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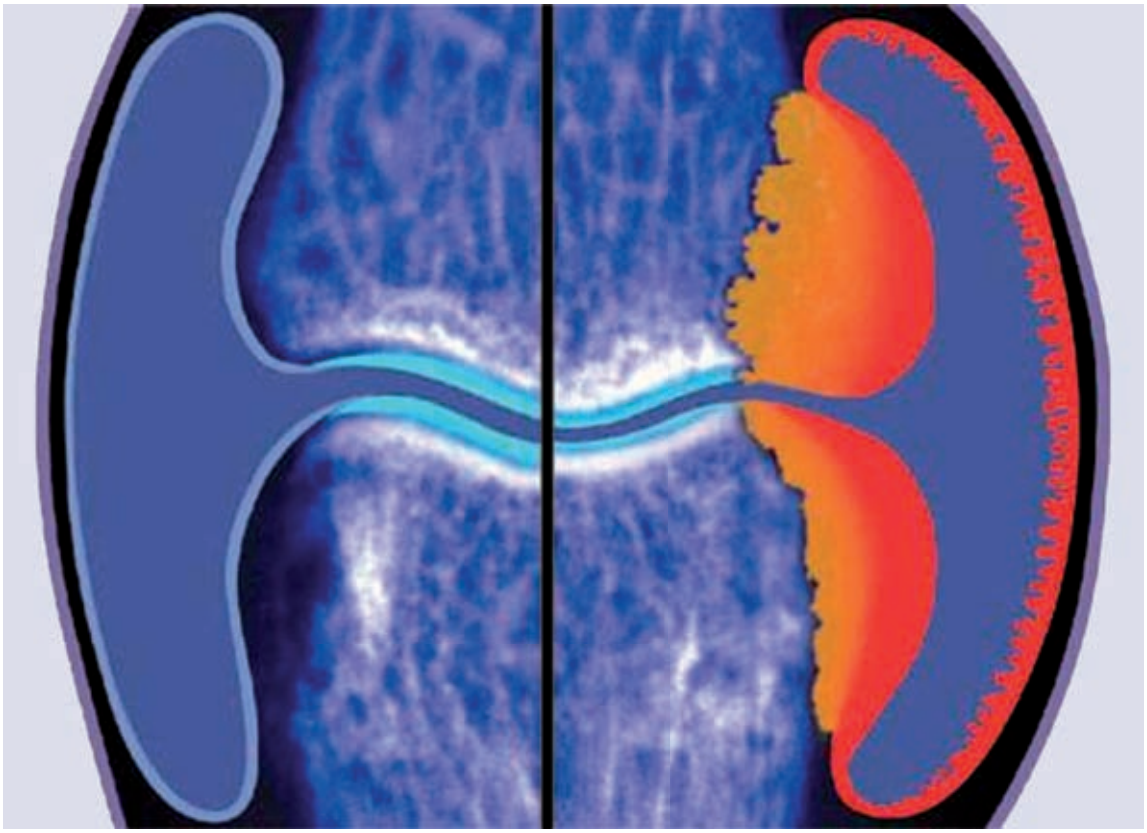

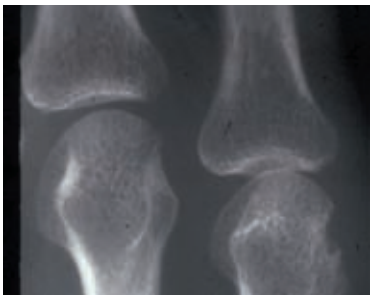


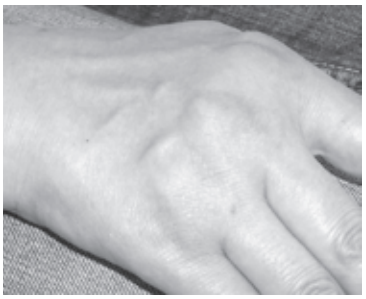

## ■ Clinical Background

Rheumatoid Arthritis – Getting the Best Outcome

## ■ Diagnostic Markers

Anti-Citrullinated Protein / Peptide Antibodies in Rheumatoid Arthritis

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Prediction of Rheumatoid Arthritis

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## Editorial

Rheumatoid arthritis (RA) is a widespread autoimmune disorder with a major impact on public health. Due to a substantial number of clinically similar diseases, diagnosis, particularly of recent-onset disease, is not always clear-cut. The classification criteria from the American College of Rheumatology were developed for established RA, but are not always useful for a differential diagnosis in the very early stage of the disease. However, an early diagnosis has been shown to be beneficial as new drugs not only improve patient wellbeing, but also influence the eventual outcome in terms of joint destruction if they are taken at an early stage of the disease. Nora Ng and Andrew Keat give a detailed overview of the clinical background and treatment, as well as the economic aspects of RA in their article on page 3.

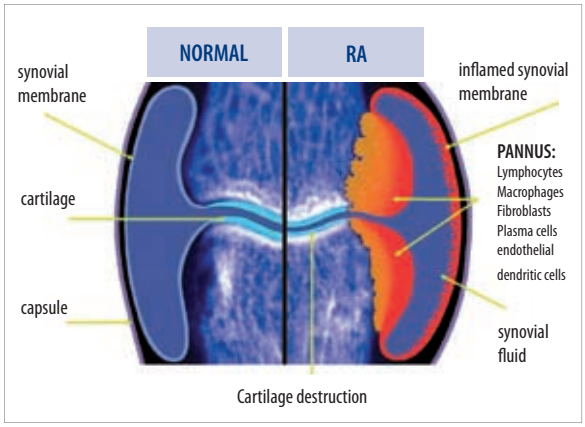
Although diagnosis of RA is mainly based on clinical features, serological markers that are highly specific and sensitive may be helpful. Rheumatoid factor is a well-established marker of RA although its specificity is limited. In contrast, the rather new marker anti-CCP is not only very sensitive but also highly specific for rheumatoid arthritis. Due to this significantly higher specificity, anti-CCP testing has the potential to better distinguish early RA from other non-RA inflammatory polyarthritides compared to rheumtoid factor. On page 9 we introduce a form which can be used to calculate a patient's prediction score for the development of RA – an easy-to-use tool to predict whether an undifferentiated arthritis is likely to develop into RA or not.

For a comprehensive review of anti-CCP and RA, please read the review from Dr. Nicola Bizzaro on page 6.

Since the first commercial anti-CCP assays were introduced to the market in 2000, many companies bought a licence or invented new kits for antibodies against citrullinated proteins or peptides. In their study on page 10, Dr. Benkhadra and Prof. Humbel from Luxembourg compared different commercial assays for the detection of anti-citrullinated peptides and proteins. EliA CCP was one of the tests which were evaluated and it showed an excellent performance.

Enjoy reading!

Nina Olschowka

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# Rheumatoid Arthritis – Getting the Best Outcome

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Rheumatoid arthritis (RA) affects around 387,000 people in the UK – roughly 0.8% of the adult population – with around 12,000 new cases per year. Principally a disease of adult women, RA may begin at any time of life: many of the 12,000 children with forms of juvenile arthritis will have arthritis which persists throughout life as RA. This is a disease of many costs – to the patient, in terms of pain, disability and spoiled opportunities, to society in terms of diminished workplace participation and to the taxpayer in terms of ever-increasing costs of medical and surgical care and social support.

## What is rheumatoid arthritis?

The central feature of RA is inflammation of synovium, the fine membrane that lines most of our joints, tendon sheaths and bursae. Synovium serves several functions, including phagocytosis of particulate matter within the joint cavity and production of synovial fluid. This proteinaceous fluid functions to minimise friction between the cartilage surfaces, ensuring a smooth range of movement of the joint, and to nourish the superficial avascular layer of cartilage which comprises the joint surfaces. Rheumatoid arthritis is slightly more common in family members of affected probands and genetic studies have demonstrated linkage between RA and the major histocompatibility complex class II antigens HLA-DR4 and DRBeta1. Although the actual initiating events are unknown, presentation of antigens by antigen-presenting cells via DR and costimulatory molecules to CD4 T cells in the joint is likely to be key to the activation of T cells with stimulation of macrophage and B cells with the release of a range of cytokines including tumour necrosis factor-α (TNF-α) and interleukins especially IL-1 and IL-6. These pro inflammatory cytokines contribute to both inflammation and the release of matrix metalloproteinases that are involved in synovial hyperplasia and the formation of pannus with damage or "erosion" of the underlying cartilage and bone. Patchy loss of cartilage leaves the underlying bone exposed. Cytokine-stimulated osteoclast activation also contributes to bone erosion. These events within the affected joints, if left unchecked, lead rapidly to chronic inflammation with progressive destruction of cartilage and bones which in turn causes pain, joint deformity and malfunction.

Rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti CCP) are autoantibodies commonly found in RA. RF, antibodies that react with the Fc portion of IgG, have been in the rheumatology world for longer than 60 years and can be detected in about 60% of RA. However, the specificity of RF as a diagnostic indicator for RA is substantially inferior compared to that of the new anti CCP antibodies, which are 97% specific. Autoantibodies in RA do not seem to play a major pathogenetic role, however, they have proven extremely useful as diagnostic tools and indicators of disease activity features.

## How does rheumatoid arthritis affect people?

RA has a variable effect on people. About 20% of patients have a mild form of the condition that is slow to progress and has relatively little impact on everyday life. On the other hand, 1 in 20 people with RA will develop rapidly progressive and destructive disease leading to persistent pain, joint deformity and substantial impairment of function. Those in between may suffer persistent pain with progressive joint damage which may continue throughout life. The first symptoms are often pain and swelling at many small joints in the hands and feet as well as associated stiffness after inactivity such as first thing in the morning. Any joint may be affected. Joint inflammation is often associated with more generalised symptoms such as malaise, tiredness and decline in general wellbeing.

Inflammation may also occur in tissues other than joints. Involvement of tendons and inflammation at bursae may also give rise to pain and dysfunction such as restricted finger movement; some individuals, especially those with high titre rheumatoid factor, develop chronic granulomas or "rheumatoid nodules" in the skin overlying bony points such as the elbow. Other organs may be involved including the lungs and pleura (leading to fibrosis and breathlessness, pleural nodules and effusion), the eyes (which occasionally leads to eyeball rupture) and even neuropathies. Extra-articular manifestations tend to be present in more aggressive forms of RA.

The anatomical distortion caused by RA often leads on to exhaustion, disabilities, especially impaired hand function, as well as difficulty with everyday tasks such as walking, climbing stairs, using a lavatory and bath. Loss of independence and inability to work and generate an income can be major problems, with 40% of people with severe RA giving up work within five years of the onset of disease. The combination of pain and stiffness, with very reasonable concerns about the future and the effects of treatment, often have a considerable psychological impact leading to low morale, anxiety and depression.

## Outcomes

The objective of treating RA is improvement of key outcomes for the patient. What outcomes? Most obviously, reduction of pain, stiffness and joint swelling is a major priority. So too is maintenance of joint and overall function, allowing people to live normal lives, stay at work and to remain independent. Key to achieving these is the prevention of joint damage. The most desirable outcome of all is complete remission or cure.

## How are outcomes measured?

There is a range of reproducible outcome measures in use. All are problematic, through being either too subjective, too limited in scope or too unwieldy for clinical practice. In consequence, composite measures that can be applied easily become the most used in everyday clinical practice. Some measures facilitate comparison between clinical trials to give a measure of a treatment's overall efficacy; the American College of Rheumatology (ACR) criteria measure improvement in the number of tender and swollen joints along with measures of acute phase response, physician and patient global assessments, assessment of pain on a visual analogue scale and a questionnaire-based assessment of function. Improvement is denoted as ACR 20, ACR 50 or ACR 70 reflecting respectively an improvement up to 20%, 50%, or 70% level in the parameters outlined. A similar composite score devised by the European League Against Rheumatism (EULAR) has given rise to a disease activity score (DAS) which is used as the basis for judging the severity of inflammatory activity of RA and response to treatment. This takes into account the number of swollen and tender joints, ESR or CRP and a subjective global health score completed by the patient. Functional status such as ability to dress and wash

can be assessed by using the Stanford health assessment questionnaire (HAQ). Health-related quality of life can also be assessed by longer, patient-completed inventories such as the SF-36 questionnaire.

More objective measures are also applied to the extent and progress of joint damage. Articular erosions can be counted on standard X-rays and these too can be recorded and measured either as erosion scores or by comparison with standard X-rays showing variable degrees of joint damage. More recently, early detection of erosions has become a critical part of assessing treatments aimed at prevention of joint damage and imaging using ultrasound or magnetic resonance imaging is playing a key role in this.

## Treatment options

A wide range of approaches and treatment modalities are available for people with RA. The acceptability of each is dependent on the likely or perceived disease outcome and on personal preferences. Treatment options include non-pharmacological, pharmacological and surgical approaches. Non–pharmacological treatment comprises of physiotherapy, occupational aids and adaptations, psychological support and patient education. Patient education is of extreme importance to enable patients genuinely to contribute to their own treatment by helping them better understand the nature of the condition.

Most people living with RA will take one or more drugs. Pain relief may be achieved by using the relatively safe analgesic compound paracetamol. Weak opioids such as codeine may help if paracetamol alone is inadequate. Combinations of these two drugs are often effective as they act on different pain modulators. Non-steroidal anti-inflammatory drugs (NSAIDs) reduce pain by damping down inflammation that causes joint swelling and stiffness. They act principally by blocking cyclooxygenase (COX) and hence, formation of proinflammatory prostaglandins. The identification of an inducible form of COX found at sites of inflammation –COX 2 – led to the development of selective NSAIDs that preferentially inhibit the COX 2 pathway leaving COX 1 to fulfil its regular "housekeeping" functions especially in gastroprotection. These agents, also known as coxibs, are associated with reduced gastrointestinal side effects often caused by traditional NSAIDs. However, reports that coxibs confer a higher risk of cardiovascular events have caused serious concern. While much remains to be done to clarify the relative risks and benefits of COX 2 inhibitors versus non-selective COX inhibitors, one recent study, the MEDAL study, has suggested that similar levels of cardiovascular risk are associated with both traditional NSAIDs and coxibs at normal therapeutic doses. Thus, rheumatologists and GPs should take care to identify and control other risk factors for cardiovascular disease and NSAIDs – like other medications – should be taken at the lowest necessary dose for the minimum necessary time. There is clearly still a valuable role for coxibs in RA especially for those at higher risk of gastrointestinal bleeding though these findings have been a useful reminder of the potential risks associated even with very familiar medications.

Although these drugs help with symptoms of pain and swelling, they do not affect the progression of structural damage. Corticosteroids and disease modifying antirheumatic drugs (DMARDs), on the other hand, do reduce structural progression but at a cost of potentially greater toxicity and a slow onset of action. Methotrexate is probably the most widely used DMARD which also serves as the best comparator for assessing the efficacy of new drugs. Although its mechanism is not entirely clear, as a cytotoxic agent it is likely that the effects of methotrexate may be mediated by reduction of cell proliferation, promotion of T cell apoptosis, increased release of endogenous adenosine and reduced production of proinflammatory cytokines. The inhibition of cellular proliferation which suppresses inflammation may also manifest as bone marrow suppression leading to sepsis, thrombocytopenia and anaemia. Other well known side effects of methotrexate include hepatotoxicity. Hence, regular blood tests are essential to monitor the potential development of these complications. However, even when patients tolerate the drugs well, a proportion of them still fail to have their arthritis adequately controlled despite maximal doses.

The introduction of biologics at the turn of the 21st century has revolutionised the treatment of RA. The discovery that TNFα plays a crucial role in the pathogenesis of RA has led to the development of specific inhibitors and their successful use in the treatment of RA. Three agents are currently available. Infliximab, a chimeric monoclonal antibody, binds specifically to TNFα and neutralizes the cytokine. Adalimumab works similarly with the difference that it is constructed from a fully human monoclonal antibody. Etanercept is a recombinant, human, soluble TNFα receptor that binds to TNFα and deactivates it. A landmark study in 1999 (ATTRACT) showed anti-TNFα drugs were remarkably effective at disease suppression in patients who have not responded to DMARDs. Moreover, it also showed that, not only was there symptomatic benefit, but that TNF blockade was effective in halting or reducing the progression of structural damage even in people whose disease had failed to be suppressed by a conventional DMARD. This dramatic outcome has changed thinking about the aims of treatment of RA, from "partial control" to prevention of damage and remission.

For those whose joints have already been damaged, surgery may still provide the prospect of salvation. Deformities may be corrected and joints replaced. The results of widespread joint replacement surgery, especially of the hip and knee, have changed the face of RA within three decades and allowed many thousands of people with RA to maintain independence and quality of life but at substantial personal and financial cost. It is not unrealistic now to expect that the effective prevention of joint damage and induction of remission should lead to a dramatic decline in the need for surgical treatment of RA in the near future albeit with a shift of expenditure from surgery and social support to medication.

