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Progress in the Use of Biochemical and Biological Markers for Evaluation of Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disorder which is predominant in females. The exact etiology remains undefined.

Recently, a large number of biochemical and biologic markers, which are useful in the diagnosis, prognosis, and monitoring therapy of RA, have been reported. The new markers include genetic markers, filaggrin, citrulline containing peptides, A2/RA 33, cytokines, joint and collagen breakdown products, and bone turnover markers. No laboratory tests in and of themselves are diagnostic of RA. The new markers have been employed in monitoring RA patients during treatment and following the course of the disease. With the development of innovative therapies for RA, many of the biochemical and biologic markers will be useful. J. Clin. Lab. Anal. 14:305–313, 2000. © 2000 Wiley-Liss, Inc.

Key words: rheumatoid arthritis; biochemical; biological and genetic markers; laboratory tests

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disorder that affects 1% of the population worldwide (1). A major unknown in the course of the disease in RA is the reason that inflammation begins and continues within joints often without involvement of other organ systems (2–4).

RA is one of many autoimmune diseases that predominate in females. The ratio of female to male patients may vary from 2:1 to 4:1 (2). Pregnancy usually is associated with remission of the disease with subsequent relapses after delivery (5).

RA patients are heterogeneous in their genetics, manner of clinical presentation, and responses to therapy (2–4). Approximately one-half of RA patients have an acute onset of joint symptoms characterized by pain and swelling. RA patients in another group of have less dramatic onset of joint symptoms that may be intermittent and are often controlled by common pain relievers such as nonsteroidal anti-inflammatory drugs (NSAIDs). Almost one-fourth of patients of each group may have spontaneous remissions. However, over one-half of each group will develop a progressive, erosive joint disease (2–4).

CLASSIFICATION CRITERIA FOR RA

In individuals with established typical disease, the diagnosis of RA can be easily made most often within the first year of disease onset; however, in many patients, the progression of symptoms may be atypical and a longer period of evaluation is needed before the diagnosis of RA can be made.

The first criteria for classification (not diagnosis) of RA were published in 1958 (6). The initial criteria were revised in 1987 and published in 1988 (7). The newer criteria are simpler to apply than the previous criteria of 1958. The revised criteria are shown in Table 1. To make a diagnosis of RA, criteria 1 through 4 must have been present for at least 6 weeks; 4 or more criteria must be present.

The new criteria demonstrate 91–94% sensitivity and 89% specificity for RA when compared with non-RA rheumatic disease control subjects (7). The diagnosis of RA should not be made by criteria alone if another systemic disease associated with arthritis is definitely present. Other diseases most likely to be confused with early-onset RA include systemic lupus erythematosus (SLE), psoriatic arthritis, and other seronegative spondyloarthropathies, mixed connective tissue disease, Reiter's syndrome, polymyalgia rheumatica, and Sjögren's syndrome with polyarthritis.

PATHOGENESIS OF RA

The exact cause of RA remains unknown. The old paradigm in regard to pathogenesis of RA was dominated by the concept that the disease manifestations are the result of an infectious disease (2,8). It was believed that the pathogenesis of RA relates to a persistent immunologic response of a genetically susceptible person to a relevant antigen that may be an infectious agent such as a virus (2,8).

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TABLE 1. The 1987 revised criteria for the classification of rheumatoid arthritis (7)

1. Morning stiffness in and around joints lasting at least 1 hour before maximal improvement
2. Soft tissue swelling (arthritis) of three or more joint areas observed by a physician
3. Swelling (arthritis) of the proximal interphalangeal, metacarpophalangeal, or wrist joints
4. Symmetric arthritis
5. Subcutaneous nodules
6. Positive test for rheumatoid factor (RF)
7. Radiographic erosions or periarticular osteopenia in hand or wrist joints
   - Four of seven criteria are required to classify a patient as having rheumatoid arthritis
   - Criteria 1 through 4 must be present for at least 6 weeks
   - Criteria 2 through 5 must be observed by a physician

Weyand and Goronzy (8) have proposed a new hypothetical disease model for RA. This model integrates the improved understanding of the genetic risk factors and new research discoveries on the complexity of the inflammatory responses. This model makes the assumption that the host's immunoresponse is not involved in the initial disease process. The various immune HLA response genes, immunoglobulin genes, and T-cell receptor genes have an impact later in the chain of pathologic events during Stage II of the disease. The initial Stage I in this complex model involves synovial tissue injury resulting from a possible infection with exposure to a wide spectrum of antigens, including many autoantigens generated by tissue injury (8).

