Observational Study

Medicine®

OPEN

Low levels of anti-cyclic citrullinated peptide (CCP) 3.1 associated with diseases other than rheumatoid arthritis

James J. Son, MD[a], Mariko Ishimori, MD[b,*], James Mirocha, MD[b], Michael H. Weisman, MD[b], Lindsy J. Forbess, MD[b]

Abstract

Our aim was to investigate the newest generation anti-cyclic citrullinated peptide (CCP) antibody 3.1 assay in diagnosing rheumatoid arthritis (RA) compared with other autoimmune and non-autoimmune diseases. We performed a retrospective observational chart review of patients with a positive CCP level over a one-year period at a single academic institution and assessed the associated diagnoses after at least six-months of follow-up. Of the 281 CCP positive patients during that period, 48% had a diagnosis of RA. The positive predictive value of RA in patients with a high CCP 3.1 assay was 0.619 compared to 0.248 with a low positive CCP 3.1 assay ($P < .0001$). Overall, there was a lower than expected positive predictive value of CCP 3.1 level with an RA diagnosis, though the likelihood of having an RA diagnosis was higher with a higher CCP level.

Abbreviations: RA = rheumatoid arthritis, CCP = anti-cyclic citrullinated peptide antibody, EHR = electronic health record, CSMC = Cedars Sinai Medical Center, ACR = American College of Rheumatology, ELISA = enzyme-linked immunosorbent assay, SD = standard deviation, IQR = interquartile range, SAS = statistical analysis software, IgA = Immunoglobulin A, IgG = Immunoglobulin G.

Keywords: ACPA, anticyclic citrullinated peptide antibodies, CCP, CCP3.1, citrullinated cyclic peptide, diagnostic performance, rheumatoid arthritis

1. Introduction

Anti-cyclic citrullinated peptide (CCP) antibodies are important serum markers used in the clinical diagnosis of rheumatoid arthritis (RA)[1]. However, it has been reported that CCP antibodies can be positive in various other autoimmune conditions[2]. Multiple studies have investigated previous generations of CCP assays (CCP 1, CCP 2, CCP 3), and several have shown CCP to be a highly specific and predictive marker in the diagnosis of RA[2–11]. Tests of sensitivity, specificity and predictive value of anti-CCP 3.1 antibodies have generally shown good discriminatory effects for the diagnosis of RA[4–10]. It is thought to be a much more improved diagnostic test compared to rheumatoid factor. There are differences in reported sensitivity, specificity, and predictive values between the various generations of the CCP test owing to the specific mixture of cyclic peptides in each. The first generation CCP 1 assay was of low sensitivity and is no longer widely distributed. The second generation CCP 2 assay has shown improved sensitivity and specificity over the CCP 1 assay[5–7,9,11]. The CCP 2 and third generation CCP 3 assays have a high specificity for RA in cohorts with already established inflammatory arthritis[2,3]. There is evidence suggesting that CCP 3 positivity may be predictive of development of future RA especially if greater or equal to 2 times the upper limit of normal (greater or equal to 40)[3]. The CCP 2 and CCP 3 test for IgG only, whereas the CCP 3.1 assay tests for both IgA & IgG. Previous studies of the CCP 3.1 assay purport that it may have a similar degree of specificity with a greater sensitivity than prior generations[2–11]. However, with the widespread and increasing use of this test in chronically ill populations we have observed quite a few false positive tests in our tertiary care clinical setting, possibly demonstrating the enthusiasm for this test as a diagnostic tool for RA vs other chronic illnesses. We queried our laboratory database for all test results of anti-CCP 3.1 antibody results obtained during a fixed time period from July 1, 2016 to July 30, 2017 for another study which was designed to discover potentially undiagnosed cases of RA in our tertiary care setting. The results of our analyses are reported herein.

2. Methods

We were able to obtain results of all anti-CCP 3.1 testing from our tertiary care center at Cedars-Sinai Medical Center (CSMC) from July 1, 2016 to June 30, 2017 during a period that all CCP

Editor: Hemant Jaiswal.

No conflicts of interest or funding disclosures to report.

Supplemental Digital Content is available for this article.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

[a] Department of Internal Medicine, [b] Cedars-Sinai Medical Center, Division of Rheumatology, Los Angeles, CA, [c] Research Institute and Clinical & Translational Science Institute (CTSI).

