JRD
Journal of Rheumatic Diseases Vol. 23, No. 6, December, 2016
https://doi.org/10.4078/jrd.2016.23.6.373

Original Article

Diagnostic Accuracies of Anti-carbamylated and Anti-citrullinated Fibrinogen Antibodies in Rheumatoid Arthritis: A Meta-analysis

Young Ho Lee

Division of Rheumatology, Department of Internal Medicine, Korea University Medical Center, Korea University College of Medicine, Seoul, Korea

Objectives. This study evaluated the diagnostic performance of anti-carbamylated protein (anti-CarP) and anti-citrullinated fibrinogen (ACF) antibodies in rheumatoid arthritis (RA). **Methods.** We searched the Pubmed, Embase, and Cochrane library databases, and performed two meta-analyses on the diagnostic accuracy of anti-CarP and ACF antibodies in patients with RA. **Results.** The meta-analysis included data from 12 studies. Of these, seven studies, which included 1,749 patients and 1,044 controls, examined anti-CarP antibody, and five studies, which included 733 patients and 1,178 controls, examined ACF antibody. The pooled sensitivities and specificities of anti-CarP antibody were 43.9% (95% confidence interval [CI], 41.6～46.3) and 94.5% (95% CI, 93.0～95.8), respectively, and those of ACF antibody were 68.3% (95% CI, 64.9～71.6) and 95.8% (95% CI, 94.5～96.9), respectively. The positive likelihood ratio (PLR) of anti-CarP antibody were 9.901 (95% CI, 5.005～19.58), negative likelihood ratio (NLR) was 0.597 (95% CI, 0.541～0.658), and diagnostic odds ratio (DOR) was 14.64 (95% CI, 8.004～34.45). For ACF antibody, PLR was 16.14 (95% CI, 10.23～25.42), NLR was 0.292 (95% CI, 0.192～0.444), and DOR was 58.61 (95% CI, 26.61～129.1). There were no significant difference in sensitivity, specificity, PLR, NLR, AUC, or Q* index between ACF and anti-cyclic citrullinated peptide (anti-CCP) in the diagnosis of RA. **Conclusion.** Our meta-analysis demonstrates that both anti-CarP and ACF antibodies are highly specific for diagnosing RA. However, while ACF and anti-CCP showed comparably high diagnostic accuracy, anti-CarP antibody showed low sensitivity in diagnosing RA. **(J Rheum Dis 2016;23:373-381)**

Key Words. Arthritis, Rheumatoid arthritis, Anti-carbamylated protein antibody, Anti-citrullinated fibrinogen antibody, Diagnostic accuracy

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of synovial joints that leads to disability and loss of quality of life [1]. Early diagnosis of RA is important to prevent joint damage and to improve prognosis by initiating treatment early [2].

Although American College of Rheumatology (ACR) criteria recommend the detection of rheumatoid factor (RF) in serological tests used to diagnose RA, these tests are nonspecific, because RF may also be present in healthy individuals or in patients with other autoimmune diseases [3]. The most specific RA autoantibodies are those that target citrullinated antigens [4], with anti-cyclic citrullinated peptide (anti-CCP) antibody being highly specific. However, several new antibodies have been studied in RA, such as those of citrullinated fibrinogen and carbamylated protein [5].

Carbamylation is a posttranslational modification similar to citrullination that is mediated by cyanate, which modifies lysine residues [6]. The level of cyanate in-

Received : October 24, 2016, **Revised :** November 10, 2016, **Accepted :** November 23, 2016

Corresponding to : Young Ho Lee, Division of Rheumatology, Department of Internal Medicine, Korea University Medical Center, Korea University College of Medicine, 73 Inchon-ro, Seongbuk-gu, Seoul 02841, Korea. E-mail : lyhcgh@korea.ac.kr

pISSN: 2093-940X, eISSN: 2233-4718

creases with smoking and inflammation, and is increased in RA [7]. Anti-carbamylated protein (anti-CarP) antibodies, like anti-CCP antibodies, have been observed in patients before the onset of clinical RA symptoms and have been associated with the development of RA [8]. Citrullinated fibrin (for which fibrinogen is the soluble precursor) is abundant in inflamed RA synovium, and it is a major target in RA synovial tissue [9]. Thus, the presence of citrullinated fibrinogen in the synovial membranes of RA patients has led to its use in assays of serum antibodies against deiminated fibrinogen [9]. Anti-citrullinated fibrinogen (ACF) antibodies have been detected in the sera of RA patients, and ACF has been compared to anti-CCP with respect to diagnostic accuracy in RA [10-13]. However, it remains uncertain whether the overall diagnostic values of ACF antibodies are comparable to anti-CCP antibodies.

Until now, the exact clinical significance of anti-CarP and ACF in the diagnosis of RA has remained unclear. Studies assessing the diagnostic value of these antibodies in RA have provided inconsistent results [10-20]. This may be because of small sample sizes, low statistical power, and/or clinical heterogeneity in studies. In order to overcome the limitations of individual studies [21-23], we performed a meta-analysis to assess the diagnostic accuracies of anti-CarP and ACF antibodies, and to compare the diagnostic values of ACF antibody with those of anti-CCP antibody in RA.