## The key to good outcomes

Early, effective treatment produces a better outcome. Given the irreversible and cumulative nature of joint damage over time, treatment should be commenced before considerable structural damage occurs. The key to this is recognition of the disease early in its metamorphosis and pre-

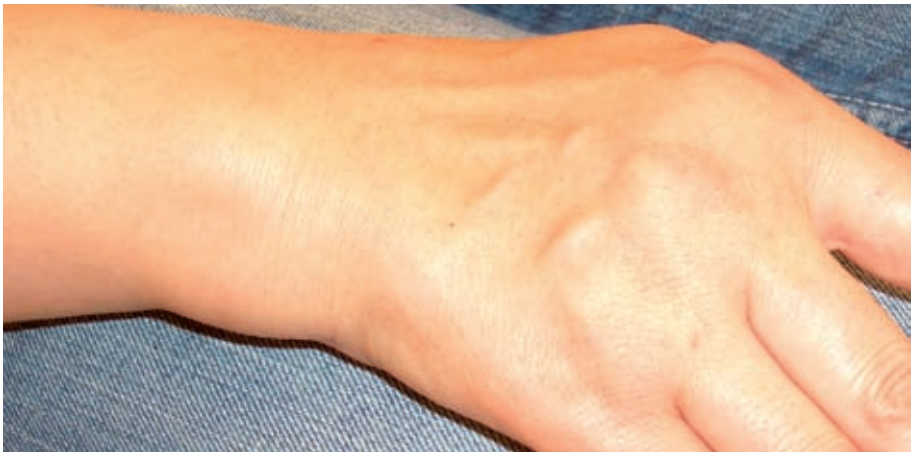

***Fig. 1:*** *hand affected by RA*

diction of those who will develop destructive disease. The advent of biomarkers has helped clinicians with this task. Three biomarkers contribute to this prediction of the likely course of untreated disease: The presence of anticyclic citrullinated peptide (anti-CCP) antibodies, rheumatoid factor antibody or shared epitope in the right clinical context is suggestive of the development of RA.

High titre rheumatoid factor has been associated with RA for many years but is relatively insensitive and non-specific as a guide to both diagnosis and prognosis. Anti-CCP antibodies have a similar sensitivity profile as rheumatoid factor but are far more specific (97%) for predicting the future development of RA. The presence of anti-CCP antibodies also predicts a more aggressive and destructive disease course**.** Shared epitope is a particular sequence of amino acids on the 3rd hypervariable region of the DRβ chain. The shared epitope confers a higher risk of developing RA and is also prognostic marker for a more severe clinical course of the disease. Other known markers of poor outcome include extensive joint involvement and radiological erosions when the patient is first seen, high levels of inflammatory markers, poor functional scores at outset, as well as poor socioeconomic circumstances and lower education level.

The duration of disease does have an affect on the response to therapy. Patients with longer disease duration respond less well than those with early disease. A delay in starting treatment of just four months allows many patients to develop irreversible structural damage. Moreover, early treatment with methotrexate in anti-CCP positive patients with undifferentiated arthritis can reduce the likelihood of evolution to full blown RA.

Good outcome from RA is linked to optimum suppression of synovitis at the outset. The "tight control" (TICORA) study has shown that intensive management of RA with DMARDs improves disease activity and increases rates of remission. However, it is clear that clinical remission does not equate to the abolition of progressive structural damage; even when RA appears in clinical remission, some patients will still develop new joint erosions and progressive structural damage. New imaging techniques using MRI and ultrasound are more sensitive in detecting synovitis and erosions than clinical examination and plain radiographs. It is thanks to these new imaging techniques that early aggressive treatment can be targeted at individuals with early joint damage which may be at a reversible stage.

Several major trials have now confirmed that anti-TNF drugs work best in combination with methotrexate so far as persisting benefit and prevention of structural damage is concerned. Hence it is logical to treat RA with this combination at the outset rather than attempt to control it in the later stages.

Aside from structural benefits, evidence is also emerging about economic benefits of early intervention with biologics. Preliminary studies suggest that, in some patients with early RA, anti-TNF agents can be withdrawn after a year of therapy whilst clinical benefit remains for at least two years. A similar approach with one TNF blocker, infliximab, combined with methotrexate, may be even better in terms of inducing lasting remission even when the infliximab is stopped after one year. Such studies suggest that the induction of truly drug-free remission is a real prospect. These are exciting data that suggest that when treating patients with early RA aggressively with anti-TNF drugs, there is a good chance they will achieve biologic free remission. However, much remains to be done both to confirm this expectation and to clarify the extent of the immediate and long-term risks so that the risks and benefits of early treatment can be properly analysed and weighed in individual cases.

For those patients who do not respond to an initial anti-TNF agent, there is now clear evidence that there may still be a response to an alternative TNF blocker. For those who still fail to respond, yet more new alternatives are being introduced. Rituximab, a B cell depleter, is available as a therapy for patients who failed anti TNF agents, abatacept, a co-stimulatory inhibitor, has just obtained its license for the same indication and a range of other biologics, including an IL-6 inhibitor, also have valuable efficacy against RA.

## The economics of RA

The direct costs of treating RA combined with indirect costs, including work-related disability, amount to about £4.7 billion (=6.5 billion Euro) per year in the UK. RA is also associated with increased cardiovascular risks and haematological malignancies, adding on to the indirect costs of treatment. At a current cost of around £10,000 (=13,800 Euro) per annum, per person, biological therapy has a lot to prove if it is to be considered good value for the NHS. Over and above the evident personal benefits to patients, several indicators of good value are emerging. Prevention of chronic disability may well lead to reductions in health and social care costs. Clear data have emerged to demonstrate that work disability is also reduced amongst anti-TNF treated patients with RA. With 9.4 million working days lost due to RA in 1999-2000 at a cost of £833 million (=1,15 billion Euro) in lost production,

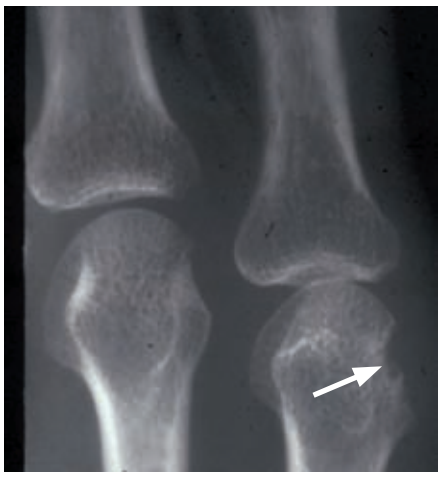

***Fig. 2:*** *X-ray of hand joints in RA showing bony erosion*

keeping people with RA at work will have potentially huge economic benefits. It is too early to know whether the need for surgical treatment will decline as a result of biologic therapy; if it does there will be a very significant reduction in overall costs associated with the care of people with RA. Moreover, if it really proves possible to identify people who will benefit from early anti-TNF therapy and to withdraw treatment after a relatively short period, the cost of treatment will be reduced very substantially.

## The future

The development of biologics has impacted on the future management of people with RA in several ways. Undoubtedly the prospects of less severe disease is real and may be improved further by newer agents still in development. Secondly, treatment is increasingly aimed at induction of complete remission with the real prospect of converting RA into a much more benign entity. Thirdly, attention can also now shift towards prevention. The finding that autoantibodies including anti-CCP may be detectable up to 10 years before presentation raises the possibility of screening for RA before any significant changes have occurred and introducing truly preventive treatment.

Advances in therapeutics, however, need to be balanced by better understanding of the risks of treatment and by improved access of patients to specialist care. The establishment of early arthritis clinics will go some way to meeting this need but care will have to be taken to ensure that changes in the delivery of healthcare recognise the huge opportunities and benefits that early diagnosis and targeted treatment are likely to bring in the near future. The issue of cost will not go away; there remains an urgent need to measure the true costs and benefits of treatment of RA in the broadest societal sense so that even the tax payer can be seen to get a fair deal.

# Antibodies to Citrullinated Peptides: A Significant Step Forward in Early Diagnosis of Rheumatoid Arthritis

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Summarized from the review in Clin Chem Lab Med 2007 (1)

**The availability of laboratory tests able to distinguish between rheumatoid arthritis (RA) and other forms of arthritis is clinically of major importance, especially in the early stages of the disease, when joint lesions that lead to irreversible damage have not yet developed and immunosuppressant treatment is effective. The development of the anti-cyclic citrullinated peptide (CCP) antibody assay, a highly disease-specific serological marker for RA, has been a great step forward. This review briefly considers the most recent data on the diagnostic accuracy of the CCP test, the diagnostic, prognostic and predictive values, and the clinical use of the assay in patients with RA.**

## Anti-filaggrin antibodies

The first description of filaggrin antibodies in the serum of patients with RA dates back to 1964, when Nienhuis and Mandema, using an indirect immunofluorescence technique, detected specific antibodies directed against unknown proteins inside the keratohyalin granules that surround the nucleus of buccal mucosa cells, and were therefore called anti-perinuclear factor antibodies (APF) (2). In view of its high specificity, the test immediately proved particularly useful, especially in the diagnosis of RA that tests negative for RF, but it has not been widely used because of the difficulty of preparing and storing cells (3).

