Biomarkers and genes predictive of disease predisposition and prognosis in rheumatoid arthritis

Rheumatoid arthritis is a debilitating disease which often progresses to relentlessly severe disease.

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Background

Rheumatoid arthritis (RA) is a debilitating disease that affects the articular joints of patients. The prognosis of the disease varies from mild cartilage degradation to progressive erosive disease of the juxta-articular bone. Progression may be cyclical or unremitting and often severity entails extra-articular manifestations such as RA nodules and vasculitis. A spontaneous remission is observed in 10 - 20% of patients while the majority of the patients progress to relentless severe disease.

Palaeopathological studies to determine the origins of RA show no evidence of typical RA lesions with marginal erosions at the bone-cartilage interface in skeletal remains from antiquity in Europe and North Africa.[1] However, skeletal remains of native American tribes dating back several thousands of years ago show unambiguous evidence of RA lesions.[2] Evidence of RA appears in Europe only in the early 17th century, when Sydenham published a case report in 1676, and was fully described by Garrod in 1859 and named 'rheumatoid' arthritis to distinguish it from rheumatic fever and gout, two recognised forms of arthritis.[3]

In an attempt to classify the disease, the Committee for Therapeutic Criteria of the New York Rheumatism Association published recommendations in 1949 on the classification of stages of RA progression, criteria for the therapeutic response of RA disease activity, and classification of functional impairment. These were updated by the American College of Rheumatology (ACR), which published certain guidelines to assist in the diagnosis of RA. In 1987 the Criteria for the Classification of Acute Arthritis of Rheumatoid Arthritis were published using 7 criteria to be considered during classification of the disease.[4] In 1991 the ACR also published revised criteria for the classification of the global functional status in RA, taking into account the limitations of the 1949 criteria. These have been widely used and are commonly referred to as the 1987 ACR classification.[5]

Recent progress in basic research, as well as improved clinical insight into the disease, have exposed the shortcomings of the 1987 ACR criteria. Aletaha *et al.* argued that the 1987 ACR criteria were developed using patients with established RA, and used radiological criteria seldom present in early disease, while the only serological marker used, rheumatoid factor

Table 1. The 2010 ACR/EULAR classification criteria for RA[9]

Classification criteria for RA
(score-based algorithm: add score of categories A-D; a score of 6/10 is needed for classification of patients as having definitive RA)

Categories	Score
Joint involvement	
• 1 large joint	0
• 2 – 10 large joints	1
• 1 – 3 small joints (with or without involvement of large joints)	2
• 4 – 10 small joints (with or without involvement of large joints)	3
• >10 joints (at least 1 small joint)	5
Serology (at least 1 test result is needed for classification)	
• Negative RF *and* negative ACPA	0
• Low-positive RF *or* low-positive ACPA	2
• High-positive RF *or* high-positive ACPA	3
Acute-phase reactants (at least 1 test result is needed for classification)	
• Normal CRP *and* normal ESR	0
• Abnormal CRP *or* normal ESR	1
Duration of symptoms	
• <6 weeks	0
• ≥6 weeks	1

RA = rheumatoid arthritis; RF = rheumatoid factor; ACPA = anti-cyclic citrullinated peptide antibodies; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate.

(RF), is less frequently (±50%) found in the early stages of RA.[6] The presence of rheumatoid nodules as one of the 5 clinical components of the ACR criteria is also not associated with early disease. Taking into consideration changes in management of the disease since 1987, the novel therapeutic drugs introduced since 1987, and the discovery of autoantibodies against citrullinated peptides, the authors were of the opinion that an updated and superior classification was necessary to assist the rheumatologist to classify RA better and earlier.[6]

Not only has a need for a better classification of RA been identified, but it is apparent that the 'early' diagnosis of RA has a positive impact on the well-being and prognosis of the patient, with rheumatologists having become more reliant on serological markers such as RF and cyclic citrullinated peptide antibodies (anti-CCP) than on the ACR criteria of 1987.[7,8]

RA is associated with the presence of several autoantibodies directed against different antigenic determinants.

A joint initiative was undertaken between the ACR and the European League against Rheumatism (EULAR), to establish a new classification which addressed the known deficiencies of the existing ACR diagnostic criteria. Not only does the new classification (established in 2010) include anti-citrullinated peptide antibodies (ACPA), but it also provides for the early detection of more severe disease, identifying those patients who would benefit most from the implementation of novel biotherapies, as seen in Table 1.[9]

Biomarkers of disease

RA is a disease that presents with a cohort of autoantibodies, biomarkers, clinical manifestations and outcomes. Indicators of disease in RA could be a gene, a product of gene expression, cytokines, acute phase reactants, autoantibodies and tissue abnormalities, such as swollen and tender joints or degradation products of cartilage. These can be investigated either by physical examination, analysis of serum and synovial fluid, synovial biopsies or imaging modalities. These indicators or markers are produced during pathological biological processes and should be of prognostic and/or diagnostic significance. The biological markers should provide an objective indication of disease activity and severity. The clinical markers are subjective in nature due to the fact that the physical variability and assessment of pain and disease severity of each specific individual being assessed is dependent on the discretion of the physician and patient, and are clearly more difficult to standardise.

