REVIEW ARTICLE

The Serological Diagnosis of Rheumatoid Arthritis

Antibodies to Citrullinated Antigens

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SUMMARY

Background: This article provides an overview of modern serological diagnostic testing for rheumatoid arthritis (RA) involving the detection of antibodies against citrullinated peptides/proteins (ACPA). Recommendations are also given for differential diagnosis and sequential testing in rheumatoid arthritis, with a view towards improving early diagnosis, so that irreparable joint damage can be avoided.

Methods: Selective literature research, with consideration of the authors' own publications.

Results: Two different, adequately evaluated testing systems, involving the detection of anti-CCP antibodies and of anti-MCV antibodies, are now commercially available and enable routine, relatively highly specific diagnostic testing for RA. Two point-of-care tests (POCT) for the early diagnosis of RA constitute the latest development in serologic diagnostic testing.

Conclusions: The two ACPA assays now on the market are equally useful for the diagnosis of rheumatoid arthritis. The correlation between RA disease activity and stratification with ACPA has only been demonstrated to date through the detection of anti-MCV antibodies.

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Key words: rheumatoid arthritis, laboratory diagnosis, antibody screening, early detection of disease, autoimmune disease

Rheumatoid arthritis (RA) has a prevalence of about 0.5% to 1% and an incidence of about 30 per 100 000 inhabitants, making it the most common chronic inflammatory autoimmune disease. The peak incidence is at about 50 years of age. Women are affected about three to four times more often than men.

RA is a systemic disease, accompanied by progressive joint destruction and deformity. Depending on the severity, there may also be extra-articular manifestations, with involvement of the skin, blood vessels, and internal organs. If inadequately treated, RA leads in the long term to a significant impairment of the quality of life; morbidity and mortality increase.

Early diagnosis and suitable therapy are therefore of decisive importance for the prognosis of RA. The three pillars for the diagnosis of rheumatological disease are the medical history, clinical findings (including imaging techniques) and serological laboratory tests.

Antibody diagnostic testing of RA

Serological diagnostic testing is of growing importance in the early detection and differentiation of rheumatoid arthritis. Apart from the traditional detection of the rheumatoid factor, new specific autoantibodies to citrullinated antigens have made a crucial contribution to the diagnosis of RA.

The rheumatoid factor is an autoantibody, which may be IgM, IgG or IgA, and which was first mentioned in 1922 (1). It recognizes domains CH2 and CH3 of the Fc segment of human IgG and is a component of the classification criteria for RA published by the American College of Rheumatology (2). Rheumatoid factor (RF) can be determined by various test methods; ELISA (enzyme-linked immunosorbent assay) and nephelometry are standardized methods. RF was determined in 3843 patients in 29 different studies, employing all current analytical methods. Analysis of the results showed that the mean specificity was only 79%, with sensitivity of about 60% (3). In recent years, new autoantibodies have been detected and characterized in the sera of RA patients. These exhibit higher specificity and can therefore help to improve serological diagnostic testing.

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FIGURE 1

Citrullination: Loss of the positive charge on arginine leads to a change in tertiary structure; possible structure of CCP

Antibodies to citrullinated antigens

One of the most important serological discoveries in rheumatology in recent years has been the characterization of autoantigens in RA containing the amino acid citrulline (4). The starting point for this discovery was the identification of the target antigen for anti-keratin antibodies (AKA). These were first described in 1979 and are highly specific for RA (4). The target antigen is fillagrin, a protein which is specifically

expressed in keratin-producing epithelial cells and which has structure forming properties. As fillagrin is only expressed in epithelial cells and not in joints or other organs, the pathogenetic significance of this finding was initially unclear. Schellekens et al. (6) showed that only citrullinated forms of fillagrin were recognized by AKA. Proteins are citrullinated by enzymatic deimination of arginine residues, to give citrulline residues. Citrullination is a posttranslational modification, which alters the charge of a protein, leading to changes in its three dimensional structure, which in turn result in changes in antigenic properties (7). Citrullination has an essential physiological and biochemical role in cell differentiation and in programmed cell death (apoptosis).

Antibodies to cyclic citrullinated peptides (anti-CCP antibodies)

As it is difficult to isolate pure fillagrin, only the relevant epitopes are used for test development. First generation ELISAs gave specificity for RA of about 85%, with sensitivity of 65% to 70%. Second generation ELISAs use synthetic peptides as antigen, with a ring structure due to intramolecular disulfide bridge formation, with the citrulline epitope in a prominent position (*figure 1*). Use of these cyclic citrullinated peptides (CCP) has improved the specificity to between 96% and 98%, without changing the sensitivity (8). Several studies have now shown that anti-CCP antibodies are not only highly specific, but also of high predictive value for an erosive course of the disease and are thus of prognostic value (9). The so-called third generation anti-CCP antibody assays have recently been brought into the market, although comparative studies have found no significant improvement in the diagnostic values of these assays in comparison with the second generation (10, 11).

