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Diagnostic Biomarkers in Rheumatoid arthritis – An update

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ABSTRACT

Rheumatoid arthritis (RA) is a prototype immune-mediated inflammatory disease, characterised by a symmetric polyarthritis usually involving the small joints of the hands and feet. RA is the commonest and the most important in socioeconomic terms, affecting 0.8 percent of the adult population worldwide. Although RF is the gold standard, more sensitive and specific autoantibodies have been under research in recent years. The new 2010 Rheumatoid Arthritis Classification Criteria include the presence of anticitrullinated protein antibodies (ACPA) as one of the criteria to be used in determining whether or not a patient should be diagnosed as having RA. The guidelines also mention that ACPAs are tested as anti-CCP. The combined application of two assays (RF and anti CCP/ or anti CCP and anti MCV) can improve the laboratory diagnostics of RA.

Keywords: rheumatoid arthritis, biomarkers, rheumatoid factor, autoantibodies, anti-cyclic citrullinated peptide.

INTRODUCTION

Rheumatoid arthritis (RA) is a prototype immune-mediated inflammatory disease, characterised by a symmetric polyarthritis usually involving the small joints of the hands and feet [1]. Other joints can also be involved. Among the inflammatory joint diseases, RA is the commonest and the most important in socioeconomic terms, affecting 0.8 percent of the adult population worldwide. Onset usually occurs between 30 and 50 years of age [2]. Female sex, a positive family history, older age, silicate exposure, and smoking are associated with an increased risk for developing rheumatoid arthritis [3]. Consumption of more than three cups of coffee daily—particularly decaffeinated coffee—also may contribute [4]. High vitamin D intake [5], tea consumption, and oral contraceptive use are associated with decreased risk.

RA causes persistent synovitis, pain, joint destruction and functional disability. Because irreversible joint destruction can be prevented by intervention during the first months of disease, early diagnosis of RA is important [6, 7].

The National Institutes of Health's Biomarkers and Surrogate Endpoint Working Group defines a biological biomarker as a characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to therapeutic intervention [8].

Although RF is the gold standard, more sensitive and specific autoantibodies have been under research in recent years.

To meet the need for improved diagnostic and prognostic tests, various serum biomarkers are being assessed for the improved diagnosis and prognosis of RA, including a wide range of autoantibodies.

Cause of release of autoantibodies:
The autoantibodies system most specific for rheumatoid arthritis is that directed to citrullinated antigens. The citrulline moiety is the essential part of the antigenic determinants in these antibodies. Citrullination is a post-translational modification, in which a peptidylarginine is converted into a peptidylcitrulline by the enzyme family of peptidylarginine deiminases (PAD). Citrullinated proteins occur at inflamed sites in healthy individuals as well as in patients [9, 10]. However, autoantibodies directed against citrullinated proteins (anti-citrullinated protein/peptide antibodies, ACPA) are very specific for rheumatoid arthritis (RA). More than 70% of RA patients display ACPA, measured via the anti-CCP2 (cyclic citrullinated peptide 2) test, in their sera [11, 12]. These antibodies are frequently present prior to disease onset and can predict the development of RA [13, 14].

Criteria for assessing the diagnostic value of a marker
An ideal marker should have 100% sensitivity, specificity and positive predictive value.

Sensitivity- It refers to the percentage of patients with disease who are correctly identified as a result of a positive test.

Specificity- It refers to the percentage of the population without disease who are correctly identified as a result of a negative test.

Positive predictive value (PPV) – Refers to the percentage of patients with a positive test that have disease (true positives).

Biochemical markers of Rheumatoid arthritis
1. Rheumatoid factor (RF)
2. Anti-cyclic citrullinated peptide (anti CCP)
3. Micro RNA (miRNA)
4. Anti-mutated citrullinated vimentin (anti MCV)
5. Anti filaggrin antibodies (AFA)
6. Interleukin -22 (IL-22)

Rheumatoid Factor (RF)
Rheumatoid factor is an antibody recognizing the Fc (crystallisable fraction) of human antibodies [15] and is present in 60%–90% of RA patients with established RA [16, 17] but in less than 50% of patients with early RA [17]. 3% to 5% of healthy adults have serum RF; this increases to 10%–30% in the elderly [18, 19].

RF is more established as a biomarker for RA than anti-CCP, having been adopted as one of the American College of Rheumatology (ACR) classification criteria for RA in 1987 [20]. The European Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT) notes that it is one of several prognostic markers used to identify patients with persistent and/or erosive disease but does not recommend RF as a diagnostic marker for RA [21] most likely at least in part due to its limited specificity. RF is also common in other autoimmune diseases, infectious diseases, and malignancies, making it a relatively nonspecific marker of RA [22].

Anti-cyclic citrullinated peptide (anti-CCP)
Anti-CCP are very specific for RA. These antibodies bind to antigenic determinant containing the unusual amino acid citrulline formed by post translational modification of arginine [11, 23]. Anti CCP is present in the serum of a portion of RA patients and has been identified in the serum of patients at all stages of RA: preclinical, early, and established. In clinical research studies, anti-CCP antibodies were found in 55%–69% of patients with RA [24, 25], 65% of RA patients with late-onset RA [26], and 10% of patients with juvenile-onset arthritis [25].