Over the past several years, studies in the role of cytokines such as IL-1 (interleukin-1) and TNF-α (tumor necrosis factor-α) in the mediation of tissue injury in RA have become important (8,9). Many new and innovative therapeutic approaches with prevention of clinical progression of RA are being developed. These include the use of soluble receptors to IL-1 (9,10) or TNF-α (9,11), monoclonal antibodies to TNF-α (12), a specific IL-1 receptor antagonist (13), and gene therapy (14,15). Other reported therapeutic approaches to treatment of RA such as IL-10 (16) and oral tolerization treatment (17) have shown promise in prevention of clinical progression of RA.

DIAGNOSIS OF RA

The diagnosis of RA is made by established criteria based on clinical history, physical examination, and laboratory tests. There is no simple feature that makes a definite diagnosis (2–4,18). The American Rheumatism Association (ARA) criteria for classification need not be used; however, the requirement for objective evidence that synovitis must be present for at least six weeks is important (7).

No laboratory tests in and of themselves are diagnostic of RA. The laboratory tests and biochemical and biological markers are useful in the determination of prognosis and response to therapy (4,18–20).

TYPES OF BIOLOGICAL MARKERS USEFUL FOR EVALUATION OF RA

There are several types of biological markers that may be useful in assessing disease activity and prognosis as well as in the treatment and management of RA patients (18–20).

The various types of markers are as follows:

1. Genetic markers
   - HLA-D4; HLA DRB-1
   - Non-HLA markers 2q34 (TNP1) and 2q35 (K812, VIL1, DES) (26)
2. Disease-associated autoantibodies
   - Rheumatoid factor
   - Antinuclear antibodies (ANA)
   - Anti-filaggrin (Anti-keratin, anti-perinuclear factor)
   - Anti-citrulline epitope containing peptides
   - Anti-A1/RA33
3. Markers of inflammatory process
   - Acute phase reactants
     i. ESR (erythrocyte sedimentation rate)
     ii. CRP (C-reactive protein)
     iii. SAA (serum amyloid-associated protein)
   - Cytokines/inhibitors including K-1, TNF-α, IL-6, IL-8, ENA 7 (epithelial neutrophil activating peptide)
4. Joint and cartilage breakdown products
   - Hyaluronic acid
   - COMP (cartilage oligomeric protein)
   - Aggrecan
5. Bone turnover
   - Bone sialoprotein
   - Pyridinoline crosslinks

GENETICS OF RHEUMATOID ARTHRITIS

There is a strong association between RA and the presence of HLA-DR4 in distinct ethnic groups including Whites, Japanese, Southern Chinese, American Blacks, and native populations in the Americas (21–23). In other ethnic groups, association between RA and other MHC (major histocompatibility center) Class II gene products have been observed (21–25). RA has been associated with DR1 in Asian Indians and Ashkenazi Jewish groups and DW16 in Yakima Indians (21–23). The association between RA and certain MHC (major histocompatibility complex) may be important in determining the pathogenesis and response to certain therapeutic modalities in the RA patients.

The following important concepts in the genetics of RA have been observed (4):

1. First-degree relatives of patients with rheumatoid arthritis develop rheumatoid arthritis at four to six times the normal rate.
2. Particular HLA-DR molecules, all of which share a common sequence in the third hypervariable region, are predisposing factors for rheumatoid arthritis.

3. The *HLA-DRB1*0401* allele (encoding the HLA-DR4Dw4 molecule), is the most commonly found allele in patients with rheumatoid arthritis.
4. Patients who are homozygous for the arthritogenic subtypes of HLA-DR4 appear to have more severe disease.