Antibodies to other citrullinated proteins

Fibrin and fibrinogen can also be regarded as citrullinated antigens. Studies with citrullinated peptide derivatives of the two proteins have now confirmed close cross-reactivity between fillagrin and citrullinated fibrin (12). Some publications have found high diagnostic specificity and sensitivity for the detection of anti-citrullinated fibrinogen antibodies in patients with RA (13). With ELISA, the sensitivity for RA was about 75%, with specificity of 98%. It follows that the diagnostic properties of this antigen in rheumatoid arthritis are comparable to the CCP antigen. Moreover, citrullinated fibrinogen and CCP are also comparable in their sensitivity and specificity for early rheumatoid arthritis (14). Both markers have been described as good predictors for the radiological progression of disease. The close association has also been confirmed with the occurrence of anti-CCP antibodies in patients with rheumatoid arthritis (14). However, there is not yet a standardized commercial test for the immunoassay to citrullinated fibrinogen which can be used in routine diagnostic testing.

FIGURE 2

Possible role of mutated citrullinated vimentin in the pathogenesis of rheumatoid arthritis (with the kind permission of Dr. H. Bang)

Another interesting citrullinated autoantigen is the citrullinated form of alpha-enolase, an enzyme which plays a role in glycolysis (15). Citrullinated alpha-enolase has been detected—together with other citrullinated antigens—in the synovial tissue of patients with rheumatoid arthritis. However, there are not yet adequate published data on the diagnostic sensitivity and specificity. Nevertheless, the specificity of 97.1% for a cohort of patients with early rheumatoid arthritis is remarkable (16).

Antibodies to citrullinated vimentin and mutated citrullinated vimentin (MCV)

Citrullinated vimentin has been described as a relevant autoantigen expressed in synovial tissue. Subsequently, it was clarified that citrullinated vimentin is identical to the formerly known antigen Sa, which stands for Savoie, the name of the patient in whom the respective autoantibody response was first identified. Anti-Sa antibodies provide a high specificity of >98%, but a limited sensitivity of 22% to 40% for patients with rheumatoid arthritis (17). Although there is currently no commercial assay for detecting anti-Sa antibodies, studies performed so far indicate that they may be of prognostic value for severe clinical courses of rheumatoid arthritis. Moreover, anti-Sa antibodies have the high predictive value of about 84% to 99% for rheumatoid arthritis and are closely associated with extra-articular manifestations and severe joint involvement (17). The most recent studies have shown that both citrullination and mutation can influence the antigenicity of vimentin. An ELISA based on mutated citrullinated vimentin (MCV) has been commercially available for the diagnosis of rheumatoid arthritis for some time and has about the same diagnostic sensitivity and specificity as anti-CCP antibodies (18, 19, 20).

Studies on 1151 RA patients found that MCV antibodies have the same specificity as anti-CCP antibodies, but with better sensitivity (82% versus 72%) (18). Moreover, a significant correlation has been established between anti-MCV antibody titers and both the severity of the rheumatoid arthritis and the disease activity score (DAS28 = internationally accepted composite score to evaluate the disease activity of the RA). In a 3-year follow-up analysis on 42 RA patients (18), the disease activity score (DAS28) in 427 individual measurements was correlated with the titers of anti-MCV antibodies and of anti-CCP antibodies. Patients with active RA exhibited significantly higher anti-MCV antibody titers than patients with milder RA. In contrast, there was no difference in the anti-CCP titer in the two groups. Moreover, the anti-MCV antibodies correlated significantly with the disease activity score in RA patients. There was no correlation between anti-CCP antibodies and the disease activity score. In analogy to anti-CCP antibodies, anti-MCV antibodies are also suitable for the early diagnosis of RA, with comparable sensitivity (55.3% versus 59.3%, respectively), specificity (92.1% versus 92.3%, respectively), and positive predictive value (95.8% versus 96.1%, respectively)

TABLE

Antibody diagnostic testing in patients with rheumatoid arthritis (RA)

	Rf IgM	Rf IgA	Anti-CCP2	Anti-MCV
RA sensitivity	60–80%	44%	39–94%	69.5–82%
RA specificity	80–95%	84%	81–100%	90.3–98%
Early RA sensitivity	15–30%	29–39%	25–58%	57–71%
Correlation with activity	doubtful	yes	no	yes
Correlation with outcome	yes	yes	yes	yes
Association with extra-articular manifestations	yes	yes	yes	unknown

Rf, rheumatoid factor

(21). Another study found that, in contrast to anti-CCP antibody-positive patients, anti-MCV-positive patients exhibited significantly lower reduction in disease activity (DAS28) and a greater number of swollen joints (22). The two anticitrullinated protein/peptide antibodies (ACPA) were equally predictive of the radiological outcome (23).