MATERIALS AND METHODS

Identification of eligible studies and extraction of data

We utilized the PubMed, Embase, and Cochrane library databases to identify articles published up to March 2016 that presented the diagnostic accuracies of anti-CarP and ACF antibodies in patients with RA. In addition, all references mentioned in these reports were reviewed to identify articles not indexed in the electronic databases. The following keywords and subject terms were used: "anti-carbamylated," "citrullinated fibrinogen," "ACF," "sensitivity," "specificity," "rheumatoid arthritis," and "RA." Studies were included in the meta-analysis if they (1) examined the diagnostic accuracy of anti-CarP and ACF antibody; (2) included sufficient data to calculate the sensitivity and specificity of anti-CarP and ACF in patients with RA; and (3) included patients with RA based on diagnostic criteria, healthy controls, and patients with non-RA rheumatic diseases. No language restriction was applied. Studies were excluded if they (1) included overlapping data or (2) did not include healthy or diseased controls. Data were extracted from the methods and results of the original studies by two independent reviewers. Discrepancies between the reviewers were resolved by consensus. The meta-analysis was conducted in accordance with the guidelines presented in the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement [24]. Information on author, publication year, demographic characteristics of participants, and sensitivity and specificity of Anti-CarP and ACF antibodies was extracted from each report. In addition, raw data on anti-CarP and ACF were extracted from primary reports to complete four cells (true positive, false positive, true negative, and false negatives values) in a diagnostic 2×2 table. The quality of each study included in the meta-analysis was assessed using Quality Assessment of Diagnostic Accuracy Studies (QUADAS) criteria [25].

Evaluation of statistical associations

Within- and between-study variation and heterogeneities were assessed using Cochran's Q statistic. This test assessed the null hypothesis that all studies evaluated the same effect. The effect of heterogeneity was quantified using I^2 with a range between 0% and 100%, representing the proportion of between-study variability attributable to heterogeneity rather than to chance [26]. I^2 values of 25%, 50%, and 75% were nominally assigned as low, moderate, and high, respectively. A fixed-effects model assumes that a genetic factor has a similar effect on disease susceptibility across all studies investigated and that the observed variation among studies are caused by chance alone [27]. A random-effects model assumes that different studies show substantial diversity and assesses both within-study sampling error and between-study variance [28]. When study groups are homogeneous, the two models are similar. However, when study groups lack homogeneity, the random-effects model provides wider confidence intervals (CIs) than the fixed-effects model. The random-effects model is the most appropriate when there is significant between-study heterogeneity [28]. In the present study, we used a random-effects model to combine estimates of the sensitivity, specificity, positive and negative likelihood ratios (PLR and NLR, respectively), and diagnostic odds ratio (DOR) and analyzed summary receiver operating characteristic (SROC) curves. DOR is a unitary measure of diagnostic performance that in-

cludes both sensitivity and specificity or both PLR and NLR that is regarded as a suitable global measure of accuracy used to compare the overall diagnostic accuracies of different tests [29]. Because sensitivity and specificity are interdependent variables, their independent calculation may result in an underestimation of these values. SROC curve analysis is more appropriate because it accounts for this mutual dependence. Area under the curve (AUC) (in this case, area under the SROC curve) provides an overall summary of test performance and shows the trade-off between sensitivity and specificity. An AUC value of 1.0 (100%) indicates perfect discriminatory ability of a diagnostic test [30]. The Q* index is another useful global estimate of test accuracy used to compare SROC curves. It is defined as the point at which sensitivity is equal to specificity on an SROC curve; moreover, it is the point on an SROC curve that is intersected by an anti-diagonal. A Q* index value of 1.0 indicates 100% accuracy (i.e., sensitivity and specificity of 100%) [30]. In the present meta-analysis, statistical manipulations were performed using Meta-DiSc version 1.4 (Hospital Universitario Ramon y Cajal, Madrid, Spain) [31].

Evaluation of heterogeneity and meta-regression

Between-study heterogeneity observed in a meta-analysis indicates variability in results across studies. A threshold effect is the most important cause of heterogeneity. Different sensitivities and specificities resulting from various study conditions lead to different threshold effects. We used the Spearman correlation coefficient between the logit of sensitivity and the logit of 1-specificity to detect the threshold effect [31]. In addition, a meta-regression analysis was performed to determine the possible source of heterogeneity in the meta-analysis using the following covariates: (i) study quality, (ii) sample size, (iii) type of control group, and (iv) ethnicities of participants.

RESULTS

Studies included in the meta-analysis

We identified 311 studies through electronic and manual searching. Of these, reports of 24 studies were selected for full-text review based on their titles and abstracts. Twelve of the 24 studies were excluded because they had duplicate or insufficient data on diagnostic accuracy. Thus, 12 studies that assessed the diagnostic accuracy of anti-CarP and ACF antibodies were included in our meta-analysis [10-20]. Of these, 7 studies, which included 1,749 patients and 1,044 controls, evaluated the diagnostic accuracy of anti-CarP antibody [14-19], 5 studies, which included 733 patients and 1,178 controls, evaluated the diagnostic accuracy of ACF antibody [10-13,20], and 4 of 5 studies on ACF antibody, which included 590 patients and 1,070 controls, evaluated the diagnostic accuracy of both anti-CCP and ACF antibodies [10-13]. Table 1 shows the characteristics of participants in the studies included in the meta-analysis and the assessments of diagnostic accuracy reported. Eleven studies had