The HLA-DR4 family is now known to include at least 22 variants with amino acid polymorphisms in positions 57, 67, 70, 71, 74, and 86 of the HLA-DDRB1 gene (21–25). All of the amino acid positions can be assigned to the third hypervariable regions of the HLA-DR chain. The variants HLA-DRB1*0401, B1*0404, B1*0405, and B1*0408 account for the HLA-DR alleles that are preferentially encountered in RA patients (21–25), whereas HLA-DRB1*0402 and B1*0403 have not been observed with increased frequency in RA patients.

It should be noted that 42% of the normal population has one or more DRB1 alleles with the "RA susceptibility sequence." Therefore, oligotyping for the various DRB1 alleles would not prove to be a specific test in clinical practice.

An important question is, "Can one exclude a diagnosis of RA if oligotyping for DRB1 sequence was found to be negative?" Currently, the answer to this question is unknown. Because of the high normal population frequency of the susceptibility sequence, DRB1 sequence oligotyping cannot be used as an inclusionary criterion for diagnosis of RA.

Recent retrospective studies suggest that the RA-associated DRB1 sequence may serve as a "severity" factor in RA. In a study by Weyand et al. (21,22) of 102 patients with seropositive erosive RA, 96% had the RA-associated sequence and 46% had inherited a "double dose." These patients who were homozygous for the sequence (especially DR4 homozygotes) were found to have more nodular disease, systemic involvement, and previous joint surgery. Thus, this study suggests that DRB1 oligotyping may be useful to identify RA patients at highest risk for the most severe disease.

There are other non-HLA genes associated with RA. Genetic predisposition to RA was associated with a particular allele of the gene encoding macrophages factor NRAMP1 (26). NRAMP1 gene regulates primary activation of macrophages for increased TNF-α and IL production. In the same study (26), they identified four other genetic loci associated with a subset of RA patients. The genes were located at 2q34 (TNP1) and 2q35 (K8R, VIL1, DE5). Further studies are needed to determine if the above non-HLA genetic markers provide useful prognostic clinical information.

RHEUMATOID FACTOR

A factor in sera of patients with rheumatoid arthritis (RA) that agglutinated red blood cells sensitized with immunoglobulin IgG antibodies was first observed in 1940 by Waaler (27). Subsequently, this factor was termed rheumatoid factor (RF). Rheumatoid factors (RFs) are antibodies directed against the Fc region (CH_2, CH_3 domains) of human IgG molecules (28). They are the hallmark autoantibodies of rheumatoid arthritis (RA), an extravascular immune complex disease (18,19,28).

The synovial fluid from the joints of rheumatoid arthritis patients contain many aggregates of immunoglobulins (Ig) and depressed levels of complement (2,8,28). These findings suggest that RFs may contribute to immune complex formation, complement consumption, and chronic tissue damage in the rheumatoid joint and synovial tissue (2,8,28).

RF is included in the 1987 laboratory criteria of RA by the American Rheumatism Association (7). The RFs are found in IgM, IgA, and IgG classes. The IgM class is the most prevalent and is found in 60–80% of RA patients. Overall, about 20% of patients with clinically apparent RA may yield negative RF results (18,19). However, RF is not specific for RA and is found commonly in systemic rheumatic diseases such as systemic lupus erythematosus (SLE), various bacterial and viral infections, cryoglobulinemia, parasitic diseases, and even in healthy subjects (18,19,28). About one-third of patients with SLE are RF positive (18).

On the basis of sensitive quantitative immunoassays for RF, it is believed that many healthy subjects may have very low levels of RF. The signs and symptoms of RA may precede development of RF for up to six months in certain RA patients. RF remains one of the key tests to subdivide RA patients in RF-positive (seropositive) and RF-negative (seronegative) patients. The absence of RF in a RA patient initiates a search for other causes of arthritis such as Reiter's syndrome, systemic lupus erythematosus, infections, and metabolic diseases. It should be noted that RF is frequently absent in juvenile onset rheumatoid arthritis. Some of the RF-negative adult RA may represent an adult onset of the juvenile RA (18,19,28).

Studies of monoclonal and polyclonal RF have shown polyreactive RF with binding specificity for a variety of substances other than IgG, such as certain nuclear components (18). The polyreactive RF is usually of low affinity and belongs predominately to the IgM class. The clinical and pathogenic significance of the cross-reactive properties is not well known (19,29).