In 1979, Young et al. described anti-keratin antibodies (4), which also possess high specificity for RA (5, 6). Later it was demonstrated that anti-keratin antibodies and APF were actually the same antibodies, and that both were directed against filaggrin (filament aggregating protein) (7–9). Since then, all these antibodies have been described as anti-filaggrin antibodies (AFA).

## Citrullinated proteins

AFA reactivity proved to be dependent on the citrullination of profilaggrin (10, 11), a biochemical process that takes place when profilaggrin, after a post-translational modification, is dephopsphorylated and some 20% of its positively charged arginine residues are deiminated to negatively charged citrulline. Following these modifications, profilaggrin is cleaved into a series of filaggrin fragments that contain citrullinated residues recognized by the specific antibodies.

Anti-citrulline antibodies are mainly produced in inflamed synovial tissue by specific plasma cells (12). However, as filaggrin is not present in synovial tissue, it is obvious that other citrullinated proteins are responsible for genesis of the autoantibodies, and that their reactivity towards citrullinated filaggrin is due to cross-reactivity (13). Different antigens are discussed as possible targets of the anti-citrulline antibodies in synovial tissue such as citrullinated fibrin or citrullinated alpha-enolase (13, 14).

Another system specific for RA is that of the anti-Sa antibodies, described by Després et al. in 1994 (15). These antibodies have a sensitivity of approximately 40% and specificity of 97%, similar to AFA (15, 16). Molecular analysis has demonstrated that the Sa antigen is actually citrullinated vimentin (17).

## Anti-citrullinated peptide antibodies and the anti-CCP test

In 1998, Schellekens et al. produced synthetic linear citrullinated peptides derived from filaggrin, with a diagnostic sensitivity for RA of about 50% and specificity of 97% in ELISA (10). To structurally improve its antigen composition and consequent antibody recognition, a cyclic peptide (cyc-cf1 peptide) was developed, which showed much better sensitivity (68%) without any loss of specificity (18). This citrullinated cyclic peptide was used in first-generation ELISA CCP tests. Second- and third-generation tests using mixtures of synthetic cyclic peptides have further increased sensitivity significantly (around 80%), while maintaining very high specificity of 98%-99% (19-27).

## Specificity of anti-CCP antibodies

Different studies confirm that the second-generation CCP test (CCP2) has the same sensitivity as RF, but with higher specificity, even in elderly patients who present a higher percentage of false positivity to RF (28). One example is the study by De Rycke et al., who compared the two tests: at predetermined specificity of 98.5%, the sensitivity of RF was only 12.8%, compared with 73.7% for the CCP2 test (29).

The CCP test is particularly useful in differential diagnosis between RA and other forms that are clinically similar to RA. For example, a significant number of patients with chronic hepatitis C present symmetrical polyarthritis, which closely resembles RA and is RF-positive, but anti-CCP negative (30). The polyarthritis present in some patients with systemic lupus erythematosus (SLE) can also be mistaken for symptoms of RA. In a group of patients with SLE, ten of whom had erosive joint lesions, Mediwake et al. found six positive for RF, and only two positive for anti-CCP. Of the patients with non-erosive arthritis, only 0.5% were anti-CCP positive, compared with 18% who were RF positive (31). Comparable findings exist for Sjögren's syndrome, where 59% and 73%, respectively, were found to have RF antibodies, whereas only 7% and 3%, respectively, were anti-CCP positive (32, 33).

## Early appearance and predictive value of anti-CCP antibodies

Recent data indicate that the anti-CCP test is not only highly specific, but also very useful for early diagnosis. A landmark study conducted in Sweden (34), which analyzed serum stored for 10 years in blood banks from 83 former blood donors who later developed RA, demonstrated that the antibodies were present up to 10 years before the appearance of the first symptoms, with a sensitivity progressively increasing and reaching 70% at the time of first examination.

Nielen et al. found anti-CCP antibodies even up to 14 years before the appearance of RA symptoms (35). RF was also present before clinical onset of the disease, but its positivity was more recent, and the percentage of patients at the time of diagnosis was lower than for anti-CCP (28% vs. 43%). Two recent longitudinal studies further confirm that anti-CCP antibodies are detectable in serum many years before clinical diagnosis of RA (36, 37). This means that citrullination of antigens in

the synovial membrane begins many years before the appearance of symptoms and development of the disease; it is therefore useful for diagnostic purposes to repeat the test for anti-citrulline antibodies in the serum of patients who tested negative at the outset.

Jansen et al. also demonstrated that the presence of anti-CCP antibodies can predict the subsequent development of RA; they found that simultaneous positivity for RF IgM and anti-CCP antibodies (55.4% sensitivity and 97% specificity) can select which patients with early arthritis will develop RA (38). In a similar study conducted by van Gaalen et al. on 318 patients with undifferentiated arthritis, 127 (40%) had developed a clinical picture of RA after 3 years follow-up (39). However, 25% of the CCP-negative patients but 93% of the patients who had tested anti-CCP positive at the outset were diagnosed with RA (odds ratio 37.8). Similar findings were also reported by Vittecoq et al. for 314 patients with early arthritis, of whom 90% of those who had tested anti-CCP positive at the outset went on to develop RA one year later (40).

## Prognostic significance of anti-CCP antibodies

Anti-CCP antibodies have shown a high predictive value for the development of erosive joint lesions.

In a prospective study conducted on 524 patients with arthritis in the initial stage, Visser et al. (41) analyzed numerous clinical and seroimmunological parameters to establish which of them most accurately predicted the development of erosive lesions in patients with arthritis 2 years before the appearance of the first symptoms. They devised a clinical model able to discriminate between three different forms of development of arthritis from the data obtained, processed with the logistical regression technique: self-limiting arthritis with spontaneous resolution, persistent non-erosive arthritis, and persistent erosive arthritis. The results of the study demonstrated that only two of the clinical and laboratory parameters predicted the development of persistent arthritis accurately: duration of the symptoms for over 6 months, and the presence of anti-CCP antibodies at the outset. However, anti-CCP antibodies proved to be the only parameter which correlated with the development of persistent erosive arthritis. This led the authors to propose a change in the international criteria to introduce positivity for anti-CCP antibodies as a diagnostic (and not only classifying) criterion for RA from the outset.

Lately a related group from Leiden University introduced a prediction model for patients with undifferentiated arthritis (42). In this study a prediction score was calculated for patients with undifferentiated arthritis based on 9 variables that are commonly assessed during the first visit: age, sex, distribution of involved joints, morning stiffness, number of tender or swollen joints, CRP level, and the presence of RF and anti-CCP antibodies. The range of possible scores is 0–14, with higher scores indicating a greater risk of developing RA. Anti-CCP antibody positivity had the biggest influence of all parameters with 2 score points.

In addition to these two studies, many other studies have confirmed the prognostic value of anti-CCP antibodies in predicting the development of RA, especially the erosive forms (29, 43, 44). Forslind et al. measured anti-CCP2 antibodies at the time of diagnosis in 379 patients with RA, and then radiologically measured the development of erosive lesions during a 2-year follow-up. They found that the presence of anti-CCP2 antibodies was associated with more serious erosive syndromes than the presence of RF or other parameters (45). Using a similar approach for a population of 242 patients with RA of recent onset, Kastbom et al. demonstrated that anti-CCP antibodies showed diagnostic sensitivity equal to that of RF, but a higher predictive value for the appearance of erosive lesions 3 years later. (43). Finally, Chan et al. found that anti-CCP positivity also indicated a fourfold risk of developing erosive arthritis in patients with SLE (44).

In conclusion, all these studies indicate that the presence of anti-CCP antibodies is correlated with the development of erosive lesions, and that this marker has considerable clinical potential in selecting patients with early arthritis who should receive aggressive treatment.