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

Study	Country	Region	Number		Control type	Antibody	Antibody		Study quality†
			RA	Control			Sensitivity*	Specificity*	
Koppejan et al., 2016 [14]	Canada	Europe	95	85	HC	Ant-CarP	0.452	0.953	11
Shi et al., 2015 [8]	Netherlands	Europe	969	305	HC	Ant-CarP	0.440	0.889	11
Alessandri et al., 2015 [15]	Italy	Europe	63	56	NRA	Ant-CarP	0.460	0.982	10
Verheul et al., 2015 [16]	Japan	Asia	268	324	NRA	Ant-CarP	0.451	0.919	9
Janssen et al., 2015 [17]	Netherlands	Europe	86	36	NRA	Ant-CarP	0.558	1.000	10
Brink et al., 2015 [18]	Sweden	Europe	192	197	NRA	Ant-CarP	0.422	0.970	10
Gan et al., 2015 [19]	United States	America	76	41	NRA	Ant-CarP	0.263	0.951	10
Cornillet et al., 2014 [10]	France	Europe	181	436	NRA	ACF	0.834	0.950	10
Zhao et al., 2008 [20]	China	Asia	183	108	HC	ACF	0.672	0.981	11
Cruyssen et al., 2008 [11]	France	Europe	86	450	NRA	ACF	0.663	0.971	10
Hill et al., 2006 [12]	Canada	America	65	63	NRA	ACF	0.815	0.952	10
Nielen et al., 2005 [13]	Netherlands	Europe	258	121	NRA	ACF	0.558	0.926	10

RA: rheumatoid arthritis, HC: healthy control, NRA: non-RA rheumatic diseases, Anti-CarP: anti-carbamylated protein, ACF: anti-citrullinated fibrinogen. *1 indicates 100% sensitivity and specificity, †Quality Assessment of Diagnostic Accuracy Studies (QUADAS) score.

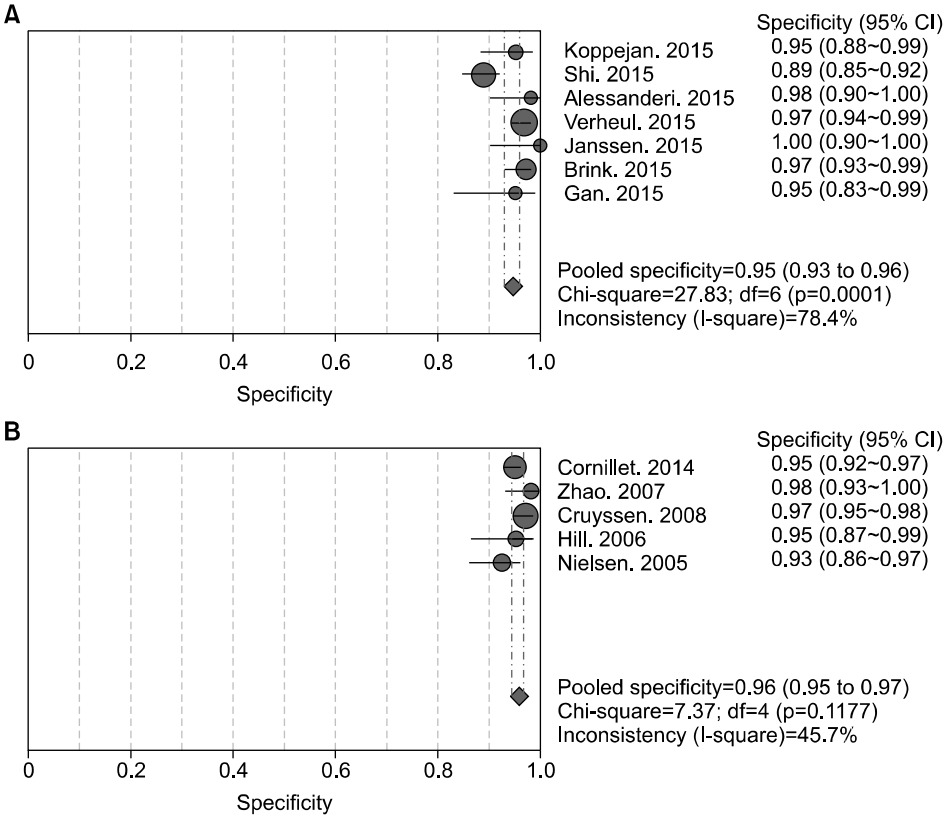

Figure 1. Estimates of the specificities of anti-carbamylated protein (A) and anti-citrullinated fibrinogen (B) antibodies in diagnosing rheumatoid arthritis. Circles and lines represent point estimates and 95% confidence intervals (CIs), respectively. Circled areas represent relative study sizes. df: degree of freedom.