Despite the lack of specificity for RA, a positive RF is an important predictor of outcome in RA. RF is associated with more active disease and development of bone erosions (18,19,30).

IgG, IgA, AND IgM RF ISOTYPES

Conventional agglutination assays usually measure the IgM-RF. Isotypes such as IgG and IgA rheumatoid factors (RFs) can be detected by enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) (31). Jonsson et al. (32,33) provided evidence that a combined elevation of IgM and IgA RF isotypes may be highly specific for RA and is

very rarely found in rheumatic diseases other than RA. In this study, RA patients had an elevation of two or more RF isotypes compared to only 16% of other RF-positive patients. A combined elevation of IgM and IgA RF was found in 52% of the RF-positive RA patients, but only in two (4%) of the other RF-positive patients. Jonsson et al. (33–35) concluded that the detection of the RA-specific RF isotype pattern may be particularly helpful early in the course of RA even before the disease is fully differentiated.

Several of the studies have suggested that IgA RF positivity is associated with more active RA, increased joint damage, and a higher frequency of extra-articular manifestations (35). However, other investigators failed to find associations between IgA RF with more aggressive RA with increased joint damage (31).

Houssien et al. (31) investigated whether the use of different antigens to measure RF alters relationships between RF isotypes and clinical variables. The IgM and IgA RF isotypes were measured with rabbit IgG and horse IgG as antigens. The RA patients positive for IgA RF and IgM RF against rabbit IgG had significantly higher disease activity and more radiological damage than negative patients. With horse IgG as antigen, these differences were smaller or absent. The patients positive for only IgM RF had milder disease than patients positive for IgA RF with or without IgM RF.

Jonsson et al. (34) studied the clinical implications of IgA RF subclasses. They observed that nearly all (31/32) RA patients with increased IgA RF had increases in both of the IgA RF subclasses compared with 67% (20/30 of non-rheumatic symptom for individuals with increased IgA RF). RA patients with extraarticular manifestations more often had increased concentrations of IgA RF and both subclasses than patients without extraarticular lesions. They found that increased concentrations of both IgA RF subclasses appear to be more specific for RA than increased IgA RF.

IgG RF is often present in sera and synovial fluids of many patients with severe RA (36). The routine assays for IgG RF presents some difficulties as the often-present polyvalent IgM RF must be removed by gel filtration or other means before IgG RF can be easily quantitated. IgG RF may precipitate in the cold; it is frequently monoclonal and is detected in cryoglobulinemia and diseases other than RA (37). In patients with rheumatoid vasculitis or the hyperviscosity syndrome, IgG RF levels may help in monitoring response to therapy (38).

ANTINUCLEAR ANTIBODIES (ANA) IN RA

Antibody to RA-associated nuclear antigen (RANA) was first demonstrated in Sjögren's syndrome patients associated with RA (39,40). The RANA was detected in WiL-2 cells (Epstein-Barr virus (EBV)-transformed lymphocytes) in a finely speckled pattern by immunofluorescence microscopy studies (39,40). There was a definite relationship between RANA and EBV. Venables et al. (41) demonstrated that RANA had epitopes in common with Epstein-Barr virus nuclear antigen-1 (EBNA-1). The main epitope of RANA is localized on a stretch of 20 amino acids of the p62 peptide, which corresponds to part of the internal repeat sequence (I4-3) of EBNA-1. The anti-RANA antibodies may represent a specific immune response to EBV-encoded antigens in patients with RA. Anti-RANA (p62) also cross-reacts with keratin, actin, and collagen and may be found in healthy persons at low titers (41,42,43). Autoantibodies to histones occur in juvenile rheumatoid arthritis (44,45), rheumatoid arthritis, and in a number of other diseases including systemic lupus erythematosus and drug-induced lupus (39,40). The presence of ANA is the hallmark in systemic rheumatic diseases. However, in RA patients, there is no specific diagnostic profile of ANA. (46)

ANTIFILAGGRIN (ANTIKERATIN AND ANTIPERINUCLEAR FACTOR)

Antikeratin and antiperinuclear factors have been studied for many years and were found to be as specific for rheumatoid arthritis as rheumatoid factor. Both antikeratin and antiperinuclear factors are sometimes present before the onset of clinical disease RA (19,20). The antigens of both of these antibodies have now been identified as epidermal filaggrin, an intermediate filament-associated protein involved in the cornification of the epidermis (47). The identification of filaggrin will allow development of more reproducible enzyme immunoassays. In the past, these antibodies have been detected by indirect immunofluorescence using buccal epithelium or rat esophagus (19,20).

