Unravelling the anti-carbamylated protein antibody response in rheumatoid arthritis

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Part I

Clinical characteristics of anti-CarP antibodies

Chapter 2

Biomarkers for rheumatoid and psoriatic arthritis

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Rheumatic diseases, such as rheumatoid and psoriatic arthritis are systemic inflammatory conditions characterized by a chronic form of arthritis, often leading to irreversible joint damage. Early treatment for patients with rheumatic diseases is required to reduce or prevent joint injury. However, early diagnosis can be difficult and currently it is not possible to predict which individual patient will develop progressive erosive disease or who may benefit from a specific treatment according to their clinical features at presentation. Biomarkers are therefore required to enable earlier diagnosis and predict prognosis in both rheumatoid arthritis and psoriatic arthritis. In this review we will examine the evidence and current status of established and experimental biomarkers in rheumatoid and psoriatic arthritis for three important purposes; disease diagnosis, prognosis and prediction of response to therapy.

Introduction

The term rheumatic musculoskeletal diseases (RMD) encompasses a large and varied group of diseases, that share a number of features such as the involvement of connective tissues, muscles and the joints. In addition to similarities, there is also significant variety across the RMD spectrum including inflammatory and non-inflammatory diseases. Rheumatoid arthritis (RA) and psoriatic arthritis (PsA) are two of the most prevalent inflammatory RMD while diseases such as osteoarthritis and fibromyalgia represent the main non-inflammatory conditions. RMD can also be classified according to duration of symptoms or impact on function. The duration may be acute, remitting or chronic persistent and the impact on the subject may vary from mild to severe, often depending on the level of inflammation or tissue damage. The level of inflammation is often quite different in patients with RA and PsA even though both may result in joint damage while fibromyalgia, which is painful, is not associated with inflammation or tissue damage. The signs and symptoms of RA and PsA may be quite similar especially at the earlier phases of disease, so it may be difficult to distinguish between them on clinical grounds, although early treatment may prevent the development of disability in both conditions if introduced appropriately[1,2].

RA occurs in 0.5-1% of the adult population globally [3]. The main characteristics of RA are stiffness and swelling of the joints as a result of inflammation of the synovium, which normally is a thin translucent membrane lining the non-articular surfaces of the joint. The synovium may proliferate and invade surrounding structures leading to damage of the articular cartilage and erosions of the periarticular bone. The cause of RA is not clear, although both genetic and environmental factors have been identified to play a role in disease initiation and progression. RA patients exhibit an increased frequency of cardiovascular disease, a higher susceptibility to infections and have an increased risk for certain malignancies [3].

PsA occurs in 10-40% of psoriasis patients [4,5]. Psoriasis is characterized by red, thickened and inflamed skin lesions and affects up to 3% of the general population.

In addition to the skin lesions, patients may develop a chronic arthritis of the peripheral and/or axial joints, characterized by inflammation of the synovium and erosions similar to but distinct from RA. Classified as one of the spondyloarthropathies, due to axial joint involvement similar to ankylosing spondylitis, patients may also exhibit enthesitis, uveitis and nail disease [4,5]. PsA patients, similar to RA, have an increased mortality due to cardiovascular disease, however there is no evidence of increased susceptibility to infections or lymphoma when compared to the general population[6].

The signs and symptoms of RA and PsA patients, including systemic features such as skin and eye manifestations, appear to respond well to anti-inflammatory drugs (corticosteroids and non-steroidal anti-inflammatories (NSAIDs)) and disease-modifying anti-rheumatic drugs (DMARDS) such as tumour necrosis factor inhibitors (TNFi) . For some other biological agents there may be a differential response when comparing RA and PsA patients[7,8].

Biomarkers

Biomarkers may be defined in several ways. A simple definition proposed by the US Food and Drugs Administration (FDA) is; 'Any measurable diagnostic indicator that is used to assess the risk or presence of disease'. However the US National Institutes of Health (NIH) has suggested a more comprehensive definition of a biomarker - 'A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to therapeutic intervention'. The NIH definition encompasses the concept of response to therapy, which is becoming more relevant and therefore more important in the context of RA and PsA.

Therapies for RA and PsA patients have developed rapidly in the past decade such that great improvements in signs and symptoms, but also in quality of life and function, have been realised. However, many patients do not respond to the first treatment that is offered, leaving room for substantial improvements [7,8]. Also, in both RA and PsA, early treatment is important in order to prevent irreversible joint damage [1,2]. In order to treat patients in an early stage of the disease, it is essential to determine which of the patients that visit the doctor with psoriasis or joint pain will eventually develop PsA or RA respectively. Only the patients that do acquire PsA or RA will benefit from the treatment, while people who do not develop severe disease might suffer from unnecessary side effects. Furthermore, not all treatments are effective in each patient and treatments are often given on basis of trial and error [7,8]. It would therefore be useful to predict which RA and PsA patients will benefit from a specific treatment.