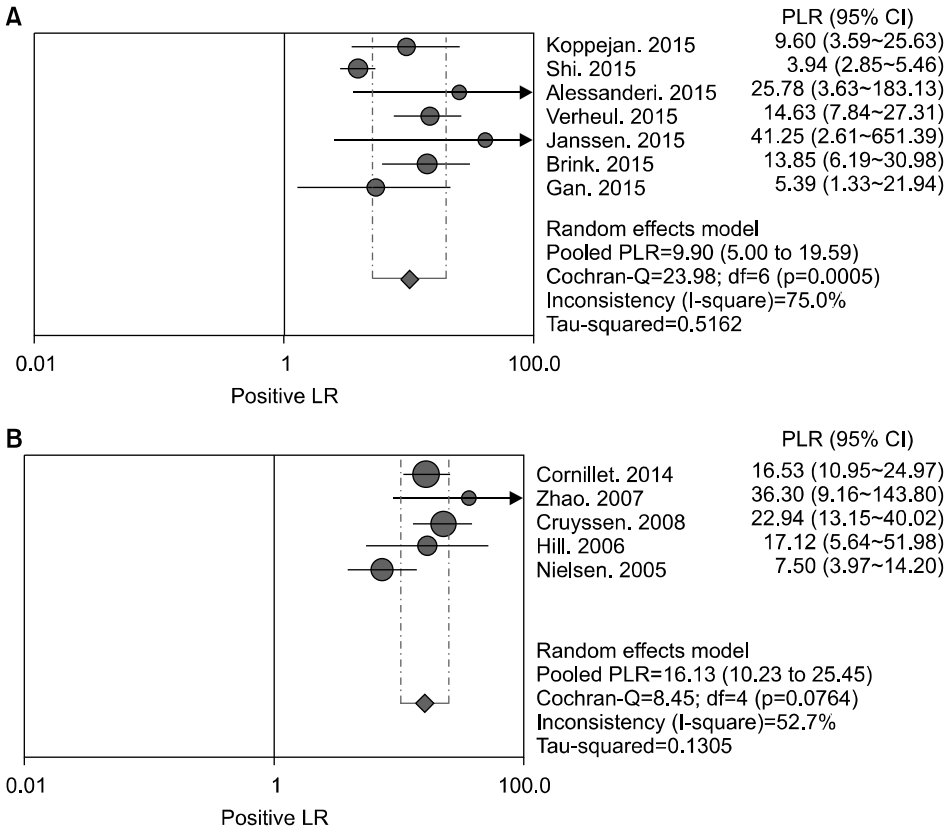

Figure 2. Estimates of the positive likelihood ratio (PLR)s of anti-carbamylated protein (A) and anti-citrullinated fibrinogen (B) antibodies in diagnosing rheumatoid arthritis. Circles and lines represent point estimates and 95% confidence intervals (CIs), respectively. Circled areas represent relative study sizes. df: degree of freedom.

a QUADAS score of >10 (Supplementary Table 1). We did not perform a subgroup analysis based on the study quality, because this meta-analysis included publications had high QUADAS scores, suggesting that the qualities of included studies were generally high and the risk of bias was a low concern.

Diagnostic accuracies of anti-CarP and ACF antibodies in RA

The sensitivities and specificities of anti-CarP antibody in detecting RA ranged from 26.3% to 55.8% and from 88.9% to 100%, respectively (Table 1, Figure 1). The sensitivities and specificities of ACF antibody ranged from 55.8% to 83.4% and from 92.6% to 98.1%, respectively (Table 1, Figure 2). The pooled sensitivities and specificities of anti-CarP antibody were 43.9% (95% CI, 41.6～46.3) and 94.5% (95% CI, 93.0～95.8), respectively, and those of ACF antibody were 68.3% (95% CI, 64.9～71.6) and 95.8% (95% CI, 94.5～96.9), respectively (Table 2, Figure 1). The PLR, NLR, and DOR of anti-CarP antibody were 9.901 (95% CI, 5.005～19.58), 0.597 (95% CI, 0.541～0.658), and 14.64 (95% CI, 8.004～34.45), respectively, and those of ACF antibody were 16.14 (95% CI, 10.23～25.42), 0.292 (95% CI, 0.192～0.444), and 58.61 (95% CI, 26.61～129.1), respectively (Table 2, Figure 2). Figure 3 shows the performance of anti-CarP and ACF tests in the form of SROC curves. The AUCs and Q* indices of anti-CarP antibody were 0.460 and 0.470, respectively, while those of ACF antibody were 0.954 and 0.896, respectively (Table 3, Figure 3).

Anti-CarP and ACF antibodies in anti-CCP antibody-negative RA patients

Anti-CarP antibody positivity ranged from 10.7%～17.3% in individuals negative for anti-CCP. The sensitivity and specificity of anti-CarP antibody for diagnosis of RA were 12% and 91% in anti-CCP-negative patients [8]. The discrepancy among anti-citrullinated protein/peptide antibodies tests including ACF and anti-CCP in RA patients was 29.5% [11], and 14.55% of the anti-CCP–negative RA patients showed ACF positivity [20].

Comparison of the accuracies of ACF and anti-CCP in diagnosing RA

Four studies, which included a total of 590 patients and 1,070 controls, evaluated the diagnostic accuracy of both anti-CCP and ACF antibodies [10-13]. The sensitivities

Table 2. Summary data from the meta-analysis

Antibody	Number of study	Number		Sensitivity* (95% CI)	Heterogeneity		Specificity* (95% CI)	Heterogeneity		PLR (95% CI)	NLR (95% CI)	DOR (95% CI)	AUC (SE)	Q* (SE)
		RA	Control		I^2	p-value		I^2	p-value					
Anti-CarP	7	1,749	1,044	0.439 (0.416～0.463)	61.4	0.014	0.945 (0.930～0.958)	78.6	<0.001	9.901 (5.005～19.58)	0.597 (0.541～0.658)	14.64 (8.004～34.45)	0.460 (0.104)	0.470 (0.070)
ACF	5	773	1,178	0.683 (0.649～0.716)	91.1	<0.001	0.958 (0.945～0.969)	45.7	0.118	16.14 (10.23～25.42)	0.292 (0.192～0.444)	58.61 (26.61～129.1)	0.954 (0.046)	0.896 (0.064)
Anti-CCP	4	590	1,070	0.668 (0.628～0.706)	85.5	<0.001	0.960 (0.946～0.971)	44.5	0.145	16.34 (10.63～25.11)	0.310 (0.213～0.452)	51.80 (28.46～94.27)	0.951 (0.059)	0.892 (0.080)