Mathsson et al. measured anti-MCV and anti-CCP antibodies in 273 patients with early rheumatoid arthritis. The specificity of the two tests was equivalent (95% for anti-MCV antibodies, versus 96% for anti-CCP antibodies), although the anti-MCV antibody

FIGURE 3

Role of ACPA in diagnostic testing for rheumatoid arthritis (RA), with consideration of the ACR criteria (modified from [24]). RF, rheumatoid factor; a-CCP, antibodies to cyclic citrullinated peptides; a-MCV, antibodies to mutated citrullinated vimentin; ACPA, anticitrullinated protein/peptide antibodies; P, positive; N, negative; ACR, criteria of the American College of Rheumatology

measurements were more sensitive (70.7% for anti-MCV antibodies, versus 57.9% for anti-CCP antibodies). In contrast to the anti-CCP antibody reactivity, anti-MCV antibody reactivity was associated both with greater disease activity and with radiological progression (23).

It thus appears that anti-MCV antibodies may have the advantage of correlating better with disease activity and patient outcome than anti-CCP antibodies.

In addition, it would be interesting to perform additional studies on the link between anti-MCV antibody reactivity and the pathogenesis of rheumatoid arthritis, as mutated and citrullinated vimentin has been identified in the synovia of patients with rheumatoid arthritis (figure 2).

The table lists diagnostic properties of rheumatoid factors, anti-CCP2 and anti-MCV. This demonstrates that anti-CCP2 and anti-MCV are tests of equal value for diagnostic testing for RA. It is an interesting observation that anti-MCV antibodies correlate with the RA disease activity score and could possibly be used for stratification (18). Figure 3 depicts the value of autoantibody determinations in diagnostic testing for RA.

Point-of-care tests (POCT)

Two serological point-of-care tests (POCT) for the early detection of RA have been very recently developed. The Rheuma-Chec test (Orgentec, Mainz, Germany) combines two biomarkers for the diagnosis of RA—rheumatoid factor and antibodies to MCV. Antibodies to CCP are detected with the CCPoint assay (Euro-Diagnostica, Malmö, Sweden) (25). The tests require only a single drop of whole blood and any general physician can perform them within minutes. The future will show whether these tests can bring additional improvements in diagnostic testing, leading to earlier therapy for RA.

Résumé

Posttranslational modification of antigens by citrullination and mutation plays an important role in the pathogenesis of rheumatoid arthritis. Detection of ACPA is of great clinical value in the early and differential diagnosis of RA. Immunoassays to detect anti-MCV and anti-CCP antibodies exhibit similar diagnostic sensitivity and specificity. Future work must be directed towards improving the mutual standardization of the tests. Moreover, further studies are needed on the correlation with disease activity score and the prognosis of RA and early RA.

Conflict of interest statement

Dr. Egerer is involved in research projects of Orgentec, EUROIMMUN, and Binding Site. Dr. Feist receives fixed fees from the firms Generic Assays and Orgentec for lectures on diagnostic testing with autoantibodies. In addition, the authors have received financial research support from the firms Euroimmun and Orgentec for their research on the evaluation of autoantibodies.

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Key messages

- According to current knowledge, diagnosis of rheumatoid arthritis (RA) should not be solely based on the determination of the rheumatoid factors.
- Anticitrullinated protein/peptide antibodies (ACPA) are extremely sensitive markers for RA, with very high specificity for the disease.
- The serological determination of the ACPAs can support the prognostic evaluation and therapeutic decisions.
- The use of POCT assays in rheumatology can lead to more rapid diagnoses and thus reduce costs in the care of RA patients.
- More large studies are needed to demonstrate to what extent specific ACPA tests (such as anti-MCV antibody measurements) correlate with the disease activity score (DAS28), with the aim of possibly using these for serological monitoring of RA.