In this review, we describe the biomarkers that are generally accepted for PsA and RA, after which we will discuss a selection of interesting biomarkers that are still under investigation.

This will include biomarkers that are used to improve diagnosis, to predict prognosis and to identify response to treatment.

Autoantibodies

For RA patients, one of the most important type of biomarkers at the moment are autoantibodies. The most recent criteria for the diagnosis of RA were described in 2010 [9]. Besides joint pain and inflammation, several serological biomarkers are used to classify RA patients. Serological biomarkers, described in the new criteria, include autoantibodies such as rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA).
Currently there is little evidence for a role of autoantibodies in PsA, as rheumatoid factor is mainly absent in PsA, so that the CASPAR classification of PsA includes rheumatoid factor negativity as an independent diagnostic criterion [10]. Indeed, also the autoantibodies to citrullinated proteins are mostly absent in 90% of the PsA patients [11,12]. There is one recent report of autoantibodies, against fibrillin 3 and desmocollin 3, crossreacting with a shared epitope common to both the skin and the joints that may suggest some as yet unidentified autoantibodies may be associated with PsA [13]. Since the role of autoantibodies as a biomarker in PsA is almost non-existent, we will now discuss the different autoantibodies that have been discovered in RA and shortly explain the relevance of these antibodies as a biomarker.

ACPA and RF

The first autoantibody that was discovered in RA patients is RF, which is present in 60-80% of the RA patients. The antigen that RF binds to is the Fc-region of an IgG molecule [14]. RF has a low specificity, since it can also be found in healthy controls and patients with other rheumatic diseases [15]. But even though RF has a low specificity, it has been used extensively to diagnose RA for a long time, since no better alternatives were available. At least, not until ACPA were discovered. ACPA bind to a different type of antigen than RF; proteins that contain the amino acid citrulline. These citrullines are formed postranslationally by the deamination of arginine, an enzymatic process facilitated by PAD enzymes. In diagnostics, ACPA have a higher specificity and sensitivity than RF [15,16]. The current diagnostic tests to identify ACPAs are mostly based on assays that use cyclic citrullinated peptides (CCP) as antigen [17].

Furthermore, ACPA have been described as a factor that is associated with disease prognosis, since RA patients that are ACPA+ develop a more severe disease compared to ACPA- patients [18,19]. Also with regards to treatment responses, ACPA+ patients seem to respond better to treatment than ACPA- RA patients in an early phase of the disease, but achieve drug-free remission less frequently [20]. Research has also been carried out with regards to the fine specificity of ACPA [21,22]. Several analytical platforms such as ELISA, chips and SPR have been developed to investigate this subject, but so far there is no clear evidence that one of the ACPA fine specificities is a superior biomarker as compared to testing for CCP [23-25].

However, the number of different epitopes recognized may provide information on the process of disease development [26,27]. Another important aspect of disease progression, involves the increased chance to develop cardiovascular diseases that has been observed in RA patients. Both ACPA and RF have been found to associate with cardiovascular disease and mortality in RA patients [28,29].

Anti-carbamylated protein antibodies

Besides RF and ACPA other autoantibodies that may function as useful biomarkers, were identified in RA patients. A first example of these autoantibodies, are anti-Carbamylated Protein (anti-CarP) antibodies, which were recently described by Shi et. al [30]. These antibodies can be found in 45% of early RA patients and are also present in 16% of the ACPA-negative RA patients. This observation, initially made in Dutch patients, was later confirmed in a large Swedish cohort [31]. Anti-CarP antibodies are present more than 10 years before disease onset [32-34] and are associated with development of RA in arthralgia patients [35]. This combination makes anti-CarP antibodies an interesting biomarker for early diagnosis of RA. However, the specificity of anti-CarP antibodies has to be determined by testing the presence of these antibodies in other rheumatic diseases.

As a biomarker associated with disease severity, anti-CarP antibodies seem to be quite promising. The antibodies were found to be associated with a more severe clinical picture, including an increase in joint damage in ACPA-negative RA patients [30]. Similar observations are also made independently in other cohorts [34,36]. Since the presence of anti-CarP antibodies can also be detected in early disease stages [33-35], it may be interesting to adjust treatment of RA patients depending on their anti-CarP status. However, research investigating the response to treatment in both anti-CarP-positive and –negative patient groups is still in progress.

