Diagnostic accuracy of combined tests of anti cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis: a meta-analysis

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Abstract

Objective

To evaluate the diagnostic properties of combined tests of anti cyclic citrullinated peptide antibody (anti-CCP) and rheumatoid factor (RF) in the diagnosis of rheumatoid arthritis (RA).

Methods

We performed an extensive research between January 2000 and January 2013 of the published literature. A random-effects model was used to summarise data from 24 studies that conformed to our inclusion criteria. Heterogeneity among studies was evaluated by threshold effect analysis and meta-regression.

Results

The summary estimates for anti-CCP antibody and RF positivity (both serum markers had to be positive) in the diagnosis of RA were: sensitivity 57% (95% confidence interval (CI), 55% to 59%), specificity 96% (CI, 96% to 97%), positive likelihood ratio (LR) 13.84 (CI, 10.56 to 18.12), negative LR 0.46 (CI, 0.40 to 0.52), diagnostic odds ratio (DOR) 33.02 (CI, 23.89 to 45.64). The pooled data for anti-CCP antibody or RF positivity (one serum marker had to be positive) were: sensitivity 78% (CI, 76% to 80%), specificity 82% (CI, 81% to 84%), positive LR 4.24 (CI, 3.61 to 4.97), negative LR 0.27 (CI, 0.22 to 0.34), DOR 16.95 (CI, 12.96 to 22.18).

Conclusion

Both anti-CCP antibody and RF positivity are useful for ruling in the diagnosis of RA, and positivity combined improves the probability of true positivity in the diagnosis. Anti-CCP antibody or RF positivity shows low specificity and positive LR, and should be integrated with other examinations to make a final diagnosis.

Key words

rheumatoid arthritis, anti-cyclic citrullinated peptide antibody, rheumatoid factor, meta-analysis

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Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disorder with inflammatory polyarthritis as the cardinal symptom, the major pathogenesis of which has been regarded as the mutual effect between immune system and environmental factors (1). The epidemiology of rheumatoid arthritis reveals that there is increasing comorbidity risk and excess mortality in patients with persistent RA (2, 3). Currently a great deal of work has demonstrated that early treatment of RA can alleviate inflammation, retard morphologic damage to joints, and ultimately improve clinical outcome (4). Therefore, the importance of early diagnosis and treatment has been strongly emphasised (5). However, there is no gold standard or specific test to indentify RA. Clinicians usually make the diagnosis by evaluating laboratory tests, clinical symptoms, and radiological examinations comprehensively (6). They resort to a set of extensively used criteria formulated by the American College of Rheumatology in 1987 (1987 ACR criteria) to classify rheumatoid arthritis (7). However, recent data have indicated that the criteria play a limited role in early detection of RA (8), since several items of the criteria were usually negative during the first few weeks of symptom onset. Consequently, it is not completely effective for indentifying RA in the early course of the disease (9).

In 2010, a new set of criteria for classification of RA was developed by the ACR and EULAR (European League against Rheumatism),which was found to be more sensitive in indentifying recent-onset RA (8). The new criteria increased the weights of small joint involvement and seropositivity in comparison with the previous one (10). Except for rheumatoid factor (RF) which was already used in the 1987 ACR criteria, anti-citrullinated protein antibodies (ACPA) were introduced into the newly developed 2010 ACR/EULAR criteria (11).

ACPA represents a family of antibodies targeting citrullinated forms peptides. The earlier discoveries of ACPA family were anti-perinuclear factor (APF) and anti-keratin antibodies (AKA). However, these two antibodies were not used extensively in the diagnosis of RA due to the subjective estimate of the indirect immunofluorescence pattern and inconsistency of the natural substrate (12). It was not until anti cyclic citrullinated peptide (anti-CCP) antibody was discovered by Schellekens *et al*. that the ACPA family aroused the interest of clinicians (13). Anti-CCP antibody has been used widely and included in research extensively because of its easy testing method and good diagnostic properties (14). Nevertheless, there are different opinions concerning the priority of anti-CCP antibody and rheumatoid factor in the diagnostic accuracy. Several studies contend that anti-CCP antibody could be used alone in the diagnosis of RA because it is more specific than RF, and comparable to RF in sensitivity (15, 16). On the other hand, some articles recommend that combined tests of anti-CCP antibody and RF would be of great help in the early classification of RA (17, 18).

In order to discuss the problem comprehensively, we performed a systematic review on the diagnostic accuracy of combined tests of anti-CCP antibody and RF. We also compared the combined tests results with the summarised data of each antibody, respectively. In addition, misdiagnoses and diagnoses missed were analysed for each single study. In the end, a stratified analysis was performed to investigate the heterogeneity of the results.

Methods

Data sources and searches

We searched MEDLINE for articles published between January 2000 and January 2013. The search strategy consisted of “rheumatoid arthritis”, “anti-CCP antibody”, “rheumatoid factor”, and logical connectives *AND OR*. In order to not omit relevant studies, we also used search terms “RA”, “anti-cyclic citrullinated peptide antibody”, “RF”, “ACPA”, “diagnostic accuracy”, “sensitivity”, “specificity” and so on. Reference lists of included studies were reviewed to obtain relevant information. Foreign language published articles were translated.

Study selection

Studies included in our meta-analysis had to conform to the following criteria:

1. Observational studies without intervention imposed by researchers.
2. Studies that evaluated the diagnostic accuracy of serum anti-CCP antibody and RF in the same participant group.
3. Studies that provided sufficient data to calculate the diagnostic accuracy when both anti-CCP antibody and RF were positive / either anti-CCP antibody or RF was positive.

The diagnostic reference was the 1987 ACR criteria (7) or the 2010 ACR/EULAR criteria (11).

We excluded studies that provided data of anti-CCP antibody/RF alone. In addition, reviews, conference abstracts, letters to editors were also excluded. Two authors evaluated the titles and abstracts independently, then full texts of potential eligible articles were retrieved.