Antiperinuclear factor (APF) was described in 1964 by Nienhuis and Mandema (48), who discovered antibodies in sera of RA patients. The antibody in RA patients was reactive with perinuclear keratohyalin granules of buccal mucosal cells. The antibody assay was performed by immunofluorescence microscopy on buccal mucosal cells. The antiperinuclear factor (APF) antibodies are present in 49–91% of RA patients with specificity between 73% and 99% (49). The immunofluorescence assay is difficult as not all buccal mucosal cells are suited as antigenic substrates. Only about 5% of donors can provide good buccal mucosal substrates for the APF assays. Thus the APF test is difficult to perform and is not a routine assay available in clinical laboratories.

All APF-positive sera tested had IgG antibodies. Studies on APF isotypes have shown that APF IgG is the predominant isotype; IgA and IgM may be present as well. However, a positive IgA-APF was found in one-third of the RA patients (50).

Antikeratin antibodies (AKA) were identified by indirect immunofluorescence in the serum of RA patients by Young et al. (51). The antibody reacted against the stratum corneum of rat esophagus and specifically recognized the 37-kDa basic epidermal filaggrin and its 40-kDa neutral/acidic isoform

(52). The occurrences of positive AKA reactions in RA sera has ranged from 36 to 59% (49).

The presence of both APF and AKA is independent of the disease duration; they appear early and may even precede clinical manifestations of RA (6,47,52). The presence and titers of antibody have been observed to correlate with each other, with the presence of rheumatoid factor (RF), and with the severity and activity of the disease (47,52). Sebbag et al. (47) has provided convincing data that APF and AKA are largely the same autoantibodies. They recognize human epidermal filaggrin- and (pro)filaggrin-related proteins of buccal epithelial cells. The major AFP antigen is a diffuse protein base of 200–400 kDa that is closely related to human epidermal (pro)filaggrin.

FILAGGRIN

Filaggrin is a 37-kDa intermediate filament associated protein involved in the aggregation of cytokeratin filaments during the cornification of the epidermis. Sebbag et al. (47) purified filaggrin and developed an immunoblot assay. This immunoblot assay for filaggrin is more sensitive and reliable than the immunofluorescence assay for APF or AKA. An assay for filaggrin by an immunoenzyme method may be the assay of the future for diagnostic clinical laboratories.

CITRULLINE-CONTAINING PEPTIDES

Schellekens et al. (52) reported that sera from RA patients contain autoantibodies that react with synthetic peptides containing citrulline, a post-transitionally modified arginine residue. With use of an enzyme-linked immunosorbent assay (ELISA) these citrulline peptide reactive antibodies were found in 76% (102/134) of RA sera with a specificity of 96% with 3.7% (13/354) of false positives. The antibodies in RA patients recognizing citrulline-containing epitopes are predominately of the IgG class and are of relatively high affinity. Schellekens et al. (52) postulate that the antibodies in RA patients reactive toward citrulline epitopes originate from a response against a gel-unidentified cross-reactive protein containing arginine residues that have similar sequences to the citrulline-containing sequences studied. The exact relationship of citrulline-containing epitopes to filaggrin remains to be determined by further studies.

ACUTE-PHASE REACTANTS

The inflammatory process plays an important role in the pathophysiology of rheumatoid arthritis. The laboratory evaluation of inflammation involves assessment of the "acute-phase response" which follows inflammatory stimulus and injury by various means. There are major changes in a heterogeneous group of plasma proteins called "acute-phase proteins." If the inflammatory stimulus is self-limited or treated, elevated acute-phase protein levels will return to normal within days or weeks (53). The levels of certain proteins such as ceruloplasmin and complement proteins rise to levels 50% greater than normal (53,54). Some other plasma proteins increase severalfold with the inflammatory stimuli and they include haptoglobulin and fibrinogen (53,54).