[*] Correspondence: Mariko Ishimori, Division of Rheumatology, Cedars-Sinai Medical Center, 8700 Beverly Blvd, Los Angeles, CA 90048, USA (e-mail: mariko.ishimori@cshs.org).

Copyright © 2021 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

How to cite this article: Son JJ, Ishimori M, Mirocha J, Weisman MH, Forbess LJ. Low levels of anti-cyclic citrullinated peptide (CCP) 3.1 associated with diseases other than rheumatoid arthritis. Medicine 2021;100:16(e25558).

Received: 8 July 2020 / Received in final form: 30 October 2020 / Accepted: 29 March 2021

http://dx.doi.org/10.1097/MD.0000000000025558

testing was done with the anti-CCP 3.1 test kit. All positive testing was further examined by a retrospective systematic chart review of the electronic health record (EHR) and stratified into low positive (≥20 and <40) and high positive (≥40). All EHR charts of CCP positive patients were available for review; they were reviewed by the study rheumatologist for diagnostic clinical information occurring approximately 6 months after the date of the testing in order to determine the diagnosis in the record. For a patient to be considered to have the diagnosis of RA, only those cases were counted as RA where the treating rheumatologist made the diagnosis of RA according to 1987 or 2010 ACR criteria. For all non-RA diagnoses, we accepted the diagnosis made by the treating physician in the medical record. Anti-CCP 3.1 antibody levels were assessed using QUANTA Lite CCP 3.1 IgA/IgG ELISA (Inova Diagnostics, Inc., San Diego, CA). The tests were performed by ETI-Max 3000 analyzer per CSMC laboratory standards. The study was approved by the institutional review board. Age was summarized by mean ± standard deviation (SD) or median and interquartile range [IQR] and was compared across two groups by the independent samples *t* test. Categorical variables were summarized by frequency and percentage and were compared across groups by the Fisher exact test. Odds ratios were reported along with 95% confidence intervals. SAS version 9.4 (SAS Institute, Cary, North Carolina) was used for statistical calculations.

3. Results

Of the 2027 CCP 3.1 assay tests performed at CSMC from July 1, 2016 to June 30, 2017, 307 positive CCP tests were reported among 281 unique patients, with some patients that had duplicate tests done. These 281 unique patients are the group that were examined by the study team. Table 1 lists the characteristics of the patient population. Of all the CCP positive patients, 48 (17.1%) were men and 233 (82.9%) were women. The mean age was 62.2 years (±14.8) and the median age was 65.0 years [54–74] for all CCP positive patients. The breakdown of ethnicities showed 59.8% Caucasian, 11.7% African–American, 11.4% Hispanic, 9.3% Asian–American, and 7.8% as other. Among the 281 patients with a CCP positive test, 135 patients (48.0%) had a diagnosis of RA, 130 patients (46.3%) had a non-RA diagnosis, and 16 patients (5.7%) had an unknown diagnosis due to lack of follow-up or lack of documentation. 105 patients (37.4%) had a low positive CCP level (≥20 and <40), and 176 (62.6%) had a high positive CCP level (≥40). 90 patients had a CCP level exceeding the limits of the assay (>250). Of those 90 patients, 80% had a diagnosis of RA, 13.3% had another diagnosis and 6.6% had an unknown diagnosis. Among the 105 patients with a low positive CCP level, only 24.8% had a diagnosis of RA, while 31.4% had another autoimmune diagnosis and 40.0% had a non-autoimmune diagnosis. Four patients (3.8%) had an unknown diagnosis. Among the 176 patients with a high positive CCP level, the diagnosis of RA increased to 61.9%, with 13.1% having another autoimmune diagnosis and 18.2% a non-autoimmune diagnosis. Twelve patients (6.8%) had an unknown diagnosis. Table 2 shows the distribution of diagnoses and low versus high CCP status.

In patients with high positive CCP, 61.9% had RA compared with 24.8% of patients with low positive CCP, and the odds ratio (OR) for RA = 4.94 (95% CI 2.89 – 8.46; $P < .0001$). Among low positive CCP patients, 81.9% were women versus 83.5% women with a high positive CCP patients ($P = .75$). The median age was 63 years in low positive CCP versus 65 years in high positive CCP patients ($P = .39$). Ethnicities were also similar among low positive vs high positive CCP positivity.