Data extraction and study quality assessment

Two reviewers independently extracted data from primary studies to acquire information on the demographic characteristics of participants and diagnostic values. The demographic information was outlined in a form that contained disease duration, female proportion, and control group composition. We also listed diagnostic values concerning misdiagnoses (false positive) and diagnoses missed (false negative) of each antibody and two antibodies combined. The methodological quality was assessed according to 14 standard items from the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) (19). Disagreements were resolved by discussing them in the whole group or consulting professionals.

Data analysis

We synthesised estimates of sensitivity, specificity, positive likelihood ratio (LR), negative LR, and diagnostic odds ratio (DOR) through a random-effect model (20). In this meta-analysis, the software Mata-Disc (version 1.4) was used to pool the data.

We assessed the diagnostic accuracy of combining anti-CCP antibody and RF through a 4x2 table of test performance. The table was comprised of the number of participants (with or without RA) who were positive for anti-CCP antibody, RF, both, or neither. The pooled sensitivity and specificity were presented in forest plots (21). In order to evaluate the combined test performance comprehensively, a summary receiver operating characteristic (SROC) curve was performed to present the relationship between sensitivity and specificity (22). An area under the curve (AUC) displays the intrinsic property of a test to discriminate diseased participants from the non-diseased. The AUCs of perfect tests generally close to 1 while that of poor tests usually close to 0.5 (23).

Heterogeneity across studies was first investigated through Cochran Q-test. The statistic I^2 was used to quantify the degree of total variation that caused by heterogeneity rather than chance. In general, substantial heterogeneity occurred when $I^2>50\%$ ($p<0.05$) (24). Then we explored the source of heterogeneity through threshold effect analysis and meta-regression. If the threshold effect existed, an inverse correlation between sensitivity and specificity appeared (25). Meta regression was carried out on the basis of the following variables: study design, disease duration, and control group type, which were considered potential sources for heterogeneity. In the end, we performed a stratified analysis to evaluate how these variables influenced heterogeneity.

Publication bias was examined by Deeks test and we used Stata (version 12.0) to explore the potential publication bias in our meta-analysis (26).

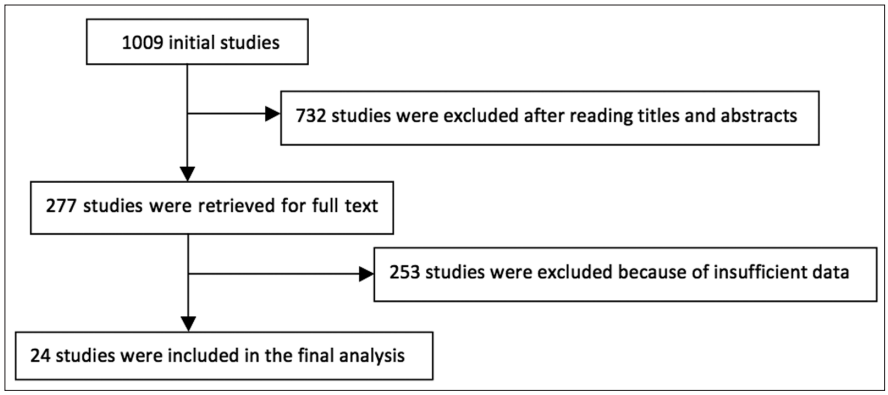

Fig. 1. The flow chart of study selection.

Results

Search results

Our searches indentified 1009 studies, 24 of which conformed to all of our inclusion criteria (Fig. 1); 732 studies were excluded after reading titles and abstracts, because they were clearly irrelevant to the subject under review; 277 full-text articles were retrieved, of which 253 were excluded because they did not report sufficient data on the combined tests results. The remaining 24 studies were eligible for meta-analysis.

Characteristics of studies

Twenty-two studies with a total of 7344 participants reported the diagnostic accuracy when both anti-CCP antibody and RF were positive (hereafter referred to as "anti-CCP and RF"); the mean number of participants was 334. Half of the studies used patients with other rheumatic diseases (ORD) asa control group. No healthy persons were reported as serving in a control group. Twelve studies reported the diagnostic accuracy of early RA (disease duration <2 years) and 10 studies reported data of established RA (disease duration >2 years). Eight studies were cohort studies, 6 were cross-sectional studies and 8 were case controls.

Thirteen studies with 4317 participants reported the diagnostic values of either anti-CCP antibody or RF was positive ("anti-CCP or RF"); the mean number of participants was 332. Seven studies used a variety of patients with other rheumatic diseases and healthy controls (ORD and HC) as the control group. Eight studies included early RA

and 5 studies included established RA. The number of each study design, respectively, was 4, 4, 5 for cohort studies, cross-sectional studies and case controls.

Study quality

Figure 2 displays the proportion of studies that accomplished each QUADAS criterion. 11 studies (45.8%) recruited an appropriate spectrum of patients (defined as patients with both early and established RA). 23 studies used 1987 ACR criteria as reference standard and 1 study used 2010 ACR/EULAR criteria. Both of the two criteria were accepted as eligible reference standards. 96% of the studies avoided partial verification bias and incorporation bias, 100% avoided differential verification bias. Practically, about 80% of the studies did not specifically mention whether the final diagnosis was blinded to the interpretation of index tests and *vice versa*. It was unclear in 71% of the studies that whether any uninterpretable data were reported and in 62% of the studies that any participants withdrew.

Publication bias was not statistical significant in our meta-analysis (data not shown).

