

Clinical Utility of the Anti-CCP Assay

Experiences with 700 Patients

ULRICH SAUERLAND, HEIDEMARIE BECKER, MATTHIAS SEIDEL,
HEIKO SCHOTTE, PETER WILLEKE, ANNETTE SCHORAT,
BERNHARD SCHLÜTER, WOLFRAM DOMSCHKE, AND MARKUS GAUBITZ

*Medizinische Klinik und Poliklinik B, Universitätsklinikum Münster,
D-48129 Münster, Germany*

ABSTRACT: Our objective was to determine the frequency of antibodies to cyclic citrullinated peptides (CCPs) in a series of patients with a variety of rheumatic diseases. Seven hundred consecutive serum samples from patients at an outpatient clinic were tested for the presence of rheumatoid factor (RF) and anti-CCP. Clinical diagnosis, radiographic information, and other laboratory data were taken from patients' charts. The sensitivity and specificity of anti-CCP reactivity for the diagnosis of rheumatoid arthritis (RA) were 74.0% and 94.5%, respectively; the corresponding results for RF were 69.7% sensitivity and 81.0% specificity. Highest rates of false-positive RF tests were found in patients with SLE (18.3% vs. 12.7% CCP), Sjögren's syndrome (73.3% vs. 3.3% CCP), and a control group with chronic hepatitis (24.7% vs. 1.3% CCP). The detection of anti-CCP is useful for the diagnosis of RA because of its similar sensitivity but higher specificity compared with RF. Anti-CCP also helps to diagnose other inflammatory and noninflammatory diseases (especially connective tissue diseases) by reducing the rate of false-positive results in comparison with RF.

KEYWORDS: cyclic citrullinated peptide (CCP); rheumatoid factor; sensitivity; specificity; rheumatoid arthritis; rheumatic diseases

Testing for the rheumatoid factor, an immunoglobulin (Ig) that binds to the constant portion of IgG, is often applied in diagnostic procedures in patients with rheumatic symptoms. However, approximately 20% of patients with rheumatoid arthritis (RA) remain RF-negative for the course of their disease. In early RA, the percentage of rheumatoid factor (RF)-positive patients may be below 50%.¹ In addition, RFs are not specific for RA. High percentages of RF-positive patients can be found in other rheumatic diseases, especially connective tissue diseases (in Sjögren's syndrome in up to 70%), other chronic inflammatory diseases such as hepatitis,² and also in elder people.³ These shortcomings of the RF assay have provided an impetus for identification of other assays for RA. Recent publications announced antibodies against cyclic citrullinated peptides (CCPs) to be a promising alternative. Anti-CCP antibodies have

Address for correspondence: Markus Gaubitz, M.D., Department of Medicine B, Münster University Hospital, Albert-Schweitzer-Strasse 33, D-48129 Münster, Germany. Voice: +49-251-83-57562; fax: +49-251-83-56429.
gaubitz@uni-muenster.de

Ann. N.Y. Acad. Sci. 1050: 314–318 (2005). © 2005 New York Academy of Sciences.
doi: 10.1196/annals.1313.033

a high specificity, mostly above 95%, for RA combined with a sensitivity comparable to the traditional RF. In addition, anti-CCP antibodies appear early in the disease, often even preceding the symptoms of RA.⁴ Finally, anti-CCP is the most reliable predictor of a progressive and erosive course of RA.⁵

With regard to limited experiences with anti-CCP, we examined the ability to distinguish RA from other chronic inflammatory rheumatic and hepatic diseases. In addition, we compared the enzyme-linked immunosorbent assay results with an automated test system.

PATIENTS

We collected blood from 700 consecutive patients from our outpatient department, mostly referred for diagnosis of rheumatic symptoms. Patient data were sampled by reviewing patient charts and medical reports, also taking account of technical tests and laboratory data. Diagnoses were made based on internationally accepted diagnostic criteria—American Rheumatism Association criteria,⁶ when possible. In this series, 231 patients had RA; 71 had systemic lupus erythematosus (SLE); 30 had Sjögren's syndrome; 38 had other forms of connective tissue disease such as scleroderma, polymyositis, or overlap syndromes; 50 had different forms of vasculitis such as Wegener's granulomatosis, giant cell arteritis, or polymyalgia rheumatica; 40 had osteoarthritis; 52 had noninflammatory myopathies/myalgias such as local tendomyalgias or fibromyalgia; and the remaining 111 patients suffered from other rheumatic diseases such as infectious arthritis, seronegative spondyloarthropathy, psoriatic arthritis, sarcoidosis, and other rare entities. Seventy-seven patients with chronic hepatitis (C and B) served as controls.

