Review

Wen-Chao Zhang[a], Han Wu[a] and Wei-Xian Chen*

Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide 2 antibody and anti-cyclic citrullinated peptide 3 antibody in rheumatoid arthritis

Abstract: The aim of this work was to assess the diagnostic value of anti-CCP-3 and anti-CCP-2 for the diagnosis of rheumatoid arthritis (RA) and determine whether anti-CCP-3 more accurately identifies patients with rheumatoid arthritis than anti-CCP-2. PubMed and CNKI databases were searched for studies published in English and Chinese that examined the use of anti-CCP-3 and anti-CCP-2 in the diagnosis of known or suspected rheumatoid arthritis from January 2006 to July 2013. Seventeen included studies of methodological quality were rated by using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tools A random-effects method was used to summarize sensitivities, specificities, positive and negative likelihood ratio (LR+ and LR–, respectively), and diagnostic odds ratio from 17 studies. The pooled sensitivity, specificity, LR+, LR– and diagnostic odds ratio for anti-CCP-3 were 0.737 (95% CI, 0.717–0.757), 0.933 (95% CI, 0.924–0.942), 11.096 (95% CI, 8.876–13.870), 0.274 (95% CI, 0.231–0.326), and 42.908 (95% CI, 33.828–54.426), respectively. For anti-CCP-2, the values were 0.719 (95% CI, 0.699–0.739), 0.960 (95% CI, 0.953–0.966), 17.485 (95% CI, 11.960–25.562), 0.294 (95% CI, 0.258–0.335) and 63.458 (95% CI, 44.214–91.078), respectively. With high specificity and moderate sensitivity, anti-CCP-2 and anti-CCP-3 played an important role in confirming the diagnosis of RA. Anti-CCP-3 did not have better diagnostic performances than anti-CCP-2, but anti-CCP-2 had evident heterogeneity compared to anti-CCP-3, especially in American patients.

Keywords: anti-CCP-3; anti-CCP-2; diagnostic accuracy; rheumatoid arthritis.

[a]Wen-Chao Zhang and Han Wu contributed equally to this work.
***Corresponding author: Wei-Xian Chen,** Department of Laboratory Medicine, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, P.R. China; and No. 74, Linjiang Road, Yuzhong District, Chongqing, 400010, P.R. China, Tel.: +86 023 63703244, Fax: +86-023-63703244, E-mail: chenweixian75@163.com

Wen-Chao Zhang: Department of Laboratory Medicine, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, P.R. China; and Department of Neurology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, P.R. China
Han Wu: Department of Neurology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, P.R. China

Introduction

Rheumatoid arthritis (RA) is a chronic, severe and systemic autoimmune inflammatory disease of unknown etiology, which affects approximately 1% of the general population [1]. It is generally progressive for irreversible joint destruction, persistent arthritis and gravis pain, which leads to significant functional disability and loss of quality of life. Since early and aggressive intervention with effective biological treatments can alter the course of the disease and improve prognosis [2], early diagnosis of rheumatoid arthritis is important. Unfortunately, there are rarely early-stage patients based primarily on history, physical examination findings, laboratory and radiographic results who meet the 1987 revised criteria of the American College of Rheumatology (ACR) [3].

So far, serological support in the diagnosis of RA is mainly based on the presence of rheumatoid factors (RF) [4]. However, the antibody directed against the Fc part of IgG is taken as a non-specific marker of RA and can also be detected in other rheumatic diseases, infectious diseases and even in healthy, especially elderly, individuals [4–6]. Therefore, several new autoantibodies have been reported in patients with RA, and their clinical value has been evaluated. Most, such as antiperinuclear factor antibodies (APF), antikeratin antibodies, anti-RA33 and anti-Sa, cannot demonstrate to have adequate sensitivity and specificity to form a basis for clinical and therapeutic decisions [7–11]. An early attempt to create an ideal RA

marker was the anti-cyclic citrullinated peptide antibody, first described by Schellekens et al. [12]. Thus the anti-CCP antibody assay has generated great interest for a long time. The first-generation of anti-CCP antibody assays were not widely used in clinical practice because of their low sensitivity [13, 14]. To improve the diagnostic value of RA, second-generation anti-CCP and anti-CCP3 antibody assays have been developed. In fact, an anti-CCP-3 assay has been introduced by INOVA and recommended as a third-generation anti-cyclic citrullinated peptide antibody; however, there are some controversies regarding its clinical performance compared to the second-generation anti-cyclic citrullinated peptide antibody.

In our systematic review, we summarized published data on the sensitivity, specificity, likelihood ratios and diagnostic odds ratio of anti-CCP-2 and anti-CCP-3 for diagnosing rheumatoid arthritis, then assessed whether anti-CCP-3 shows an evident improvement compared with anti-CCP-2.