RA: rheumatoid arthritis, CI: confidence interval, PLR: positive likelihood ratio, NLR: negative likelihood ratio, DOR: diagnostic odds ratio, AUC: area under the curve, SE: standard error, Anti-CarP: anti-carbamylated protein, ACF: anti-citrullinated fibrinogen, Anti-CCP: anti-cyclic citrullinated peptide. *1 indicates 100% in diagnostic values.

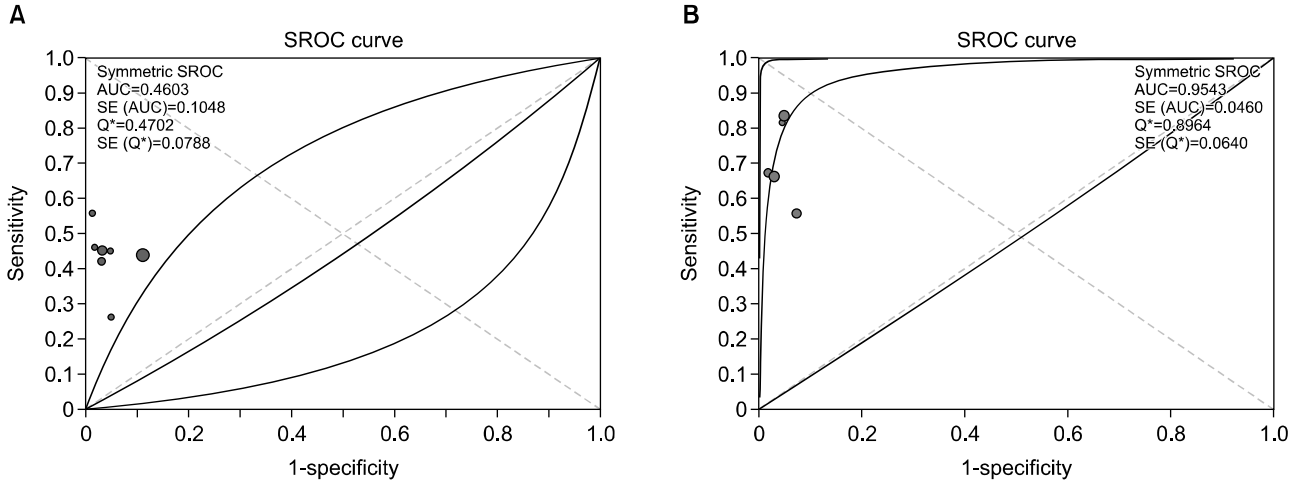

Figure 3. Summary receiver operating characteristic (SROC) curves of anti-carbamylated protein (A) and anti-citrullinated fibrinogen (B) antibodies in diagnosing rheumatoid arthritis. Solid circles represent individual studies included in the meta-analysis. The curve shown is a regression line that summarizes overall diagnostic accuracy. SE: standard error, AUC: area under the curve. Q*, index defined by a point on the SROC curve where sensitivity is equal to specificity; SE (Q*), standard error of the Q* index.

and specificities of ACF were comparable with those of anti-CCP in the diagnosis of RA (68.6% vs. 66.8%, difference of 0.018, p=0.517; 95.6% vs. 96.0%, difference of −0.004, p=0.658) (Table 3). Similarly, there was no difference in the PLRs, NLRs, AUCs, or Q* indices between the ACF and anti-CCP groups in diagnosing RA (Table 3).

Table 3. Paired comparison (ACF vs. anti-CCP) of accuracy in diagnosing rheumatoid arthritis

Diagnostic accuracy	Number of study	Number		ACF* (95% CI)	Anti-CCP* (95% CI)	Difference for ACF vs. anti-CCP	p-value
		RA	Control				
Sensitivity	4	590	1,070	0.686 (0.647～0.724)	0.668 (0.628～0.706)	0.018	0.517
Specificity	4	590	1,070	0.956 (0.942～0.968)	0.960 (0.946～0.971)	−0.004	0.658
PLR	4	590	1,070	14.93 (9.161～24.33)	16.34 (10.63～25.11)	−1.410	0.792
NLR	4	590	1,070	0.278 (0.150～0.516)	0.310 (0.213～0.452)	−0.032	0.774
AUC	4	590	1,070	0.973 (0.034)	0.951 (0.059)	0.022	0.747
Q* index	4	590	1,070	0.925 (0.057)	0.892 (0.080)	0.033	0.730

ACF: anti-citrullinated fibrinogen, Anti-CCP: anti-cyclic citrullinated peptide, RA: rheumatoid arthritis, PLR: positive likelihood ratio, NLR: negative likelihood ratio, AUC: area under the curve. *1 indicates 100% in diagnostic values.