## Anti-CCP antibodies in the monitoring of treatment

Despite their high predictive value of erosive arthritis, it is not yet clear whether anti-citrulline antibodies are a useful parameter in monitoring treatment that has proven particularly effective in limiting or reducing the progression of osteolytic lesions and improving patients' quality of life (46–48). Bobbio-Pallavicini et al. prospectively monitored a group of patients with RA during treatment with infliximab, a chimeric monoclonal antibody that acts by inhibiting tumor necrosis factor-alpha (TNF-alpha), and found that while RF levels fell during the treatment and as the clinical picture improved, anti-CCP levels returned to pre-treatment values after an initial decline, despite improvements in the clinical condition of patients (49). In the above mentioned study by Kastbom et al., levels of anti-CCP remained unchanged for 3 years after diagnosis and during treatment with anti-rheumatic drugs (43). Both De Rycke et al. (50) and Caramaschi et al. (51), who studied patients treated with infliximab who failed to respond to conventional treatment, observed a definite reduction in RF, but no change in anti-CCP levels, whereas Alessandri et al. (52) observed a significant reduction in anti-CCP antibodies from the 43rd week of treatment. Finally, in a study by Mikuls et al. (53) it was observed that anti-CCP antibodies fell by 25%-50% in the first year of the disease, after which they remained stable, and that the reduction was not correlating with the type of treatment or clinical course, whereas RF declined progressively and correlated with the clinical course.

In general, these findings indicate that anti-CCP antibodies are not useful in monitoring treatment; they also suggest that RF and anti-CCP are two different antibody systems, and that while RF production can be modulated through the inhibition of TNF-alpha, anti-CCP production is probably independent of TNF-alpha.

## Clinical use of anti-CCP antibodies

Anti-CCP antibodies are undoubtedly among the most interesting antibodies to have been identified in recent years. Their high specificity and high positive predictive value, associated with their prognostic significance for the development of progressive erosive arthritis, allow their use for both diagnostic and prognostic purposes in RA patients. According to the new recommendations of EULAR (European League Against Rheumatism) for RA diagnosis and management, CCP antibodies should be measured in every patient presenting with early arthritis as a factor to predict persistent and erosive disease (54). The fact that this test is positive in some 20% of RF-negative RA patients (20, 24, 26, 55, 56) suggests its use as a first-level test in patients with suspected RA.

Equally, 15%-20% of patients with RA only test positive for RF, and not for anti-CCP (33, 57, 58); the combined use of the two tests therefore significantly increases the diagnostic sensitivity of the immunoassays. Simultaneous positivity for RF and anti-CCP antibodies also increases the specificity and positive predictive value to almost 100% (19). This means that a patient who tests positive for RF and anti-CCP has an approximately 99% probability of suffering from RA, and a high probability of developing erosive lesions during the disease (59).

## Conclusions

Emerging data strongly suggest that anti-CCP antibodies have the power to predict the development of RA in patients with early arthritis, the severity of disease in patients with established RA, and the possibility of future onset of RA in high-risk populations. These findings have important diagnostic and therapeutic implications because clinicians are now using more aggressive forms of therapy early in the course of disease to reduce the long-term morbidity associated with RA. Automation of the assay and worldwide use in clinical laboratories will further increase the diagnostic potential of this test, thereby improving the early serological diagnosis of RA and eventually its clinical management.

