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The Diagnostic Specialist

Rheumatoid Arthritis

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Víctor Manuel Martínez-Taboada
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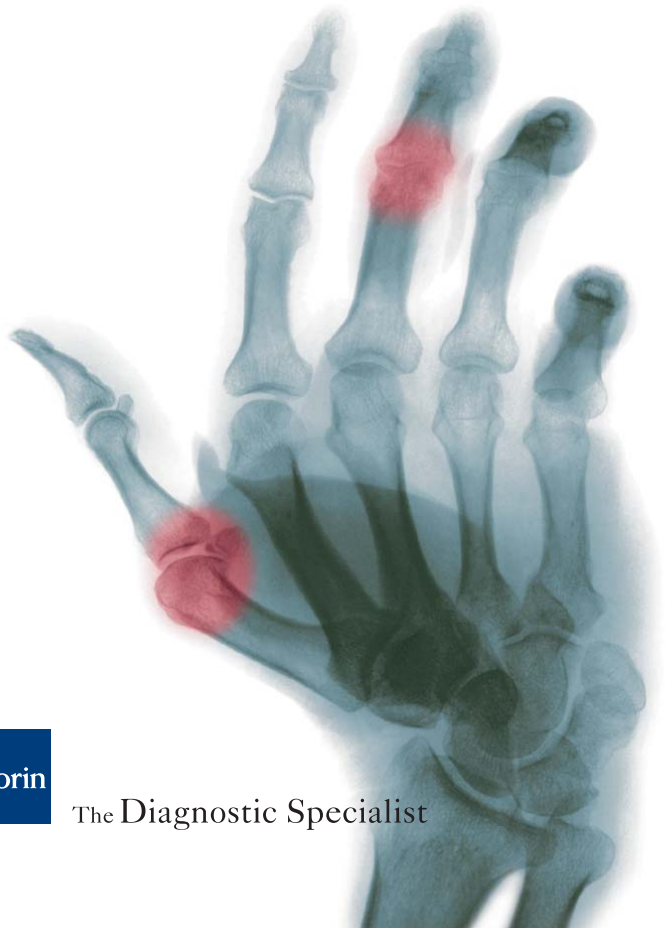

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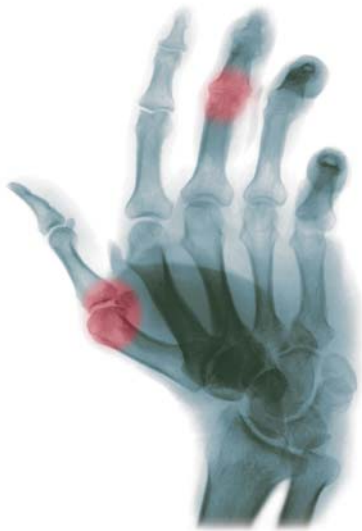

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Presentation

Rheumatoid arthritis is an invalidating disease that most frequently affects individuals when young. This autoimmune disease has been known for decades and is found very widely, affecting 1% of the world's population. There is evidence of the existence of this disease in archaeological remains dating from pre-Columbian America. The disease does not seem to be present in Europe until after the discovery of America; the first evidence of the presence of rheumatoid arthritis comes from 16th century pictures.

Though a relatively old disease, widespread attention has been given to rheumatoid arthritis only in the past decade. This is partly because progress in immunology has made rheumatoid arthritis a model of a complex autoimmune syndrome in which multiple genetic risk determinants participate together with other non-hereditary factors, producing persistent and destructive chronic immune response.

Progress in knowledge has triggered a true revolution in the way the disease is followed up, and this hinges on two fundamental aspects. First, the classic rheumatoid arthritis treatment pyramid has been replaced by a new approach in which
biological agents play an essential role in blocking the activity of cytokines such as tumor necrosis factor (TNF).

Then, from the time of the rheumatoid factor description in the 1940s – observed in the serum of one of Rose's lab technicians, who suffered from rheumatoid arthritis and produced agglutination – no further sufficiently specific laboratory markers were discovered until 1998, when citrullinated peptide autoantibodies were demonstrated to be highly specific for rheumatoid arthritis and quickly introduced into laboratory routines. Over the past decade it has been found that this autoantibody can be detected many years before the onset of the disease and its presence has therefore taken on a high prognostic value, permitting introduction of a new diagnostic criterion in addition to those established by the American College of Rheumatology.

In short, the discovery of an excellent serological marker along with the introduction of a new therapeutic tool has brought about a true revolution in rheumatoid arthritis follow-up. This manual aims to summarize and update all these concepts.

Dr. Marcos López Hoyos

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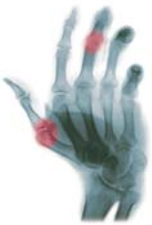
Summary



Rheumatoid arthritis

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Epidemiology

Rheumatoid arthritis (RA) is a chronic inflammatory disease with universal distribution (Table I). It is a common disease, characterized by the development of persistent articular pain and inflammation, occasionally accompanied by extra-articular manifestations of variable severity. The disease can produce articular destruction with the consequent loss of physical function and quality of life by the affected patient.

TABLE I. General characteristics of rheumatoid arthritis.

- Chronic inflammatory disease
- Unknown etiology
- Complex pathogenesis (multifactorial)
- Prevalence: 0.5%
- Characterized by:
  - Pain and progressive articular destruction
  - Loss of physical function
  - Decreased quality of life

According to the EPISER study, the most ambitious epidemiological study ever conducted in Spain on rheumatic pathology, the prevalence of rheumatoid arthritis in people over 20 years old is 0.5%. Altogether it has been calculated that there may be around 200,000 patients with rheumatoid arthritis in Spain. The disease is more frequent in women (0.6%) than in men (0.2%), and mainly affects working-age individuals although it can affect any age group.

Importance of rheumatoid arthritis

Rheumatoid arthritis is a chronic disease that has major impact not only on the affected patient, but also on society.

Importance for the patient

Rheumatoid arthritis has significant impact on a patient's daily activities, not only in physical areas (work or recreational activities), but also in psychological and economic areas, and in terms of social relations. In addition, the mortality rate associated with rheumatoid arthritis is greater than that of the general population and is directly proportional to the severity of the disease.

Importance for society

Rheumatoid arthritis is a common disease; as mentioned above it affects an estimated 200,000 people in Spain. Ten years after the onset of rheumatoid arthritis, more than 50% of patients suffer serious disability, and a significant percentage of these patients are forced to quit their work as a result of the disease. At the same time, patients require specialized medical

follow-up involving use of frequent laboratory tests and expensive drugs, while a significant percentage require reconstructive orthopedic surgery due to articular destruction. It is therefore clear that rheumatoid arthritis generates very significant costs for the public health-care system and for society as a whole, given its frequency and the costs associated directly and indirectly with the disease.