Materials and methods

Data sources and searches

We developed a protocol for the review and followed standard reporting guidelines [15]. We searched PubMed and CNKI databases for studies published in English and Chinese from January 2006 to July 2013 that examined the use of both anti-CCP-2 and anti-CCP-3. Our searches were based on combinations of the following index terms: rheumatoid arthritis; CCP; anti-CCP antibodies; anti-CCP antibody; anti-cyclic citrullinated peptide antibodies and anti-cyclic citrullinated peptide antibody; second-generation assays of anti-cyclic citrullinated peptide antibodies; anti-CCP-2 and anti-CCP3; and CCP-2 and CCP-3. We also reviewed the reference lists of retrieved studies and review articles.

Study selection

Two reviewers independently scanned abstracts that met the inclusion criteria. We included studies that evaluated the utility of assaying anti-CCP-2 and anti-CCP-3 for the diagnosis of confirmed or suspected rheumatoid arthritis and that provided enough data to allow the calculation of sensitivity and specificity for the diagnosis of rheumatoid arthritis. We used the ACR criteria as the reference standard of rheumatoid arthritis [3, 16–19]. The following articles were not included in the current study: reviews; publications without valid data to obtain the sensitivity and specificity of anti-CCP; researches with the anti-CCP collected from synovial fluid but not blood; publications not related to the diagnostic values of anti-CCP for RA.

Data extraction and study quality assessment

We extracted data by using a standard form that included the author, publication year, demographic characteristics of the participants, assays of index tests, true-positive results, false-negative results, true-negative results, false-positive results, sensitivity and specificity. Two investigators independently assessed the methodological quality of each study by using 14 standard items from the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool [20]. We resolved any item discrepancies through discussion.

Data analysis

We used a random-effects model to combine estimates of sensitivity, specificity, positive and negative likelihood ratios, and diagnostic odds ratio [21, 22]. Threshold analyses and meta-regression were conducted to assess whether threshold effect and heterogeneity among studies existed [23]. Heterogeneity was investigated by using stratified analyses for different assays and different races of RA patients. Funnel plots were examined for diagnostic odds ratios to explore the possibility of publication bias [24]. For analyses, we used the software applications MetaDiSc version 1.4 and Review Manager version 5.0.

Results

Search results and characteristics of the studies

We identified 797 articles, of which 17 met the inclusion criteria [25–41]. There were 1952 RA patients selected from Europe, Americas and Asia in the remaining 17 articles that were eventually available for this meta-analysis (see Table 1). Of these, 9 (52.9%) were studies involving European RA patients, 5 (29.4%) American and 3 (17.7%) Asian. For the assay methods, all anti-CCP-3 assay data used the products of INOVA, whereas 9 (52.9%) studies used INOVA and 8 (47.1%) studies used non-INOVA products for the anti-CCP-2 assays.

Table 1 Characteristics of the studies using anti-CCP-3 and anti-CCP-2.

Study	Patients' race	Setting	Assay manufacturer (cut-off: U/mL)	RA patient	Women,%	Mean[c] or median[d] age (SD), years	Age range	Average disease duration, (SD), years	Control participants
[25]	Germans	A single clinical center for rheumatic diseases	INOVA[a] (20)	141	69.5	62.3[c] (12.0)	Not referred	8.5 (8.8)	Non-RA (n=154)
			Phadia[b] (10)						
[26]	Belgians	Ghent University Hospital	INOVA[a] (20)	86[g]	65.2[g]	55[2g]	22–85[g]	3.0[g]	Non-RA (n=450)[g]
			Pharmacia[b] (10)	155[h]	71.7[h]	62.6[d,h]	30–80[h]	9.0[h]	
[27]	Spanish	Hospital Virgen del Rocio	INOVA[a] (20)	124	77.4	56.7[c] (1.4)	Not referred	Not referred	Non-RA (n=110), HC (n=48)
			INOVA[b] (20)						
[28]	Belgians	Rheumatology clinic	INOVA[a] (20)	85	74.1[i]	51.5[d,i,j]	26–84[i]	Not referred	Non-RA (n=157), AI (n=39)
			Phadia[b] (7)	17		54.0[d,i,k]			
[29]	Japanese	Not referred	INOVA[a] (20)	227	78.4	60.2[c] (13.1)	Not referred	10.3 (8.8)	Non-RA (n=173)
			Axis-Shield[b] (5)						
[30]	Japanese	Not referred	INOVA[a] (20)	106	84.0	59.3[c] (13.4)	Not referred	7.6 (7.8)	Non-RA (n=57)
			Axis-Shield[b] (4.5)						
[31]	Belgians	University Hospital of Liège	INOVA[a] (20)	120	71.0	56.0[d]	20–79	Not referred	Non-RA (n=170)
			INOVA[b] (20)						
[32]	Brazilian	Hospital Universitario of the Universidade Federalde Santa Catarina Florianopolis	INOVA[a] (20)	70	91.0	49.1[c] (12.3)	26–72	9.0 (6.23)	SLE (n=34), HC (n=54)
			INOVA[b] (20)			47.0[d]			
[33]	Portuguese	Not referred	INOVA[a] (20)	86	Not referred	Not referred	Not referred	Not referred	Non-RA (n=60), infections (n=30)
			INOVA[b] (20)						
[34]	Brazilian	Not referred	INOVA[a] (20)	48	Not referred	Not referred	Not referred	Not referred	SSc (n=74), PBC (n=80), HC (n=40)
			INOVA[b] (20)						
[35]	Trukese	3 different rheumatology clinics	INOVA[a] (20)	93	83.0	53.8[c]	26–76	Not referred	Non-RA (n=56), HC (n=17)
			INOVA[b] (20)						
[36]	301 Italians and 1 Chinese	4 hospital settings of the authors	INOVA[a] (20)	100	90.0	64.0[c]	40–86	Not referred	Non-RA (n=51), AI (n=21), infections (n=39), cancer (n=17), HC (n=74)