Diagnostic accuracy

When both anti-CCP antibody and RF were positive, the included studies showed excellent specificities that varied moderately. The summarised specificity was 96%, ranged 89% to 100% (Chi-square, 67.83, p=0.0000, I^2=69.0%). By contrast, the sensitivity estimates were low and variable. The pooled sensitivity was 57% with a range of 33% to 80%, which presented a considerable heterogeneity (Chi-square, 273.24, p=0.0000, I^2=92.3%). When either anti-CCP or RF was positive, both the estimates of sensitivity and specificity revealed a statistically significant heterogeneity across studies. The summarised sensitivity was 78% with a range of 52% to 90% (Chi-square, 113.21, p=0.0000, I^2=89.4%). The pooled specificity was 82%, ranged 79% to 96% (Chi-square, 62.83, p=0.0000, I^2=80.9%) (Fig. 3). Figure 4 shows the SROC curves for the combined tests. For "anti-CCP and RF", the AUC was 0.90, Q^*=0.83; for "anti-CCP or RF", the AUC was 0.88, Q^*=0.81. The synthesised data suggested that both anti-CCP and RF positivity yielded a higher level of diagnostic accuracy. Table I summarises the pooled results of combined tests and each test respectively. The pooled sensitivity was much lower for "anti-CCP and RF" (57%, CI [55% to 59%]) than for anti-CCP alone (67% CI [65% to 68%]). While the pooled specificity of anti-CCP and RF positivity combined (96%, CI [96% to 97%]) increased slightly over that of anti-CCP (94%, CI [93% to 94%]). In addition, the diagnostic odds ratio (DOR) barely increased (33.0 *vs*. 29.8). However, the summarised positive LR increased markedly from 9.8 (CI, 7.8 to 12.3) for anti-CCP positivity alone to 13.8 (CI, 10.6 to 18.1) for two antibodies positivity combined. When either of the two antibodies was positive, the pooled sensitivity (78%, CI [76% to 80%]) improved over RF positivity alone (71%, CI [69% to 72%]). But the summarised specificity decreased considerably in comparision with that of anti-CCP antibody (82% *vs*. 94%). In addition, the positive LR (4.24, CI [3.61 to 4.97]) and DOR (16.9, CI [12.9 to 22.2]) were also reduced.

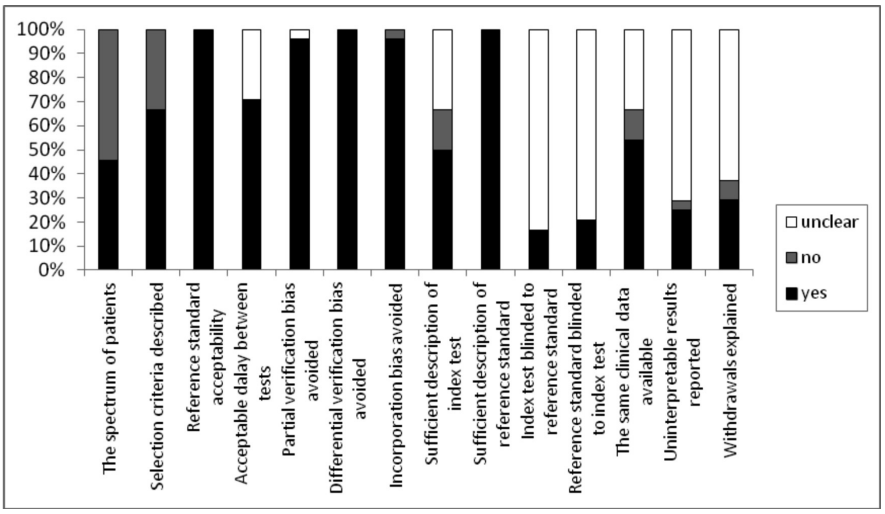

Fig. 2. Study assessment with QUADAS criteria.
Horizontal axis was each QUADAS criterion; vertical axis was the percentage of included studies that fulfilled each QUADAS criterion.

Results of misdiagnosis and diagnosis missed

Table II summarises the data of misdiagnoses (false positive) and diagnoses missed (false negative) for each antibody and two antibodies combined. In general, RF could be more frequently detected in the non-RA group than anti-CCP. When RF was positive in diagnose-waiting people, there was a higher probability of misdiagnosis. False positive of RF usually appeared in Sjögren's syndrome, systemic lupus erythematosus (SLE), systemic sclerosis and healthy elderly people. Anti-CCP antibody was more specific than RF, was only occasionally detected in osteoarthritis, spondylarthropathy, SLE, etc., and sometimes virus infected disease. In addition, the titers of anti-CCP antibody in these non-RA diseases were rather low, usually slightly over cut-off values. For "anti-CCP and RF", misdiagnosis was defined as both two markers being positive in the non-RA group (false positive), which ranged from 0% to 11.2%, and fluctuated around 4%. The false positive of "anti-CCP and RF" was much lower than that of anti-CCP and RF, respectively. The combined tests improved the probability of making a correct RA diagnosis.

The percentage of anti-CCP false negative represented the proportion of RA patients whose anti-CCP tests were negative (miss-diagnosis). There were 10 studies of which miss-diagnosis proportions were between 20% and 40%, 7 studies above 40% and 7 studies below 20% for anti-CCP antibody. This was due to a number of reasons,