The major acute-phase reactants are C-reactive protein (CRP) and serum amyloid-associated protein (SAA). CRP and SAA concentrations will increase up to 1,000-fold or more above normal in severe inflammatory states, usually infections. In contrast, concentrations of other proteins such as albumin will decrease in inflammatory conditions (53–56).

The most widely used laboratory tests to monitor the "acute-phase response" are the erythrocyte sedimentation rate (ESR) and the serum CRP concentration (57,68). These tests have been used to monitor the diseases and also to gauge the presence and degree of inflammation.

The detection of an elevated ESR to distinguish RA from other forms of arthritis such as osteoarthritis is of limited value for a variety of reasons. Not all patients with RA have an elevated ESR. Also, an elevated ESR found in other patients with osteoarthritis is a result of associated diseases. The assessment of ESR is more helpful in the management of the RA patient and only of occasional value in diagnosis (18). The ESR and CRP levels are used to monitor the disease activity of RA patients.

ERYTHROCYTE SEDIMENTATION RATE (ESR)

The erythrocyte sedimentation rate (ESR) is often a reflection of the level of acute-phase reactants and predominately reflects increased fibrinogen production in the liver. It is believed to correlate with severity of the inflammatory process (18).

The Westergren ESR is used often as a simple marker for inflammatory activity in RA, polymyalgia rheumatica, temporal arteritis, and the vasculitides (18). The ESR can also be elevated in conditions unrelated to rheumatic disease, such as aging, anemia, infection, pregnancy, trauma, and tumors. However, the ESR, if well standardized, is useful in monitoring disease activity and management of RA.

ESR is dependent on fibrinogen, albumin, and globulin levels and is a reflection of the acute-phase response. However, the ESR is also influenced by other unrelated factors such as size, shape, and number of erythrocytes, i.e., anemia and age.

C-REACTIVE PROTEIN (CRP)

Human C-reactive protein (CRP) was discovered as a protein that precipitated the C-polysaccharides from pneumococci in 1930 (59). It is one of the most responsive acute-phase serum reactants. The CRP levels in patients with RA have been correlated with clinical disease activity, radiologic progression, and response to therapy (54–57). CRP levels are closely related to IL-1 and tumor necrosis factor (TNF-α) production.

Serial CRP levels have been shown to correlate significantly with radiographic findings in patients with early RA (less than 1 year) subsequently followed for 3 years (18,57). In patients with RA for an average of 14.6 years (18), CRP levels corresponded significantly and more closely than other serologic parameters with radiographic deterioration (18).

SERUM AMYLOID-ASSOCIATED PROTEIN (SAA)

Serum amyloid-associated protein (SAA), similar to CRP, correlates with a number of clinical parameters in inflammatory disease (53,55,60). SAA is a precursor protein for amyloid A. Migita et al. (61) has shown that SAA is a potent inducer of matrix metalloproteinases in the synovial tissue fibroblasts of RA patients. Therefore, SAA may play an important role in the degradation of extracellular matrix in the joint tissues of RA patients. SAA may not only be a marker for inflammation but may also be involved in the pathogenesis or perpetuation of the disease process (53,55,60).

There are two classes of SAA: acute phase (A-SAA) and constituted (C-SAA). Hepatic syntheses of A-SAA are unregulated by inflammatory cytokines. Constituted C-SAA is produced in the absence of inflammation. The measurement of SAA does not provide any more significant clinical useful information than CRP measurements (60).

CYTOKINES

Proinflammatory cytokines such as IL-1, TNF-α, and IL-6 are present at higher levels in the synovial fluid and blood of RA patients than of normal patients (8–10). There are immunoassays available for cytokines that have been used to monitor synovial fluid neutrophil activity (62), joint erosions (63), and various drug effects (64). The cytokines IL-I and IL-6 are correlated with the pathogenic process of RA. However, the direct measurement of these cytokines is currently being studied in clinical trials and research. There are numerous therapeutic drugs and agents being developed and tested in the cytokine area. Significant therapeutic drugs such as monoclonal antibodies to TNF-α (10,62,65) and TNF-α receptor agent (11) show great promise for new treatment protocols in RA patients.