Initially RA is characterised by synovial hyperplasia, marked by numerous giant cells appearing in the synovial lining, induced by the up-regulation of cytokines produced by immune and structural cells, vessel proliferation and angiogenesis, all of which are driven by an abnormal antibody and T-cell response to the putative auto-antigens shown in Table 2.[10]

Table 2. Autoantigens defined by experimental serum and B-cell analysis in RA syndromes in animals and humans[10]

Type/nature	Specificity
Autoantigen	Citrulline-containing peptide
	Keratin
	Perinuclear factor
	Savoy antigen
	Filaggrin
	Human leukocyte antigen
	Calpastatin
	Immunoglobulin (rheumatoid factor)
	Calreticulin
	Antineutrophil cytoplasmic antigen
	Antinuclear antibody
	Immunoglobulin heavy-chain protein / p68
	Heteronuclear ribonucleoprotein A2 (RA33)
	Glucose-6-phosphate isomerase
Cartilage (organ specific)	Collagen type II
	Chondrocyte antigen 65
	Large aggregating chondroitin sulfate proteoglycan (aggrecan)
	Human chondrocyte glycoprotein 39
	Cartilage oligomeric matrix protein
Non-autoantigens	Bacterial heat shock protein

Laboratory procedures

Autoantibodies

RA is associated with the presence of several autoantibodies directed against different antigenic determinants. The most important

Table 3. Autoantigens and antibodies present in RA (compiled from references 22 - 27)

Antigen	Antibody	Sensitivity (%)	Specificity (%)
Immunoglobulin G	Rheumatoid factor	70 - 75	NRADA and healthy individuals
Citrullinated proteins (fibrin, filaggrin, vimentin)	ACPA and anti-CCP	50 - 91	>97*
Heterogeneous nuclear ribonucleoprotein A2	Anti-RA33	≤36	NRADA
Collagen II	Anti-collagen	≤30	NRADA
Stress proteins	Anti-BiPp68, anti-hsp90	≤68	71 - 99*
Calpastatin	Anti-calpastatin	24 - 57 (83)*	Not verified (96)*
Citrullinated vimentin	Anti-Sa	≤40	85 - 95
α-Enolase (citrullinated)	Anti-α-enolase	46	*
Glucose-6 phosphate isomerase (GPI)	Anti-GPI	≤64	>95*
PAD4	Anti-PAD4	36 - 42*	*

*Requires confirmation.
NRADA = non-RA-differentiated arthritis.

of these, which have variable sensitivity and prognostic value, are shown in Table 3. Nevertheless, RF and anti-CCP remain the cornerstones of the serodiagnosis and monitoring of disease progression.[11-14] The advent of testing for the presence of different immunoglobulin isotypes has resulted in emphasis being placed on the positivity of RF and anti-CCP IgG in combination with IgA to assist in the identification of severity of disease.[15-18]

A more recent development is the advocacy of using a profile of RA-associated autoantibodies for serodiagnosis. Steuer *et al.* reported that the combined use of latex-enhanced RF nephelometry combined with testing for anti-CCP enhances the specificity of RA diagnosis in the primary care setting.[19] Tedesco *et al.* suggest a multiplex strategy utilising cytofluorometric methods to detect anti-CCP as well as RF isotypes. A combination of a RF multiplexed cytofluorometric test combined with anti-CCP detection gave a sensitivity of 100% and specificity of 87%.[20] Conrad *et al.* propose taking autoantibody profiling even further by including autoantibodies other than RF isotypes and anti-CCP. They suggest including anti-MCV (mutated citrullinated vimentin), anti-RA33 (heterogeneous nuclear ribonucleoprotein A2) and ACPA, the rationale being that the identification of a range of autoantibodies may improve prediction of outcome, as well as responses to chemotherapy/immunotherapy as these agents may have selective effects on autoantibody production.[12] A systematic review in 2010 by Whiting *et al.* utilising 10 databases representative of 151 studies, concluded that although the sensitivity of RF and anti-CCP is similar (56% v. 58%), anti-CCP had a higher sensitivity than RF (96% v. 86%) and a positive likelihood ratio of 12.7 and a negative likelihood ratio of 0.45.[21]

However, skeletal remains of native American tribes dating back several thousands of years ago show unambiguous evidence of RA lesions.