Heterogeneity and meta-regression analysis

Between-study heterogeneity was observed in the meta-analyses of anti-CarP and ACF tests. Cut-off values for anti-CarP and ACF antibodies were different among the studies. A typical "shoulder arm" pattern in the SROC space suggests the presence of a threshold effect. However, this pattern was not seen in the SROC curve. Moreover, the Spearman rank correlation test did not detect a threshold effect in the meta-analyses of anti-CarP and ACF antibodies (anti-CarP: Spearman correlation coefficient=−0.679, p=0.094; ACF: Spearman correlation coefficient=−0.100, p=0.878). Next, we explored heterogeneity arising from factors other than a threshold effect. Meta-regression analysis showed that study quality, sample size, type of control group, publication year, and ethnicities of participants were not the sources of heterogeneity in the meta-analyses of anti-CarP and ACF tests.

DISCUSSION

In this meta-analysis, we analyzed the combined evidence of diagnostic accuracies of anti-CarP and ACF antibodies in RA patients and compared the diagnostic accuracies of ACF and anti-CCP tests. This meta-analysis of 12 studies compared the diagnostic values of anti-CarP and ACF antibodies in the diagnosis of RA, and showed

that both anti-CarP and ACF antibodies had high specificities and that the sensitivity and AUC of ACF antibody was comparable to those of anti-CCP tests, but that the sensitivity and AUC of anti-CarP antibody was low. When sensitivity and specificity were considered independently, the sensitivity and specificity of anti-CarP antibody were found to be 43.9% and 94.5%, respectively, while those of ACF antibody were 69.0% and 94.6%, respectively. Antibodies against citrullinated antigens are considered the most specific antibodies for diagnosing RA. However, our data showed that anti-CarP antibody was as specific as ACF antibody. Our data also suggested that anti-CarP antibody was less sensitive than ACF. The Q* index is a useful global estimate of the discriminatory ability of a test. When sensitivity and specificity were considered simultaneously, the Q* index of anti-CarP was 0.460, while that of ACF antibody was 0.880. Our meta-analysis indicated that the overall diagnostic performance of anti-CarP antibody was not good, mainly because of its relatively low specificity, while the diagnostic value of ACF antibody was as good as anti-CCP antibody when we compared both antibodies against citrullated protein.

RA-specific antibodies can be detected several years before the onset of clinical symptoms [32]. Thus, sensitive and specific tests are needed for application during this pre-clinical phase. Like citrullination, carbamylation is another post-translational modification of proteins where cyanate modifies lysine to form homocitrulline through a non-enzymatic process [6]. Carbamylation is not restricted to RA, similar to citrullination, but the generation of antibodies against these modified proteins can precede clinical onset of RA and is associated, independently of anti-CCP antibody, with an increased risk of RA [8].

Previous studies have shown that anti-CarP antibody was present years prior to the onset of RA symptoms [32]. The sensitivity of detecting anti-CarP antibody in RA patients seems to be slightly lower than that of anti-CCP antibody, but the specificity of detecting anti-CarP antibodies is similar to anti-CCP antibody [8]. Anti-CarP antibodies were also found in anti-CCP–negative RA patients. The presence of anti-CarP antibodies overlapped in most anti-CCP-positive patients, and ~17.3% of the anti-CCP–negative RA patients displayed anti-CarP antibodies. Thus, the detection of anti-CarP antibody in anti-CCP–negative RA patients may be a useful additional test that identifies anti-CCP-negative patents with RA.

Recently, citrullinated fibrin was found in the inflamed synovium of RA patients, while ACF antibody was detected in the sera of these patients [9]. Fibrin accumulation may be harmful to a patient with RA, because fibrin can enhance the production of chemokines and inflammatory cytokines from monocytes [33], and inhibition of fibrin formation showed beneficial effects in collagen-induce arthritis [34]. Our meta-analysis showed that ACF is a sensitive and specific serologic biomarker for RA: ACF is as sensitive and specific as anti-CCP in diagnosing the disease. ACF and anti-CCP perform similarly in the diagnosis of RA. The ACF and anti-CCP test had a moderate agreement in RA. ACF was found in 14.55% of the anti-CCP–negative RA patients [20], and 38% of RF-negative RA patients were ACF positive [20]. In addition, both the ACF and the anti-CCP tests were the best predictors for diagnosing RA compared to individual test [13]. Thus, the ACF test may have a potential to be especially valuable in diagnosing seronegative RA.

The present study has some limitations that should be considered. First, between-study heterogeneity was observed in this meta-analysis. This between-study heterogeneity may have affected the results of the meta-analysis, and this effect may have been compounded by limited information on the clinical status and disease severity of participants in these studies. We tried to overcome this limitation by using a random-effects model that incorporates uncertainties arising from between-study variation and by performing a meta-regression analysis. Second, the diagnostic accuracy of anti-CarP and ACF antibodies may differ based on their cut-off values in specific assays. Although the Spearman rank correlation test did not detect threshold effects in the meta-analyses of anti-CarP and ACF antibodies, the cut-off values for these antibodies must be optimized. Moreover, further research is required to examine the diagnostic accuracies of anti-CarP and ACF antibodies based on their cut-off values. Third, the studies analyzed included patients with RA of various durations. The diagnostic values of anti-CarP and ACF antibodies may differ in patients with early vs. long-standing RA. Therefore, further research is required to examine changes in the diagnostic accuracy of anti-CarP and ACF antibodies with prolonged disease.