ANTI-A22/RA 33

Hassfeld et al. (66,67) observed a new autoantibody to a 33-kDa antigen (anti RA-33) in 30% of Austrian RA patients but in none of the patients with ankylosing spondylitis or psoriatic arthropathy. The assay consisted of immunoblast analysis using soluble nuclear extracts from HeLa cells. The nuclear autoantigen RA 33 was recently demonstrated to be identical with the A2 protein of the heterogeneous nuclear riboprotein (hnRNP-A2) complex (68,69).

This A2/RA33 antibody is found in less than 50% of RA patients and is also found in systemic lupus erythematosus and mixed connective tissue disease (70). The specificity of anti-A2/RA33 antibody was already present in sera from 23.5% of 18 patients with RA of less than 1 year's duration. Anti-A2/RA33 was the only positive immunological marker in 3/20 cases of seronegative adult RA. There was no correlation between anti-A2/RA33 and antikeratin or antiperinuclear factor (70). Isenberg et al. (71) has presented evidence that antibodies to A2/RA33 may be associated with the presence of erosive arthritis in SLE.

JOINT AND COLLAGEN BREAKDOWN PRODUCTS

Hyaluronic Acid

Markers of joint and connective tissue destruction with erosion of articular cartilage and release of polysaccharides and related products may be useful in monitoring RA patients. Soluble fragments of hyaluronic acid (HA) can be elevated 10- to 20-fold in the sera of RA patients and correlate with clinical disease activity (72). Higher HA levels were found in the aggressive disease group. In another study, the diagnostic value of hyaluronic acid assays was questioned because levels of HA varied significantly during the daytime; peak levels corresponded to times of physical activity (73). Further studies on the usefulness of HA levels are required.

Aggrican and COMP (Cartilage Oligomeric Protein)

Increased concentrations of circulating COMP were observed in RA patients with rapid joint destruction and the COMP levels correlated with acute-phase reactants such as CRP (74). Aggrican and COMP are both released into synovial fluid in early RA. High aggrican to COMP ratios in knee joint exudates in early disease was a strong predictor of joint destruction and need for replacement surgery (75).

There are several other cartilage, bone, and synovial markers in early disease. YKL-40 is a secreted protein that has been isolated from human synovial cells (76). Serum levels and synovial fluid levels of YKL-40 reflect and correlate with joint disease. The assays for YKL-40 may be useful in the evaluation of connective tissue injury and repair in patients with rheumatic joint diseases (76).

Bone Turnover

Information on bone formation can be assayed by serum analysis of osteocalcin, bone-specific alkaline phosphatase, and collagen 1 propeptides. The concentrations of an osteoblast-derived calcium-binding protein, bone sialoprotein, in synovial fluid correlated with the degree of destruction. The serum levels of bone sialoprotein were elevated regardless of knee joint destruction (77).

Bone degradation and breakdown can be monitored by urine concentration of pyridinoline cross-links (20,78–80). The as-

say may be done by HPLC (high-performance liquid chromatography) or by a specific immunoassay. Degradation of collagen I with increased urine concentrations of pyridinoline cross-links was observed to correlate with disease activity.

SUMMARY

Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disorder that affects 1% of the population worldwide. RA is predominant in females, and the exact etiology remains undefined.

During the past several years, a large number of biochemical and biological markers that may help in the diagnosis, prognosis, and monitoring therapy and course of RA have been reported. Besides the traditional markers in RA, there are now genetic markers, filaggrin, citrulline-containing peptides, A2/RA33, cytokines, joint and collagen breakdown products, and bone turnover markers.

No laboratory tests in and of themselves are diagnostic of RA. However, many of the biochemical and biological markers are helpful in confirming diagnosis, determination of prognosis, and management of therapy in RA patients.

Recently, there has been development of many new and novel therapeutic approaches to treat and prevent clinical progression of RA. These include the use of soluble receptors to IL-1, or TNF-α, monoclonal antibodies to TNF-α, specific IL-I receptor antagonists, and gene therapy.

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