Potential Biomarkers for Diagnosis of Rheumatoid Arthritis

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Abstract

Background: Rheumatoid arthritis (RA) is a chronic, progressive, inflammatory autoimmune disease characterized by painful, swollen joints. Some patients with RA may present or later develop disease manifestations in other organs, such as interstitial lung disease, pericarditis, pleural effusion, or bronchiectasis. As with other autoimmune rheumatic diseases, the diagnosis depends upon the aggregation of characteristic symptoms, signs, laboratory data and radiological findings. Although nonspecific, is an important predictor of outcome in RA. RF is an autoantibody directed against the Fc portion of immunoglobulin G (IgG). This autoantibody can belong to any of the three main Ig classes G, A or M but the classical RF is pentameric IgM. RF is produced by intra-synovial B-lymphocytes and reacts against IgG molecules that are abnormal in their carbohydrate moieties, a feature that probably renders them immunogenic. Anti-citrullinated peptide/protein antibodies significantly improve the diagnosis of RA especially in the RF negative population. They present in 23% of patients with early stage RA, in about 50% of patients at diagnosis and in about 53% to 70% of patients 2 years after diagnosis. A positive anti-CCP result also predicts joint erosion and radiographic progression in RA. Anti-MCV Ab is a very useful diagnostic test for RA. It is a good marker for early diagnosis of RA with higher sensitivity and specificity when compared to other markers. AKA/APF antibodies recognized epitopes that contained the amino acid citrulline. These two antibodies appeared to have a highly similar specificity for RA patients. In daily practice, Disease Activity Score 28 (DAS28) combining clinical parameters with erythrocyte sedimentation rate (ESR) or C reactive protein (CRP) is used. ESR and CRP are inflammatory biomarkers, but not specific to the joint.

Keywords: Rheumatoid Arthritis, Biomarkers

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Introduction

Rheumatoid arthritis (RA) is a chronic, progressive, inflammatory autoimmune disease characterized by painful, swollen joints. Some patients with RA may present or later develop disease manifestations in other organs, such as interstitial lung disease, pericarditis, pleural effusion, or bronchiectasis **(1).**

The prevalence of RA in most populations is around 1%, while Prevalence of RA in Egypt is around 0.29%**.** The incidence and prevalence increase with age, RA generally manifests between the ages of 30 and 65 **(2).** The incidence of rheumatoid arthritis in women is 4-5 times higher than men below the age of 50 years, but the women/men ratio is only about 2:1 above 60-70 years **(3).**

RA usually has an insidious, slow onset over weeks to months in 55 to 65% of cases, 8 to 15% of patients have an acute onset of symptoms within a few days and are often less symmetric than those with insidious onset,15 to 20% of patients have an intermediate type of onset with symptoms developing over days or weeks, other patterns of presentation include mono articular disease, palindromic (short-lived and episodic) attacks of extra-articular features, such as nodules**.** Whatever the onset is, the subsequent course may be brief or episodic, prolonged and progressive or something intermediate,a monocyclic course is a single cycle with remission for at least 1 year, seen in 10 % of patients, a polycyclic course is seen in 70 % of patients, with either intermittent or continuing subtypes, a progressive pattern with increasing joint damage and extra-articular manifestations is seen in about 10% of patients **(4).**

Conistitional manifestations: Constitutional features such as aching muscles, tiredness, generalized weakness, low mood, fever, weight loss and loss of appetite can accompany the initial onset of RA. **(4).**

Articular manifestations:

RA usually presents as symmetrical and polyarticular (an arthritis affecting more than 3 joints) with the predilection for the wrists, the metacarpophalengeal joints and the proximal interphalengeal joints **(5).**

Arthritis of joints involves inflammation of the synovial membrane. Joints become swollen, tender and warm and stiffness particularly early in the morning on waking or following prolonged activity. Increased stiffness early in the morning is often a prominent feature of the disease and typically lasts for more than an hour. Gentle movements may relieve symptoms in early stages of the disease, Most commonly involved are the small joints of the hands, feet and cervical spine, but larger joints like the shoulder and knee can also be involved**.** The most common deformities are boutonniere deformity, swan neck deformity and ulnar deviation of fingers **(6).**

Extra-articular manifestations:

1-Cutaneous manifestations:

They are characterized by classical rheumatoid nodules, rheumatoid vasculitis and granulomatous dermatitis **(7).**

Classical rheumatoid nodules are the most common extra-articular manifestation in patients with RA; About 25% to 35% of people with rheumatoid arthritis develop nodules in different parts of the body. They are subcutaneous lesions, skin-like colored movable, typically appear in the advanced phase and there is a strict association with HLA DR4 and DRB1 haplotypes **(8).**