Circulating cytokines

Cytokines are small protein or glycoprotein molecules which form an integral part of the immune system and have regulatory functions. They are secreted by, and act upon, various cells of the immune system. Cytokines are critical mediators of the immune-biological process, being essential for cell growth, development, repair, fibrosis, inflammation and immunity. In general, cytokines are beneficial, but if their production is dysregulated, they are also involved in disease immunopathogenesis.[28]

It was already suspected in the mid-1980s that tumour necrosis factor (TNF) played a key role in RA when it was shown that this cytokine had the potential to degrade bone and cartilage.[29,30] When isolated RA synovial mononuclear cell cultures were incubated over five days, they produced TNF and other proinflammatory cytokines, including interleukin-1 (IL-1), IL-6, granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-8. It was also noted that if TNF bioactivity was blocked, IL-1 bioactivity was neutralised.[31] The role of TNF in RA has now been clearly established and biological therapeutics directed against TNF have been successfully used in the treatment of RA, with arrest or retardation of erosions.[32]

Notwithstanding TNF, other cytokines have also been targeted for development of biological therapies, clearly underscoring the role of cytokines in the immunopathogenesis of RA. Various biological interactions are involved, especially cytokine-mediated destruction of cartilage and bone via initiation of the cascade of proteolytic and metalloproteinase enzymes and osteoclast

activity. In addition, the IL-1 and IL-1 superfamily members IL-18 and IL-33, the IL-6, IL-23 families and putative downstream mediator IL-17, downstream targets of the IL-12/IL-23 family, IL-17 and IL-22, as well as cytokines that bind to γc (gamma chain) receptors, have all been identified as potential targets for biological therapeutic agents against RA.[33]

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Interestingly, qualitative measurement of pro-inflammatory cytokines and CRP in combination with ACPA and RF has been reported to predict disease severity,[34] but also time to onset of clinical disease.[35,36] In the South African RA population certain cytokines (IFN-γ, IL-1γ, IL-1Ra, TNF, GM-CSF and VEGF) were significantly correlated (p<0.04 - 0.001) with high disease activity (HDA), defined as RA patients with a DAS28 score of ≥5.1. Circulating cytokines in RA reflect a multifaceted increase in immune reactivity encompassing Th1 and Th2 cells, monocytes/macrophages, and synovial fibroblasts, underscored by strong correlations between these cytokines, as well as their relationships with RF, aCCP, and aMCV, with some cytokines showing promise as biomarkers of HDA.[34]

Molecular biomarkers

There are several genetic polymorphisms that are associated with RA, but none has been conclusively identified as being the primary predisposing genetic determinant of RA. Of these, the 'shared epitope' was the first to be convincingly associated with RA. The shared epitope (SE) concept in relation to genetic predisposition was first described in 1986 and has evolved from the classic HLA-DRB*01, - *04, and -*10 association, to the identification of the RAA amino acid motif at positions 72 - 74 of third hypervariable region of the different HLA-DRβ chains as being the definitive SE.[37-41] This concept has been extended by Gao *et al.* to include the amino acid residues at positions 71 and 76 and, recently, to a new classification which incorporates the modulatory activities of the amino acids at positions 70 and 71, assigning severity (S2, S3P) or protective (S1, S3D) effects, in addition to the RAA motif at positions 72 - 74.[42-44] A recent study on the new classification by du Montcel has shown that not only is the RAA amino acid sequence present, but the alleles associated with severity (S2, S3D) are more prevalent in the South African RA population and are associated with anti-CCP positivity.[27]

Single nucleotide polymorphism (SNP) studies clearly confirm that a genetic susceptibility may contribute to the risk for the development of RA. Firstly, the central portion of the ancestral 8.1 HLA haplotype, identified as A1-B8-DRB1*03, has been identified to carry certain risk factors or SNPs. The TNF gene complex is also present in this haplotype and high production of TNF is associated with certain TNF alleles in the 8.1 haplotype, which may explain the association with RA. Secondly, RA susceptibility has been identified within the HLA class II region of certain HLA-DRB1*0404 haplotypes, not associated to the SE amino acid sequence.[45] Furthermore, SNP mapping of the MHC region of anti-CCP positive RA patients has identified additional independent risk in the centromeric region of the HLA-DRB1 locus, which includes the HLA-DOB (beta chain), antigen peptide transporter 2 (TAP2), HLA-DPB1 and collagen type X1 alpha2 (COL11A2) genes.[46]

Of further note is a missense SNP (R620W) in PTPN22, an intracellular protein tyrosine phosphatase non-receptor type 22 gene, which has a negative regulatory effect on T-cell activation through binding to intracellular kinases, and has been shown to have a very strong association in a genome-wide functional SNP screen in RA.[47] This was later confirmed by Criswell and Gregerson.[48] Other moderate risk associations have been revealed by SNP studies of RA patients.