(**Table 1** Continued)

Study	Patients' race	Setting	Assay manufacturer (cut-off: U/mL)	RA patient	Women,%	Mean[c] or median[d] age (SD), years	Age range	Average disease duration, (SD), years	Control participants
[37]	American	Morris and Metzger rheumatologist group in Los Angeles	INOVA[b] (20) INOVA[a] (20)	115	Not referred	Not referred	Not referred	Not referred	Non-RA (n=346), CREST (n=52), HC (n=51)
[38]	American	University of Utah institutional review board	INOVA[b] (20) INOVA[a] (20)	137	Not referred	Not referred	Not referred	Not referred	SLE (n=50), HC (n=100)
[39]	Belgians	Brugmann University Hospital	INOVA[b] (20) INOVA[a] (20)	112	80.4	57.0[d,e]	41–69	Not referred	Non-RA (n=53), non-AI (n=65), infections (n=18)
[40]	American	University of North Carolina Hospitals	Elecsys[b] (17) INOVA[a] (20)	41	80.0	58.0[d,f] 51.5[d]	18–88	Not referred	Non-RA (n=87), non-AI (n=35)
[41]	Japanese	Not referred	Phadia[b] (7 or 10) INOVA[a] (20)	89	84.3	51.2[c] (15.5)	Not referred	5.9 (7.2)	Non-RA (n=148), HC (n=168), infections (n=142)
			Axis-Shield[b] (4.5)						

[a]Assay for detecting anti-CCP-3. [b]Assay for detecting anti-CCP- 2. [c]Mean. [d]Median age. [e]Median age of male. [f]Median age of female. [g]Characteristics of the study in population 1 (from study [42]). [h]Characteristics of the study in population 2 (from study [43]). [i]This study only gave these 85 patients of characteristics. [j]Median age of early RA. [k]Median age of established RA. Anti-CCP-2/3, anti-cyclic citrullinated peptide 2/3 antibody; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; CREST, Calcinosis cutis, Raynaud's, esophageal dysfunction, sclerodactyly and telangiectasia; SSc, systemic sclerosis; PBC, primary biliary cirrhosis; AI, autoimmune diseases; QUADAS, Quality Assessment of Diagnostic Accuracy Studies.

Table 1 summarizes the characteristics of the included articles. The characteristics of the control groups varied. Among these articles, only eight used a mix of healthy individuals and patients with other non-RA diseases, such as systemic lupus erythematosus, systemic sclerosis, psoriatic arthritis, mixed connective tissue disease, primary biliary cirrhosis, immune thrombocytopenic purpura and infectious disease.

Study quality

Most of the studies included in the meta-analysis had high quality with more than 9 satisfied items of 14 items using the QUADAS tool, and the median score for quality was 11. None of the studies satisfied all the criteria of the quality checklist. Only one study satisfied 13 items of 14 standard items, three studies satisfied 12 items, eight studies satisfied 11 items, three studies satisfied 10 items and two studies satisfied 9 items (Figure 1).

Diagnostic accuracy of anti-CCP-3

The summary positive and negative likelihood ratios were 11.096 (95% CI, 8.876–13.870) and 0.274 (95% CI, 0.231–0.326), respectively. The pooled sensitivity and specificity were 0.737 (95% CI, 0.717–0.757) and 0.933 (95% CI, 0.924–0.942), respectively (Table 2). Data that were calculated from anti-CCP-3 provided a high diagnostic value of rheumatoid arthritis. The summary diagnostic odds ratios were 42.908 (95% CI, 33.828–54.426), and the area under the curve was 0.9148 with a Q^* of 0.8474 (Figure 2). But a significant heterogeneity was found among included studies. Therefore, we next probed the reasons for the heterogeneity through meta-regression analysis and discovered heterogeneity from the different races of patients. We did a stratified meta-analysis for each subgroup.