Among those with a diagnosis of RA, 49.4% were females and 41.7% were male ($P = .35$). The median age of patients with RA (65 years) was similar to non-RA patients (64 years) with CCP positivity ($P = .22$). Among RA patients, the median age with low positive CCP was 61.5 years compared to 65 years among high positive CCP RA patients ($P = .46$). There were no significant differences in ethnicity between low versus high positive CCP RA patients ($P = .91$).

Among the non-RA diagnoses, 56 patients (19.9%) were diagnosed with another autoimmune disease (Table 3). The most common autoimmune diagnoses included systemic lupus erythematosus (20 patients), primary Sjogren's syndrome (11 patients) and polymyalgia rheumatica (5 patients). The most common non-autoimmune diagnoses were osteoarthritis (18 patients), pulmonary disease (16 patients, of which 10 had interstitial lung disease with equal numbers with low vs high positive CCP) and malignancy (9 patients). The full list of diagnoses is listed in Table 3. For additional data on patients age, sex, ethnicity, CCP level and diagnosis, see supplemental table, http://links.lww.com/MD2/A64.

Table 1

Characteristics of patient population (n = 281).

Age	
Median [IQR]	65.0 [54–74], years
Mean (SD)	62.2 (± 14.8), years
Sex	
Male	48 (82.9%)
Female	233 (17.1%)
Ethnicity	
Caucasian	168 (59.8%)
African–American	33 (11.7%)
Hispanic	32 (11.4%)
Asian–American	26 (9.3%)
Other	22 (7.8%)
CCP status	
Low positive CCP	105 (37.4%)
High positive CCP	176 (62.6%)
Diagnosis	
Rheumatoid arthritis	135 (48.0%)
Other autoimmune	56 (19.9%)
Non-autoimmune	64 (22.8%)
Malignancy	10 (3.6%)
Unknown	15 (5.7%)

CCP = anti-cyclic citrullinated peptide, IQR = interquartile range, SD = standard deviation.
*Low +CCP level defined as ≥ 20 and <40 Units and high +CCP level defined as ≥ 40 Units.

Table 2.

Diagnoses by grouping, among all patients, low and high +CCP levels*.

	All +CCP (n = 281)	Low +CCP (20-39) (n = 105)	High +CCP (≥ 40) (n = 176)
Rheumatoid arthritis	135 (48.0%)	26 (24.8%)	109 (61.9%)
Other autoimmune	56 (19.9%)	33 (31.4%)	23 (13.1%)
Non-autoimmune	74 (26.4%)	42 (40.0%)	32 (18.2%)
Unknown	16 (5.7%)	4 (3.8%)	12 (6.8%)

CCP: anti-cyclic citrullinated peptide.
P-value < .0001 by the Fisher exact test.
*Low +CCP level defined as ≥ 20 and <40 Units, and high +CCP level defined as ≥ 40 Units.

Table 3.

Number of other autoimmune and non-autoimmune diagnoses, among all patients, low and high +CCP levels*.

	Diagnosis	All +CCP	Low +CCP	High +CCP
Other autoimmune	Systemic lupus erythematosus	20	12	8
	Primary Sjogren's syndrome	11	7	4
	Polymyalgia rheumatica	5	3	2
	Undifferentiated connective tissue disease	4	2	2
	Psoriatic arthritis	3	3	0
	Scleroderma	3	1	2
	Inflammatory bowel disease	2	0	2
	Ankylosing spondylitis	1	1	0
	Juvenile idiopathic arthritis	1	0	1
	Polymyositis	1	1	0
	Still's disease	1	0	1
	Antiphospholipid syndrome	1	1	0
	Autoimmune hepatitis	1	1	0
	Mixed connective tissue disease	1	1	0
	Giant cell arteritis	1	0	1
Non-autoimmune	Osteoarthritis	18	11	7
	Pulmonary disease†	16	9	7
	Malignancy‡	9	3	6
	Fibromyalgia	5	4	1
	Gout	4	2	2
	Rheumatic heart disease	2	1	1
	Neuropathy	2	0	2
	Viral illness	2	2	0
	Unspecified arthralgia	2	1	1
	Monoclonal gammopathy of unknown significance	1	0	1
	Lumbar stenosis	1	1	0
	Cervical myofascial pain syndrome	1	1	0
	Costochondritis	1	1	0
	Pregnancy	1	1	0
	Liver transplant	1	1	0
	Membranous nephritis	1	0	1
	Rosacea	1	1	0
	Optic neuritis	1	0	1
	Thrombophilia	1	0	1
	Meniere's disease	1	1	0
	Diabetic myopathy	1	1	0
	Tendonitis	1	1	0
	Carpal tunnel syndrome	1	0	1

CCP = anti-cyclic citrullinated peptide.