## References


1. **Bizzaro N (2007)** Antibodies to citrullinated peptides: a significant step forward in the early diagnosis of rheumatoid arthritis. Clin Chem Lab Med 45:150–157
2. **Nienhuis RL, Mandema EA (1964)** A new serum factor in patients with rheumatoid arthritis. The antiperinuclear factor. Ann Rheum Dis 23:302–305
3. **Youinou P, Le Goff P, Maran R (1996)** Perinuclear factor (profilaggrin) autoantibodies. In: Peter JB, Shoenfeld Y (eds). Autoantibodies. Elsever Science BV, Amsterdam, pp 618-623
4. **Young BJ, Mallya RK, Leslie RD et al (1979)** Antikeratin antibodies in rheumatoid arthritis. Br Med J 2:97–99
5. **Serre G, Vincent C (1996)** Filaggrin (keratin) autoantibodies. In: Peter JB, Shoenfeld Y (eds). Autoantibodies. Elsever Science BV, Amsterdam, pp 271-276
6. **Miossec P, Youinou P, Le Goff P, Moineau MP (1982)** Clinical relevance of antikeratin antibodies in rheumatoid arthritis. Clin. Rheumatol 1:185–189
7. **Simon M, Girbal E, Sebbag M et al (1993)** The Cytokeratin Filament-Aggregating Protein Filaggrin Is the Target of the So-Called Antikeratin Antibodies, Autoantibodies Specific for Rheumatoid Arthritis. J Clin Invest 92:1387-1393
8. **Sebbag M, Simon M, Vincent C et al (1995)** The antiperinuclear factor and the so-called antikeratin antibodies are the same rheumatoid arthritis-specific autoantibodies. J Clin Invest 95:2672-2679
9. **Hoet RM, Boerbooms AM, Arends M et al (1991)** Antiperinuclear Factor, a Marker Autoantibody for Rheumatoid Arthritis - Colocalisation of the Perinuclear Factor and Profilaggrin. Ann Rheum Dis 50:611-618
10. **Schellekens GA, De Jong BA, Van den Hoogen FH et al (1998)** Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. J Clin Invest 101:273-281
11. **Girbal Neuhauser E, Durieux JJ, Arnaud M et al (1999)** The Epitopes Targeted by the Rheumatoid Arthritis Associated Antifilaggrin Autoantibodies are Posttranslationally Generated on Various Sites of (Pro)Filaggrin by Deimination of Arginine Residues. J Immunol 162:585-549
12. **Masson Bessiere C, Sebbag M, Durieux JJ et al (2000)** In the rheumatoid pannus, anti-filaggrin autoantibodies are produced by local plasma cells and constitute a higher proportion of IgG than in synovial fluid and serum. Clin Exp Immunol 119:544-552
13. **Vossenaar ER, Nijenhuis S, Helsen MM et al (2003)** Citrullination of synovial proteins in murine models of rheumatoid arthritis. Arthritis Rheum 48:2489-2500
14. **Kinloch A, Tatzer V, Wait R et al (2005)** Identification of alpha-enolase as a candidate autoantigen in rheumatoid arthritis. Arthritis Res Ther 7:R1421-R1429
15. **Despres N, Boire G, Lopez Longo FJ et al (1994)** The Sa system: A novel antigen-antibody system specific for rheumatoid arthritis. J Rheumatol 21:1027-1033
16. **Hayem G, Chazerain P, Combe B et al (1999)** Anti-Sa antibody is an accurate diagnostic and prognostic marker in adult rheumatoid arthritis. J Rheumatol 26:7-13
17. **Asaga H, Yamada M, Senshu T (1998)** Selective deimination of vimentin in calcium ionophore-induced apoptosis of mouse peritoneal macrophages. Biochem Biophys Res Commun 243:641-646
18. **Schellekens GA, Visser H, De Jong BA et al (2000)** The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide (and comment by Berthelot,J.M. et al.). Arthritis Rheum 43:155-163
19. **Bizzaro N, Van Venrooij WJ (2002)** Anti-citrulline antibodies: higher sensitivity of second generation immunoassays. Riv Med Lab 2:255
20. **Van Venrooij WJ, Hazes JM, Visser H (2002)** Anticitrullinated protein/peptide antibody and its role in the diagnosis and prognosis of early rheumatoid arthritis. Neth J Med 60:383-388
21. **Suzuki K, Sawada T, Murakami A et al (2003)** High diagnostic performance of ELISA detection of antibodies to citrullinated antigens in rheumatoid arthritis. Scand J Rheumatol 32:197-204
22. **Lee DM, Schur PH (2003)** Clinical utility of the anti-CCP assay in patients with rheumatic diseases. Ann Rheum Dis 62:870-874
23. **Pinheiro GC, Scheinberg MA, da Silva MA, Maciel S (2003)** Anti-cyclic citrullinated peptide antibodies in advanced rheumatoid arthritis. Ann Intern Med 139:234-235
24. **Vasishta A (2002)** Diagnosing early-onset rheumatoid arthritis: The role of anti-CCP antibodies. Am Clin Lab Aug/Sep:34-36
25. **van Gaalen FA, Visser H, Huizinga TW (2005)** A comparison of the diagnostic accuracy and prognostic value of the first and second anti-cyclic citrullinated peptides (CCP1 and CCP2) autoantibody tests for rheumatoid arthritis. Ann Rheum Dis 64:1510-1512
26. **Greiner A, Plischke H, Kellner H, Gruber R (2005)** Association of anti-cyclic citrullinated peptide antibodies, anti-citrulline antibodies, and IgM and IgA rheumatoid factors with serological parameters of disease activity in rheumatoid arthritis. Ann N Y Acad Sci 1050:295-303
27. **Fernandez Suarez A, Reneses S, Wichmann I et al (2005)** Efficacy of three ELISA measurements of anti-cyclic citrullinated peptide antibodies in the early diagnosis of rheumatoid arthritis. Clin Chem Lab Med 43:1234-1239
28. **Palosuo T, Tilvis R, Strandberg T, Aho K (2003)** Filaggrin related antibodies among the aged. Ann Rheum Dis 62:261-263
29. **De Rycke L, Peene I, Kruithof E et al (2004)** Rheumatoid factor and anticitrullinated protein antibodies in rheumatoid arthritis: diagnostic value, associations with radiological progression rate, and extra-articular manifestations. Ann Rheum Dis 63:1587-1593
30. **Leone N, Pellicano R, Ariata Maiocco I et al (2002)** Mixed cryoglobulinaemia and chronic hepatitis C virus infection: the rheumatic manifestations. J Med Virol 66:200-203
31. **Mediwake R, Isenberg DA, Schellekens GA, Van Venrooij WJ (2001)** Use of anti-citrullinated peptide and anti-RA33 antibodies in distinguishing erosive arthritis in patients with systemic lupus erythematosus and rheumatoid arthritis. Ann Rheum Dis 60:67-68
32. **Gottenberg JE, Mignot S, Nicaise Roland P 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
33. **Sauerland U, Becker H, Seidel M et al (2005)** Clinical Utility of the Anti-CCP Assay - Experiences with 700 Patients. Ann N Y Acad Sci 1050:314-318
34. **Rantapää Dahlqvist S, De Jong BA, Berglin E et al (2003)** Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 48:2741-2749
35. **Nielen MM, Van Schaardenburg D, Reesink HW et al (2004)** Specific Autoantibodies Precede the Symptoms of Rheumatoid Arthritis. A Study of Serial Measurements in Blood Donors. Arthritis Rheum 50:380-386
36. **Mandl LA, Costenbader K, Chibnik L et al (2005)** Anti-cyclic citrullinated peptide (anti-CCP) antibodies are strongly associated with risk of rheumatoid arthritis after adjusting for hormonal and behavioral factors. Arthritis Rheum 52:S732
37. **Deane KD, Majka DS, Parrish LA et al (2005)** RA-related antibodies appear earlier in the pre-clinical period in subjects with an older age at disease onset. Arthritis Rheum 52:S154
38. **Jansen LM, Van Schaardenburg D, van der Horst Bruinsma IE et al (2003)** The Predictive Value of Anti-Cyclic Citrullinated Peptide Antibodies in Early Arthritis. J Rheumatol 30:1691-1695
39. **van Gaalen FA, Linn Rasker SP, Van Venrooij WJ et al (2004)** Autoantibodies to Cyclic Citrullinated Peptides Predict Progression to Rheumatoid Arthritis in Patients With Undifferentiated Arthritis. A Prospective Cohort Study. Arthritis Rheum 50:709-715
40. **Vittecoq O, Incaurgarat B, JouenBeades F et al (2004)** Autoantibodies recognizing citrullinated rat filaggrin in an ELISA using citrullinated and non-citrullinated recombinant proteins as antigens are highly diagnostic for rheumatoid arthritis. Clin Exp Immunol 135:173-180
41. **Visser H, le Cessie S, Vos K et al (2002)** How to diagnose rheumatoid arthritis early: a prediction model for persistent (erosive) arthritis. Arthritis Rheum 46:357-365
42. **van der Helm van Mil AH, le Cessie S, van Dongen H et al (2007)** A Prediction Rule for Disease Outcome in Patients With Recent-Onset Undifferentiated Arthritis. Arthritis Rheum 56:433-440
43. **Kastbom A, Strandberg G, Lindroos A, Skogh T (2004)** Anti-CCP antibody test predicts the disease course during 3 years in early rheumatoid arthritis (The Swedish TIRA project). Ann Rheum Dis 63:1085-1089
44. **Chan MT, Owen P, Dunphy J et al (2005)** Anti-cyclic citrullinated peptide antibodies are associated with erosive arthritis in SLE. Arthritis Rheum 52:S611
45. **Forslind K, Ahlmen M, Eberhardt K et al (2004)** Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (Anti-CCP). Ann Rheum Dis 63:1090-1095
46. **Lard LR, Visser H, Speyer I et al (2001)** Early versus delayed treatment in patients with recent-onset rheumatoid arthritis: comparison of two cohorts who received different treatment strategies. Am J Med 111:446-451
47. **Nell V, Machold KP, Eberl G et al (2004)** Benefit of very early referral and very early therapy with disease-modifying anti-rheumatic drugs in patients with early rheumatoid arthritis. Rheumatology 43:906-914
48. **Maini RN, Breedveld FC, Kalden JR et al (2004)** Sustained improvement over two years in physical function, structural damage, and signs and symptoms among patients with rheumatoid arthritis treated with infliximab and methotrexate. Arthritis Rheum 50:1051-1065
49. **Bobbio Pallavicini F, Alpini C, Caporali R et al (2004)** Autoantibody profile in rheumatoid arthritis during long-term infliximab treatment. Arthritis Res Ther 6:R264-R272
50. **De Rycke L, Verhelst X, Kruithof E et al (2005)** Rheumatoid factor, but not anti-cyclic citrullinated peptide antibodies, is modulated by infliximab treatment in rheumatoid arthritis. Ann Rheum Dis 64:299-302
51. **Caramaschi P, Biasi D, Tonolli E et al (2005)** Antibodies against cyclic citrullinated peptides in patients affected by rheumatoid arthritis before and after infliximab treatment. Rheumatol Int 26:58-62
52. **Alessandri C, Bombardieri M, Papa N et al (2004)** Decrease of anti-cyclic citrullinated peptide antibodies and rheumatoid factor following anti-TNF alpha therapy (Infliximab) in rheumatoid arthritis is associated with clinical improvement. Ann Rheum Dis 63:1218-1221
53. **Mikuls TR, ODell JR, Stoner JA et al (2004)** Association of rheumatoid arthritis treatment response and disease duration with declines in serum levels of IgM rheumatoid factor and anti-cyclic citrullinated peptide antibody. Arthritis Rheum 50:3776-3782
54. **Combe B, Landewe R, Lukas C et al (2007)** EULAR recommendations for the management of early arthritis: report of a task force of the European Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT). Ann Rheum Dis 66:34-45
55. **Kroot EJ, De Jong BA, Van Leeuwen MA et al (2000)** The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. Arthritis Rheum 43:1831-1835
56. **Sivhonen S, Korpela M, Mustila A, Mustonen J (2005)** The predictive value of rheumatoid factor isotypes, anti- cyclic citrullinated peptide antibodies, and antineutrophil cytoplasmic antibodies for mortality in patients with rheumatoid arthritis. J Rheumatol 32:2089-2094
57. **Vossenaar E R, Van Venrooij WJ, Pruijn GJ (2002)** Anti-CCP antibodies in (early) rheumatoid arthritis. Conrad K, Fritzler M, Meurer M, Sack U, Shoenfeld Y (eds) From proteonomics to molecular epidemiology: relevance of autoantibodies, Lengerich, Pabst Science Publishers, pp 454-462
58. **Bizzaro N, Mazzanti G, Tonutti E et al (2001)** Diagnostic accuracy of the anti-citrulline antibody assay for rheumatoid arthritis. Clin Chem 47:1089-1093
59. **Vencovsky J, Machacek S, Sedova L et al (2003)** Autoantibodies can be prognostic markers of an erosive disease in early rheumatoid arthritis. Ann Rheum Dis 62:427-430

# Prediction of Rheumatoid Arthritis

## A prediction model described in Van der Helm-van Mil AHM et al. Arthritis Rheum 2007; 56: 433-440

Making individualized decisions regarding treatment is one of the most important challenges in medicine. In rheumatology practices, the majority of patients who present with recent-onset arthritis have undifferentiated arthritis, which is a form of arthritis that does not fulfil the classification criteria for a more definitive diagnosis. About 40 to 50 % of patients with undifferentiated arthritis experience spontaneous remission, whereas one third of patients will go on to be classified as having rheumatoid arthritis (RA). New treatment protocols have put greater importance on the need for early diagnosis and therapy of RA patients but their costs and side-effects means that they must be restricted to those where RA diagnosis and probable progression can be confirmed. There are different approaches to introduce a prediction model for patients with undifferentiated arthritis. Van der Helm-van Mil et al. developed a prediction rule for disease outcome which proved to be easy-to-use and of high clinical utility (see figure 1).

None of the patients who had a prediction score below 3 progressed to RA during the 1-year follow-up period, and all of the patients who had a score above 11 did experience progression to RA. Among the patients with a score between 3 and 11, the frequency of a progression to RA increased with rising scores (see table 1).

In an external validation cohort, the pogression score at baseline was calculated for 36 patients with undifferentiated arthritis. If treatment decisions were based on the prediction rule using cut-off levels of 8 or higher for initiating treatment and 6 or less for withholding treatment, treatment would have been withheld inaccurately from only 6% of the patients, and no patient would have received treatment inaccurately.