METHODS

Anti-CCP activity was determined by an enzyme-linked immunosorbent assay using a commercial assay (Euroimmun, Lübeck, Germany). The rheumatoid factor was measured by agglutination in the Waaler-Rose test and by Latex-Immuno-nephelometry (BN II, Dade Behring, Marburg, Germany). A positive Waaler-Rose test and/or a concentration of the Latex-RF above 20 IU/mL was considered RF-positive. The first 500 samples were additionally tested in an automated test system (EliA; Pharmacia Diagnostics, Freiburg, Germany).

RESULTS

In this cohort of 700 patients with a variety of rheumatic diseases (herein 231 patients with RA) and hepatitis, 250 samples (35.7%) tested positive for RF, and 197 (28.1%) tested positive for anti-CCP (TABLE 1).

In the subgroup of 231 patients with RA diagnoses according to the American Rheumatism Association criteria, the sensitivity of anti-CCP was 74% compared with 69.7 for RF. From 171 anti-CCP-positive sera, 11 (6.4%) were between 5 and 10 units, 21 (12.3%) were between 10 and 30 units, 56 (32.7%) were between 30 and

TABLE 1. Patients in different diagnosis groups and positive results for RF and anti-CCP, respectively

Diagnosis	<i>n</i>	RF-positive	Anti-CCP-positive
RA	231	161 (69.7%)	171 (74.0%)
SLE	71	13 (18.3%)	9 (12.7%)
Sjögren's syndrome	30	22 (73.3%)	1 (3.3%)
Other connective tissue disease	38	4 (10.5%)	0
Vasculitis	50	7 (14%)	1 (2%)
Osteoarthritis	40	5 (12.5%)	3 (7.5%)
Noninflammatory myalgia	52	10 (19.2%)	4 (7.7%)
Others	111	9 (8.1%)	7 (6.3%)
Hepatitis	77	19 (24.7%)	1 (1.3%)
Total	700	250 (35.7%)	197 (28.1)
Non-RA	469	89 (19.0%)	26 (5.5%)

TABLE 2. Sensitivity and specificity of RF and anti-CCP for presence of rheumatoid arthritis

	Rheumatoid factor	Anti-CCP antibodies
Sensitivity	69.7%	74.0%
Specificity	81.0%	94.5%

100 units, and 83 (48.5%) were above 100 units; the proposed cutoff recommended by the company was 5 units.

Ten sera were found to be RF-positive but anti-CCP-negative; vice versa, 20 sera were anti-CCP-positive but RF-negative.

In the non-RA rheumatic diseases, RF and anti-CCP were rarely found with the expected exception of sera from patients with Sjögren's syndrome, in which the RF could be detected in 73.3%, anti-CCP in 3.3%. In patients with chronic hepatitis, 24.7% were RF-positive, but only 1.3% were anti-CCP-positive.

TABLE 2 summarizes the sensitivity and specificity of RF and anti-CCP.

The first 500 sera were tested in parallel with the EliA CCP (Pharmacia Diagnostics). Controversial results were found in only 8 sera, distributed over several diseases. All controversial results were close to the cutoff of the tests. In 6 of these 8 sera, the EliA-CCP correlated with the diagnosis according to accepted criteria.

DISCUSSION

In our cohort of 700 patients with different rheumatic diseases and a subgroup with chronic hepatitis, testing for anti-CCP was confirmed to be very helpful in diagnosing patients with different rheumatic symptoms. Anti-CCP was moderately more sensitive, but clearly more specific than was testing by traditional RF.

In patients with RA, our results correlate with published data. Lee and Schur⁷ found a sensitivity of 66% for anti-CCP and 71.6% for RF, whereas specificity of

anti-CCP and RF was 90.4% and 80.3%, respectively. Dubucquoi *et al.*⁸ described a sensitivity of 85% for anti-CCP and 94% for RF, whereas specificity was 90.9% for anti-CCP but only 53% for RF. The decision to take either positive Waaler-Rose agglutination test and/or positive testing in Latex-Immunonephelometry as a positive result obviously did not decrease the specificity of RF in our series.

