Antibodies to cyclic citrullinated peptides: how they assist in the diagnosis of rheumatoid arthritis

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Summary

New assays for antibodies against artificially generated cyclic citrullinated peptides are of importance in the assessment of patients with suspected rheumatoid arthritis, especially during the early stages of the disease. These assays have similar sensitivity but are more specific for rheumatoid arthritis than the traditional rheumatoid factor test. The combined use of these assays and tests for rheumatoid factor provides more information than either test alone, particularly with respect to differentiating potential cases of rheumatoid arthritis from early cases of undifferentiated arthritis.

Key words: anti-keratin antibodies, rheumatoid factor.

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Introduction

Around 80% of patients with rheumatoid arthritis have a positive test for rheumatoid factor, but the test may take many years to become positive. The test for rheumatoid factor therefore has a low sensitivity in the early stages of rheumatoid arthritis. Furthermore, tests for rheumatoid factor may be positive in some patients with other inflammatory diseases (including Sjogren's syndrome), infections (bacterial or chronic viral, such as viral hepatitis) and haematological disorders (including cryoglobulinaemia and some plasma cell disorders). Rheumatoid factor therefore also has a relatively low specificity so it is not an ideal test in the early detection and confirmation of rheumatoid arthritis.

Alternatives to rheumatoid factor

In view of the limitations in interpreting rheumatoid factor results, there has been interest in developing better tests for the diagnosis of rheumatoid arthritis. It has been known for many years that senescent (ageing) cells display antigens that are not present on other cells, and that patients with rheumatoid arthritis may generate antibodies against these antigens. This was first reported in 1964 with the test for anti-perinuclear factor antibodies that were directed against senescent buccal mucosal cells. However, this test was challenging to perform and interpret. Buccal mucosal cells were later found to express filament aggregating protein (filaggrin) and in 1979, antibodies directed against keratin (anti-keratin antibodies) in senescent oesophageal cells were identified.

It now appears that anti-perinuclear factor, anti-filaggrin and anti-keratin antibodies are essentially the same antibody detected by different assays. Of these, only assays for anti-keratin antibodies are currently performed by a limited number of Australian pathology laboratories.

Antibodies to citrullinated peptides

As cells age, some of their structural proteins undergo 'citrullination' under the direction of cellular enzymes. Arginine residues undergo deimination to form the non-standard amino acid citrulline. Citrullinated peptides fit better into the HLA-DR4 molecules that are strongly associated with rheumatoid arthritis development, severity and prognosis. It is also known that many types of citrullinated peptides are present in the body, both in and outside joints.

In the late 1990s, antibodies against citrullinated peptides were 'discovered'. Sera from patients with rheumatoid arthritis contain antibodies that react against different citrullinated peptides, however the antibodies from each individual do not react against all possible citrullinated peptides. Artificial cyclic citrullinated peptides (CCP) have therefore been developed to mimic the range of conformational epitopes present *in vivo*. These artificial peptides are used in the current assays for antibodies against CCP (anti-CCP assays). The patient's serum is mixed with these peptides and if it contains anti-CCP antibodies they will bind together. This binding can be detected by an enzyme-linked immunosorbent assay.

Anti-CCP assays can be considered as alternatives to assays for anti-keratin antibodies. Table 1 compares assays for anti-CCP antibodies, anti-keratin antibodies and rheumatoid factor.

Table 1

Comparison of antibody assays for rheumatoid arthritis

	Assay type		
	Anti-CCP* antibodies	**Rheumatoid factor**	**Anti-keratin antibodies**
Sensitivity (%) †	39–94% (64%)	25–95% (60%)	23–47% (42%)
Specificity (%) †	89–98% (94%)	31–95% (79%)	94–97% (96%)
Availability	Offered by many laboratories in Australia	Widely available	Limited availability
Comments	Results (including numerical values) may vary between different laboratories depending on assay used	False positive results occur in a range of inflammatory, infectious and haematological diseases	Less sensitive than anti-CCP assays which can be considered as a replacement for this test

* CCP cyclic citrullinated peptides

† range of values from various studies (mean value)

Clinical utility of anti-CCP assays

Anti-CCP assays are offered by many, if not the majority, of private and public pathology services in Australia. The assay requires 5 mL of clotted serum which can also be used to test for rheumatoid factor. The turnaround time from these laboratories is generally less than two weeks.

Diagnosis of rheumatoid arthritis and prediction of disease severity

Anti-CCP assays have a sensitivity of 39–94% (mean 64%) in patients with established rheumatoid arthritis, with a specificity of 89–98% (mean 94%).[1] This means that anti-CCP antibodies are more specific than rheumatoid factor for the presence of rheumatoid arthritis but have similar sensitivity (Table 1). A positive result for anti-CCP antibodies also appears to be a better predictor of greater disease severity than a positive result for rheumatoid factor. The combined use of anti-CCP assays and rheumatoid factor tests also provides better prognostic information than using anti-CCP assays alone.