Etiopathogenesis

The etiology of rheumatoid arthritis is still unknown, although both genetic and environmental factors have been blamed. It has traditionally been assumed that certain exogenous factors, such as infections or, more recently, tobacco, trigger an uncontrolled immune response in a genetically predisposed host, that gives rise to inflammation of the joints and perpetuate it in time (Fig. 1). Although macrophages, synoviocytes and fibroblasts, among other cells, are demonstrated to largely contribute to the process of inflammation of the joints, the existing data support a central role for T lymphocytes in both the start and the continuation of the disease. Activation of T lymphocytes following antigen presence is probably accompanied by proliferation of specific T lymphocytes which, in turn, activate the various cell types involved in the disease pathogenesis (Fig. 2). Activation of the immune system is accompanied by imbalance in the synthesis of pro-inflammatory and anti-inflammatory cytokines, by alteration of the balance between metalloproteases and their natural inhibitors (Fig. 3),

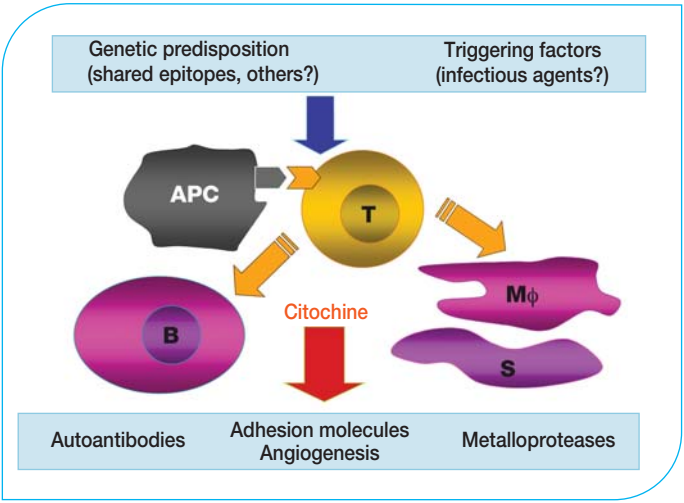


Figure 1. Etiopathogenesis of rheumatoid arthritis. APC: Antigen-presenting cell; T: T lymphocyte; B: B lymphocyte; Mø: macrophage; S: synoviocyte.

and, as a result, by a chronic inflammatory process. Inflammation causes destruction of the articular cartilage, and later of the subchondral bone, giving rise to the structural injuries characteristic of rheumatoid arthritis, namely decreased articular space and appearance of juxta-articular osteoporosis and erosions, where osteoclasts seem to play a key role (Fig. 4 and Table II).

Clinical manifestations

Articular manifestations

Although the disease can evolve in various different ways,

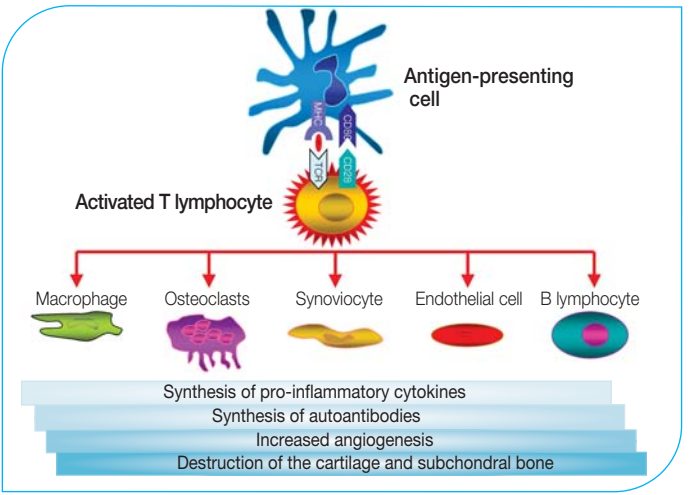
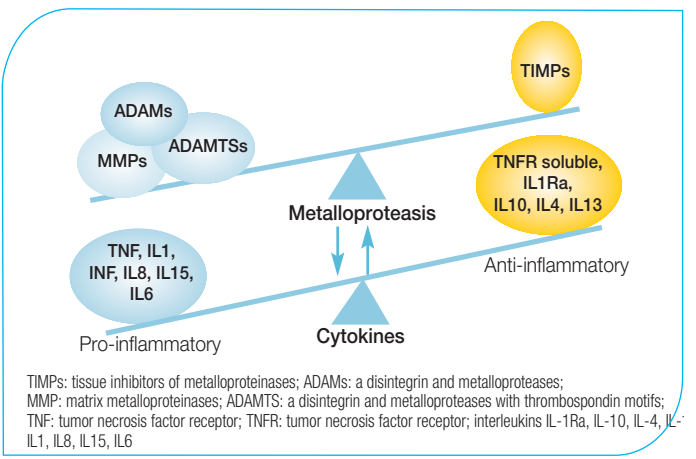


Figure 2. Main cells involved in pathogenesis of rheumatoid arthritis.



TIMPs: tissue inhibitors of metalloproteinases; ADAMs: a disintegrin and metalloproteases; MMP: matrix metalloproteinases; ADAMTS: a disintegrin and metalloproteases with thrombospondin motifs; TNF: tumor necrosis factor receptor; TNFR: tumor necrosis factor receptor; interleukins IL-1Ra, IL-10, IL-4, IL-1, IL8, IL15, IL6

Figure 3. Cytokines and metalloproteases in rheumatoid arthritis.

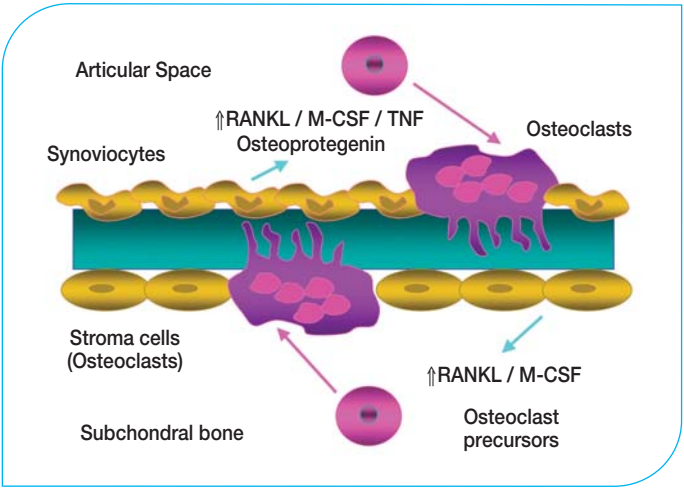


Figure 4. Role of osteoclasts in the structural damage of rheumatoid arthritis.

TABLE II. Structural damage in rheumatoid arthritis.

- Osteoporosis
  - Systemic damage
  - - Juxta-articular damage
- Decreased articular space
  → Involvement of cartilage
- Erosion
  → Involvement of cartilage and subchondral bone

rheumatoid arthritis usually begins in an early insidious form with pain and swelling in one or more joints, especially the hands, knees and feet. Symptoms are usually symmetrical and evolve

TABLE III. Main clinical manifestations of rheumatoid arthritis.

Articular manifestations	Extra-articular manifestations
• Pain	• Rheumatoid nodules
• Swelling	• Involvement:
• Stiffness	- Ocular
• Functional impotency	- Pulmonary
	- Cardiac
Systemic manifestations	- Neurological
• Asthenia	- Cutaneous
• Anorexia	- Renal
• Fever or low-grade fever	

from an additive form to conversion to full-blown polyarthritis (Table III and Fig. 5). The patient normally also suffers from intense pain at night and while at rest, and these symptoms are accompanied by prolonged morning stiffness. The pain and swelling are accompanied by functional impotence, which for sufferers with major polyarthritis can be extremely invalidating.

The disease generally evolves with oscillations in symptomatology, which may occasionally be spontaneous but are generally due to treatment administered to the patient. In patients with persistent inflammatory activity, irreversible structural damage is present, accompanied by limitations in mobility, articular deformities, tendinous injuries and muscular atrophy due to disuse.