2-Ocular manifestations:

Keratoconjunctivitis sicca (eye dryness) is common in RA. Episcleritis and scleritis may occur **(8).**

3-Respiratory manifestations:

Lung fibrosis is a consequence of therapy with methotrexate and leflunomide. Exudative pleural effusions are also associated with RA **(9).**

4-Cardiac manifestations:

Pericarditis can occur in almost 50% of patients. It is manifested by pain or pericardial effusion. It may progress to right sided heart failure **(10).**

5-Gastrointestinal (GIT) manifestations:

Dry mouth or ischemic bowel disease as a complication of rheumatoid vasculitis. Gastritis and peptic ulcer occur as a complication of non- steroidal anti- inflammatory drugs (NSAID) therapy **(11).**

6-Renal manifestations

The kidney is rarely involved directly in RA. Proteinuria develops either due to drug toxicity or secondary to renal amyloidosis. Interstitial renal disease occurs with Sjogren's syndrome or related to exposure to NSAIDs or acetaminophen. Membranous nephropathy is related to therapy with gold salt and D-penicillamine. Phenacetin abuse causes renal papillary necrosis **(12).**

7-Neurological manifestations:

The most dangerous neurological complication is cervical spine or neck inflammation with compression on the spinal cord causing numbness, tingling, weakness and pain in the arms or whole body along with severe headaches **(.** Also, entrapment neuropathy as carpal tunnel syndrome, peripheral neuropathy and rheumatoid vasculitis may cause transient ischemic attacks, stroke, quadriplegia or paraparesis **(5).**

8-Haematologic manifestations:

Anemia of chronic disease due to increased hepcidin levels, poor iron absorption and iron sequestration into macrophages **(13).**

Diagnosis of rheumatoid arthritis:

There is no single clinical, radiologic or serologic test which enables a diagnosis of RA. Early diagnosis and treatment of RA can prevent progression of joint damage in up to 90% of patients, thereby preventing irreversible disability. Although no diagnostic criteria exist, classification criteria that include clinical manifestations and serological assays (autoantibody and acute-phase reactant levels) inform clinical diagnosis **(14).**

The 2010 classification criteria of RA requires presence of at least 1 clinically swollen joint and at least 6 of 10 points from a scoring system. Sensitivity of the new classification criteria was 11% greater and specificity 4% lower compared with the 1987 criteria **(15).**

Table (2): American College of Rheumatology/European League against Rheumatism Classification Criteria 2010 for rheumatoid arthritis, the classification criteria should be restricted to individuals with ≥1 swollen joint. A score of ≥6 points is required for classification as definite rheumatoid arthritis **(15).**

To be applied to patients: (1) who have ≥ 1 joint with definite synovitis, excluding the DIP joints, first MTP joints, and first CMC joints, and (2) in whom the synovitis cannot be explained by another disease.

Criteria	Score
A. Joint involvement:	
1 large joint	0
2 - 10 large joints[a]	1
1 - 3 small joints (with or without involvement of large joints)	2
4 - 10 small joints[b] (with or without involvement of large joints)	3
> 10 joints (at least 1 small joint)	5
B. Serology (at least 1 test result is needed for classification):	
Negative RF and negative anti-CCP antibodies	0
Low-positive RF or low-positive anti-CCP antibodies[c]	2
High-positive RF or high-positive anti-CCP antibodies[d]	3
C. Acute phase reactants:	
Normal CRP level and normal ESR	0
Abnormal CRP level or abnormal ESR	1
D. Duration of symptoms:	
< 6 weeks	0
≥ 6 weeks	1

ACR, American College of Rheumatology; EULAR, European League Against Rheumatism; RA, rheumatoid arthritis; DIP, distal interphalangeal; MTP, metatarsophalangeal; CMC, carpometacarpal; MCP, metacarpophalangeal; PIP, proximal interphalangeal; IP, interphalangeal; RF, rheumatoid factor; anti-CCP, anti–cyclic citrullinated protein; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

[a] Large joints = shoulders, elbows, hips, knees, ankles.

[b] Small joints = MCPs, PIPs, second - fifth MTPs, thumb IPs, wrists.

[c] Low-positive is ≤ 3 times the upper limit of normal.

[d] High-positive is > 3 times the upper limit of normal.