* Low +CCP level defined as ≥20 and <40 Units and high +CCP level defined as ≥40 Units.

† Pulmonary diseases include: interstitial lung disease, cryptogenic organizing pneumonia, asthma, hypersensitivity pneumonitis, pulmonary nodule and bronchiectasis.

‡ Malignancy types include: colon, breast, lung, papillary thyroid, diffuse large B-cell lymphoma and VIPoma (vasoactive intestinal peptide).

4. Discussion

In our population, less than half of all CCP 3.1 positive patients were found to have a diagnosis of RA. For low CCP positive patients, only one quarter of the patients had a diagnosis of RA, and 40% had a non-autoimmune diagnosis. For high CCP positive patients, 61.9% had a diagnosis of RA. The positive predictive value of RA in patients with a high positive CCP 3.1 was 2.5-fold higher than those with a low positive CCP 3.1 ($P < .0001$). This increased to 80% when the CCP level was >250, above the detectable limit of the assay. There were no significant differences in age, sex or ethnicity among RA patients who were CCP positive. Additionally, there were no differences in age, sex or ethnicity among those with low versus high positive CCP. Thus we found that only CCP level ($P < .0001$) and not sex, age or race related to the development of RA.

Overall, there were similar rates of non-RA diagnoses with patients with low versus high positive CCP 3.1 levels. Almost one fifth of patients with any positive CCP 3.1 level had a different autoimmune diagnosis besides RA, the most common being systemic lupus erythematosus and primary Sjogren's syndrome (Table 3). It has been previously reported that patients with other rheumatic diseases, notably systemic lupus erythematosus and primary Sjogren's syndrome can have an elevated CCP level[12,13]. Interestingly, certain autoimmune conditions, such as systemic lupus erythematosus, primary Sjogren's and psoriatic arthritis more frequently had a lower positive CCP 3.1 level than a higher one in our study. Osteoarthritis, pulmonary diseases and malignancy were other common diagnoses associated with a positive CCP 3.1 level (Table 3). It should be noted that these patients did not have a diagnosis of RA or another autoimmune disease. Because the majority of CCP positive patients were female, sex varied across all diagnosis categories (RA, autoimmune, non-autoimmune and malignancy) ($P = .009$) but age and ethnicity did not.

It has been shown that there are differences in sensitivity, specificity and predictive value between the 1st, 2nd and 3rd generations of the anti-CCP test[2–11]. Each new generation of the

CCP assay has showed improved sensitivity and specificity over the previous generation in cohorts with already established rheumatoid arthritis[2,3]. Previous reviews of the CCP 3.1 assay reported greater sensitivity with a similarly high degree of specificity than the previous generations, thought to be due to the incorporation of IgA antibody testing in addition to the IgG in the previous assays[4–6]. Unfortunately, the CCP 3.1 assay does not specify IgA versus IgG in the test results to make this distinction. It has been reported that both IgG and IgA anti-CCP isotypes can pre-date the onset of RA by several years, and there seems to be an association with anti-CCP positivity and increasing age as well[14,15]. However, the role of circulating IgA anti-CCP antibodies are not fully understood at this time.

While previous studies examined CCP levels in patients with known RA compared to other known autoimmune conditions[4–12], we hoped to emulate real-world environments where physicians check CCP levels in testing for RA or other conditions. In our study, there was a lower than expected positive predictive value of test CCP 3.1 test for a diagnosis of RA compared to the reports in the literature[5,9], leading us to question whether the CCP 3.1 assay, when used in clinical practice, is in fact superior to previous generations. This may indicate that the CCP 3.1 assay may have sacrificed specificity for sensitivity. When the CCP level was high positive (>40), the odds of RA were found to be 4.94 times as high as compared to a low positive CCP, and this was highly significant ($P < .0001$). It should be noted that these CCP 3.1 tests were ordered by physicians from all specialties for any indication, not just by rheumatologists. In a setting where there are many chronically ill patients in the population tested, the CCP 3.1 test, at a low positive value, does not reliably correlate with a diagnosis of RA.