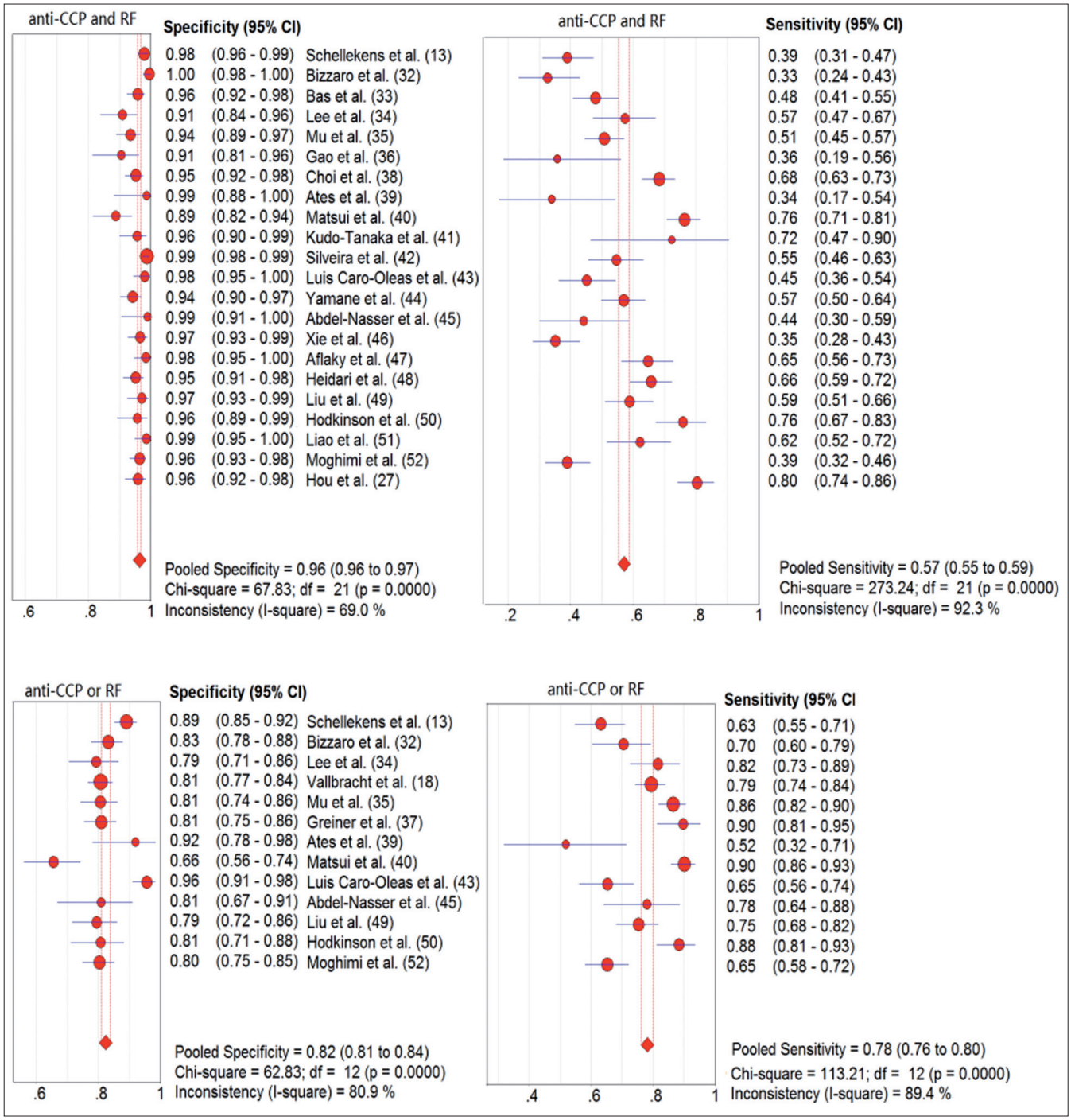

Fig. 3. Forest plots for "anti-CCP and RF", "anti-CCP or RF".
Each solid circle represents for the sensitivity or specificity estimates of each single study. Error bars indicate 95% confidence interval. The pooled sensitivity and specificity estimates are synthesised by random effect model. I square (I^2) is a statistic to quantify the degree of inconsistency. Anti-CCP and RF, both anti-CCP antibody and RF are positive. Anti-CCP or RF, either anti-CCP antibody or RF is positive.

for example, the technique for measuring anti-CCP, selection of RA patients, study design and sample size could result in a miss-diagnosis rate that varied widely. The false negative proportions of RF were smaller/larger than those of anti-CCP in 13/6 studies and more or less comparable to (within 3% difference) the anti-CCP false negative proportions in 5 studies. Six studies had a miss-diagnosis rate for RF over 40% (13, 27, 28, 29, 30, 31), and all of the 6 studies included only early RA patients (disease duration <2 years). Studies which included established RA patients (disease duration >2 years) generally had a miss-diagnosis rate of about 20% (32, 33, 34, 35, 36, 37). RA patients who were both anti-CCP and RF negative constituted "anti-CCP or RF" false negative results. The situation that both two antibodies were negative was relatively rare among patients who really developed into RA.