Nevertheless, this study has some strengths of meta-analysis. First, we performed this meta-analysis systematically to evaluate the diagnostic performance of anti-CarP and ACF antibodies for diagnosing patients with RA. The number of patients with RA in individual studies ranged from 63 to 969. However, the pooled analyses included up

to 1,749 patients and 1,044 controls. Compared with individual studies, this meta-analysis presents a more accurate assessment of the performance of diagnostic tests by pooling the results of independent analyses for greater statistical power and resolution. Second, this meta-analysis also analyzes variations in the results of different studies and quantifies result inconsistency (heterogeneity) across studies. Thus, it may be an objective and quantitative method, which provides a less biased estimate on the topic.

CONCLUSION

Our meta-analysis demonstrates thatboth anti-CarP and ACF antibodies are highly specific. However, the sensitivity of anti-CarP antibody is low, while the diagnostic value of ACF is comparable to that of anti-CCP. We conclude that, despite the fact that anti-CarP antibody shows low sensitivity in diagnosing RA, both anti-CarP and ACF antibodies are highly specific in diagnosing RA.

ACKNOWLEDGMENTS

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

SUPPLEMENTARY MATERIALS

Supplementary material can be found with this article online at https://doi.org/10.4078/jrd.2016.23.6.373.

REFERENCES

1. Lee DM, Weinblatt ME. Rheumatoid arthritis. Lancet 2001;358:903-11.
2. Lard LR, Visser H, Speyer I, vander Horst-Bruinsma IE, Zwinderman AH, Breedveld FC, et al. Early versus delayed treatment in patients with recent-onset rheumatoid arthritis: comparison of two cohorts who received different treatment strategies. Am J Med 2001;111:446-51.
3. Dörner T, Egerer K, Feist E, Burmester GR. Rheumatoid factor revisited. Curr Opin Rheumatol 2004;16:246-53.
4. Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van Venrooij WJ. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. J Clin Invest 1998; 101:273-81.
5. van Boekel MA, Vossenaar ER, van den Hoogen FH, van Venrooij WJ. Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. Arthritis Res 2002;4:87-93.
6. Shi J, van Veelen PA, Mahler M, Janssen GM, Drijfhout JW, Huizinga TW, et al. Carbamylation and antibodies against carbamylated proteins in autoimmunity and other pathologies. Autoimmun Rev 2014;13:225-30.
7. Wang Z, Nicholls SJ, Rodriguez ER, Kummu O, Hörkkö S, Barnard J, et al. Protein carbamylation links inflammation, smoking, uremia and atherogenesis. Nat Med 2007;13: 1176-84.
8. Shi J, van Steenbergen HW, van Nies JA, Levarht EW, Huizinga TW, van der Helm-van Mil AH, et al. The specificity of anti-carbamylated protein antibodies for rheumatoid arthritis in a setting of early arthritis. Arthritis Res Ther 2015;17:339.
9. Masson-Bessière C, Sebbag M, Girbal-Neuhauser E, Nogueira L, Vincent C, Senshu T, et al. The major synovial targets of the rheumatoid arthritis-specific antifilaggrin autoantibodies are deiminated forms of the alpha- and beta-chains of fibrin. J Immunol 2001;166:4177-84.
10. Cornillet M, Sebbag M, Verrouil E, Magyar A, Babos F, Ruyssen-Witrand A, et al. The fibrin-derived citrullinated peptide β 60-74Cit60,72,74 bears the major ACPA epitope recognised by the rheumatoid arthritis-specific anticitrullinated fibrinogen autoantibodies and anti-CCP2 antibodies. Ann Rheum Dis 2014;73:1246-52.
11. Vander Cruyssen B, Nogueira L, Van Praet J, Deforce D, Elewaut D, Serre G, et al. Do all anti-citrullinated protein/peptide antibody tests measure the same? Evaluation of discrepancy between anti-citrullinated protein/peptide antibody tests in patients with and without rheumatoid arthritis. Ann Rheum Dis 2008;67:542-6.
12. Hill JA, Al-Bishri J, Gladman DD, Cairns E, Bell DA. Serum autoantibodies that bind citrullinated fibrinogen are frequently found in patients with rheumatoid arthritis. J Rheumatol 2006;33:2115-9.
13. Nielen MM, van der Horst AR, van Schaardenburg D, van der Horst-Bruinsma IE, van de Stadt RJ, Aarden L, et al. Antibodies to citrullinated human fibrinogen (ACF) have diagnostic and prognostic value in early arthritis. Ann Rheum Dis 2005;64:1199-204.
14. Koppejan H, Trouw LA, Sokolove J, Lahey LJ, Huizinga TJ, Smolik IA, et al. Role of anti-carbamylated protein antibodies compared to anti-citrullinated protein antibodies in indigenous North Americans with rheumatoid arthritis, their first-degree relatives, and healthy controls. Arthritis Rheumatol 2016;68:2090-8.
15. Alessandri C, Bartosiewicz I, Pendolino M, Mancini R, Colasanti T, Pecani A, et al. Anti-carbamylated protein antibodies in unaffected first-degree relatives of rheumatoid arthritis patients: lack of correlation with anti-cyclic citrullinated protein antibodies and rheumatoid factor. Clin Exp Rheumatol 2015;33:824-30.
16. Verheul MK, Shiozawa K, Levarht EW, Huizinga TW, Toes RE, Trouw LA, et al. Anti-carbamylated protein antibodies in rheumatoid arthritis patients of Asian descent.