There are several limitations to the results and conclusions drawn. The autoimmune diagnoses were made by rheumatologist and non-autoimmune diagnoses are based on what is reported in the EHR. In addition, the minimum follow-up interval in our study was 6 months after a positive CCP test, which could bias the diagnoses given the short follow-up and may need to be extended for a longer duration to determine if any of these patients develop RA in the future. Further research needs to be done to investigate the utility of the CCP 3.1 assay in diagnosing RA versus other conditions, as well as to investigate the significance of IgA versus IgG isotype of anti-CCP in the diagnosis of RA.

Author contributions

Data curation: James Mirocha.
Supervision: Mariko Ishimori, Lindsy J Forbess.
Writing – original draft: James Jaewon Son.
Writing – review & editing: James Jaewon Son, Mariko Ishimori, Michael H Weisman, Lindsy J Forbess, James Mirocha.

References

[1] Alamanos Y, Drosos AA. Epidemiology of adult rheumatoid arthritis. Autoimmun Rev 2005;4:130–6.
[2] Van Venrooij WJ, van Beers JJ, Pruijn GJ. Anti-CCP antibodies: the past, the present and the future. Nat Rev Rheumatol 2011;7:391–8.
[3] Demoruelle MK, Parish MC, Derber LA, et al. Performance of anti-cyclic citrullinated peptide assays differs in subjects at increased risk of rheumatoid arthritis and subjects with established disease. Arthritis Rheum 2013;65:2243–52.
[4] Szekanecz Z, Szabó Z, Zeher M, et al. Superior performance of the CCP3.1 test compared to CCP2 and MCV in the rheumatoid factor-negative RA population. Immunol Res 2013;56:439–43.
[5] Dos Anjos LM, Pereira IA, d 'Orsi E, et al. A comparative study of IgG second- and third-generation anti-cyclic citrullinated peptide (CCP) ELISAs and their combination with IgA third-generation CCP ELISA for the diagnosis of rheumatoid arthritis. Clin Rheumatol 2009;28:153–8.
[6] Bizzaro N, Tonutti E, Tozzoli R, et al. Analytical and diagnostic characteristics of 11 2nd- and 3rd-generation immunoenzymatic methods for the detection of antibodies to citrullinated proteins. Clin Chem 2007;53:1527–33.
[7] Webb T, Lakos G, Swart A, et al. Clinical evaluation of a novel chemiluminescent immunoassay for the detection of anti-citrullinated peptide antibodies. Clin Chim Acta 2014;437:161–7.
[8] Innala L, Kokkonen H, Eriksson C, et al. Antibodies against mutated citrullinated vimentin are a better predictor of disease activity at 24 months in early rheumatoid arthritis than antibodies against cyclic citrullinated peptides. J Rheumatol 2008;35:1002–8.
[9] Infantino M, Manfredi M, Meacci F, et al. Anti-citrullinated peptide antibodies and rheumatoid factor isotypes in the diagnosis of rheumatoid arthritis: an assessment of combined tests. Clin Chim Acta 2014; 436:237–42.
[10] Damjanovska L, Thabet MM, Levarth EW, et al. Diagnostic value of anti-MCV antibodies in differentiating early inflammatory arthritis. Ann Rheum Dis 2010;69:730–2.
[11] Bizzaro N, Tampoia M. Diagnostic accuracy of immunoassays for the detection of antibodies to citrullinated proteins. Clin Rev Allergy Immunol 2008;34:16–20.
[12] Lee DM, Schur PH. Clinical utility of the anti-CCP assay in patients with rheumatic diseases. Ann Rheum Dis 2003;62:870–4.
[13] Van Noord C, Hooijkaas H, Dufour-van den Goorbergh BC, et al. Diagnostic value of anti-cyclic citrullinated peptide antibodies to detect rheumatoid arthritis in patients with Sjögren's syndrome. Ann Rheum Dis 2005;64:160–2.
[14] Kokkonen H, Mullazehi M, Berglin E, et al. Antibodies of IgG, IgA and IgM isotypes against cyclic citrullinated peptide precede the development of rheumatoid arthritis. Arthritis Res Ther 2011;13:R13.
[15] Berens HM, Polinski KJ, Mikuls TR, et al. Anti-CCP3.1 and Anti-CCP-IgA are associated with increasing age in individuals without rheumatoid arthritis. J Rheumatol 2019;46:1556–9.