REFERENCES

1. Dörner T, Egerer K, Feist E, Burmester GR: Rheumatoid factor revisited. *Curr Opin Rheumatol* 2004; 16: 246-53.
2. Arnett FC, Edworthy SM, Bloch DA et al.: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
3. Schur PH: Anti-cyclic citrullination peptide antibodies: diagnostic, predictive and monitoring value in RA. *Int J Adv Rheumatol* 2005; 3: 77-83.
4. Van Venrooij WJ, Pruijn GJ: Citrullination; a small change for a protein with great consequences for rheumatoid arthritis. *Arthritis Res* 2000; 2: 249-51.
5. Yölinou P, Serre G: The anti-perinuclear factor and antikeratin antibody systems. *Int Arch Allergy Immunol* 1995; 107: 508-18.
6. 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.
7. Schellekens GA, Visser H, de Jong BA et al.: The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum* 2000; 43: 155-63.
8. van Venrooij WJ, Zendman AJ, Pruijn GJ: Autoantibodies to citrullinated antigens in (early) rheumatoid arthritis. *Autoimmun Rev* 2006; 6: 37-41.
9. van Venrooij WJ, Hazes JM, Visser H: Anticitrullinated protein/peptide antibody and its role in the diagnosis and prognosis of early rheumatoid arthritis. *Neth J Med* 2002; 60: 383-8.
10. Coenen D, Verschueren P, Westhovens R, Bossuyt X: Technical and diagnostic performance of 6 assays for the measurement of citrullinated protein/peptide antibodies in the diagnosis in the diagnosis of rheumatoid arthritis. *Clin Chem* 2007; 53: 498-504.
11. Bizzara N, Tonutti E, Tozzoli R, Villalta D: 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.
12. Sebbag M, Moirand N, Auger I et al.: Epitopes of human fibrin recognized by the rheumatoid arthritis-specific autoantibodies to citrullinated proteins. *Eur J Immunol* 2006; 36: 2250-63.

13. Vander Cruyssen B, Cantaert T, Nogueira L et al.: Diagnostic value of anti-human citrullinated fibrinogen ELISA and comparison with four other anti-citrullinated protein assays. *Arthritis Res Ther* 2006; 8: R122.
14. Nielen MM, van der Horst AR, van Schaardenburg D et al.: Antibodies to citrullinated human fibrinogen (ACF) have diagnostic and prognostic value in early arthritis. *Ann Rheum Dis* 2005; 64: 1199-204.
15. Kinloch A, Tatzer V, Wait R et al.: Identification of citrullinated alpha-enolase as a candidate as a candidate autoantigen in rheumatoid arthritis. *Arthritis Res Ther* 2005; 7: R1421-9.
16. Saulot V, Vittecoq O, Charlionet R et al.: Presence of autoantibodies to the glycolytic enzyme alpha-enolase in sera from patients with early rheumatoid arthritis. *Arthritis Rheum* 2002; 46: 1196-201.
17. Vossenaar ER, Despres N, Lapointe E et al.: Rheumatoid arthritis specific anti-Sa antibodies target citrullinated vimentin. *Arthritis Res Ther* 2004; 6: R142-50.
18. Bang H, Egerer K, Gauliard A et al.: Mutation and citrullination modifies vimentin to a novel autoantigen for rheumatoid arthritis. *Arthritis Rheum* 2007; 56: 2503-11.
19. Dejaco C, Klotz W, Larcher H, Duftner C, Schirmer M, Herold M: Diagnostic value of antibodies against a modified citrullinated vimentin in rheumatoid arthritis. *Arthritis Res Ther* 2006; 8: R119.
20. Soós L, Szekanez Z, Szabo Z et al.: Clinical evaluation of anti-mutated citrullinated vimentin by ELISA in rheumatoid arthritis. *J Rheumatol* 2007; 34: 1658-63.
21. Usum J, Nielen MM, van Schaardenburg D et al.: Antibodies to mutated citrullinated vimentin and disease activity score in early arthritis: a cohort study. *Arthritis Res Ther* 2008; 10: R12.
22. 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 citrullinated peptides. *J Rheumatol* 2008; 35: 1002-8.
23. Mathsson L, Mullazehl M, Wick MC et al.: Antibodies against citrullinated vimentin in rheumatoid arthritis. *Arthritis Rheum* 2008; 58: 36-45.
24. Feist E, Egerer K, Burmester GR: Autoantikörperprofile bei der rheumatoiden Arthritis. *Z Rheumatol* 2007; 66: 212-8.
25. Snijders GF, den Broeder AA, Bevers K et al.: Measurement characteristics of a new rapid anti-CCP2 test compared to the anti-CCP2 ELISA. *Scand J Rheumatol* 2008; 151-4.

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