Thus, with this easy-to-use tool, the risk of developing RA can be predicted, thereby allowing individualized decisions regarding the initiation of treatment with disease-modifying antirheumatic drugs.

| | | |
|---|---|---|
| Age in years; multiple by 0.02 | age x 0.02 points | _____ |
| Sex female | 1 point | _____ |
| Distribution of involved joints | | |
| In case small joints hands/feet | 0.5 point | _____ |
| In case symmetric | 0.5 point | _____ |
| In case upper extremities | 1 point | _____ |
| In case upper and lower extremities: | 1.5 points | _____ |
| Score for morning stiffness on a 100-mm VAS | | |
| In case 26-90 mm | 1 point | _____ |
| In case >90 mm | 2 points | _____ |
| Number of tender joints | | |
| In case 4-10 | 0.5 points | _____ |
| In case >10 | 1 point | _____ |
| Number of swollen joints | | |
| In case 4-10 | 0.5 points | _____ |
| In case >10 | 1 point | _____ |
| C-reactive protein level | | |
| In case 5-50 mg/liter | 0.5 points | _____ |
| In case >50 mg/liter | 1.5 points | _____ |
| Rheumatoid factor positive | 1 point | _____ |
| CCP antibodies positive | 2 points | _____ |
| | **Total score:** | _____ |

*Figure 1. Form used to calculate a patient's prediction score. The range of possible scores is 0 to 14, with higher scores indicating a greater risk of developing RA. VAS = visual analog scale; anti-CCP = anti-cyclic citrullinated peptide antibodies.*

| Prediction score | No progression to RA (n= 387) | | Progression to RA (n= 175) | |
|---|---|---|---|---|
| | Number of patients | % | Number of patients | % |
| 0 | 1 | 100 | 0 | 0 |
| 1 | 8 | 100 | 0 | 0 |
| 2 | 42 | 100 | 0 | 0 |
| 3 | 58 | 100 | 0 | 0 |
| 4 | 78 | 93 | 6 | 7 |
| 5 | 73 | 85 | 13 | 15 |
| 6 | 63 | 74 | 22 | 26 |
| 7 | 37 | 49 | 38 | 51 |
| 8 | 16 | 33 | 33 | 67 |
| 9 | 6 | 14 | 36 | 86 |
| 10 | 5 | 23 | 17 | 77 |
| 11 | 0 | 0 | 8 | 100 |
| 12 | 0 | 0 | 1 | 100 |
| 13 | 0 | 0 | 1 | 100 |
| 14 | 0 | 0 | 0 | 0 |

*Table 1. Prediction scores and progression or nonprogression to RA*

## Reference:

**Van der Helm-van Mil AHM, le Cessi S, van Dongen H, et al (2007)** A prediction rule for disease outcome in patients with recent-onset undifferentiated arthritis. Arthritis Rheum 56: 433-440

# Anti-Citrullinated Protein / Peptide Antibodies in Rheumatoid Arthritis

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The best known and most frequently used biological marker for rheumatoid arthritis (RA) is the rheumatoid factor (RF), which still constitutes one of the 7 classification criteria for RA established by the American College of Rheumatology (ACR). While these markers are sensitive and present in extra-articular and severe forms of RA, they lack specificity. The recently discovered antibodies against citrullinated proteins or peptides feature excellent specificity for RA, while also displaying a sensitivity which is equivalent to RF. Furthermore, they enable the prediction of the more aggressive forms of the disease which require individualized management. Recent progress in the detection of these antibodies, i.e. progress connected with the identification of antigen targets, has made it possible to improve the analytical and diagnostic performance of reagent kits.

## Background

In 1964, Nienhuis et al., a group of Dutch researchers, detected antibodies in the serum of RA patients, using the indirect immunofluorescence method. The antibodies reacted with keratohyaline granules in the cytoplasm of epithelial cells in the human buccal mucous membrane. These antibodies were named the "perinuclear factor".

Fifteen years later, Young et al. from London described antibodies that bind to the corneous layer of squamous epithelium of rats' oesophagus. Due to this histological localization, these antibodies were named "anti-keratin antibodies".

Twenty years later, a French group under Guy Serre identified the target antigen of these antibodies. The antigen was not keratin, as previously assumed, but filaggrin, a protein that enables cytokeratin filaments to aggregate. This major discovery led researchers to use filaggrin obtained from purified extracts of the human epidermis or from rats' oesophagus to search for corresponding antibodies. The test results were disappointing because the sensitivity of the tests was very low and results varied greatly. In 2000, Schellekens et al. from the Netherlands demonstrated that the antigenic determinants present on filaggrin and recognized by the antibodies were actually citrulline residues.

Citrulline is an amino acid which is not coded by DNA, but results from the post-translational modification of arginine. This modification represents a deiminization stage governed by an enzyme known as peptidylarginine deiminase (PAD). Different isoforms of PAD exist, of which PAD2 and PAD4 are present in leukocytes. PAD activity is heavily dependent on $Ca^{++}$ ions. The physiological intracellular concentration of $Ca^{++}$ ions is too low to sustain enzyme activity. However, in case cell integrity is reduced (inflammation, necrosis, apoptosis), a major influx of calcium into the interior of the cell leads to the activation of PAD, resulting in increased "citrullination" of certain proteins. This modification leads to increased hypdrophobicity as well as unrolling of the citrullinated protein subsequent to the modification of charges. Whereas arginine is positively charged, citrulline is neutral. Many organic proteins, such as filaggrin, myelin basic protein, vimentin, and fibrinogen, undergo citrullination. Of these proteins, only vimentin and fibrinogen occur in inflamed synovial membranes. Therefore, the immunoreaction with filaggrin is a consequence of a cross-reaction with citrullinated epitopes common to these proteins.

More recently, it has also been demonstrated that, subsequent to citrullination, collagen, fibronectin and α-enolase can react with the serum antibodies of patients suffering from RA.

Citrullination of proteins at the synovial membrane level is a phenomenon that occurs during inflammation. Nevertheless, the production of antibodies to citrullinated residues is completely specific to RA. There seems to be a link between the production of these antibodies and HLA alleles associated with an increased risk of RA (in cases of shared epitopes in haplotypes HLA-D1 and DR4).

Figure 1 summarizes the development of analytical methods for the detection of anti-citrullinated protein / peptide antibodies.

## Citrullinated Anti-Protein Antibodies

Different citrullinated proteins are used for detecting anti-citrullinated residue antibodies. Recombinant rat filaggrin, citrullinated in vitro, is used in an ELISA test, marketed under the name "CPA".

