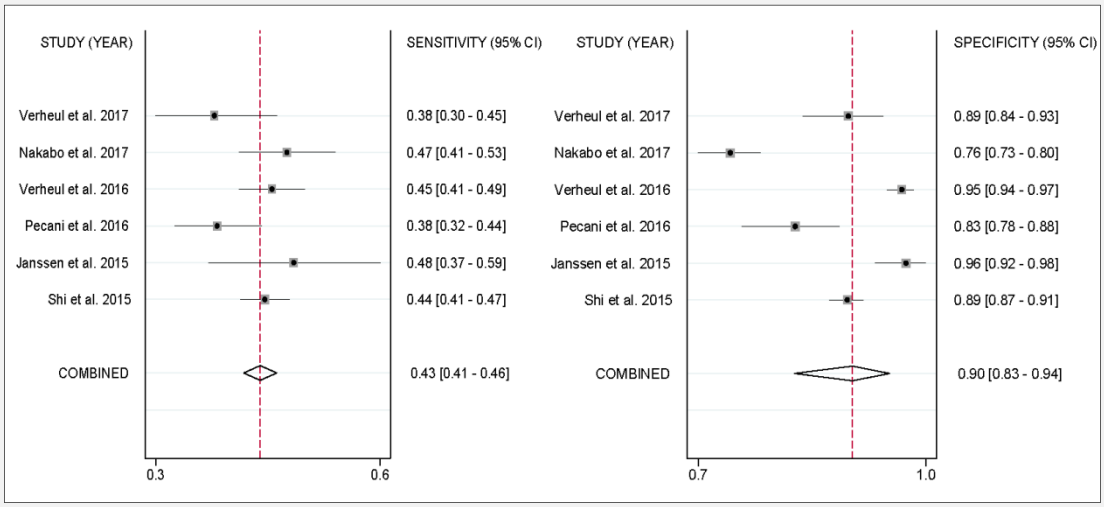

Figure 5. Forest plot of the estimates of sensitivity and specificity for the anti-CarP antibody in the disease control group.

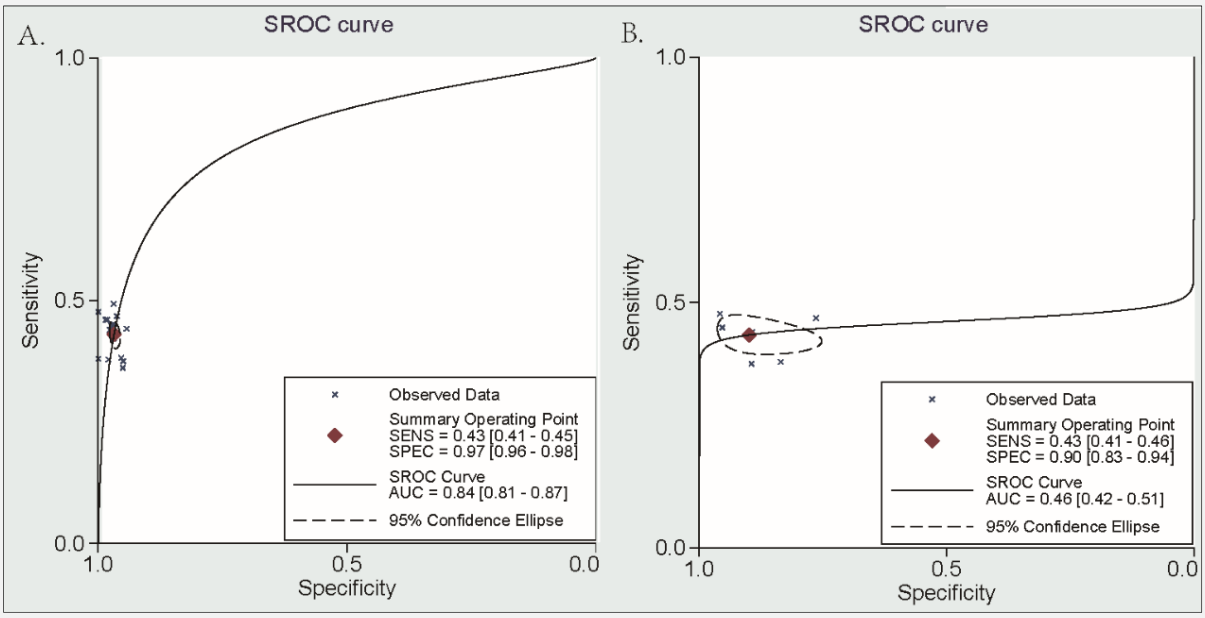

Figure 6. Summary receiver operating characteristic curves for the anti-CarP antibody in the subgroups of patients.

A: the healthy control group, B: the disease control group.

DISCUSSION

Rheumatoid arthritis (RA) is a highly heterogeneous autoimmune disease, and there are a wide variety of antibodies in patients with RA. These antibodies have been used as special serological biomarkers and constitute a significant part of the laboratory testing used to make a diagnosis of RA. Early diagnosis and accurate treatment clearly lead to less joint erosion and better disease outcomes, so studies of RA often focus on the discovery of new diagnostic biomarkers. This meta-analysis analyzed the diagnostic accuracy of a novel separate antibody, the anti-CarP antibody. The evidence shows that the presence of the anti-CarP antibody is a risk factor that is independent of the ACPA status. It is not only associated with the development of RA in persons with arthralgia but also with more severe joint destruction, radiological damage, and increased mortality in RA patients [21,32,33].

