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EDITORIAL

Gauging rheumatoid arthritis

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The ideal biomarker is a simple test that reliably recognises a disease when present, can predict the disease when not yet present, provides prognostic information, predicts response to treatment and reflects disease activity or actual response to treatment. In most instances, different biomarkers will be needed to cover the different aspects one wants to measure in a disease, such as for instance blood glucose, HbA1c and microalbuminuria in diabetes. Biomarkers are mostly thought of as biochemical measurements, but can equally well be clinical measurements or imaging features. In complex diseases such as rheumatoid arthritis, molecular and clinical biomarkers are often combined for the purpose of classification[1] or in order to create an index of (low) disease activity.[2] This editorial will briefly deal with molecular biomarkers of prediction, diagnosis and prognosis of rheumatoid arthritis, as measured in blood. For reasons of space, the emerging field of prediction of treatment response is left out.

Nowadays, quite a lot is known about the pathogenesis of rheumatoid arthritis. Acquiring this knowledge was facilitated by the easy accessibility of the main site of the pathology of this disease, the peripheral joints, and the accompanying immunological abnormalities that can be detected in the blood. That is a different situation compared with other immune diseases such as spondyloarthropathy or multiple sclerosis, which have a centrally located pathology and hardly any signs of autoimmunity. The discovery of the rheumatoid factor in the middle of the past century triggered an interest in pathophysiological research and even helped rheumatology to become a separate speciality. However, research on the rheumatoid factor has not led to a solution for rheumatoid arthritis, as it was also found in several other conditions involved with infections or tissue damage, and its pathogenic significance has remained uncertain.

Already in 1964, another serological hallmark of rheumatoid arthritis was discovered and named the anti-perinuclear factor.[3] It took more than 30 years to determine the antigen that these antibodies targeted, namely citrulline residues present in a large variety of intra- and extracellularly occurring proteins in the context of inflammation, e.g. due to infections or cigarette smoking.[4] The corresponding antibodies are collectively referred to as anti-citrullinated protein antibodies (ACPA). ACPA have clearly replaced rheumatoid factor as the main autoantibody in rheumatoid arthritis. ACPA not only have a higher diagnostic and prognostic value than rheumatoid factor,[5] they most likely play an important role in the pathogenesis of rheumatoid arthritis for several reasons, some of which are listed here: 1) ACPA production is linked to the presence of the strongest genetic risk factors for rheumatoid arthritis located on the HLA-DRB1 region, and the PTPN22 gene;[6] 2) ACPA appear earlier than rheumatoid factor in the asymptomatic phase, up to 15 years before the first symptoms;[7] 3) in persons at risk for rheumatoid arthritis, their presence is associated with an increase in the risk of the onset of clinical arthritis as the titre increases and a higher number of epitopes are recognised;[8,9] 4) they can exacerbate arthritis in animal models of arthritis. At present it is unknown why rheumatoid arthritis (pre-)patients develop an antibody response to the widely occurring citrulline. The practical value of ACPA testing is that a positive test greatly facilitates the early recognition of rheumatoid arthritis and at the same time defines the patient subset that has the highest likelihood of developing erosive disease, which in the long term is associated with functional deterioration. ACPA-positive early arthritis patients are thus the main candidates for early intervention. Naturally, ACPA testing should be restricted to persons with suspected rheumatoid arthritis.

The rheumatology research group from Leiden recently discovered a new group of autoantibodies in rheumatoid arthritis, called anti-carbamylated protein antibodies or anti-CarP, which they describe in this issue of the Journal in relation to ACPA.[10] The pathophysiology is similar to that of ACPA, in that the naturally occurring amino acid lysine is modified post-translationally – in an inflammatory environment – into homocitrulline, which

then acts as a neo-epitope for autoantibody formation, in the same manner as the amino acid arginine after conversion to citrulline in the case of ACPA. The reaction producing homocitrulline is a chemical one, however, whereas citrullination is an enzymatic process. Although homocitrulline is nearly identical to citrulline, the authors have shown that anti-CarP antibodies are not just cross-reacting ACPA. Furthermore, these are also found in some of the ACPA-negative patients and are associated with radiographic joint damage. The authors mention that the value of anti-CarP testing could be to recognise those ACPA-negative patients who have a poor prognosis. However, the question is whether we really need additional biomarkers for ACPA-negative rheumatoid arthritis, since even in the time before modern effective treatment of rheumatoid arthritis was the norm, the average radiographic damage was already extremely low in ACPA-negative patients.[11]