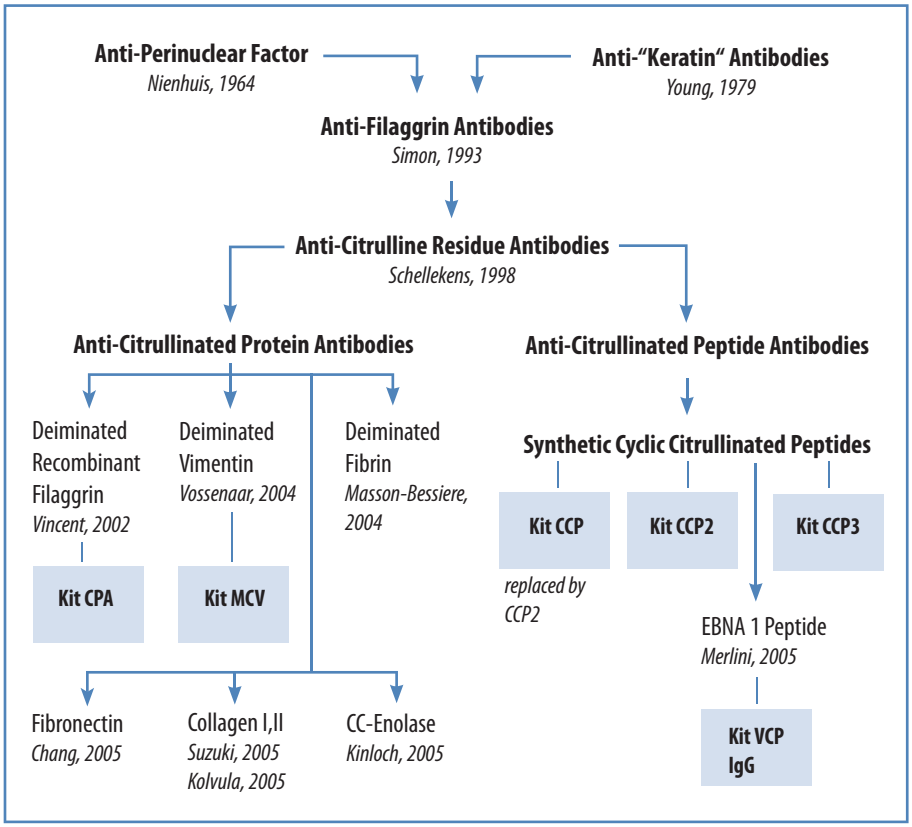


***Fig. 1:*** *Anti-citrullinated protein / peptide antibodies*

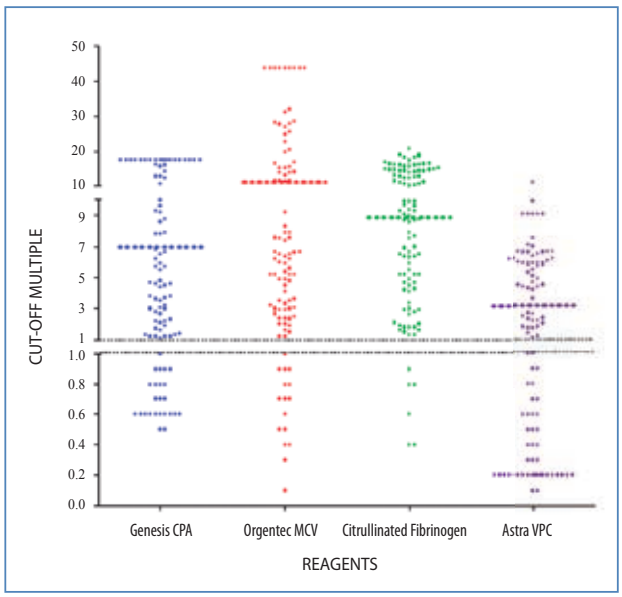


*Fig. 2: Distribution histogram of Ab values for reagent kits CPA/MCV/Fibrinogen/VPC*

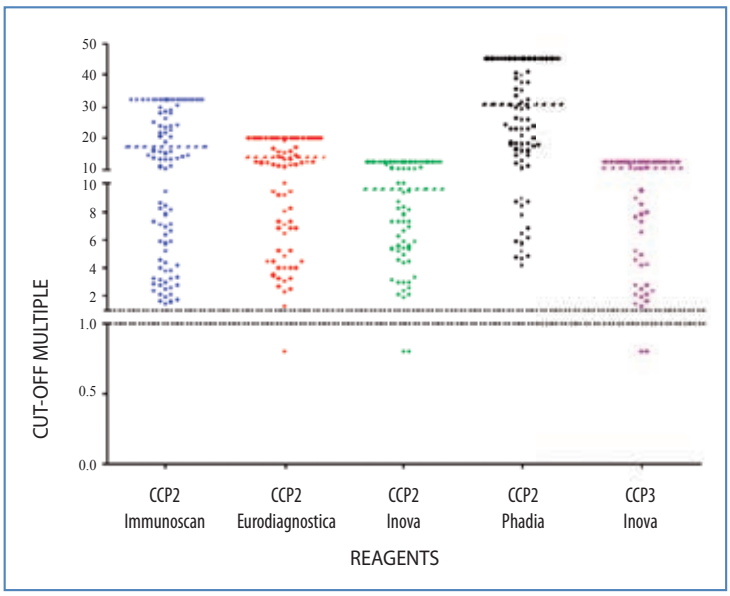


*Fig. 3: Distribution histogram of Ab values for CCP2 and CCP3 kits as a function of the cut-off multiple*

A mutant form of vimentin which, following citrullination, reacts in a specific manner with antibodies of sera affected by RA is used for the so-called MCV test. The other citrullinated protein, which is of particular interest because of its presence in the rheumatoid synovial membrane, is fibrin. α and β chains of fibrin carry citrullinated epitopes analogous to those carried by filaggrins. An ELISA test, using citrullinated human fibrinogen, was developed by Guy Serre's team in Montpellier. Italian researchers have successfully demonstrated that one of the proteins of the Epstein-Barr virus, EBNA 1, contains a sequence of arginine-rich amino acids in its N-terminal region. This sequence has been synthesized by substituting arginine residues with citrulline. The new peptide is used in a commercially available ELISA VPC test. There are currently no commercial reagents using citrullinated collagen, citrullinated fibronectin or citrullinated α-enolase.

The study carried out in our laboratory on a group of 102 patients with RA showed a major difference in sensitivity with regard to citrullinated antigenic substrates (see figure 1), although specificity was satisfactory (data not shown). These differences can be attributed to the nature and composition of the antigenic substrates employed, more particularly to the number of citrullinated residues and their localization within the peptide chain.

## Synthetic citrullinated Anti-Peptide Antobodies

Starting from information obtained from the antigenic structure of human filaggrin, peptides were synthesized, citrullinated, subsquently cyclisized, and then selected on the basis of their high reactivity with antibodies from patients affected with RA (CCP1). A second experiment involved the preparation of new peptides that no longer have any homology to filaggrin or other known proteins (CCP2 and CCP3). These peptides were selected from a peptide library for their strong reactivity with the serum of patients with RA. The majority of reagent kits for detecting anti-CCPs, currently on the market, use exactly the same CCP2 peptide substrate.

We carried out a comparative study of 4 reagent kits that use the same CCP2 citrullinated peptide, on a group of 102 patients with RA. The tested kits were from Immunoscan, Eurodiagnostica, Inova and Phadia. Additionally, Inova's CCP3 was tested. This ELISA contains another peptide mixture which is uniquely used in this commercial test. We noted that the qualitative results obtained are highly consistent among the different kits. However, the value distribution in relation to the analytical cut-off for each of the kits is very variable.

It was noted that Phadia's EliA CCP on ImmunoCAP is the most discriminating, with the lowest value observed amounting to more than 4 times the cut-off, as compared to twice the cut-off for the other kits (see figure 2).

There is no association between rheumatoid factors (RF) and anti-CCP antibodies. Some RA samples are RF positive but anti-CCP negative, others are uniquely positve for anti-CCP. Both biological markers should be used for diagnosing RA. Current treatments do not significantly alter the titres of anti-CCP antibodies. However, with effective treatment, RF levels drop very rapidly, thus enabling a short-term follow-up of RA progression.

At present, anti-citrullinated residue antibodies are the most specific markers for RA, since they are only rarely found in other rheumatic diseases. They have proven to be early markers for RA, thus enabling the identification of the disease at a very early stage. Moreover, the presence of these antibodies is associated with severe forms of RA. The frequency of erosions is 10 times higher in patients with anti-CCPs.

Rheumatoid arthritis is the most common form of chronic inflammatory rheumatism. It is characterized by inflammation of synovial tissue which may lead to irreversible destruction of bony and cartilageous joint tissue. It is now recognized that an aggressive treatment, initiated during the first three months after the onset of the disease, can prevent or limit the articular destruction characteristic for this disease. New drugs are available for this kind of treatment, in particular biotherapies based on TNF-α inhibitors, which enable the reduction of inflammation and the control of disease activity. Due to their impact on the immune system and because of their substantial cost, these drugs should only be given to patients at risk of developing severe RA. Anti-CCP antibodies are a highly valuable tool in this context because they characterize precisely those cases of RA with the potential for developing a severe form of the disease, more particularly involving bone destruction.

## References

1. **Humbel RL, Olsson NO (2005)** Update on anti-protein and anti-citrullinated peptide antibodies. Biotribune 17:34-36
2. **Benkhadra F, Hila I, Foerster G, Pierrard V, Humbel RL** (to be published soon) Techniques au quotidien (Current techniques): Comparative study of 9 reagent kits for detecting anti-protein antibodies or anti-citrullinated peptide antibodies. CORATA Immuno-analyse et Biologie spécialisée (IBS)

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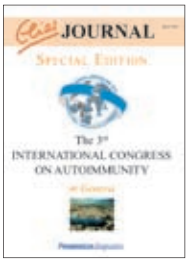
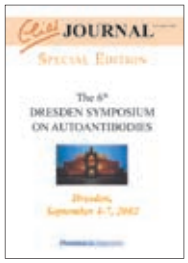



Special Ed.
No.1, 2002


No. 2, 2002

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No.2, 2002


No. 1, 2003


No. 2, 2003


No. 3, 2003

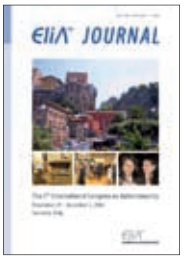

No. 1, 2004


No. 2, 2004


Special Ed.
No.1, 2004


No. 1, 2005


No. 1, 2006

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No.1, 2007

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