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These include:

- 2 independent alleles at chromosome 6q23, which is nearest to the gene encoding TNFAIP3 (tumour necrosis factor, alpha-induced protein 3) and OLIG3 (oligodendrocyte transcription factor 3)[49,50]
- TRAF1-C5, a novel 100kb region on chromosome 9
- a TNF receptor-associated factor gene[51]
- a variant allele of STAT4 (signal transducer and activator transcription 4) which encodes a transcription factor, utilised to transmit signals induced by several key cytokines[52]
- PADI4 (peptidylarginine deiminase type 4) polymorphisms, regulating PAD4 (peptidylarginine deiminase 4), an enzyme which is responsible for the formation of citrulline from arginine, have been identified in the Japanese population as a RA susceptibility locus[53] and associated with anti-CCP antibodies.[54] It was also identified in North American and Swedish populations, [55] but could not be replicated in other European populations [56] with the exception of the German population.[57]

Duroux-Richard *et al.* describe the function of MicroRNA (miRNA) as '... small noncoding RNA molecules that modulate the expression of multiple protein-

encoding genes at the posttranscription level. They likely participate in nearly every developmental and physiologic process. Although the function of most mammalian miRNAs has yet to be determined, it appears that their aberrant expression may play a role in the pathogenesis of several pathological conditions, including immune-mediated inflammatory disorders.'[58] miRNA expression studies represent a novel and growing field of study, with recent publications providing novel insights into the immunopathogenesis and diagnosis of RA, and possibly identifying those that would benefit from early initiation of biological therapies. Several dysregulated miRNAs have been identified in RA associated with different immune regulatory functions and hold potential value as a biomarker in RA.[58-61]

Recommendations

Combinations of all of these objective and subjective markers may predict disease outcome, from remission to disability and, in the extreme, premature mortality. Ongoing research is attempting to identify the best combinations of prognosticators in RA by examining circulating biological and clinical markers. A recent South African study underscored the need for better disease management and understanding the relation between disease severity and treatment.[62] To date the correlation of markers with disease manifestations, joint destruction or physical impairment as a predictive/prognostic indicaton has been sub-optimal for the majority of them. This may be for several reasons. Firstly, what is measured in the circulation may not be a true reflection of the micro-environment of the joint. Secondly, the elevated concentrations of circulatory biomarkers measured might not be indicative of functionality or may reflect a state of equilibrium between the pro- and anti-inflammatory markers. Thirdly, the pathogenesis of RA may be so variable that different markers may play a more dominant role in subsets of patients. Fourthly, the mechanisms of disease pathogenesis may vary broadly during the course of the disease. Finally, fluctuations and genetic differences in biomarker expression in different patients may render them uninterpretable. However, with ongoing research into these associated biomarkers, a body of evidence has been published which emphasises certain biomarkers as indicative of possessing diagnostic as well as prognostic value.

In conclusion, a suggested arthritis screening profile consisting of the acute phase reactant CRP, circulating cytokines (IFN-γ, IL-1β, IL-1Ra, TNF, GM-CSF, and VEGF), autoantibodies RF and anti-CCP and HLA-DRB1 high-resolution molecular typing shows promise to identify those patients who may benefit from a more aggressive treatment regimen or immediate biological biotherapy. The cost of such an extensive panel may be justified by the benefits to the patients and management of disease burden in the long term.

References available at www.cmej.org.za

In a nutshell

- The ACR 1987 criteria are inadequate for early RA diagnosis.
- The 2010 EULAR/ACR classification can assist in detection of 'early' onset of disease.
- RF and anti-CCP are the predominant biomarkers for assisting in diagnosis of RA.
- RF and anti-CCP collectively, especially IgG and IgA isotypes, are more sensitive and specific.
- Cytokines and associated receptors are not only biomarkers for novel biotherapies but can assist in diagnosis of high disease activity in RA.
- The HLA shared epitope (SE), especially the new du Montcel classification, is relevant in the South African setting and is indicative of disease severity.

Single suture

Liposuction used to create blood vessels for bypass

A bit of bulge could one day save your life. Stem cells extracted from fat tissue after liposuction may one day be used to create blood vessels to replace faulty arteries in the heart.

Fat tissue is a plentiful source of stem cells. Matthias Nollert at the University of Oklahoma in Norman and his colleagues coaxed liposuction-derived stem cells into forming smooth muscle cells found in arteries and veins.

They then grew these cells along a thin collagen membrane, which was rolled into a tube the size of a small blood vessel. As the smooth muscle cells grew, the team subjected them to a battery of mechanical stresses that mimic the expansion and collapse that such a vessel would ultimately experience in the heart. The team hopes that this will increase the vessel's robustness in the body.

Unlike artificial stents, which restore blood flow through narrow or once-blocked arteries, vessels made from your own stem cells wouldn't run the risk of being rejected by the immune system. Side-effects that can occur when damaged vessels are replaced with those taken from other parts of the body would also be avoided.

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