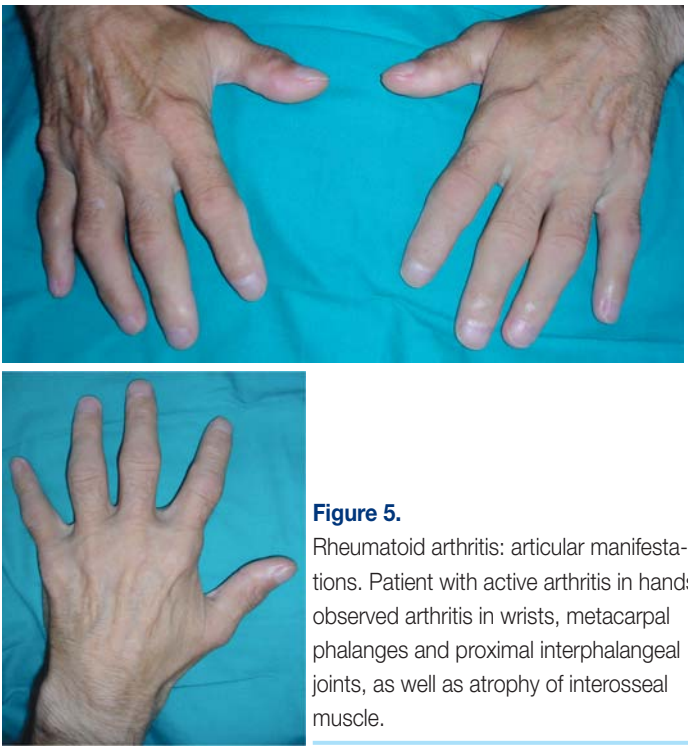


Figure 5.
Rheumatoid arthritis: articular manifestations. Patient with active arthritis in hands, observed arthritis in wrists, metacarpal phalanges and proximal interphalangeal joints, as well as atrophy of interosseal muscle.

Systemic and extra-articular manifestations

As seen from Table III, patients with rheumatoid arthritis may show a wide variety of clinical manifestations, underlying the systemic character of the disease. Asthenia is rather frequent, and rheumatoid arthritis is sometimes accompanied by low-grade or even high fever.

Rheumatoid arthritis is often accompanied by extra-articular

Figure 6.
Rheumatoid arthritis: extra-articular manifestations. 1. The elbows are the commonest site of rheumatoid nodules: these usually appear in long-standing arthritis patients, in the presence of rheumatoid factor and poor control of the disease.

manifestations, mainly in the advanced stages of the disease in patients who have not received adequate treatment for prolonged periods of time (Figs. 6 and 7). On the other hand, it may be also difficult to differentiate between extra-articular manifestations of the disease and secondary complications of the treatments used.

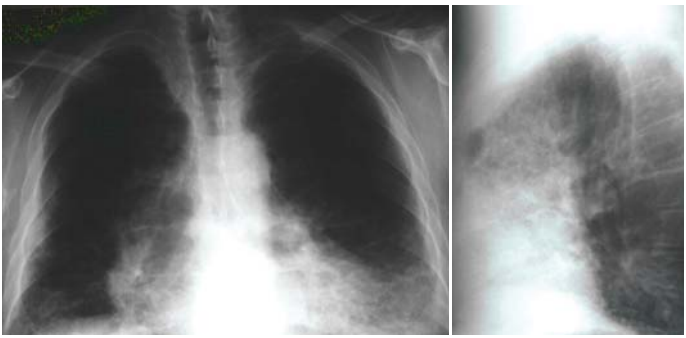


Figure 7. Rheumatoid arthritis: extra-articular manifestations. 2. As in the case of other extra-articular manifestations, pulmonary fibrosis only appears in long-standing arthritis patients, in the presence of rheumatoid factor and poor control of the disease. Prognosis is affected by the appearance of these extra-articular manifestations.

Comorbidity in rheumatoid arthritis

Patients with rheumatoid arthritis show a great number of co-morbidities, the control of which must be part of the overall care for these sufferers. Although some of these co-morbidities may be independent of the disease or the various courses of treatment, most complications appearing during the course of rheumatoid arthritis are directly related to the continuing inflammatory process affecting these patients. Control of the inflammatory disease consequently often results in control or prevention of comorbidity. Figure 8 shows the main co-morbidities in rheumatoid arthritis.

A good example of comorbidity associated with rheumatoid arthritis is the accelerated arteriosclerosis that these patients

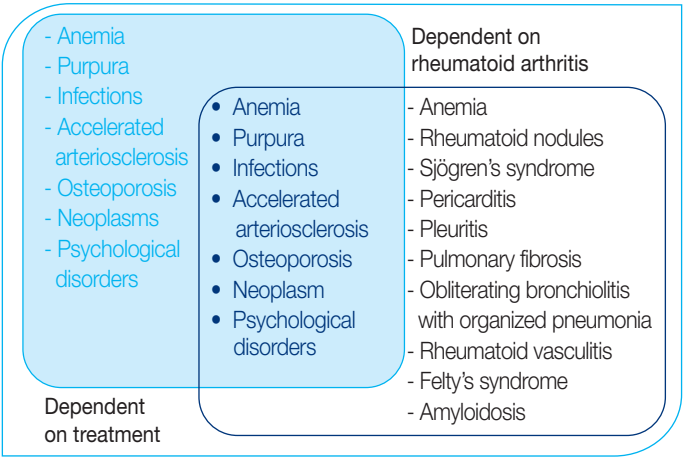


Figure 8. Comorbidity in rheumatoid arthritis.

TABLE IV. Basic laboratory studies for diagnosis of rheumatoid arthritis.

Parameter	Time of evaluation
Rheumatoid factor	Basal in order to establish diagnosis. Repeat 6-12 months after the onset of rheumatoid arthritis if negative
Anti-CCP antibodies	Basal in order to establish diagnosis. Repeat 6-12 months after the onset of rheumatoid arthritis if negative
Blood tests + VSG	Basal
Biochemical tests + PCR	Basal
Elemental and urine sediment	Basal
Synovial fluid analysis	Basal, in order to rule out other diseases. During exacerbation of the disease, in order to rule out septic arthritis

endure. Of all the causes of mortality, mortality of cardiovascular origin appears most significant in patients with rheumatoid arthritis. Mortality of cardiovascular origin cannot be explained completely by the traditional cardiovascular risk factors, and is clearly related to the activity of rheumatoid arthritis and therefore with the underlying chronic inflammatory process. In this sense, it is supposed that the decreased mortality observed in patients with more recent diagnosis is partially attributable to better control of the inflammatory process thanks to the more effective therapeutic agents used in recent decades.

Diagnosis

Diagnosis of rheumatoid arthritis is based fundamentally on clinical judgment. Anamnesis and physical exploration are key approaches, while laboratory tests, specifically analytical (Table IV) and radiological (Table II and Fig. 9) are used to rule out other processes and confirm the disease.

Diagnosis of the disease in its early stages is fundamental for controlling rheumatoid arthritis: the sooner a specific treatment for rheumatoid arthritis begins, the higher the probability of controlling the inflammatory process and, therefore preventing structural damage and patient disability. Therefore, any suspicion of rheumatoid arthritis must be considered by the primary attending physician as a diagnostic priority, and the patient should be referred to a rheumatologist as soon as possible.

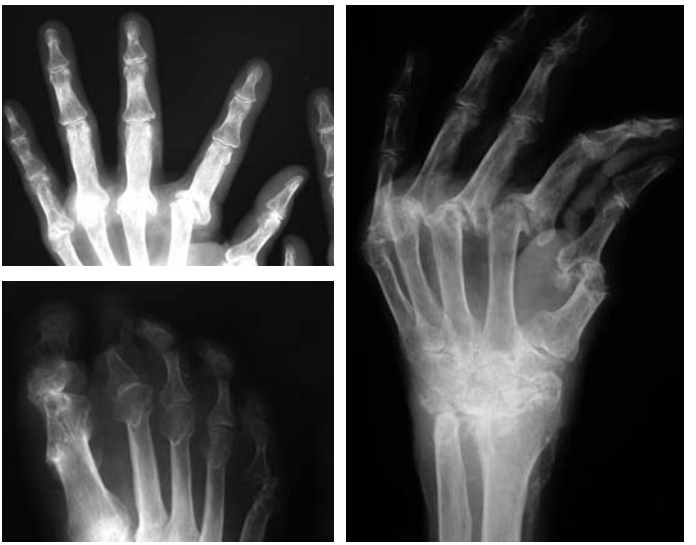


Figure 9. Radiology in rheumatoid arthritis. The main radiological injuries that can be observed in rheumatoid arthritis are juxta-articular osteoporosis, decreased articular space, and, especially, development of erosions.