In Figure 3A, the red solid circles show the forest plot of sensitivity and specificity estimates from nine studies that used patients with RA from Europe. This subgroup showed high specificity estimates with a pooled specificity of 0.944 (95% CI, 0.932–0.955). By contrast, sensitivity estimates were moderate, with a pooled sensitivity of 0.700 (95% CI, 0.672–0.726). Table 2 shows the results of the meta-analysis. The summary values from the nine studies were almost as heterogeneous as those from the meta-analysis of all 17 studies, but the Spearman's correlation coefficient and the p value were changed, 0.450 and 0.224 for this subgroup, and 0.522 and 0.032 for all 17 studies, respectively. It suggests that there were no remarkable threshold effects in these nine studies.

The yellow solid circles show the forest plot of sensitivity and specificity estimates from five studies that used patients with RA from the Americas. As in the subgroup of Europeans, high specificity estimates (0.939, 95% CI, 0.922–0.953) and moderate sensitivity estimates (0.769, 95% CI, 0.725–0.809) were achieved. However, we could see that there was no heterogeneity between them (Table 2).

The green solid circles show the forest plot of sensitivity and specificity estimates from three studies that used patients with RA from Asia. These were the highest

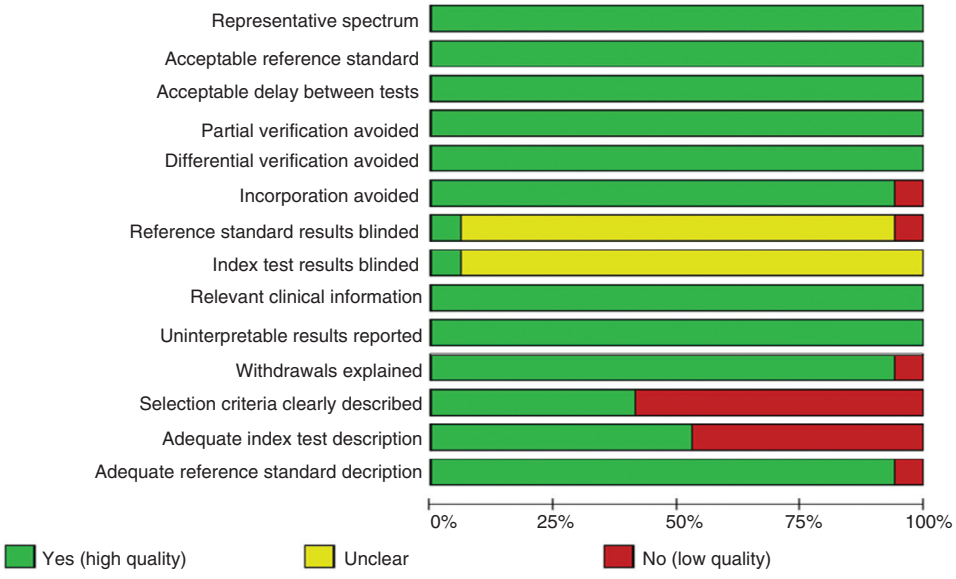

Figure 1 A cumulative bar plot of methodological quality items across all studies.

Table 2 Summary values of anti-CCP-3 and anti-CCP-2 for all studies and different subgroups.

	Groups (n)	Sensitivity	Specificity	LR+	LR−	DOR	Spearman correlation coefficient
Anti-CCP-3							
Summary accuracy (95% CI)[h]	1 (17)	0.737 (0.717–0.757)	0.933 (0.924–0.942)	11.096 (8.876–13.870)	0.274 (0.231–0.326)	42.908 (33.828–54.426)	0.522
	1[a] (9)	0.700 (0.672–0.726)	0.944 (0.932–0.955)	12.868 (8.966–18.468)	0.313 (0.249–0.394)	43.337 (31.450–59.716)	0.45
	1[b,j] (5)	0.769 (0.725–0.809)	0.939 (0.922–0.953)	13.478 (10.197–17.816)	0.247 (0.207–0.295)	54.383 (37.877–78.081)	-0.100
	1[c] (3)	0.806 (0.765–0.842)	0.901 (0.877–0.922)	7.388 (4.879–11.187)	0.207 (0.138–0.313)	34.391 (18.643–63.441)	-0.500
Heterogeneity p Value[i]							
	1 (17)	0.000	0.000	0.001	0.000	0.079	0.032
	1[a] (9)	0.000	0.001	0.005	0.000	0.188	0.224
	1[b, j] (5)	0.619	0.221	0.203	0.622	0.268	0.873
	1[c] (3)	0.016	0.090	0.065	0.036	0.089	0.667
Anti-CCP-2							
Summary accuracy (95% CI)[h]	2[k] (17)	0.719 (0.699–0.739)	0.960 (0.953–0.966)	17.485 (11.960–25.562)	0.294 (0.258–0.335)	63.458 (44.214–91.078)	0.517
	2[a,k] (9)	0.695 (0.667–0.722)	0.968 (0.958–0.976)	20.507 (12.650–33.245)	0.316 (0.265–0.377)	68.619 (42.432–110.97)	0.417
	2[b] (5)	0.708 (0.661–0.752)	0.958 (0.944–0.970)	16.723 (9.108–30.704)	0.309 (0.264–0.362)	61.911 (40.439–94.785)	0.800
	2[c] (3)	0.794 (0.752–0.831)	0.945 (0.925–0.961)	12.129 (3.558–41.351)	0.219 (0.150–0.320)	58.200 (15.033– 225.31)	0.500
	2[f] (9)	0.665 (0.633–0.696)	0.961 (0.950–0.970)	17.133 (11.367–25.824)	0.344 (0.298–0.398)	54.948 (40.024–75.436)	0.767
	2[g,k] (8)	0.765 (0.738–0.790)	0.959 (0.949–0.968)	17.588 (9.066–34.118)	0.253 (0.222–0.289)	73.918 (37.659–145.09)	0.357
Heterogeneity p Value[i]	2[k] (17)	0.000	0.000	0.000	0.000	0.000	0.034
	2[a,k] (9)	0.000	0.001	0.003	0.000	0.028	0.265
	2[b] (5)	0.256	0.003	0.007	0.371	0.379	0.104
	2[c] (3)	0.016	0.000	0.000	0.040	0.000	0.667
	2[f] (9)	0.010	0.003	0.016	0.019	0.489	0.016
	2[g,k] (8)	0.043	0.000	0.000	0.227	0.000	0.385