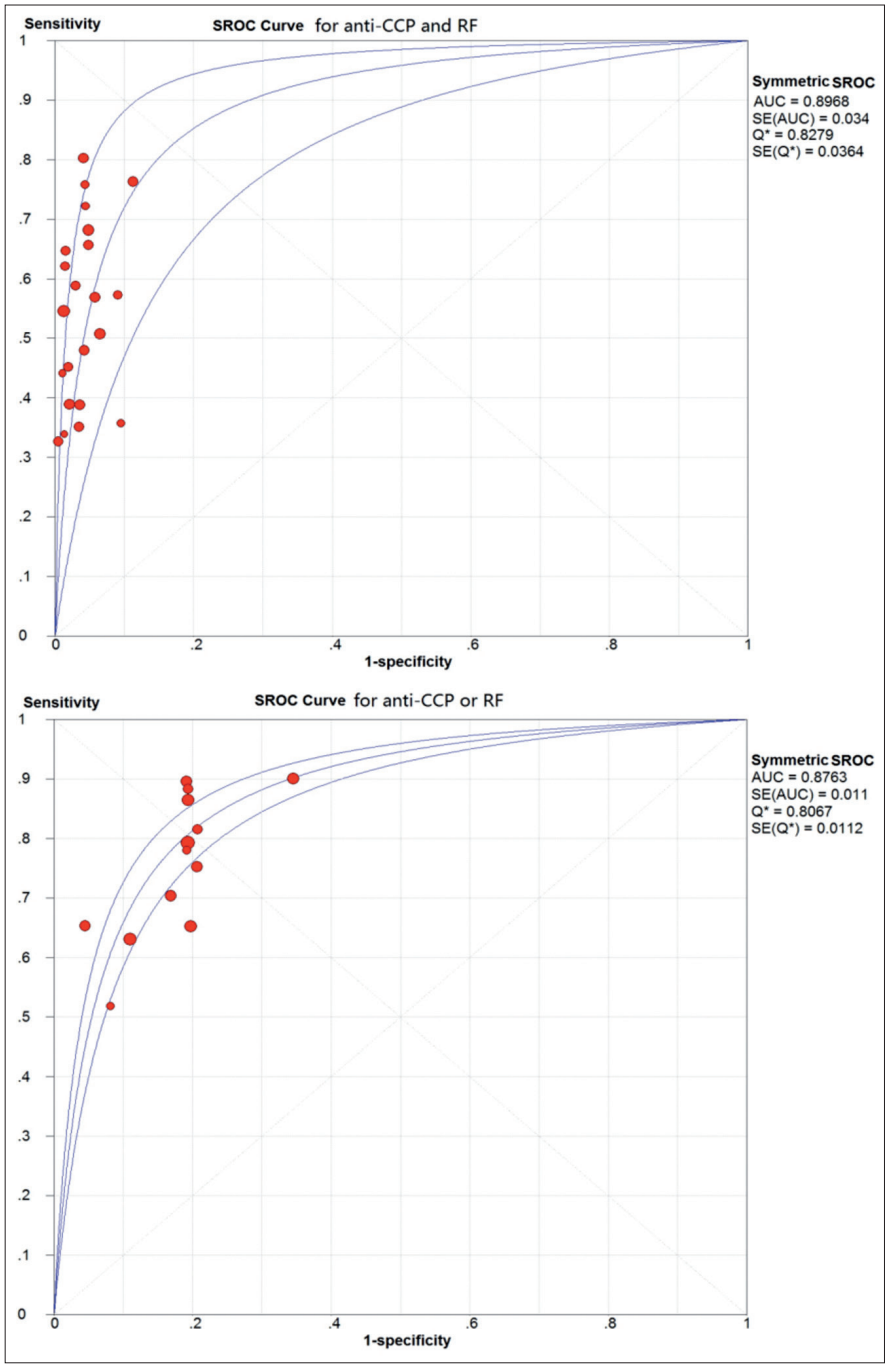

Fig. 4. Summary receiver operating characteristic curve for "anti-CCP and RF", "anti-CCP or RF" Each solid circle represents each single study in the meta-analysis. The size of solid circle indicates the sample size of each study. AUC, the area under the curve.

Results of meta-regression

The Spearman correlation coefficients were 0.415 for "anti-CCP and RF" and 0.575 for "anti-CCP or RF", respectively, indicating that heterogeneity was not caused by threshold effect. Meta-regression was then performed on the basis of composite variables, "study design", "disease duration" and "control group type" (Table III).

When both anti-CCP and RF were positive, the summary sensitivity of case control studies (67%, [CI 65% to 69%]) was much higher than that of cohort studies (54%, [CI 51% to 57%]) and cross-sectional studies (40%, [CI 36% to 43%]). Pooled specificity was comparable across the three study designs. The case control group had a higher AUC (0.90 Q*=0.83) than cohort studies did (0.84, Q*=0.77). In the group classified by disease duration, specificity of early RA and established RA were identical (96% *vs*. 96%), but sensitivity was considerably higher for the established RA group (60%, [CI 58% to 63%]) than for the early RA group (53%, [CI 50% to 56%]). However, the early RA group displayed a better trade-off relationship between sensitivity and specificity, the AUC of which (0.95, Q*=0.895) was much better than that of established RA (0.84, Q*=0.77). Across the strata of control group type, the two groups remained the same in sensitivity and specificity and showed no major difference in positive LR or negative LR. While the AUC of the ORD and HC group (0.92, Q*=0.86) was higher than that of the ORD group (0.87, Q*=0.80).

For "Anti-CCP or RF", case control group had the largest pooled sensitivity (86%, [CI 84% to 88%]) but the smallest specificity (78%, [CI 75% to 81%]). When specificities were pooled in cross-sectional studies, I^2 was 0.00% (p=0.8457>0.05). Although the diagnostic odds ratios (DOR) of different study designs varied considerably, the internal of each study design presented good homogeneity. The DOR was, respectively, 18.3 (p=0.1655>0.05), 11.8 (p=0.0809>0.05), 21.9 (p=0.0635 >0.05) for cohort study, cross-sectional study and case control group. These results suggested that stratified analysis decreased the degree of heterogeneity to some extent. In the second sub-group, early RA had greater pooled specificity (85%, [CI 83% to 87%]) but smaller sensitivity (72%, [CI 69% to 75%]). The DORs (17.4 *vs*. 17.2) /AUCs (0.88 *vs*. 0.87) for early RA and established RA were comparable. Across the strata of control group type, all of the pooled diagnostic values for ORD and HC group were slightly better than those for ORD group, indicating that diversity of control group could yield a higher level of diagnostic accuracy.

Discussion

We have identified several reviews that did not address past relevant studies quantitatively (50, 51). Basically, they admitted the superior diagnostic

Table I. Diagnostic accuracy of combined tests and each test.