- Clinical suspicion in primary health care.

  The Spanish Society of Rheumatology recommends that patients suspected of having rheumatoid arthritis be sent to a rheumatologist as soon as possible. In this sense, the goal of establishing early arthritis diagnosis units is ensuring that any patient suspected of rheumatoid arthritis be seen by a rheumatologist in no more than two weeks. Although there are various different views on the criteria triggering the need to consult a rheumatologist, the

best known are those of P. Emery et al., shown in Table V. However, patients with persistent arthritis lasting more than four weeks should in any case be referred to a rheumatologist.

TABLE V. Criteria of early identification in patients with suspicion of rheumatoid arthritis.

Patients should undergo rheumatological assessment if there is clinical suspicion of rheumatoid arthritis supported by the presence of some of the following signs or symptoms:

- Three or more inflamed joints
- Involvement of metacarpal and metatarsal phalanges: positive *squeeze test*
- Morning stiffness for 30 minutes or more

Emery P, et al. Ann Rheum Dis 2002: 61; 290-297.

- Diagnosis of rheumatoid arthritis. The 1987 classification criteria of the *American College of Rheumatology* (Table VI) are still applied. These criteria have more than acceptable sensitivity and specificity in patients with full-blown disease, but their utility is controversial in patients in the very early stages of the disease. Although these criteria do include a combination of clinical manifestations, radiological and laboratory tests, as listed here, the appearance of other very specific serological markers of rheumatoid arthritis can provide clear diagnosis in the early stages of the disease (e.g., cyclic citrullinated peptide antibodies

TABLE VI. Criteria for classification of rheumatoid arthritis.

Diagnosis of rheumatoid arthritis
American College of Rheumatology (ACR) Criteria

A minimum of four of the following criteria:

- Morning stiffness for at least one hour
- Arthritis in three or more articular areas
- Arthritis in the hands
- Symmetrical arthritis

} These must be present for at least six weeks

- Rheumatoid nodules
- Rheumatoid factor in blood
- Typical radiographic changes

Arnett FC, et al. Arthritis Rheum 1988; 31:315-24.

(anti-CCP), or development of readily accessible and more sensitive imaging techniques, such as ultrasound scan).

Treatment

The person directly responsible for overall treatment of rheumatoid arthritis is the rheumatologist, working in close contact with the primary physician, and in specific situations in collaboration with other specialists such as the traumatologist or physiotherapist. The main goal is to bring about full remission of the rheumatoid arthritis and prevent structural damage (Table VII). The considerable number of drugs available today allows

this goal to be reached in a significant proportion of patients (Fig. 10). Patient treatment must be personalized and adjusted to the various prognostic factors (Table VIII); it must also take into account the various comorbidities that may arise in these patients.

TABLE VII. Rheumatoid arthritis. Objectives of treatment.

- To achieve full clinical remission and to prevent articular destruction
- If full remission is not achieved, to obtain minimum possible progression:
  - To alleviate the articular symptoms, including swelling, stiffness and pain
  - To delay articular destruction, loss of articular function, deformity and disability
  - To improve quality of life as far as possible

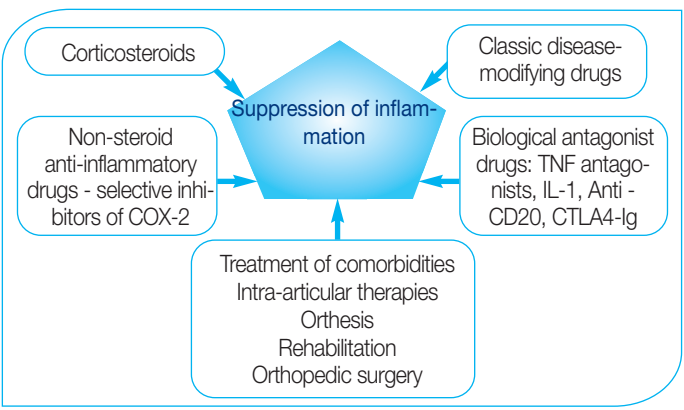


Figure 10. Rheumatoid arthritis: treatment.

TABLE VIII. Rheumatoid arthritis: prognostic factors.

- Sociodemographic factors
  - Female sex
  - Low level of education
- Genetic markers
  - Shared Epitope (HLA-DRB1)
- Psychological factors
  - Depression
- Social factors
  - Social support
- Dependent on rheumatoid arthritis
  - Presence of rheumatoid factor and CCP antibodies
  - High number of highly inflamed joints
  - Elevated acute-phase reagents
  - High health assessment questionnaire (HAQ)
  - Presence of early erosions
  - Involvement of large joints
  - Extra-articular manifestations
- Dependent on treatment
  - Delay in treatment start with disease-modifying drugs
  - Duration of treatment with disease-modifying drugs

Because the time elapsed from disease onset to beginning of correct medical treatment is one of the few variables the doctor can modify, it is currently recommended that all patients with rheumatoid arthritis be treated with disease-modifying drugs as soon as the diagnosis of the disease is confirmed, whether or not they meet the *American College of Rheumatology* criteria for classification of rheumatoid arthritis. Although a significant number of drugs are now available (Table IX), a great number of these agents have been disregarded fundamentally as a result of their limited clinical effectiveness, their questionable ability to prevent structural damage, and their safety characteristics.

TABLE IX. Rheumatoid arthritis: classic disease-modifying drugs.

Treatment of rheumatoid arthritis: disease-modifying drugs	
• Methotrexate	• Azathioprine
• Leflunomide	• Gold salts
• Salazopyrin	• Cyclosporine
• Antimalaric drugs	• D-penicillamine
	• Cyclophosphamide
	• Chlorambucil
	• Minocycline

On the other hand, rheumatologists' growing awareness of the aggressive nature of rheumatoid arthritis, combined with better use of drugs (some such as methotrexate are relatively old) has shifted the therapeutic strategy for rheumatoid arthritis towards a much more aggressive approach, in particular using early treatment of the disease, with the aim of bringing about full remission of rheumatoid arthritis and preventing the structural damage it causes (Table VII and Fig. 11).

We now know that almost half of rheumatoid arthritis patients develop erosions in the first six months after the onset of disease, and that more than two thirds of patients with rheumatoid arthritis suffer structural damage during the first two years of the disease. If treatment is started as soon as possible, the probability of controlling the disease and curbing structural

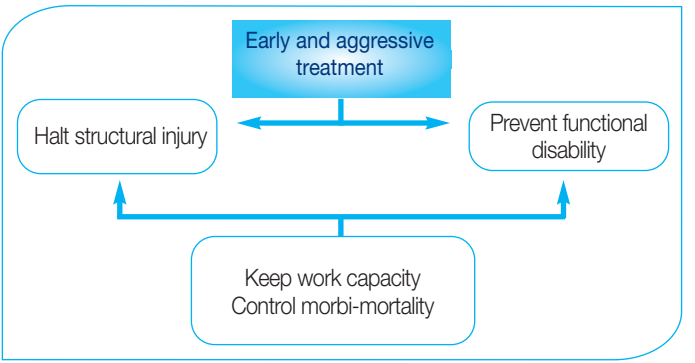


Figure 11. Rheumatoid arthritis: current trends in treatment.

damage is enhanced. This concept is referred to as a therapeutic window of opportunity, reflecting the importance of early diagnosis and immediate aggressive treatment capable of limiting the damage produced by rheumatoid arthritis and its long-term consequences (Fig. 12).