Group 1, Anti-CCP-3 group. Group 2, Anti-CCP-2 group. [a–c]European, American and Asian patients with rheumatoid arthritis, respectively. [f]Subgroup for INOVA assay for detecting anti-CCP-2. [g]Subgroup for non-INOVA assay for detecting anti-CCP-2. [h]Random-effects model; [i]Random-effects heterogeneity. [j]Fixed-effects model. [k]The SROC curve was asymmetric. n, number of included articles; anti-CCP-2/3, anti-cyclic citrullinated peptide 2/3 antibody, respectively; LR+/−, positive/negative likelihood ratio, respectively; CI, confidence interval; RA, rheumatoid arthritis; SROC, summary receiver operative curves; DOR, diagnostic odds ratio.

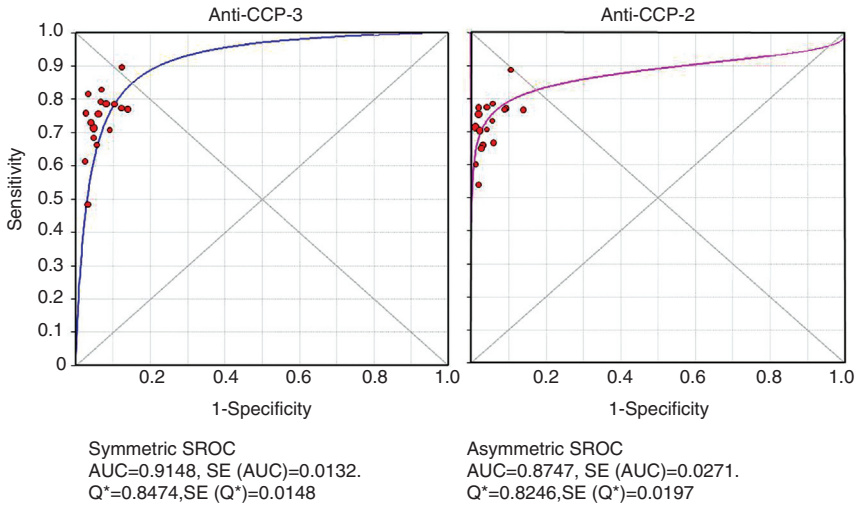

Figure 2 SROC of the anti-CCP-3 and anti-CCP-2 assays.
Each solid circle represents each study in the meta-analysis. The size of each study is indicated by the size of the solid circle. Anti-CCP-2/3, anti-cyclic citrullinated peptide 2/3 antibody; SROC, summary receiver operative curves; AUC, area under the curve; SE, standard error; *Q**, Cochran *Q*.

sensitivity (0.806, (95% CI, 0.765–0.842)) but the lowest specificity (0.901, (95% CI, 0.877–0.922)) compared to the other two subgroups. The results of the meta-analysis from this subgroup showed heterogeneity but no remarkable threshold effects since the Spearman's correlation coefficient was −0.5 and the p value was 0.667 (Table 2).

Summing up the above data, we could see that heterogeneity was changed after a stratified analysis, especially in the American patients, followed by the Asian patients. In contrast, there were no threshold effects in all subgroups. Thus we conjectured that the diagnostic accuracy of anti-CCP-3 for RA diagnosis may depend on a patient's race [44]. In our review, the results were limited by the finite included reports.