Anti-malondialdehyde and anti-malondialdehyde acetaldehyde antibodies

Besides citrullination and carbamylation, other autoantibodies against posttranslational modifications were identified. In this case, the posttranslational modifications are due to lipid peroxidation, This can result in the presence of malondialdehyde (MDA) and malondialdehyde-acetaldehyde (MAA) – adducts. Antibodies against MDA-adducts, especially MDA-LDL, were identified quite some time ago and were found to associate with cardiovascular problems in RA patients [37], but very little follow-up work has been carried out on this subject. Interestingly, a recent study also investigated the presence of autoantibodies against MAA adducts, which are more stable than MDA-adducts alone and are therefore more likely to be present in vivo. It was found that both MAA adducts and antibodies directed against these adducts were increased in RA patients [38]. However, MAA-antibodies are not very specific, since they have also been detected in people with liver disease and type 2 diabetes [39,40]. Therefore, these antibodies are most likely not very suitable for diagnostic purposes. Furthermore, a positive correlation between the presence of ACPA and anti-MAA antibodies was observed [38].

However, to what extent the presence of anti-MAA adds clinically relevant information on top of ACPA in ACPA-positive patients or in their own right in ACPA-negative patients remains to be established.

Anti-PAD4 antibodies

Autoantibodies directed against PAD4 have also been identified [41,42]. PAD4 is a peptidylarginine deiminidase, one of the proteins that is responsible for the conversion from arginine to citrulline. Anti-PAD4 antibodies were increased in RA when compared to disease-controls and present in 22% - 45% of the measured RA patients [41,42]. Anti-PAD4 antibodies can be detected in 14% of SLE patients [42], but seem to be absent in spondyloarthritis patients [43]. The diagnostic value of anti-PAD4 antibodies, may not be very high since the specificity seems to be lower than 50% [41]. Furthermore, a specific group of anti-PAD4 antibodies that cross-reacts with anti-PAD3 antibodies has also been described [44]. These antibodies can be found in 12%-18% of the RA patients. Anti-PAD3 antibodies can only be found in anti-PAD4 positive RA patients. Interestingly, the cross-reactive antibodies were associated with the severity of radiographic damage [44], so these anti-PAD3/4 antibodies may serve as biomarker for disease prognosis, although they can only be detected at low frequency in RA patients.

BRAF

PAD4 was one of the proteins identified in a proteomic approach aimed at the identification of autoantigens [43]. In the same study, a second antigen, BRAF, a serine-threonine kinase involved in the MAPK pathway, was identified [43]. Of the RA patients, 21 - 32% have been found positive for anti-BRAF antibodies [43,45]. But again, specificity seems to be a limitation, since anti-BRAF antibodies were also present in SLE and primary Sjogren's syndrome in almost similar percentages. The use of these autoantibodies for other purposes has been suggested, but requires more research as well.

RA-33

Another type of autoantibody targeting an intracellular molecule is RA-33, which binds to heterogeneous nuclear protein (hnRNP) A_2, a part of the splicosome. RA-33 is found in one third of RA patients and does not seem to correlate with ACPA or RF, but the frequency of RA-33 in patients negative for ACPA or RF is relatively low. [46,47]. In the RF and ACPA-negative patients, the amount of RA patients that could be identified was 13%, while 9% of the non-RA patients were also positive for RA-33. Since the window between RA and non-RA is very small, it may not be useful as a clinical biomarker for diagnosing RA. Interestingly, patients that are positive for RA-33 do often show a less severe disease development than RA-33-negative patients, so RA-33 might serve as a good marker for prognostic purposes [46].

Other autoantibodies

There are many other autoantibodies that have been suggested as potential diagnostic biomarkers. However, many of these studies lack power, due to small patient numbers. For example, autoantibodies against transthyretin, a hormone carrier, were found to be slightly increased in RA patients when compared to healthy controls, but this was only tested with samples derived from 60 patients [48]. Similar numbers were used in a study that proposed that tryptase was another autoantigen recognised in RA patients [49]. A larger cohort on the other hand, was used to measure anti-agalactosyl IgG antibodies in RA patients [50]. These types of studies are very interesting with regards to the identification of new autoantibodies in RA patients, but should be expanded or require follow-up in order to gain enough power for solid conclusions.