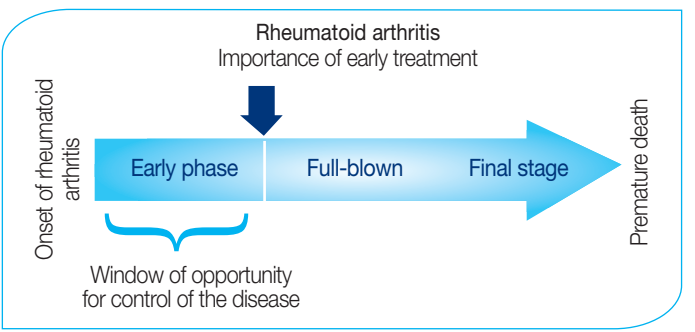


Figure 12. Rheumatoid arthritis: importance of early treatment.
The therapeutic window of opportunity.

Currently, methotrexate is the basic drug used for treating rheumatoid arthritis, especially in its early stages. Methotrexate has very well-known safety characteristics, weekly administration makes it very convenient for the patient and its efficacy has been confirmed in various different clinical conditions (early and full-blown rheumatoid arthritis), especially if the rapid scaling guidelines recommended in recent years are adopted.

In certain clinical conditions, such as the onset of rheumatoid arthritis without unfavorable prognostic signs or in older patients with rheumatoid arthritis of polymyalgic origin, the use of other drugs (whether or not combined with corticoids) with lower toxicity and more convenient monitoring, such as anti-malaric drugs, may be of value. On the contrary, in patients with multiple unfavorable prognostic signs, more aggressive treatment with combined therapy with different drugs or including biological agents may be recommended from the start.

Effectiveness of the therapy undertaken in patients with rheumatoid arthritis must be evaluated periodically in accordance with the parameters currently widely accepted (number of inflamed and painful joints, acute phase reagents, and *Health Assessment Questionnaire* – HAQ). If a satisfactory clinical response is not obtained, treatment should be changed. In the event of lack of response to methotrexate or toxicity, current

recommendation is use of other drugs that have been demonstrated effective in rheumatoid arthritis, such as leflunomide or salazopyrin, or of biological therapy with antagonists of tumor necrosis factor (TNF).

There are currently available a number of different biological agents (Table X) with different mechanisms of action (Fig. 13). All TNF antagonists have demonstrated their effectiveness in full-blown rheumatoid arthritis refractory to drugs and in early arthritis. They are characterized by initial rapid action, elevated clinical efficiency and outstanding ability to halt structural damage. In the use of these therapeutic agents special care must be taken in the

TABLE X. Rheumatoid arthritis: biological therapies.

Monoclonal antibodies
- Chimeric:
  - Infliximab
  - Rituximab
- Human:
  - Adalimumab

Fusion proteins
- Etanercept
- Abatacept

Receptor antagonists
- Anakinra

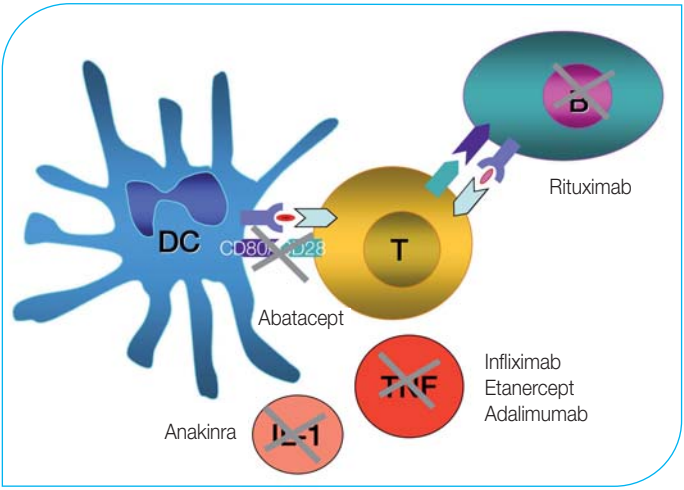


Figure 13. Rheumatoid arthritis: main mechanisms of action of biological therapies.

prevention and treatment of infections, including tuberculosis. The availability of biological agents with a range of structures and different mechanisms of action now offers the possibility of targeted therapeutic options for patients experiencing insufficient or toxic response to conventional treatment.

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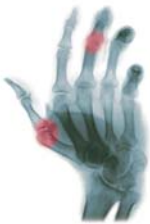

Serological markers and autoantibodies in the diagnosis of rheumatoid arthritis

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Until a few years ago, the only laboratory test used in clinical practice for the diagnosis of rheumatoid arthritis (RA) was determination of rheumatoid factor. Nevertheless, its diagnostic specificity is very low, although it is one of the diagnostic criteria for rheumatoid arthritis selected by the *American College of Rheumatology (ACR)*. However, it must be clear that these criteria have a classification rather than diagnostic function, especially as regards early diagnosis. As RA is an autoimmune disease, the search for specific autoantibodies in serum of patients with rheumatoid arthritis has been a priority. Since the 1960s, a number of different autoantibodies linked with rheumatoid arthritis have been described, some more specific than others (Table I). Problems of specificity of these autoantibodies and the technical difficulties involved in detecting them have hindered introduction of these tests in clinical laboratories. It is therefore essential to make available a laboratory test (or combination of tests) that helps diagnosis of rheumatoid arthritis in its early stages in all patients with early arthritis. In addition, the ideal characteristic of that test would be the prognosis of rheumatoid arthritis in a specific patient.

Luckily, anti-citrullinated cyclical peptide antibodies (anti-CCP antibodies) have been described as useful and early markers of rheumatoid arthritis. Next to rheumatoid factor, anti-CCP antibodies are now considered the most important tool for diagnosis of rheumatoid arthritis in any laboratory dedicated to diagnosis of autoimmune diseases.

TABLE I. Autoantibodies studied as serological markers of rheumatoid arthritis.

Non-specific antibodies	Specific antibodies
Rheumatoid factor	Anti-Glycoprotein antibodies
Antinuclear antibodies (ANA)	Anti-Sa antibodies
Anti-cardiolipin antibodies	Anti-Keratin antibodies
Neutrophile granulocyte cytoplasm antibodies	Anti-Perinuclear factor antibodies
Collagen II antibodies	Anti-Filaggrin antibodies
RA33 antibodies	Anti-Citrullinated peptide antibodies

Rheumatoid factor

Rheumatoid factor has to date been the only laboratory test available for serological diagnosis of rheumatoid arthritis, and as such is included in the *American College of Rheumatology's* qualifying criteria for rheumatoid arthritis. Since its first description as an agglutination factor of sheep erythrocytes by Wahler in 1939, confirmed by Rose ten years later, the rheumatoid factor test has undergone a number

of modifications, today's version most commonly used in health-care laboratories being the technique of latex particle agglutination and measurement by means of nephelometry/turbidimetry. Rheumatoid factor is an autoantibody of IgM class directed against the Fc domain of autologous IgG, with agglutination properties in laboratory tests. Independently of the technique employed, detection of rheumatoid factor in serum of patients with rheumatoid arthritis has never reached high sensitivity (60-90%) and, above all, its specificity has been always moderate, as it can be detected in numerous pathologies (as seen further on) and even in healthy people (prevalence of 3-25%). Although it can certainly be detected up to ten years before the clinical onset of rheumatoid arthritis, its lack of specificity clearly does not aid in early diagnosis of the disease. The utility of rheumatoid factor in the diagnosis of rheumatoid arthritis depends on its serum titer. This means that the sensitivity of a low-titer test is moderate, with specificity of 80% in most cases. However, if we consider high rheumatoid factor titers, sensitivity drops below 20%, but its specificity peaks to above 90%. Unlike its low diagnostic specificity, rheumatoid factor can have prognostic value in articular erosions, as well as in extra-articular manifestations of rheumatoid arthritis.