Diagnostic accuracy of anti-CCP-2

As shown in Table 2, the pooled sensitivity, specificity, positive and negative likelihood ratios, and diagnostic odds ratio for anti-CCP-2 were 0.719 (95% CI, 0.699–0.739), 0.960 (95% CI, 0.953–0.966), 17.485 (95% CI, 11.960–25.562), 0.294 (95% CI, 0.258–0.335) and 63.458 (95% CI, 44.214–91.078), respectively. We could see that the values were changed when comparing anti-CCP-3 with anti-CCP-2. The summary values from anti-CCP-3 deteriorated the specificity, positive likelihood ratios and diagnostic odds ratio of the diagnosis of rheumatoid arthritis, except for the improved sensitivity and negative likelihood ratios to a little extent. Data from anti-CCP-2 were still evidently heterogeneous. Meanwhile, the SROC curve was asymmetric (Figure 2) and did not show a clear trade-off between sensitivity and specificity. In anti-CCP-2, we defined three subgroups on the basis of the different races of the RA patients and then conducted another two subgroups analysis based on the various assay products: INOVA tests and non-INOVA tests.

The blue, purple and orange solid circles in Figure 3B provide the forest plot of sensitivity and specificity estimates from nine, five and three studies that used patients with RA from Europe, the Americas and Asia, respectively. The results showed high specificity estimates and moderate sensitivity estimates. Although all summary values were grossly heterogeneous (Table 2), threshold effects were negative and SROC curves became symmetric in the American and Asian groups (data not shown). In contrast, we could see a lower inconsistency of the pooled sensitivity in the American groups vs. the other two subgroups (p=0.256).

If anti-CCP-3 is compared with anti-CCP-2 in the same race, such as in the American subgroup, anti-CCP-3 may be a better index to diagnose rheumatoid arthritis because of its profound low inconsistency than anti-CCP-2 (Table 2). In the Asian subgroup, we yielded the conclusion that anti-CCP-3 was not superior to anti-CCP-2 in diagnosing rheumatoid arthritis. The conclusion was the same as those from all 17 studies, but still needed to be investigated for its substantial heterogeneity (Table 2). Nevertheless, in the European subgroup, we inferred a different conclusion that the SROC curve was asymmetric in anti-CCP-2 and that this assay still has a higher diagnostic accuracy than anti-CCP-3. This was the reason why different kinds of anti-CCP-2 assays were used in the European patients.

The pink and cyan solid circles in Figure 3C show the forest plot of sensitivity and specificity estimates from