Conventional radiography remains the imaging modality of choice in evaluating the efficacy of treatments. The evolution of radiographic findings ranges from joints with minimal abnormalities to severe destructive changes seen as bony erosions and joint space narrowing reflecting cartilage changes **(14).**Positive anti-CCP is associated with a higher larsen score at baseline and at follow up and it predicts radiological joint damage in early RA, change in the Larsen score and poor functional response in patients who were RF negative **(16).**

Assessment of disease activity:

1-Modified Larsen system for grading RA:

Modified Larsen scores were calculated to assess joint destruction. Postero-anterior radiographs were taken of hands, wrists and feet at enrolment and after two years. Radiographic damage was classified by comparison with standard reference films according to the modified method of Larsen ***(17).***

Each joint is graded 0–5, as follows:

Grade	Definition
0	Normal
1	Soft tissue swelling, slight joint space narrowing (< 25% of the original joint space), periarticular osteoporosis
2	Definite early abnormality, one or several small erosions
3	Medium destructive abnormality, marked erosions
4	Severe destructive abnormality, large erosions
5	Gross deformity, the bony outlines of the joint have disappeared

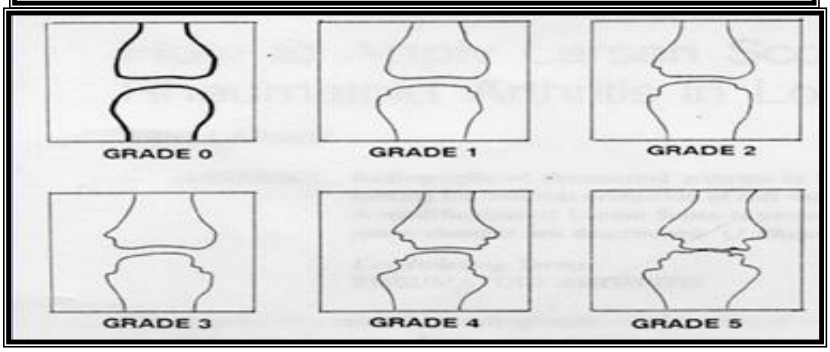


Figure (): Modified larsen scoring **(18).**

2-Disease Activity Score 28 (DAS-28):

DAS-28 is an index derived from the original DAS with fewer joints included. DAS28 consists of a 28 tender joint count (range 0-28) which include right and left {4 PIPs, thumb interphalangeal (IP), 5 MCPs, wrists, elbows, shoulders and Knees}, a 28 swollen joint count (range 0-28), ESR and global health (GH) in the Disease Activity Score (DAS) and the DAS-28 indices. Global assessment of overall well being measured on an anchored horizontal scale (range 0-100 mm), visual analogue scale for measurement of pain (VAS) with "very well" at one end (scored 0) and "very poor" at the other end (scored 100) **(19).**

DAS-28 is a continuous index ranging from 2 to 10 **(20)**.

DAS-28= 0.56*√(TJC28) + 0.28*√(SJC28)+ 0.70*ln(ESR) +0.014* (general health)

Grading: (van Gestel et al., 1998).

1. **Low disease activity** is defined as DAS-28 ≤ 3.2.
2. **Moderate** as 3.2 < DAS-28 ≤ 5.1.

3. **High** as DAS-28 > 5.1
4. **Remission** corresponds to DAS-28<2.6.

3-Simplified Disease Activity Index (SDAI):

Is the numerical sum of 5 sensitive assessment measures, tender and swollen joint count (28-joint count), patient and physician global assessment of disease activity (VAS) and level of CRP. An SDAI score ≤3.3 denotes remission, > 3.3 and ≤ 11 denotes low activity, >11 and >26 denotes moderate activity, or >26 denotes high disease activity**(14).**

4-Clinical Disease Activity Index (CDAI):

The CDAI is an abbreviated version of the SDAI and does not include the CRP value**.** A CDAI of ≤ 2.8 denotes remission, values > 2.8 and ≤ 10 denote low disease activity, values >10 and ≤ 22 denote moderate disease activity and values >22 denote high disease activity **(14).**

5-American College of Rheumatology/EULAR remission criteria for rheumatoid arthritis: 2022 revision

A joint ACR/EULAR guideline on remission criteria for RA, first published in 2011 and updated in 2022, includes two definitions, one a Boolean-based definition and the other based on a composite index of RA activity, either the SDAI or the CDAI (the latter omits CRP). The Boolean-based definition requires that the patient satisfy all of the following to be considered in remission:

- Tender joint count of 1 or less
- Swollen joint count of 1 or less
- Patient global assessment of 2.0 or less (on a 0-10 scale)
- Optional: CRP 1 mg/dL or lower

To be considered in remission using the traditional index definition, the patient must have an SDAI score of less than 3.3, or a CDAI score of 2.8 or lower **(18).**