Tests	Study n	Sen% (CI %)	Spe % (CI %)	Positive LR(CI %)	Negative LR(CI %)	DOR (CI %)	AUC
Anti-CCP	24	67 (65-68)	94 (93-94)	9.8 (7.8-12.3)	0.35 (0.30-0.41)	29.8 (22.5-39.5)	0.91
RF	24	71 (69-72)	83 (82-84)	3.96 (3.43-4.59)	0.37 (0.32-0.42)	11.7 (9.5-14.3)	0.84
Anti-CCP and RF	22	57 (55-59)	96 (96-97)	13.8 (10.6-18.1)	0.46 (0.40-0.52)	33.0 (23.9-45.6)	0.90
Anti-CCP or RF	13	78 (76-80)	82 (81-84)	4.24 (3.61-4.97)	0.27 (0.22-0.34)	16.9 (12.9-22.2)	0.88

RA: rheumatoid arthritis; Anti-CCP: anti-cyclic citrullinated peptide antibody; RF: rheumatoid factor; "anti-CCP and RF": both anti-CCP antibody and RF were positive; "anti-CCP or RF": either anti-CCP antibody or RF was positive; CI: confidence interval; Sen: sensitivity; Spe: specificity; LR: likelihood ratio; DOR: diagnostic odds ratio; AUC: area under the curve (summary receiver operating characteristic curve).

performance of anti-CCP antibody but few of them compare it with rheumatoid factor systematically. Nishimura K *et al*. (16) have conducted a meta analysis to compare anti-CCP antibody with RF on the diagnostic accuracy of RA. However, the number of inluded studies was limited and the combination test accuracy was not assessed by stratified analysis. Our meta-analysis conducted extensive searches and were less likely to be affected by missing relevant studies.

In general, positivity of both anti-CCP and RF tests can rule in (positive LR 13.8) the diagnosis of RA, but they became less sensitive in detecting RA patients. The sensitivity was reduced

Table II. Characteristics of false positive and false negative.

Author, year (ref.)	Anti-CCP false positive (n/NRA) (%)	RF false positive (n/NRA) (%)	"Anti-CCP and RF" false positive (n/NRA) (%)	Anti-CCP false negative (n/RA) (%)	RF false negative (n/RA) (%)	"Anti-CCP or RF" false negative (n/RA) (%)
Schellekens *et al*., 2000 (13)	14/312 (4.5%) UA (n=10), palindromic rheumatism (n=2), sarcoidosis (n=1), pseudogout (n=1)	28/312 (8.9%) UA (n=14), others (n=14) [JRA, PsA, septic arthritis, crystal arthropathy, osteoarthritis, sarcoidosis]).	6/312 (1.9%) Detail not reported	77/149 (51.7%)	69/149 (46.3%)	55/149 (36.9%)
Bizzaro *et al*., 2001 (27)	5/232 (2.2%) HCV-infected (n=1), Lyme disease (n=3), autoimmune thyroid disease (n=1)	36/232 (15.5%) Detail not reported	1/232 (0.43%) Detail not reported	58/98 (59.2%)	37/98 (37.8%)	32/98 (32.7%)
Bas *et al*., 2002 (28)	24/196 (12.2%) Matched healthy donors (n=2), healthy elderly donors (n=0), data of disease control group were not reported	43/196 (21.9%) Matched healthy donors (n=12), healthy elderly donors (n=5), data of disease control group not reported.	10/196 (5.1%) Healthy blood donor (n=0), data of disease control group not reported.	86/239 (36.0%)	53/239 (22.2%)	not reported
Lee *et al*., 2003 (29)	14/146 (9.6%) Inflammatory arthritis(n=4), PsA (n=2), SLE (n=1), fibromyalgia (n=1), JRA (n=6)	22/146 (15.1%) Detail not reported.	10/146 (6.8%) Detail not reported.	35/103 (34.0%)	29/103 (28.2%)	19/103 (18.4%)
Vallbracht *et al*., 2004 (30)	12/420(2.9%) Inflammatory joint diseases (n=4), CTD (n=7), HC (n=1).	75/420 (17.9%) Inflammatory joint diseases (n=20), CTD (n=44), HC (n=11).	Data not reported.	105/295 (35.6%)	99/295 (33.6%)	61/295 (20.7%)
Mu *et al*., 2005 (31)	14/186 (7.5%) Detail was not reported.	34/186 (18.3%) Detail not reported.	12/186 (6.5%) Detail not reported.	101/266 (38.0%)	76/266 (28.6%)	36/266 (13.5%)
Gao *et al*., 2005 (32)	10/74 (13.5%) Detail was not reported.	16/74 (21.6%) Detail not reported.	7/74 (9.5%) Detail not reported.	16/28 (57.1%)	15/28 (53.6%)	not reported
Greiner *et al*., 2005 (33)	5/246 (2.0%) Including osteoarthritis, SpA, SLE	44/246 (17.9%) Detail not reported	Data not reported.	17/87 (19.5%)	12/87 (13.8%)	9/87 (10.3%)
Choi *et al*., 2005 (34)	20/251 (8.0%) BD (n=1), fibromyalgia (n=1), gout (n=2), JRA (n=6), osteoarthritis (n=6), SLE (n=1), SpA (n=2), reactive arthritis (n=1).	54/251 (21.5%) Detail not reported	12/251 (4.8%) Detail not reported	88/324 (27.2%)	63/324 (19.4%)	not reported