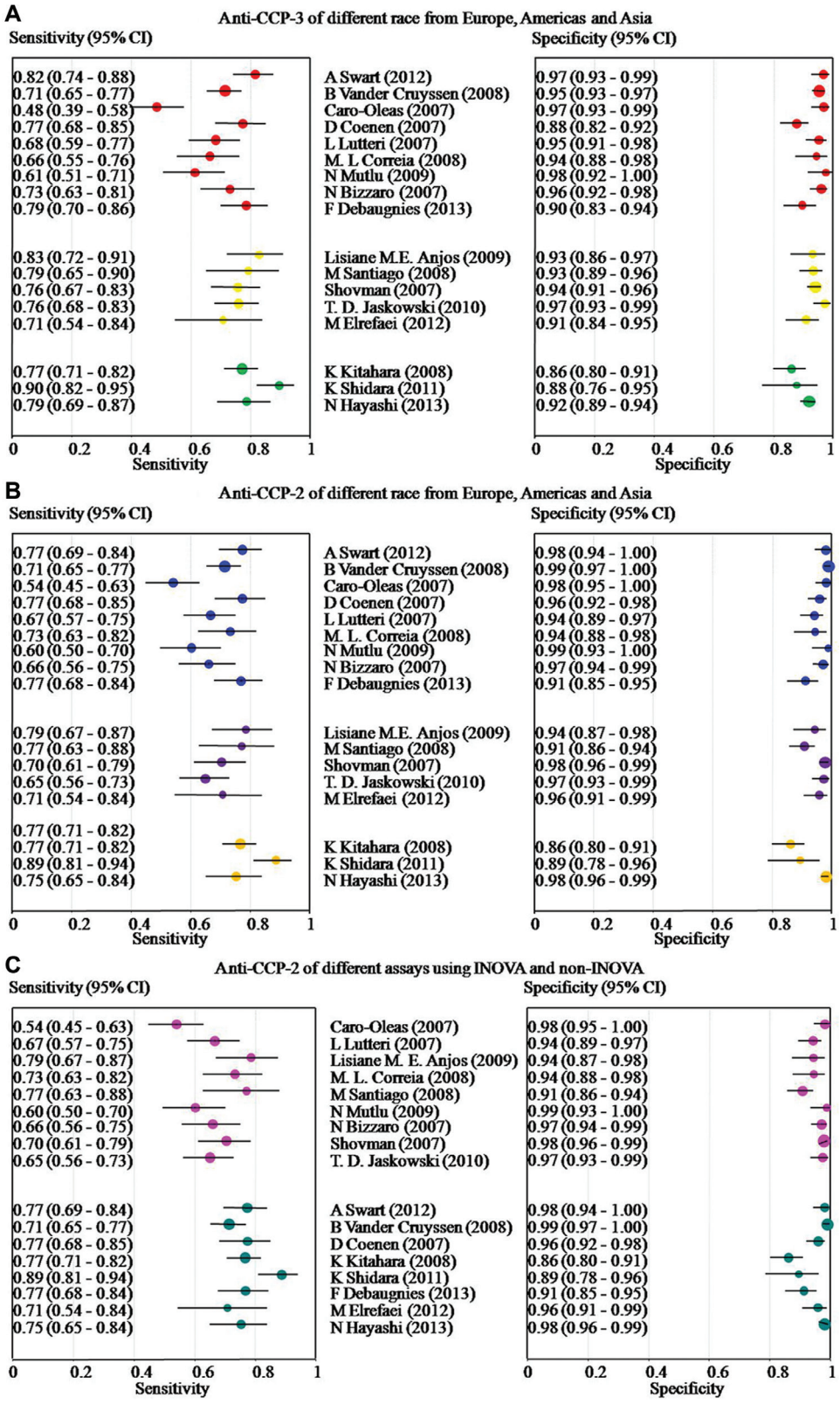

Figure 3 Forest plot of estimates of sensitivity and specificity for different subgroups.
Each solid circle indicates the point estimate of sensitivity and specificity from each study in the meta-analysis. Error bars indicate 95% confidence intervals. The different colors represent subgroups. As for the different races, red is Europe, yellow is the Americas and green is Asia for anti-CCP-3, and blue, purple and orange for anti-CCP-2, respectively. Meanwhile, pink is INOVA and cyan is non-INOVA for different assays in anti-CCP-2. Anti-CCP-2/3, anti-cyclic citrullinated peptide 2/3 antibody.

nine studies that used INOVA tests and from eight studies that used non-INOVA tests, respectively. What surprised us was that the diagnostic efficacy from the former stratified group was overshadowed vs. all 17 studies. Yet, this subgroup introduced a symmetric SROC curve. Data from the latter subgroup demonstrated a little higher diagnostic performance than those in all 17 studies, and there still existed an evident inconsistency (Table 2).

Publication bias

The funnel plots of anti-CCP-3 and anti-CCP-2 for publication bias showed some asymmetry, indicating a potential publication bias (Figure 4).

Discussion

It is well known that rheumatoid arthritis is a long-term inflammatory disorder and not curable by drugs. Early and accurate diagnosis of rheumatoid arthritis could decrease the morbidity of functional disability and improve quality of life. With the desire to resolve the problem, most rheumatologists recommend measuring anti-CCP antibody and RF for early rheumatoid arthritis [45, 46]. Because it is costly to treat persons who were misdiagnosed with rheumatoid arthritis, we need to consider the risks and benefits of such an approach. Also, rheumatologists proposed to revise the 2010 ACR criteria [16]. Despite the high sensitivity, the 2010 ACR criteria still miss some RA patients, especially symmetrical seronegative arthritis and limited joint involvement [47].

Because the anti-CCP antibody has a high specificity but moderate sensitivity in the diagnosis of rheumatoid arthritis [48], as in our results, anti-CCP antibody assays are developed further to maximize sensitivity. Anti-CCP-2 and anti-CCP-3 assays offer slightly improved sensitivity over that of anti-CCP-1 assays [49, 50], although they have no significant changes in specificity for RA (0.924–0.942 for CCP-3 and 0.953–0.966 for CCP-2 from our data).

The accuracies of anti-CCP-2 and anti-CCP-3 for RA diagnosis in our review seem to be extraordinarily high (diagnostic odds ratio was 42.908 for anti-CCP-3 and was 63.458 for anti-CCP-2), but these assays are restricted by their excessive heterogeneity. We reviewed the quality of the included articles that are of high level in terms of QUADAS rating. However, items 10 and 11 (index test results blinded and reference standard blinded to index test) of all studies except for one article [27] are "unclear". The two antibodies in eight articles [26, 27, 29, 32–34, 37, 38] were not described in detail, so they did not reach item 8 (adequate index test description). There were 10 articles [25, 26, 28–30, 33, 37–39, 41] that did not meet item 2 (selection criteria clearly described). One article [26] did not accord with item 14 as it did not explain withdrawals. Meanwhile, only two articles [27, 40] were prospective studies and had different kinds of reference standard [3, 16–19]. It is reported that various characteristics of the