Laboratory diagnosis of RA:

As with other autoimmune rheumatic diseases, the diagnosis depends upon the aggregation of characteristic symptoms, signs, laboratory data and radiological findings **(15).**

Potential biomarkers for rheumatoid arthritis:

Table (3): Autoantigens in RA **(21).**

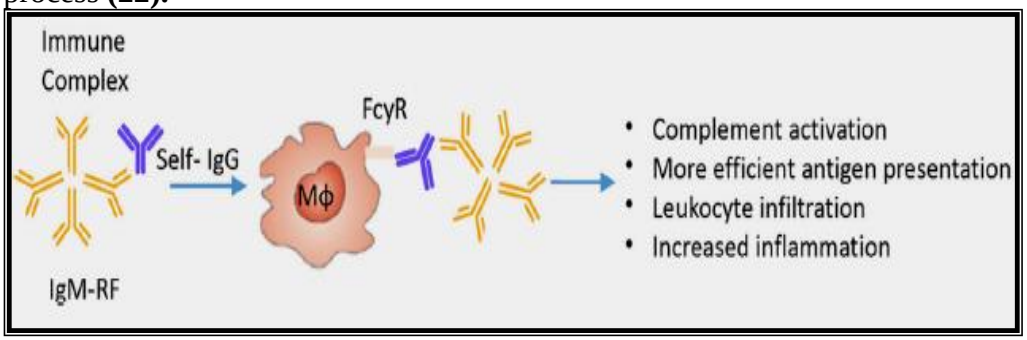
Established	T or B Cell[a]	Molecular Specificity	Assay
Immunoglobulin G	B	Human Fc IgG	Rheumatoid factor
Cyclic peptides	T and B	Citrullinated peptides	Anti-CCPs
Fibrin	T and B	α- and β-chain epitopes	Research[b]
Fibrinogen	T and B	Multiple epitopes	Research[b]
Enolase	T and B	CEP-1 dominates	Research[b]
Vimentin	T and B	Citrullinated vimentin	MCV assay
Collagen II	T and B	Multiple epitopes	Research[b]
HnRNPA2	B	Multiple epitopes	Research[b]
Aggrecan	T and B	Multiple epitopes	Research[b]
HCgp-39	T	Multiple epitopes	Research[b]
Glucose-6-phosphate isomerase	B	Multiple epitopes	Research[b]

Autoantibodies:

1. ***Rheumatoid factor:***

Although nonspecific, is an important predictor of outcome in RA. RF is an autoantibody directed against the Fc portion of immunoglobulin G (IgG). This autoantibody can belong to any of the three main Ig classes G,

A or M but the classical RF is pentameric IgM. RF is produced by intra-synovial B-lymphocytes and reacts against IgG molecules that are abnormal in their carbohydrate moieties, a feature that probably renders them immunogenic. The resulting immune complex is likely to participate in the perpetuation of the inflammatory process **(22).**



Figue (14): Schematic presentation of IgM-RF **(22).**

RF is found in 75 to 80 % of RA patients at some time during the course of their disease. Higher titer of IgM RF is relatively specific for the diagnosis of RA. In addition, RF may have some prognostic values as regards disease manifestations, activity and the severity of joint erosions. Seropositive RA with IgM RF is often associated with more aggressive joint disease and is commonly complicated by extra-articular manifestations than seronegative RA **(23).**

There is about 15% of RA patients are seronegative. During the first year of illness, rheumatoid factor is more likely to be negative with some individuals converting to seropositive status over time. RF is also seen in other illnesses for example Sjögren's syndrome, Hepatitis C, chronic infections and in approximately 10% of the healthy population, therefore the test is not very specific **(23).**

The decrease in IgM RF levels was accompanied by a decrease in ESR and CRP values suggesting that IgM RF can act as a marker of inflammatory activity **(24).**

RF is a strong predictor of clinical and radiographic progression in patients with RA. High RF titers are further predictive of patients at greatest risk of erosive damage, combined increase in IgM and IgA RFs is found almost exclusively in patients with RA**(24).**

2. *Anti–cyclic citrullinated peptide:*

Anti-citrullinated peptide/protein antibodies significantly improve the diagnosis of RA especially in the RF negative population. They present in 23% of patients with early stage RA, in about 50% of patients at diagnosis and in about 53% to 70% of patients 2 years after diagnosis. A positive anti-CCP result also predicts joint erosion and radiographic progression in RA **(25).**