Rheumatology (Oxford) 2015;54:1930-2.
17. Janssen KM, de Smit MJ, Brouwer E, de Kok FA, Kraan J, Altenburg J, et al. Rheumatoid arthritis-associated autoantibodies in non-rheumatoid arthritis patients with mucosal inflammation: a case-control study. Arthritis Res Ther 2015;17:174.
18. Brink M, Verheul MK, Rönnelid J, Berglin E, Holmdahl R, Toes RE, et al. Anti-carbamylated protein antibodies in the pre-symptomatic phase of rheumatoid arthritis, their relationship with multiple anti-citrulline peptide antibodies and association with radiological damage. Arthritis Res Ther 2015;17:25.
19. Gan RW, Trouw LA, Shi J, Toes RE, Huizinga TW, Demoruelle MK, et al. Anti-carbamylated protein antibodies are present prior to rheumatoid arthritis and are associated with its future diagnosis. J Rheumatol 2015;42:572-9.
20. Zhao X, Okeke NL, Sharpe O, Batliwalla FM, Lee AT, Ho PP, et al. Circulating immune complexes contain citrullinated fibrinogen in rheumatoid arthritis. Arthritis Res Ther 2008;10:R94.
21. Lee YH, Rho YH, Choi SJ, Ji JD, Song GG. PADI4 polymorphisms and rheumatoid arthritis susceptibility: a meta-analysis. Rheumatol Int 2007;27:827-33.
22. Lee YH, Woo JH, Choi SJ, Ji JD, Song GG. Associations between osteoprotegerin polymorphisms and bone mineral density: a meta-analysis. Mol Biol Rep 2010;37:227-34.
23. Lee YH, Choi SJ, Ji JD, Song GG. Candidate gene studies of fibromyalgia: a systematic review and meta-analysis. Rheumatol Int 2012;32:417-26.
24. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6:e1000097.
25. Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. BMC Med Res Methodol 2003;3:25.
26. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539-58.
27. Egger M, Smith GD, Phillips AN. Meta-analysis: principles and procedures. BMJ 1997;315:1533-7.
28. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177-88.
29. Lijmer JG, Bossuyt PM, Heisterkamp SH. Exploring sources of heterogeneity in systematic reviews of diagnostic tests. Stat Med 2002;21:1525-37.
30. Walter SD. Properties of the summary receiver operating characteristic (SROC) curve for diagnostic test data. Stat Med 2002;21:1237-56.
31. Zamora J, Abraira V, Muriel A, Khan K, Coomarasamy A. Meta-DiSc: a software for meta-analysis of test accuracy data. BMC Med Res Methodol 2006;6:31.
32. Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 2004;50:380-6.
33. Liu X, Piela-Smith TH. Fibrin(ogen)-induced expression of ICAM-1 and chemokines in human synovial fibroblasts. J Immunol 2000;165:5255-61.
34. Marty I, Péclat V, Kirdaite G, Salvi R, So A, Busso N. Amelioration of collagen-induced arthritis by thrombin inhibition. J Clin Invest 2001;107:631-40.

Journal of Rheumatic Diseases Vol. 23, No. 6, December, 2016
https://doi.org/10.4078/jrd.2016.23.6.373

Supplementary Table 1. QUADAS quality evaluation of studies

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Sum
Koppejan, 2016 [14]	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	N	Y	11
Shi, 2015 [15]	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	N	Y	11
Alessanderi, 2015 [16]	Y	Y	Y	N	Y	Y	Y	N	Y	N	Y	Y	N	Y	10
Verheul, 2015 [17]	Y	U	Y	N	Y	Y	Y	N	Y	N	Y	Y	N	Y	9
Janssen, 2015 [18]	Y	Y	Y	N	Y	Y	Y	N	Y	N	Y	Y	N	Y	10
Brink, 2015 [19]	Y	Y	Y	N	Y	Y	Y	N	Y	N	Y	Y	N	Y	10
Gan, 2015 [20]	Y	Y	Y	N	Y	Y	Y	N	Y	N	Y	Y	N	Y	10
Cornillet, 2014 [10]	Y	Y	Y	N	Y	Y	Y	N	Y	N	Y	Y	N	Y	10
Zhao, 2008 [21]	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	N	Y	11
Cruyssen, 2008 [11]	Y	Y	Y	N	Y	Y	Y	N	Y	N	Y	Y	N	Y	10
Hill, 2006 [12]	Y	Y	Y	N	Y	Y	Y	N	Y	N	Y	Y	N	Y	10
Nielsen, 2005 [13]	Y	Y	Y	N	Y	Y	Y	N	Y	N	Y	Y	N	Y	10

QUADAS: Quality Assessment of Diagnostic Accuracy Studies, Y: Yes, N: No, U: Unclear. Item 1. Was the spectrum of patients representative of the patients who will receive the test in practice? 2. Were selection criteria clearly described? 3. Is the reference standard likely to correctly classify the target condition? 4. Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests? 5. Did the whole study population or a random selection of the sample, receive verification using a reference standard for diagnosis? 6. Did patients receive the same reference standard regardless of the index test result? 7. Was the reference standard independent of the index test? 8. Was the execution of the index test described in sufficient detail to permit replication of the test? 9. Was the execution of the reference standard described in sufficient detail to permit its replication? 10. Were the index test results interpreted without the knowledge of the results of the reference standard? 11. Were the reference standard results interpreted without knowledge of the index test results? 12. Were the same clinical data available when test results were interpreted as would be available when the test is used in practice? 13. Were uninterpretable / intermediate test results reported? 14. Were withdrawals from the study explained?