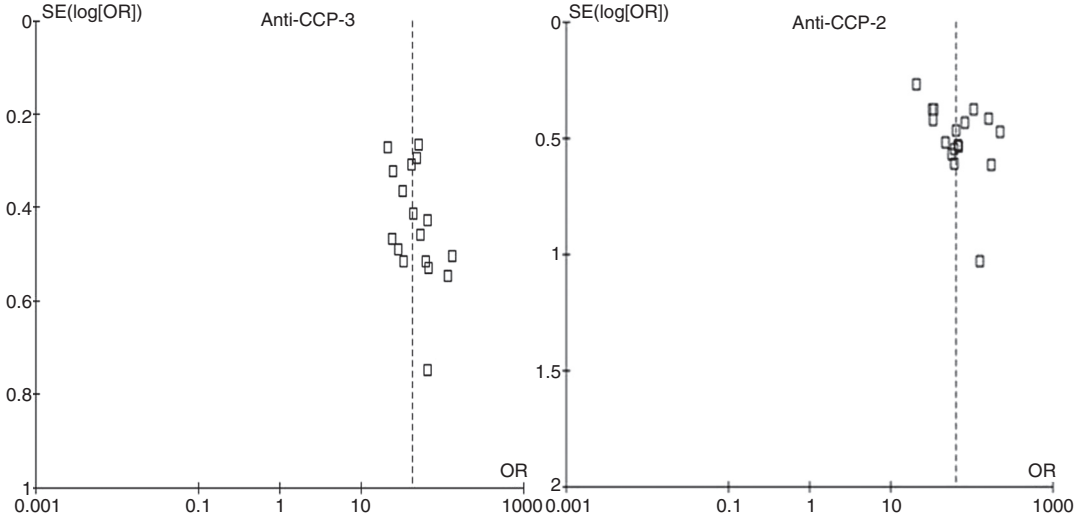

Figure 4 Funnel plots for the assessment of potential publication bias in anti-CCP-3 and anti-CCP-2.
The funnel graph plots the log of the DOR against the standard error of the log of the DOR (an indicator of sample size). Each open box represents each study in the meta-analysis. The line in the center indicates the summary DOR. Anti-CCP-2/3, anti-cyclic citrullinated peptide 2/3 antibody; DOR, diagnostic odds ratio.

control groups play important roles in sensitivity and specificity since the prevalence of anti-citrullinated peptide antibody differs from non-RA rheumatic diseases and from common infections and healthy individuals. These aspects would cause spectrum bias, review bias, selection bias or affect the replication of anti-CCP antibody assays. In this case, the analysis of the accuracy of anti-CCP-2 and anti-CCP-3 would be influenced.

We probed inconsistency through meta-regression and Spearman's correlation coefficients and conducted a stratified analysis step by negative threshold effects but still distinct non-threshold effects in the same race. We have shown that the specificity and sensitivity of anti-CCP-3 and anti-CCP-2 for RA diagnosis depend on a patient's race. Further we found that anti-CCP-3 showed superior diagnostic accuracy for RA than anti-CCP-2 in American patients, but more supportive data is needed for this conclusion due to the fact that only five publications were used for this analysis in the present study. We discovered that there was a significant heterogeneity for sensitivity, specificity, LR+, LR– and diagnostic odds ratio after a stratified analysis of different kinds of assays in anti-CCP-2. Thus, there were additional improvements for both anti-CCP-3 and anti-CCP-2 assays to be more reliable and better diagnostic accuracy.

Even though we tried to minimize bias as much as possible by performing a full-scale search strategy by including articles with high levels of rating and by using data extraction independently, there are still some inevitable limitations. First, scanty reports (only 17 studies included) lead to result bias. Second, we could only integrate the available published results and might miss some important ongoing/unpublished research data and the language capability could only allow us to choose the publications in English and Chinese. All of these reasons might produce publication selection bias. Third, as we abstracted data on varied assays of anti-CCP-2, it may have resulted in information bias.

In conclusion, both anti-CCP-2 and anti-CCP-3 are considerable and novel indicators in the diagnosis of RA with high specificity and moderate sensitivity, and anti-CCP-3 did not have better diagnostic performances than anti-CCP-2, whereas anti-CCP-2 had evident heterogeneity compared to anti-CCP-3, especially in American patients. Only a crucial revolution was happened on optimizing sensitivity and reducing inconsistency, it should be true that they were taken into clinical practice.

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Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Research support played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
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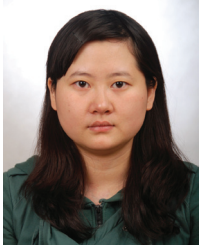
Wen-Chao Zhang is a postgraduate student at Chongqing Medical University. Her major is Laboratory Medicine and Internal Medicine (Neurology). She is currently doing her clinical practice in the Department of Laboratory Medicine, at the Second Affiliated Hospital of Chongqing Medical University and the First Affiliated Hospital of Chongqing Medical University.



Han Wu is a postgraduate of the Chongqing Medical University. Her major is Internal Medicine (neurology). She is currently doing her clinical practice at the First Affiliated Hospital of Chongqing Medical University.

Wei-Xian Chen is the Director of the Department of Laboratory Medicine, the Second Affiliated Hospital of Chongqing Medical University, China. He holds a Medical Doctor Degree and has been a Master student tutor. He is mainly engaged in infection and immunity research, especially hepatitis virus and autoimmunity. He has published more than 30 original and review articles.