A positive anti-CCP result means RA is likely but a negative result does not rule out RA. Anti-CCP demonstrate a higher sensitivity of 78.5% when compared to RF and a much higher specificity of 95.9% **(26).** Testing for the combination of anti-CCP antibodies and the IgM-RF was found to improve drastically the diagnostic sensitivity and specificity in patients with early RA than by testing either antibody alone. So the presence of either RF or anti-CCP increases the sensitivity to 85% and the presence of both RF and anti-CCP shows a specificity of 100% **(27).**

Anti-CCP can also differentiate RA from hepatitis C infection associated with rheumatological manifestations and positive RF **(27).**

Van Venrooij et al.(28) made a cyclic peptide (substituting the terminal serine residues with cysteines and cyclizing the peptide through the formation of a disulfide bond) to which anti-CCP antibodies reacted with higher affinity. Cyclic citrullinated peptides were subsequently used as antigens in the first generation of CCP test (named CCP1).

To improve the CCP1 test, libraries of citrullinated peptides were used to construct the second-generation antiCCP assay (CCP2), which was broadly adopted for clinical use. This CCP2 test has slightly better performance in term of characteristic than anti-CCP1 antibodies**.** Anti CCP2 antibodies is the most widely use anticitrullinated peptide assay. A third generation of anti–cyclic citrullinated peptide (CCP3) has been

reported to recognize additional citrullinated epitopes that are not identifiable with the second-generation CCP assays **(29).**

3. ***Anti-mutated citrullinatedvimentin (Anti-MCV)Ab:***

It is a member of ACPA family. Anti-MCV Ab is a very useful diagnostic test for RA. It is a good marker for early diagnosis of RA with higher sensitivity and specificity when compared to other markers. The use of anti-MCV and anti-CCP collectively give the best result for the diagnosis of rheumatoid disease **(30).**

4. ***Antiperinuclear factor and antikeratin antibodies:***

Anti-perinuclear factor (APF) is an autoantibody which reacts with the keratohyaline granules scattered around the perinuclear region of the human buccal epithelium. The target molecule of APF was profilaggrin (the precursor molecule of filaggrin). Antikeratin antibodies (AKA) are also RA–specific autoantibodies. The antigen targeted by AKA is the intermediate filament-aggregating protein filaggrin which is expressed exclusively in keratinizing epithelial cells. **Andrade et al. (29)** showed that the AKA/APF antibodies recognized epitopes that contained the amino acid citrulline. These two antibodies appeared to have a highly similar specificity for RA patients **(31).**

The sensitivity and specificity of APF and AKA vary depending on the laboratory means of detection. APF detected by indirect immunofluorescence (IIF) using buccal epithelium has a reported specificity for RA of 73% to 99% and a sensitivity of 49% to 91%. Moreover, the combination of testing AKA, APF and RF in the diagnosis of RA has shown to have a sensitivity and specificity of 75% and 82 % respectively, if at least one test was positive **(29)**

Markers of inflammation:

In daily practice, Disease Activity Score 28 (DAS28) combining clinical parameters with erythrocyte sedimentation rate (ESR) or C reactive protein (CRP) is used. ESR and CRP are inflammatory biomarkers, but not specific to the joint **(32).**

1. ***C-reactive protein:***

C-reactive protein is an acute-phase reactant serum protein that is present in low concentration in normal serum. It is produced by the liver during periods of inflammation and is detectable in the serum of patients with various infectious and inflammatory diseases **(33).**

In RA, an elevated level of CRP correlates with disease activity in most patients. It is also shown to be elevated in patients with extra-articular manifestations. CRP levels are found to drop after the use of disease modifying anti-rheumatic drugs rather than after non-steroidal anti-inflammatory drugs **(34).**

2. Erythrocyte sedimentation rate:

ESR is not specific as it rises with anemia, use of cholesterol-lowering drugs. It also rises with age and is of extremely limited value in the elderly. A normal ESR excludes active inflammatory disorders including RA. It can be used to monitor the inflammatory activity during therapy **(35).**

Many outstanding clinical questions remain that better biomarker identification could help answer. Does seronegative RA truly exist, or have we simply not yet identified the antibodies this subset of patients makes? Can we identify a biomarker that permits earlier RA diagnosis, widening the golden three- to six-month "window of opportunity" to therapeutically intervene? Can a universal biomarker be found that accurately identifies ongoing subclinical disease activity, permitting better titration of RA therapies? Can we identify biomarkers that allow for the personalized selection of RA therapies, permitting more rapid and effective disease control? Future work should pursue answers to these questions. Better biomarkers could lead to earlier diagnosis, treatment, and outcomes. Once a better biomarker is identified, the cost and feasibility of testing will need to be considered in order to ensure clinical utility on a worldwide scale.

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