Finally, some studies postulate the usefulness of determining other rheumatoid factor isotypes, such as IgA and IgG, although the isotype that nephelometry techniques measure is IgM, which is used in diagnosis with a specific pathogenic role. These studies show how the combination of

the three rheumatoid factor isotypes can increase sensitivity of rheumatoid factor determination, and how the IgA isotype above all can have prognostic value in certain disease manifestations. In any case, rheumatoid factor detection with the IgA or IgG isotype can only be achieved by means of other techniques such as ELISA or indirect immunofluorescence.

Anti-Citrullinated antigen antibodies

With the exception of glucose-6-phosphate isomerase antibodies, the other specific rheumatoid arthritis antibodies listed in Table I have a common characteristic, in that they react with antigens with high content of citrulline residues (Table II). Table II demonstrates how all the non-specific rheumatoid arthritis antibodies listed in Table I react with a variety of autoantigens that have nothing to do with citrullination. Citrulline is an unusual amino acid resulting from enzymatic modification (deimination) of the arginine residue. The peptidylarginine deiminase (PAD) is the responsible enzyme, and transforms an NH_2 basic arginine residue into a neutral citrulline residue. This modification seems to be fundamental to creation of the antigenic specificity which is recognized by keratin, anti-perinuclear factor and anti-filaggrin antibodies (Table II).

There are three fundamental reasons for considering immunity to citrullinated peptides as essential in rheumatoid arthritis.

TABLE II. Autoantibodies and autoantigens in rheumatoid arthritis.

Antigen	Antibody
Immunoglobulin antigens	
- Fc domain of IgG	- Rheumatoid factor
- Final glycosilation products	- Anti-AGE antibodies
Citrullinated antigens	
- Citrullinated peptides	- Anti-CCP antibodies
- Pro-filaggrin	- Anti-Keratin, perinuclear factor antibodies
- Filaggrin	- Anti-Filaggrin antibodies
- Citrullinated vimentin	- Anti-Sa antibodies
Cartilage antigens	
- Type II collagen	- Anti-Collagen antibodies
- Glycoprotein 39 of cartilage	- Anti-gp39 human antibodies
- Aggrecan	- Anti-Aggrecan antibodies
Glycolytic enzymes	
- Glucose 6-phosphate isomerase	- Anti-Glycoprotein 1 antibodies
- Enolase	- Anti-Enolase antibodies
- Creatinine kinase	- Anti-Creatinine kinase antibodies
Other antigens	
- A2 protein of heterogeneous ribonuclear protein (hnRNP)	- Anti-RA33 antibodies
- Calpastatin	- Anti-Calpastatin antibodies
- Immunoglobin binding protein (BiP) (vegetable binding protein)	- Anti-p68 antibodies
- Fibronectin	- Anti-Fibronectin antibodies
- Actin, Myosin	- Anti-Cytoskeleton antigen antibodies
- Pituitary-specific factor 1a	- Anti-Pituitary antibodies
- High mobility protein group (HMG1 and HMG2)	- Anti-Non-histone protein antibodies
- Lactoferrin, MPO, cathepsin G	- ANCA
- Cardolipins	- Anti-Phospholipid antibodies

First, various citrullinated antigens (such as fibrin) are present in the inflamed joint which is the target organ. In second place, antibodies directed against citrullinated proteins are detected before the onset of disease, as seen later. And the third and probably most important reason as regards diagnosis is the high specificity of these antibodies in the diagnosis of rheumatoid arthritis.

The citrulline residue in the antigen is so important that practically any peptide or citrullinated protein is recognized by the autoantibodies present in the serum of a patient with rheumatoid arthritis. The original technique with nine linear peptides derived from sequences of filaggrin is not very sensitive: 30-45% of sera of patients with rheumatoid arthritis react with most peptides, whereas around 75% of sera react with only one. Overall, this technique shows a sensitivity of 48% with a specificity of 98%.

Why has detection of filaggrin antibodies not been established as the diagnostic method for rheumatoid arthritis? The specificity described for the ELISA or immunoblot methods using filaggrin as an antigenic substrate is similar to that of the anti-citrullinated peptide antibodies (98-99%). Nevertheless, sensitivity is low and very variable (31-47%), and agreement between tests is moderate. In short, it is very difficult to obtain sufficient amounts of purified recombinant filaggrin, and even more difficult with a reproducible amount of citrulline residues. This makes test standardization difficult.

Anti-Citrullinated cyclical peptide antibodies (CCP antibodies)

Given the limited sensitivity of the early citrullinated linear peptides, Van Venrooj's group developed a first-generation anti-citrullinated cyclical peptide (anti-CCP-1), derived from the sequence of filaggrin, which displayed citrulline residues with optimal conformation for recognition by the anti-CCP antibodies. When using this anti-CCP-1, the ELISA technique considerably increased its sensitivity to 68% or beyond, while maintaining its specificity close to 100%. Nevertheless, the increased sensitivity of ELISA test with anti-CCP-1 is not superior to that described with some ELISA tests for filaggrin antibodies nor to that for rheumatoid factor.

More recently, new peptides with citrulline residues have been selected from a collection of peptides that react with the serum of patients with rheumatoid arthritis (CCP-2 or second-generation peptides). These new peptides have no similarity to filaggrin or any other known proteins. ELISA tests using CCP-2 significantly increased sensitivity over use of CCP-1 (at levels similar to that of rheumatoid factor), while maintaining specificity. All ELISA tests present on the market use CCP-2s, and the most recent studies validating the detection of CCP antibodies as a diagnostic method in rheumatoid arthritis use ELISA tests with CCP-2.

Very recently a new commercial ELISA test has been introduced that uses a third-generation citrullinated peptide

as an antigen; however, this is not a further modification of the CCP-1 or CCP-2 peptides, but rather a different citrullinated peptide. The validity of this ELISA test remains to be established; it has been presented at conferences, but as yet there are no related published papers.

New citrullinated antigens

In recent years, new methods for antibody detection have been developed for other citrullinated antigens different from cyclic citrullinated peptides. Synthetic citrullinated peptides derived from type I and II collagen have thus been developed. The study in which ELISA tests are used to detect antibodies to the above peptides shows a correlation with anti-CCP antibodies. A series of patients, however, showed the presence of antibodies that react with the collagen carboxi-terminus, independently of its citrullination state, and do not with CCP. These antibodies have a pathogenic role. Also, ELISA methods have been developed that use citrullinated fibrinogen and have a sensitivity similar to that of anti-CCP antibodies and greater than of rheumatoid factor with high specificity. Other antigens involved previously in rheumatoid arthritis have shown great specificity to citrullination, as in the case of alpha-enolase. Nevertheless, the improvement that has acquired the greatest importance and has been most commercially developed in the last few years has been detection of Anti-Sa antibodies, where Sa is another citrullinated antigen, citrullinated vimentin. Anti-Sa antibodies have diagnostic sensitivity and specificity

very similar to that of CCP antibodies, although the team that developed this method claims that Sa antibodies offer a greater diagnostic advantage in terms of persistence of rheumatoid arthritis, severity and fulfillment of the *American College of Rheumatology* criteria 30 months after onset of early arthritis. All these are different approaches to the same goal that contribute very few or no advantages over the established method used throughout the world for detection of anti-CCP2 antibodies (commented on above), which is available in the form of a commercial ELISA kit. In addition, all these examples once again emphasize the importance of citrullinated residues in the different antigens used for antibody detection. Finally, the use of other citrullinated antigens as a diagnostic method involves the additional problem of test reproducibility, due to difficult standardization of citrullination, which makes perfect purification of the protein before and after the citrullination essential.