We combined the evidence regarding anti-CarP antibodies in RA patients and evaluated the diagnostic accuracy of the anti-CarP test. This meta-analysis included 16 studies, and the results indicated that anti-CarP antibodies had a high specificity of 94.4% and a positive LR of 7.69, indicating that compared with the controls without RA, RA patients had a 7-fold higher chance of having anti-CarP antibodies. The sensitivity was only 43.1%, and the negative LR was 0.603, which suggest that RA cannot be excluded if the anti-CarP antibody test is negative (NLR < 0.1). The DOR, ranging from 0 to infinity, reflects the links between the results of diagnostic tests and diseases, and a higher value suggests that the diagnostic test has a stronger discriminatory ability. The DOR in our analysis was 12.752, which demonstrated that the anti-CarP antibody is helpful in the diagnosis of RA. The AUC was 0.55, which indicated that the overall diagnostic performance of anti-CarP antibody might not be as good as that of anti-CCP antibody.

The diagnostic accuracy of the anti-CarP antibody for RA in our results was similar to that reported in the previous meta-analysis [34], but an important difference was that we compare the diagnostic effect of anti-Carp antibody between healthy persons and no-RA patients in this study, while the previous one focused mainly on healthy persons. To explore the causes of that heterogeneity in specificity, we performed a subgroup analysis. To explore the causes of that heterogeneity, we performed a subgroup analysis. There were some differences between the healthy control group and the other diseases group. The sensitivity (43% vs. 43.4%) and NLR (0.588 vs. 0.63) were similar, but the specificity, PLR, and AUC of the other diseases group (89.8%, 4.263, 0.84) were lower than those of the healthy control group (96.8%, 13.56, 0.46), indicating that the anti-CarP antibody might not be useful to exclude RA from other diseases. The anti-CarP antibody could be detected in patients with renal failure [27], periodontitis [22] and other connective tissue diseases (Sjögren syndrome, systemic lupus erythematosus, systemic sclerosis, vasculitis, psoriatic arthritis, and inflammatory osteoarthritis) [18,24,28]. Furthermore, anti-CarP antibodies were also more prevalent in healthy first-degree relatives of RA patients than in healthy controls [20,25]. It was clear that the different diseases in the control groups constituted a factor that resulted in the bias in this meta-analysis, which was inevitable and existed in the meta-analyses of anti-CCP antibody and anti-MCV antibody as well [35,36]. Although there were differences between the subgroups, we can still conclude that the diagnostic accuracy of the anti-CarP antibody for RA patients is high.

Most studies used carbamylated fetal calf serum (Ca-FCS), a complex mixture of proteins, as an antigen for the ELISA assay because the precise target antigen of anti-CarP antibodies is unknown, which is a gap in knowledge that needs to be further explored. Recently, some studies attempted to utilize other proteins as target antigens, such as fibrinogen (49%) [37], vimentin (77%) [38], type I (22%) and II (19%) collagen [39], and synthetic citrulline-rich peptide (57%) [40], which are known to be the target antigens of ACPA and have moderate sensitivity and high specificity for the anti-CarP antibody. Like anti-CCP antibody and RF, the anti-CarP antibody has three forms in the serum of RA patients, namely, IgG, IgA, and IgM. The sensitivities of IgA [5,27,29] and IgM [29] were 40.8 - 43% and 16.4%, respectively, and the specificity was 94.3 - 94.9% and 95.9%, respectively. To assess the further diagnostic value of anti-CarP for RA, we used single rate analysis to generalize the sensitivity in anti-CCP antibody positive/negative or RF IgM positive/negative RA patients (supplementary material 2 - Table 4). The summary estimate sensitivities for the anti-CarP antibody in the anti-CCP antibody positive/negative or the RF IgM positive/negative RA patients were 59%, 16%, 52%, and 25%, respectively, indicating that the anti-CarP antibody, ACPA or RF IgM might not coexist in the serum of some patients with RA. Therefore, an anti-CarP antibody might be helpful for identifying RA in some CCP-negative or RF-negative patients, who may benefit from early and accurate interventions.

However, this meta-analysis had some limitations. First, some articles published in other databases may have been missed. Second, when assessing the quality of the included studies by QUADAS-2, there are some points that should be considered. The design method was case-control, and it was unclear whether the anti-CarP antibody assay was assessed while blinded to the reference standard, which might have introduced bias with regard to overestimating the index test performance. In addition, although the most common reason for the heterogeneity in our meta-analysis was the cross-reaction with anti-CarP antibodies generated as a result of the presence of other diseases in the control group, the region, clinical status, RA duration and disease severity of the participants might also have affected the results. Thus, further research is needed to clarify these issues.

CONCLUSION

With high specificity but relatively low sensitivity, the anti-CarP antibody is a meaningful serological biomarker for RA.

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Ethical Approval:
This article does not contain any studies with human participants or animals performed by any of the authors.

Declaration of Interest:
The authors have no competing interests to declare.

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