Table II. *continued*

Author, year (ref.)	Anti-CCP false positive (n/NRA) (%)	RF false positive (n/NRA) (%)	"Anti-CCP and RF" false positive (n/NRA) (%)	Anti-CCP false negative (n/RA) (%)	RF false negative (n/RA) (%)	"Anti-CCP or RF" false negative (n/RA) (%)
Ates *et al*., 2006 (35)	2/55 (3.6%) UA (n=1), vasculitis (n=1)	6/55 (10.9%) UA (n=2), vasculitis (n=4).	0/55 (0%) Detail not reported	15/27 (55.6%)	16/27 (59.3%)	13/27 (48.1%)
Matsui *et al*., 2006 (36)	20/116 (17.2%) SLE (n=4), CTD (n=2), SSc (n=4), pSS (n=3), PM/DM (n=5), vasculitis (n=2)	34/116 (29.3%) Detail not reported	13/116 (11.2%) Detail not reported	46/262 (17.6%)	42/262 (16.0%)	26/262 (9.9%)
Kudo-Tanaka *et al*., 2007 (37)	8/114 (7.0%)	27/114 (23.7%)	5/114 (4.4%)	3/18	4/18	not reported
Silveira *et al*., 2007 (38)	22/636 (3.5%)	65/636 (10.2%)	8/636 (1.3%)	50/132 (37.9%)	48/132 (36.4%)	not reported
Luis Caro-Oleas *et al*., 2007 (39)	3/158 (1.9%) UA (n=3), HC (n=0)	7/158 (4.4%) Detail not reported	3/158 (1.9%) UA (n=3), HC (n=0)	56/124 (45.2%)	54/124 (43.5%)	43/124 (34.7%)
Yamane *et al*., 2008 (40)	21/226 (9.3%) Other rheumatic disease (n=7), non-rheumatic disease (n=13), HC (n=1)	69/226 (25.9%) Detail not reported	13/226 (4.9%) Detail not reported	72/209 (34.4%)	65/209 (31.1%)	not reported
Abdel-Nasser *et al*., 2008 (41)	4/47 (8.5%) SLE (n=1), PsA (n=2), AS (n=1).	5/47 (10.6%) SLE (n=2), osteoarthritis (n=2), HC (n=1).	0/47	15/50 (30%)	24/50 (48%)	11/50 (22%)
Xie *et al*., 2009 (42)	10/176 (5.7%) pSS (n=4), PM/DM (n=2), SpA (n=2), HC (n=2)	36/176 (20.5%) SLE (n=8), osteoarthritis (n=4), SSc (n=2), pSS (n=8), PM/DM (n=6), HC (n=8)	6/176 (3.4%) Detail not reported	86/168 (51.2%)	36/168 (21.4%)	not reported
Aflaky *et al*., 2009 (43)	12/131 (9.2%) SLE (n=4), scleroderma (n=1), JRA (n=1), overlap disease (n=5), osteoarthritis (n=1).	18/131 (13.7%) SLE (n=5), scleroderma (n=4), SpA (n=1), osteoarthritis (n=1), vasculitis (n=2), CIA (n=1), overlap disease (n=2)	2/131 (1.5%) Detail not reported	24/139 (17.3%)	42/139 (30.2%)	not reported
Heidari *et al*., 2009 (44)	26/208 (12.5%)	28/208 (13.5%)	10/208 (4.8%)	37/201 (18.4%)	49/201 (24.4%)	not reported
Liu *et al*., 2009 (45)	5/136 (3.7%) SLE (n=2), pSS (n=2), SSc (n=1).	27/136 (19.9%) SLE (n=5), pSS (n=6), SSc (n=3), AS (n=2), HC (n=10), HBV (n=1).	4/136 (2.9%) Detail not reported	65/170 (38.2%)	47/170 (27.6%)	42/170 (24.7%)
Hodkinson *et al*., 2010 (46)	14/93 (15.1%) SSc (n=2), SLE (n=11), HC (n=1).	9/93 (9.7%) Detail was not reported	4/93 (4.3%) Detail not reported	21/120 (17.5%)	2/120 (18.3%)	14/120 (11.7%)
Liao *et al*., 2011 (47)	5/140 (3.6%) SLE (n=3), gout (n=1), PsA (n=1).	22/140 (15.7%) SLE (n=9), gout (n=4), CTD (n=5), AS (n=1), osteoarthritis (n=2), PsA (n=1).	2/140 (1.4%) Detail not reported	20/95 (21.1%)	31/95 (32.6%)	not reported
Moghimi *et al*., 2012 (48)	18/254 (7.1%)	41/254 (16.1%)	9/254(3.5%)	102/193 (52.8%)	83/193 (43%)	67/193 (34.7%)
Hou *et al*., 2012 (49)	10/172 (5.8%) SLE (n=3), pSS (n=3), myositis (n=2), CTD (n=2).	51/172 (29.7%) Detail not reported	7/172 (4.1%) Detail not reported	38/198 (19.2%)	21/198 (10.6%)	not reported

n: number; RA: rheumatoid arthritis; NRA: non-rheumatoid arthritis; anti-CCP: anti-cyclic citrullinated peptide antibody; RF: rheumatoid factor; "anti-CCP and RF": both anti-CCP antibody and RF were positive; "anti-CCP or RF": either anti-CCP antibody or RF was positive; UA: undifferentiated arthritis; PsA: psoriatic arthritis; HC/BV: hepatitis C/B virus; SLE: systemic lupus erythematosus; JRA: juvenile rheumatoid arthritis; CTD: connective tissue disease; HC: healthy controls; SpA: spondylarthropathy; BD: Behçet's disease; SSc: systemic sclerosis; PM/DM: polymyositis/dermatomyositis; pSS: primary Sjögren's syndrome; AS: ankylosing spondylitis; CIA: crystal induced arthritis.

Table III. Results of subgroup analysis.