Diagnostic features of anti-CCP antibodies

The ideal serological marker of a disease must show the following features: high sensitivity and specificity, early detection, and prognostic value. In the case of rheumatoid arthritis it would be desirable that the marker should also monitor treatment, given the number of biological therapies introduced, such as those that interfere with signalling through TNF. The following describes how

anti-CCP antibodies fulfill these requirements.

Specificity and sensitivity of anti-CCP antibodies

The importance of specificity for differentiation of rheumatoid arthritis from many other forms of arthritis has been clear from the start. The ELISA method using CCP-1 showed specificity ranging from 95 to 98.5% which is maintained with the present ELISA test using CCP-2 targeting rheumatoid factor (Table III). Some data have shown somewhat lower specificities (90%), although this effect is attributable to the positivity for anti-CCP antibodies observed in approximately 30% of patients with juvenile chronic arthritis. Juvenile chronic arthritis sufferers with positive test for anti-CCP antibodies normally have unusual clinical symptoms: polyarticular onset, presence of the rheumatoid factor and erosive arthritis.

The importance of the specificity of the anti-CCP antibodies lies in distinguishing rheumatoid arthritis from other diseases which may initially seem similar (Table IV). For

TABLE III. Comparison of diagnostic value of anti-CCP-1 and anti-CCP-2 antibodies according to published data.

Antibody	N	Sensitivity	Specificity
Anti-CCP-1	2,234	53-54%	96-97%
Anti-CCP-2	6,125	68-68,5%	95-97%

TABLE IV. Sensitivity and specificity of anti-CCP-2 antibodies and rheumatoid factor.

Illness	CCP-2	Rheumatoid factor
Rheumatoid arthritis	77%	74%
Healthy control group	0,4%	11%
Arthrosis	9%	18%
Juvenile rheumatoid arthritis	29%	15%
Psoriasic arthritis	8%	19%
Palindromic rheumatism	44%	42%
Systemic lupus erythematosus	9%	30%
Sjögren's syndrome	5%	70%
Other inflammatory diseases	1%	9%
Rheumatic polymyalgia	0%	7%
Viral infections	1%	25%
Bacterial infections	1%	12%
Parasitic infections	2%	22%
Total for pathologies other than RA	3%	22%
SPECIFICITY	97%	78%

example, CCP antibodies distinguish rheumatoid arthritis from the less frequent forms of systemic lupus erythematosus with rheumatoid factor and erosive arthritis. Arthropathy is one frequent extra-hepatic complication (up to 20%) in hepatitis C virus infections, in which rheumatoid factor does not distinguish it from rheumatoid arthritis (up to 75% test positive for rheumatoid factor, especially if cryoglobulinemia concurs). But anti-CCP antibodies test negative in all cases of hepatitis C without cryoglobulinemia and in only 2% of cases studied

with mixed cryoglobulinemia. A high percentage of patients suffering from palindromic rheumatism (PR) with the presence of anti-CCP (as opposed to CCP-1) antibodies has been found. PR consists of acute arthritis attacks of short duration that affect a single joint and that do not cause clinical or radiological changes. It has been suggested that anti-CCP antibodies would identify those forms of palindromic rheumatism that evolve into rheumatoid arthritis. Anti-CCP antibodies also help in differential diagnosis of rheumatoid arthritis in the elderly or of polymyalgic origin. This form of rheumatoid arthritis is very difficult to differentiate from rheumatic polymyalgia (RPM), although both are pathologies with onset in advanced age (over 50 years) and that show similar articular manifestations. Published data demonstrate that anti-CCP antibodies are absent in 100% of rheumatic polymyalgia patients. Psoriasic arthropathy may be the only form of arthritis in which a relatively high percentage of patients with anti-CCP antibodies is observed, although they do not attain statistical significance. In any case, CCP antibodies are never observed in the exclusively cutaneous forms of psoriasis. When they are detected in psoriasic arthritis they indicate the seriousness of the disease (erosive arthritis and involvement of multiple joints) and are usually associated with the shared HLA-DRB1*04 epitope.

An important aspect of anti-CCP antibodies specificity is the cut-off value used to establish test positivity. The ELISA tests available on the market have a zone of results defined

as grey or borderline. Although the correct practice is to establish a cut-off value in each laboratory for the treated population, it is possible to generalize, stating that anti-CCP antibodies specificity lies in selection of a cut-off value just above the grey zone. In addition, rheumatoid arthritis diagnosis is practically concrete in cases showing titers of anti-CCP antibodies above the detection limit of the tests.

Since anti-CCP antibodies were first described, the goal has been to increase their sensitivity, since specificity consistently borders on 100%. The first ELISA tests performed with linear peptides based on filaggrin identified ca 45% of rheumatoid arthritis cases. Later, the system based on CCP-1 detected 68% of all cases. At present, published papers show a sensitivity of 80%, ranging between 67 and 90%. From our experience we may conclude that the ELISA tests available on the market have a sensitivity of 75% of rheumatoid arthritis cases identified by a consultant specialist.

An additional use of anti-CCP antibodies is diagnosis of seronegative rheumatoid arthritis, in which rheumatoid factor is absent. Given the low sensitivity of the initial tests with CCP-1, the method is of little use in seronegative rheumatoid arthritis, with an approximate sensitivity of 15%. Even so, 40-60% of rheumatoid arthritis cases which do not present rheumatoid factor now test positive for anti-CCP antibodies (anti-CCP-2). In these cases, diagnosis

of rheumatoid arthritis is certain if in line with the clinical case history. Diagnosis is very probable in cases in which rheumatoid factor is not found, the titers of anti-CCP antibodies are low and clinical evidence is suggestive of rheumatoid arthritis. Seronegative subjects with doubtful clinical evidence but with medium to high titers of anti-CCP antibodies with respect to the titration curve must be considered to be affected by rheumatoid arthritis and their evolution carefully followed up. In the last two cases it would be advisable to confirm anti-CCP antibodies titers at a later visit (Fig. 1).

Finally, it should be emphasized that detection of anti-CCP antibodies in synovial fluid does not contribute

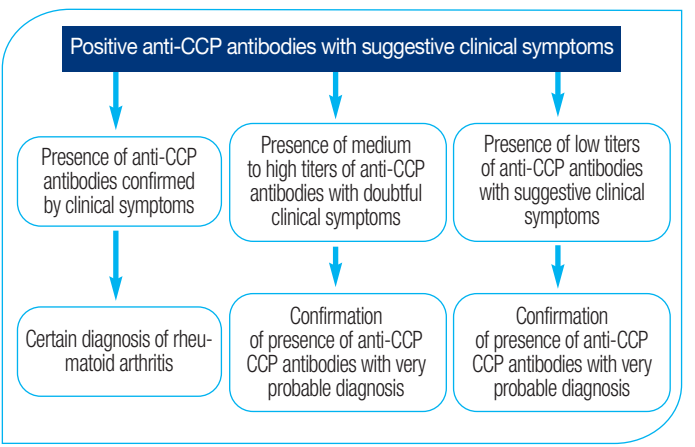

Figure 1. Study of anti-CCP antibodies in the diagnosis of seronegative rheumatoid arthritis.

any diagnostic advantage with regard to detection of serum antibodies even although rheumatoid arthritis is an inflammatory pathology of the synovial joint. However, serum and synovial fluid titers of anti-CCP antibodies maintain good positive correlation.

Anti-CCP antibodies detected in the very early stages

In addition to its specificity, the importance of anti-CCP antibodies also depends on their early appearance. The prevalence of CCP antibodies in patients who undergo rheumatological consultations is 2-5%. Evidence exists for evolution into rheumatoid arthritis in these positive cases.