The anti-CCP assays appear to be of particular value in the evaluation of patients with early-onset arthritis. They have a sensitivity of 50–60% and specificity of 95–98% for the development of rheumatoid arthritis. This is useful during the early phase of rheumatoid arthritis, when patients may have milder and non-specific symptoms which make a definitive clinical diagnosis difficult. Making a definitive diagnosis of rheumatoid arthritis during this early phase is important, as early aggressive therapy within the first three months of the development of joint symptoms may decrease the probability of developing severe joint disease. A prospective study of 318 patients with early undifferentiated arthritis reported that within one year 83% and within three years 93% of patients who were positive for anti-CCP antibodies developed symptoms and signs that enabled a diagnosis of rheumatoid arthritis, compared with 25% of patients who were negative for anti-CCP antibodies.[2]

Anti-CCP antibodies have been shown to pre-date the development of clinical disease. However, neither rheumatoid factor nor anti-CCP assays should be used to screen for rheumatoid arthritis in healthy individuals in the absence of clinical symptoms.

Several studies have shown that while the majority of patients with rheumatoid arthritis will be positive for rheumatoid factor and anti-CCP antibodies at some point during their disease, these tests may not be positive at the same time. For example, while patients may initially have a positive anti-CCP assay, it may take many years to become rheumatoid factor positive. In addition, a minority of patients will only be positive for either rheumatoid factor or anti-CCP antibodies. This is another reason why, ideally, both tests should be performed in the assessment of a patient with suspected rheumatoid arthritis, including all patients with persistent arthritis of more than six weeks duration.

Uncertain role in monitoring disease activity

At present, there are conflicting data regarding the utility of serial anti-CCP assays to monitor the activity of rheumatoid arthritis and its response to therapy. Some studies have suggested that the correlation between anti-CCP antibodies and disease activity was stronger than for rheumatoid factor, but at least one study found the reverse. Furthermore, studies looking at patients who have responded to disease-modifying antirheumatic drugs or tumour necrosis factor inhibitors have not shown a consistent fall in concentrations of anti-CCP antibodies or rheumatoid factor. Based on the available data, serial monitoring of anti-CCP antibodies is not currently recommended. Clinical assessment and serial measurements of inflammatory markers, such as C-reactive protein and erythrocyte sedimentation rate, are better established methods of monitoring.

Comparison of results between different laboratories

While the majority of currently available anti-CCP assays are based on one particular manufacturer's assay (for patent reasons), other manufacturers are actively developing their own anti-CCP assays (likely to be marketed as 'third or subsequent' generation assays). Such assays will probably produce different results and numerical values from the currently available assays. We therefore recommend caution when comparing the results (particularly numerical values) of anti-CCP antibody testing from different laboratories.

Conclusion

Assays that detect antibodies to CCP are a new and important development in the diagnosis of patients with rheumatoid arthritis, particularly during the early phases of the disease when making a definitive diagnosis on clinical grounds may be difficult. The use of anti-CCP assays and rheumatoid factor in combination provides better diagnostic and prognostic information than either test alone.

References

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Further reading

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Conflict of interest: none declared

Self-test questions

The following statements are either true or false (answers on page 171)

9. Some patients with rheumatoid arthritis do not have a positive test for antibodies to cyclic citrullinated peptides.
10. The response to treatment of patients with rheumatoid arthritis is best assessed by serial assays of antibodies to cyclic citrullinated proteins.

Medicines Australia Code of Conduct: breaches

Medicines Australia has a code of conduct to guide the promotion of prescription drugs by pharmaceutical companies in Australia.[1] Complaints are reviewed by the Code of Conduct Committee and the results are published in its annual report. The report for 2005–06 is available on the Medicines Australia website.[2]

There were 27 new complaints in 2005–06. Seven are unresolved, but the report includes three complaints held over from the previous year. The Code of Conduct Committee found breaches in 11 of the complaints it finalised (Table 1).

The number of complaints coming from health professionals almost equalled the number made by companies about their competitors. In one case eight pharmaceutical companies were alleged to have breached the Code of Conduct with their advertisements in electronic prescribing software.[3] The Code of Conduct Committee required six of these companies to revise their advertising.

During the year the Code of Conduct Committee had to consider whether a venue was of more than 'reasonable quality'. It also judged if the hospitality offered to specialists was 'sumptuous' or 'simple and modest'. Probably for the first time the Code was applied across the Tasman. A cruise vessel on Auckland harbour was not considered to be an appropriate place for an educational event.

In total 11 complaints were found to have identified breaches of the Code of Conduct. Details of the complaints can be found in the annual report.[2] Analysis of these complaints should lead to improvements in the Code. The 15th edition of the Code of Conduct should be available in 2007.

References

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