Grouping	Study number	Sensitivity% (I^2)	Specificity% (I^2)	Positive LR (I^2)	Negative LR (I^2)	DOR (I^2)	AUC
			Anti-CCP and RF				
Study design							
Cohort	8	54 (81.1%)	97 (74.2%)	15.5 (64.1%)	0.49 (79.8%)	33.9 (53.5%)*	0.84
Cross-sectional	6	40 (48.5%)*	97 (70.6%)	10.7 (55.5%)*	0.63 (39.7%)*	17.7 (47.9%)*	0.53
Case control	8	67 (89.9%)	95 (63.0%)	14.8 (60.8%)	0.34 (89.6%)	46.9 (66.3%)	0.90
Disease duration							
Early RA	12	53 (93.1%)	96 (24.3%)*	13.3 (30.0%)*	0.49 (91.3%)	30.5 (59.5%)	0.95
Established RA	10	60 (90.8%)	96 (83.1%)	14.8 (73.9%)	0.43 (90.4%)	36.1 (68.4%)	0.84
Control group type							
ORD and HC	11	57 (93.2%)	96 (56.4%)	14.7 (33.8%)*	0.44 (93.0%)	39.1 (59.3%)	0.92
ORD	11	57 (92.1%)	96 (77.7%)	12.7 (70.9%)	0.48 (90.5%)	28.4 (67.2%)	0.87
			Anti-CCP or RF				
Study design							
Cohort	4	68 (79.5%)	89 (83.2%)	6.32 (79.3%)	0.37 (65.6%)	18.3 (41.0%)*	0.86
Cross-sectional	4	74 (76.4%)	81 (0.00%)*	3.89 (0.00%)*	0.33 (75.8%)	11.8 (55.5%)*	0.88
Case control	5	86 (79.3%)	78 (66.6%)	3.87 (68.4%)	0.18 (77.1%)	21.9 (55.1%)*	0.89
Disease duration							
Early RA	8	72 (71.1%)	85 (80.8%)	4.75 (64.0%)	0.31 (80.7%)	17.4 (71.7%)	0.88
Established RA	5	83 (84.2%)	79 (72.9%)	3.77 (61.7%)	0.22 (76.1%)	17.2 (16.7%)*	0.87
Control group type							
ORD and HC	7	79 (82.8%)	83 (78.3%)	4.48 (51.9%)*	0.26 (76.1%)	18.7 (54.3%)*	0.88
ORD	6	77 (93.5%)	82 (85.6%)	3.97 (73.3%)	0.29 (89.0%)	15.1 (65.6%)	0.87

LR: likelihood ratio; DOR: diagnostic odds ratio; AUC: area under the curve; ORD: other rheumatic disease; HC: healthy control.

(57% *vs*. 71%) because positivity for both anti-CCP antibody and RF could be a more stringent criterion than positivity for each antibody alone. The pooled specificity of "anti-CCP and RF" was not markedly better than that of anti-CCP (96% *vs*. 94%). This available direct evidence indicated that anti-CCP antibody and RF positivity combined provided limited benefit on screening non-RA patients in comparison with anti-CCP positivity. Put another way, if the purpose is to exclude the possibility of getting RA, anti-CCP test alone is almost as effective as anti-CCP and RF tests combined. However, this does not mean that measuring RF has no value. In fact, adding RF detection upon anti-CCP antibody test could provide additional benefit. The markedly increased positive LR (13.8 *vs*. 9.8) indicated that positivity of RF and anti-CCP combined improved the probability of true positivity in the diagnosis of RA. Which means that patients being positive for both two antibodies are more likely to develop into RA. In practice, if the purpose is to determine whether a clinically suspected patient does suffer from RA, both anti-CCP and RF tests are recommeded.

"Anti-CCP or RF" included three types of combination, "anti-CCP positive and RF negative" "anti-CCP negative and RF positive" "anti-CCP positive and RF positive". We did not synthesize the diagnositc accuracy of the first two types of combination because of insufficent data. On the whole, the sensitivity for "anti-CCP or RF" was much higher than that for "anti-CCP and RF" because two antibodies complemented each other with false negative results. However, the significantly reduced positive LR (4.24) suggested that anti-CCP or RF positivity could not rule in the diagnosis of RA. Either anti-CCP or RF positivity should be incorporated with other examinations to make a final diagnosis.

In any case, comparison between measuring two antibodies with measuring one involves a balance between sensitivity and specificity. Anti-CCP antibody has a better specificity than RF, which indicates that it is less likely to be detected in non-RA patients. Unlike RF which usually appears in healthy elderly people, anti-CCP antibody is barely detected in healthy controls. However, the detection of anti-CCP is less sensitive than that of RF, the reason for which is diverse. In-house ELISA instead of the commercially available ELISA kit, can decrease the detection rate of anti-CCP due to its limited skill of coating plates and reagents prepartion. Small sample analysis is not as representative as large sample, so the results of which are usually outside confidence interval. Selecting established or early RA patients influences the sensitivity directly. In general, studies enrolling established RA patients have a higher sensitivity than those with only early RA patients, especially for RF. Sometimes, RF is negative during the early course of disease, reducing the sensitivity for early RA patients.

The substantial heterogeneity among the results of 24 included studies was addressed by stratified analysis. The heterogeneity was not significant when the statistic was I^2<50% (p>0.05). Our stratified analysis partially reduced the percentage of total heterogeneity which can be seen from decreased I^2 when the diagnostic values were pooled in each subgroup. In general, case control studies overestimated sensitity both when two antibodies were positive or either one was positive. Evidences

have pointed out that such study design could overrate test performace in clinical practice (52). In a different stage of RA, the summarised sensitivity was markedly higher in long-term RA than in early RA. However, the sensitivty and specificity displayed a better trade-off in early stage of the disease, especially for anti-CCP and RF positivity combined. When the control group was comprised of both healthy people and patients with other rheumatic diseases, the overall diagnostic accuracy was better. In order to improve the quality of reporting diagnostic test accuracy, future studies should avoid carrying out case control studies. In addition, we recommend enrolling suspected RA patients and adding healthy people to the control group to improve the quality of the study.

The ACR and EULAR issued new criteria for classification of RA in 2010, which recruited the detection of anti-CCP antibody. According to the criteria, the weights for anti-CCP antibody and RF varied according to the titers (10). However, few studies have reported sufficient information concerning the relationship between antibody titers and the diagnostic accuracy. Further investigation is needed on this issue. Another worthwhile issue is to explore the potential for anti-CCP antibody and RF to predict the development of RA even before symptom onset, which can provide additional help in treating RA timely and effectively.

In conclusion, positivity for anti-CCP and RF combined are useful for establishing the diagnosis of RA, especially in early stage of the disease. The combined positivity maximises the probability of true positivity in classifying RA. Because of the decreased diagnostic accuracy, positivity for anti-CCP antibody or RF should be incorporated with other examinations to make a final diagnosis.

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