The length of time that anti-CCP antibodies are detectable in patient serum prior to disease onset appears to be age related. Serum anti-CCP is detectable in the serum of older patients well before the developmental of clinical symptoms, while in younger patients, the detection of serum anti-CCP occurs closer to the time of disease onset [27].

Three generations of anti-CCP tests are currently available commercially. Of the three generations, the second generation tests currently appear to provide the best performance [28].

In 2007 the European League Against Rheumatism (EULAR) recommended that all patients diagnosed with early arthritis be tested for the presence of anti-CCP [21], and the 2008 RA treatment guideline published by the American College of Rheumatology (ACR) includes positive anti-CCP as one of a number of measures of poor patient prognosis to be used in treatment selection [29]. Over the past three years, the ACR and EULAR jointly developed an updated set of criteria for RA to replace the outdated 1987 ACR classification criteria for RA. The new 2010 Rheumatoid Arthritis Classification Criteria include the presence of anticitrullinated protein antibodies (ACPA) as one of the criteria to be used in determining whether or not a patient should be diagnosed as having RA [30]. The guidelines also mention that ACPAs are tested as anti-CCP.

miRNAs

miRNAs are an abundant class of endogenous, short non-coding, single-stranded RNA molecules (19-23 nucleotides) that function as negative regulators of the expression of protein-encoding genes. Human miRNA genes are located in all chromosomes except Y chromosome and they are non randomly distributed in the human genome [31]. The majority of miRNA genes (70%) are found in the introns, in the sense orientation, and approximately 30% are located in intergenic regions. Approximately 50% of known human miRNAs are found in clusters and are transcribed by RNA polymerase II as mono- or poly-cistronic long primary transcripts, namely primary miRNA or pri-miRNA [32], ranging from approximately 200 nucleotides (nt) to several kilobases (kb) in length and folded into hairpin structures containing imperfectly base-paired stems. miR-146a and miR-155 are the most frequently reported miRNAs deregulated in RA samples including blood, plasma [33].

Increased miR-146a expression levels are correlated with active disease in RA patients [34]. They are both involved in the development of innate and adaptive immune cells, and numerous studies report their upregulation in inflammatory conditions [35, 36].

Anti MCV

Antibodies directed against citrullinated vimentin are members of the family of autoantibodies reactive with citrullinated proteins and are among the most specific serological markers for the diagnosis of rheumatoid arthritis (RA) [37].

Vimentin is an intermediate filament that is widely expressed by mesenchymal cells and macrophages and easy to detect in the synovium. Modification of the protein occurs in macrophages undergoing apoptosis, and antibodies to citrullinated vimetin may emerge if the apoptotic material is inadequately cleared [38].

Citrullinated vimentin has been identified as potential autoantigen in the pathophysiology of RA and an enzyme-linked immunosorbent assay (ELISA) for the detection of Abs directed against a mutated citrullinated vimentin (anti-MCV) was developed [39].

Anti filaggrin antibodies (AFA)

Anti-filaggrin antibodies are a subset of a large panel of antibodies, previously called anti perinuclear factor (APF) when detected in buccal mucosal cells, or anti keratin antibodies (AKA) when detected in epithelial cells of the stratum corneum of rat oesophagus [40].

Filaggrin is an intermediate filament associated protein involved in the aggregation of cytokeratin filaments during the cornification of the epidermis. It is synthesized in the stratum granulosum as a large and heavily phosphorylated precursor, profilaggrin, which is accumulated in keratinocyte-specific-cytoplasmic organelles, the keratohyalin granules. During the late stages of epidermal keratinocyte differentiation, profilaggrin molecules are dephosphorylated and cleaved, to release functional filaggrin units [41].

Both AKA and AFP have been demonstrated to be highly specific serological markers of the disease and therefore are increasingly used for the diagnosis of RA [42]. In RA, the appearance of AFA may precede disease onset and are present in some RA seronegative patients and appear to be more specific. AFA can be useful in the clinical management of RA, particularly in patients with suspected early RA [43].

Of 40-60% RA patients exhibit autoantibodies against epidermal flaggrin (anti keratin, anti-perinuclear antibody) in the serum. In the recent years, it has been shown that a rarely found amino acid citrulline, which is present in flaggrin, is a substantial component of the antigenic epitope [44].

Interleukin-22 (IL-22)

IL-22 is a cytokine produced primarily by CD4 T cells. The receptor for IL-22 is expressed in various joint tissues. Earlier studies demonstrated that IL-22 activates synovial fibroblasts. These, in turn, secrete cartilage-destroying proteinases that immediately participate in the destruction of cartilage. In addition, interleukin-22 promotes the formation of osteoclasts, which are substantially involved in bone erosion and joint destruction [45].

IL-22 is elevated in the serum of half of the patients with RA. Elevated serum IL-22 allows discrimination between patients with different radiographic progression and indicates a possible involvement of IL-22 in the pathophysiology of RA [45].

IL-22 concentration in the synovial fluid as well as serum was higher in RA patients and this correlated with serum titers of rheumatoid factor and anti-cyclic citrullinated peptide antibodies in the study of Kim et al [46].

CONCLUSION

Clinical and biologic biomarkers have led to advances in the case of patients with RA. Novel biomarkers like anti-CCP and RF assays in combination or anti CCP and anti MCV in combination promise better assessment of disease. The combined application of two assays can improve the laboratory diagnostics of RA.

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