Altogether, we now have three autoantibody systems associated with the (prediction of the) more severe forms of rheumatoid arthritis: rheumatoid factors,[12] ACPA[5] and anti-CarP antibodies.[13] A common pathogenic denominator may be their local production in the inflamed synovium and their ability to bind complement and thereby enhance the level of inflammation. However, it is likely that this will not be the end of the rheumatoid arthritis autoimmunity story. Various proteases are active in the inflamed synovium, which through cleaving proteins might produce new epitopes with a potential for further autoantibody formation, and thereby further activation of the inflammatory cycle. Indeed, antibodies to Fab fragments of IgG molecules cleaved at the hinge region between the Fab and Fc portions were recently described in the serum of rheumatoid arthritis patients.[14]

Looking beyond autoantibodies, what other molecular biomarkers might be useful in rheumatoid arthritis? There are now over 30 confirmed genetic susceptibility loci for rheumatoid arthritis. However, even when combined these have only low predictive ability by themselves.[6] One can also study general inflammation markers such as acute-phase reactants and cytokine profiles. Acute-phase reactants, mainly C-reactive protein, are well established as markers of disease activity and perform well in composite measures of disease activity or remission in rheumatoid arthritis.[2] In the preclinical phase of the disease, levels become elevated from the appearance of ACPA onwards;[15] however, they do not provide additional predictive ability for future rheumatoid arthritis.[16] Similarly, various cytokines are elevated before clinical rheumatoid arthritis appears, which probably reflects increasing inflammation in this phase, rather than an initiating pathogenic event.[17,18] Recently, 14-3-3 proteins reflecting activated signal transduction pathways were identified as another specific biomarker of rheumatoid arthritis disease activity.[19]

A relatively novel approach is to analyse gene expression activity as opposed to the mere presence or absence of specific genetic traits. Using this approach, an increased expression of a number of interferon-related genes in combination with low activity of B-cell genes was discovered in the blood of persons at risk for rheumatoid arthritis,[20] which was also present in patients with active rheumatoid arthritis.[21] The so-called 'interferon signature' was predictive of future rheumatoid arthritis, independent of ACPA positivity.[20] A drawback of this technique is that it is dependent on qPCR, which is not readily available. Obviously, the new findings on biomarkers such as anti-CarP antibodies and the interferon signature need to be validated in other populations and tested in different phases of the disease. In parallel, in order to be clinically useful they will have to demonstrate additional value over testing of ACPA alone as the most potent single biomarker in rheumatoid arthritis to date.

Emerging technology will soon allow the testing of a large number of biomarkers in only a few drops of blood, essentially measuring rheumatoid arthritis-specific autoimmunity, biochemical inflammation and possibly also genetic susceptibility. In combination with a careful clinical examination and imaging results, the properties of such a blood-based biomarker set may prove to be better than the current evaluation in the following situations: prediction of future rheumatoid arthritis in persons at increased risk for rheumatoid arthritis, and prediction of the disease course in patients newly diagnosed with rheumatoid arthritis, including the preferred treatment regime. In order to be useful for the monitoring of treatment, a blood-based biomarker set should be cheaper than a visit to the clinic and be able to reliably predict or reflect a state of remission or minimal disease activity, which is the present goal of treatment. In spite of the usual proclamation of another step towards 'personalised medicine' for every new association of a single biomarker with a measure of disease, all these wishes are still far from being fulfilled at present. For quite some time now, molecular biomarker sets for rheumatoid arthritis have been more of a promise than a reality, and it will take some more time before we have a good 'rheumachip' for use in the clinic.

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