Presence of anti-CCP antibodies (CCP-1) has been detected two to five years before the appearance of symptoms. In addition, anti-CCP antibodies are detected before (up to 14 years before) the rheumatoid factor (up to 10 years before the onset of symptoms) and with greater specificity. Later, similar data were reported with ELISA tests using CCP-2, with better sensitivity than that of rheumatoid factor.

Anti-CCP antibodies are an essential tool in the early clinical diagnosis of arthritis, now being established in all rheumatology units. The capacity of anti-CCP antibodies to predict later development of rheumatoid arthritis in early arthritis is very high, with a sensitivity of 60% and a specificity of 95% (considering pathologies unrelated to rheumatic arthritis) to 99% (considering healthy subjects).

The probability (odds ratio) of developing rheumatoid arthritis in patients with undifferentiated forms of arthritis is 25 if at the initial vi it to the specialist the presence of serum anti-CCP antibodies is demonstrated. In comparison, the probabilities for the shared HLA-DRB1*04 epitope and the rheumatoid factor are 2.35 or 8.8, respectively. When anti-CCP antibodies are combined with the presence of the shared epitope, the probability of developing rheumatoid arthritis reaches 66.8 with respect to patients who have neither of these two factors.

Prognostic role of anti-CCP antibodies

Anti-CCP antibodies now have such great diagnostic importance that they have been proposed for inclusion as diagnostic criterion in the *American College of Rheumatology's* classification. This is largely due to the discriminative power of the predictive models of persistent erosive arthritis that include anti-CCP antibodies in regression analysis. In this analysis the area under the ROC curve that differentiates between autolimited and persistent arthritis is 0.84, whereas the area under the curve that differentiates between erosive and non-erosive forms is 0.91. In addition, patients with early rheumatoid arthritis with circulating anti-CCP antibodies show greater radiological damage, greater levels of acute-phase reagents and greater number of affected joints after three years of follow-up than those without circulating antibodies. More recent data show similar predictive value for radiological progression between the presence of anti-CCP antibodies and persistent articular inflammation or poor response to treatment.

Also, rheumatoid arthritis with bony erosion shows higher positivity for anti-CCP antibodies with greater average titers. Finally, titers of CCP antibodies above the upper limit of detection of ELISA tests have been observed in 12 patients with Felty's syndrome (a severe form of rheumatoid arthritis with leukopenia and splenomegaly).

In conclusion, anti-CCP antibodies are related to the development of erosive articular injuries and, more recently, they have been associated with development of extra-articular manifestations, although by an approach method less powerful than rheumatoid factor.

Monitoring anti-CCP antibodies during treatment of rheumatoid arthritis

Despite the prognostic value of the presence of anti-CCP antibodies in rheumatoid arthritis, it is not clear that monitoring this presence during treatment (with FAME or with alpha-TNF inhibitors) is useful. Currently, some studies are reporting a reduction, whereas others show no variation. On the contrary, in all studies rheumatoid factor titers drop with biological therapies. In any case, the available evidence involves a small number of patients being monsitored for less than a year, although our experience also suggests that it is not a useful marker in monitoring rheumatoid arthritis treatment. No variation of serum concentration of anti-CCP antibodies has been demonstrated in relation to clinical or radiological aggravation of arthritis. We may deduce from all

this that serial measurements of CCP antibodies are not useful for following up rheumatoid arthritis evolution and that determination need never be repeated beyond the second confirmed result. Finally, there are no described cases of seroconversion of the CCP antibodies, except when the titers are very close to the cut-off value of the test used.

It follows that rheumatoid factor and CCP antibodies are two different types of antibody; rheumatoid factor is affected by biological therapy, whereas CCP antibodies are not.

Conclusions (Table V)

Anti-CCP antibodies represent an essential tool for follow-up of rheumatoid arthritis, which should be available in any autoimmunity laboratory. The serological study of early arthritis in specialist consultations should include testing for rheumatoid factor and for anti-CCP antibodies. The clinical symptoms of arthralgia and arthritis are among the most frequent emerging in primary health-care consultations. Nevertheless, there is as yet no information about adopting anti-CCP antibodies as a rheumatoid arthritis screening method. Figure 2 illustrates an algorithm for using anti-CCP antibodies in the diagnosis of rheumatoid arthritis in primary health care.

TABLE V. CCP antibodies: conclusions.

1. Anti-CCP antibodies are excellent serological markers of rheumatoid arthritis.
2. Anti-CCP antibodies allow early diagnosis of rheumatoid arthritis.
3. Anti-CCP antibodies differentiate rheumatoid arthritis from other forms of arthritis that occur with articular erosions and positive rheumatoid factor.
4. Anti-CCP antibodies are very useful in diagnosis of seronegative rheumatoid arthritis.
5. Anti-CCP antibodies help to identify forms of rheumatoid arthritis with poor prognosis demanding more aggressive treatment.
6. Titers of anti-CCP antibodies are not useful for follow-up treatment of rheumatoid arthritis.
7. There is no reason for quantification of anti-CCP antibodies while monitoring rheumatoid arthritis.
8. The specificity of anti-CCP antibodies hinges on correct selection of a cut-off value. Grey or borderline zones should not be used.
9. Available commercial kits for detecting anti-CCP-2 antibodies have similar diagnostic features.
10. The *American College of Rheumatology's* diagnostic criteria for rheumatoid arthritis will probably include anti-CCP antibodies in the future, although their relevance lies in their early diagnostic capacity rather than classification capacity.

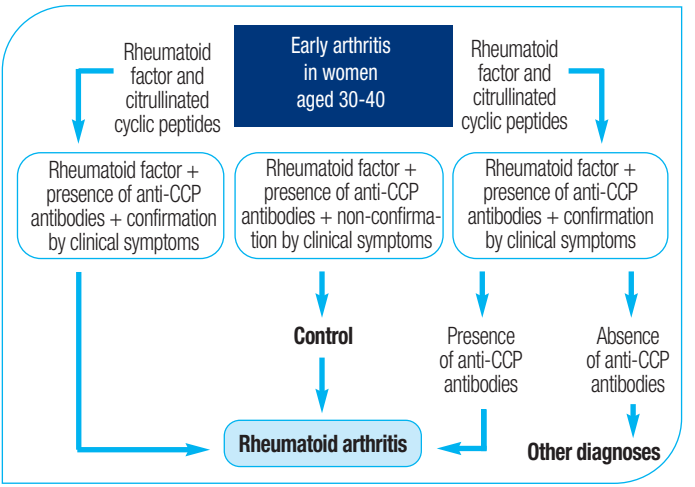

Figure 2. Diagnostic algorithm with serological markers available in clinical history of early arthritis.

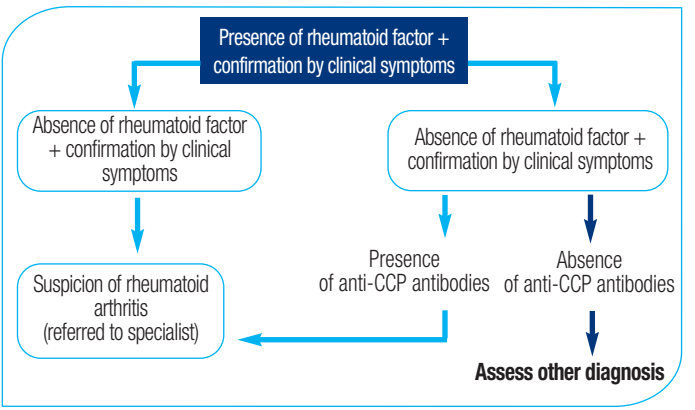

Figure 3. Working algorithm for diagnosis of rheumatoid arthritis in a primary health-care consultation.

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