A general problem of testing these diagnostic tools is, of course, the fact that RF is part of the American Rheumatism Association criteria for the classification of RA. It seems not unrealistic that the sensitivity of RF might be slightly overrated when accepting a diagnosis of RA which eventually is made in part by the diagnostic tool we want to evaluate. The presence of RF as a classification criterion will also make it more difficult for anti-CCP testing to substitute for RF testing, although there are some evident advantages that argue for anti-CCP testing.

In our opinion, the high specificity of anti-CCP is especially valuable for the rheumatologist. Patients with arthralgias or arthritis showing a combination of antibodies and RF herein are still a frequent diagnostic problem. The differential diagnosis between early rheumatoid arthritis versus systemic lupus erythematosus versus Sjögren's syndrome versus rheumatoid arthritis with secondary Sjögren's syndrome also has therapeutic impact. In our series, the anti-CCP-positive patients with SLE were less frequent than those with RF; however, especially in patients with Sjögren's syndrome, other connective tissue diseases, and vasculitic disorders, we found positive anti-CCP extremely rare. In a recent article, Gottenberg *et al.*³ confirmed this observation. Correlating with our results, they found 59% of their patients with Sjögren's syndrome to be RF-positive, but only 7.5% anti-CCP-positive.

In patients with hepatitis who frequently show RF positivity as an immunological epiphenomenon, arthralgias may be explained as an extrahepatic feature of hepatitis; on the other hand, early RA cannot be excluded. Thus, a positive RF is puzzling. In our hepatitis patients, only one was found to be anti-CCP-positive but 24.7% were RF-positive. Werner *et al.*⁹ recently described 79 patients with chronic hepatitis C virus infection, of whom 44% were RF-positive, but anti-CCP could not be observed in any patient.

In summary, the detection of anti-CCP is useful for the diagnosis and exclusion of RA. It seems to be advantageous compared with testing for traditional RFs. Automated test systems provide a reliable cost-effective means of testing for anti-CCP antibodies.

REFERENCES

1. STEINER, G. 2003. Autoantibodies in rheumatoid arthritis. *In* Rheumatology, pp. 833–841. Mosby, Edinburgh/London/New York.
2. BOMBARDIERI, M., C. ALESSANDRI, G. LABBADIA, *et al.* 2004. Role of anti-cyclic citrullinated peptide antibodies in discriminating patients with rheumatoid arthritis from patients with chronic hepatitis C infection-associated polyarticular involvement. *Arthritis Res. Ther.* **6**: R137–R141.
3. GOTTENBERG, J-E., S. MIGNOT, P. NICAISE-ROLLAND, *et al.* 2005. Prevalence of anti-cyclic citrullinated peptide and anti-keratin antibodies in patients with primary Sjögren's syndrome. *Ann. Rheum. Dis.* **64**: 114–117.
4. NIELEN, M.M.J., D. VAN SCHAARDENBURG, H.W. REESINK, *et al.* 2004. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum.* **50**: 380–386.

5. KROOT, E.-J.J.A., B.A.W. DE JONG, M.A. VAN LEEUWEN, *et al.* 2004. The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. *Arthritis Rheum.* **43**: 1831–1835.
6. ARNETT, F.C., S.M. EDWORTHY, D.A. BLOCH, *et al.* 1988. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum.* **31**: 315–324.
7. LEE, D.M. & P.H. SCHUR. 2003. Clinical utility of the anti-CCP assay in patients with rheumatic diseases. *Ann. Rheum. Dis.* **62**: 870–874.
8. DUBUCQUOI, S., E. SOLAU-GERVAIS, D. LEFRANC, *et al.* 2004. Evaluation of anti-citrullinated filaggrin antibodies as hallmarks for the diagnosis of rheumatic diseases. *Ann. Rheum. Dis.* **63**: 415–419.
9. WERNER, M.H., K. HUTCHINSON, C. MORISHIMA, *et al.* 2004. Absence of antibodies to cyclic citrullinated peptide in sera of patients with hepatitis C virus infection and cryoglobulinemia. *Arthritis Rheum.* **50**: 2305–2308.