Combining several antibodies, may potentially be a better method to identify those patients most at risk for the development of RA. Therefore, another method that has been used to identify autoantibodies is the use of a cDNA phage display library of RA synovium, which was screened for antigen reactivity, using pooled RA plasma samples [51] Eventually, 11 RA-specific sequences were identified, the combination of which, resulted in reactivity of 50 – 58% of the RA patients plasma samples, from RA patients. Also, increased positivity for these autoantibodies was also associated with higher CRP levels, indicating an increase of inflammation [51]. The most widely used autoantibody combination in the clinic consists of IgM-RF and anti-CCP as these are part of the 2010-RA criteria [9] but currently significant efforts are ongoing to look into combinations of the above described antibodies in providing optimal clinical utility regarding the diagnosis, prognosis and prediction of medication efficacy.

Other serum biomarkers

Besides autoantibodies, there are of course many other factors that have been investigated in RA patients. Cytokine levels for example were measured in order to investigate the pathogenesis of RA, although these levels appear to vary widely at different time points with little relationship to disease activity, but some may be useful as a possible biomarker. Also, many different proteins have been found increased or down regulated in RA patients, which might also make these interesting biomarkers. Therefore, in this part we will describe some examples of other proteins besides antibodies that may be relevant biomarkers for the clinical management of RA patients.

Type 1 IFN-signature

Besides the direct measurement of cytokines, the effects of these cytokines on gene expression can also be measured. In RA patients, peripheral blood was isolated in order to measure gene expression levels. A subset of RA patients with a specific phenotype could be identified based on the expression levels of interferon (IFN) type I response genes [52]. This so-called Type 1 IFN-signature can also be adjusted to predict which patients will not respond to rituximab treatment [53].

14-3-3η
The 14-3-3 protein family consists of 7 isoforms that are intracellular chaperonin, present in eukaryotic cells. The levels of one of these isoforms, 14-3-3η is increased in RA patients and may therefore serve as an additional diagnostic marker for RA patients. It has a sensitivity of 73% and a specificity of 93%, which are quite promising numbers [54]. Futhermore, the presence of these proteins appear to be associated with increased joint damage [54,55].

Combinations of serum biomarkers
Different combinations of biomarkers have been commercially developed as a diagnostic test, for example the multi-biomarker disease activity (MBDA) test developed by Crescendo. This test measures 12 serum components using a multiplex format [56]. An algorithm is used to calculate the MBDA score from the acquired data. This score shows a correlation with DAS28 levels [57]. Furthermore, it has been suggested that these biomarkers may predict joint damage in RA patients [58].

In addition, serum proteins have been identified as possible markers for diagnostic purposes. Some proteins or posttranslational modifications thereof have been reported together with their respective autoantibodies. There are many more proteins that are increased in RA patients, but most of these are only slightly elevated when compared to healthy controls, which leaves them unsuitable for diagnostic purposes. L-Ficolin, for example was increased in RA patients, however there was a large overlap between healthy controls and RA patients [59]. Interestingly, M-ficolin has been reported to associate with disease activity and may predict remission in RA patients. The overlap between healthy controls and RA patients seems to be smaller for this ficolin [60]. It would certainly be interesting to measure both ficolins in the same cohort in order to compare the two.

Synovial tissue biomarkers
The antibodies and proteins measured above are mostly measurable by serological tests and do not require invasive methods for analysis. There are, however, other potential biomarkers, such as biomarkers that can be found in the synovial tissue at biopsy. Biomarkers that are derived from the inflamed tissue may be more reliable in predicting the local disease status and response to therapy, since the biomarkers are derived from the target tissue of the disease.

Cellular infiltrates
Extensive studies of synovial tissue markers have been performed over the last 2 decades that have revealed some specificity in relation to PsA and RA patients [61]. The first observations focused on differences in cellular infiltrate and a marked increase in vascularity [62,63] and vascular growth factors expressed in PsA synovial tissue, generally at significantly higher levels than in RA [64]. In RA synovial tissue several multi-centre studies have identified the monocyte/macrophage cells to be a significant and validated biomarker of disease activity and of response to therapy[65].

In PsA there are significantly less macrophages and the lining layer appears to be normal in depth compared to the hyperplastic features in RA [62]. The exact reasons for these differences have not yet been defined, however the changes first noted at a cellular level have been supported with studies of molecular pathways suggesting that molecular expression of vascular growth factors is significantly higher in PsA synovium [64].

In RA the most well-defined and validated tissue biomarker is the CD68 molecule expressed on the surface of activated macrophage cells in the synovial lining layer and in the sub-lining stroma [66]. Expression of CD68 has been validated in several proof-of-concept studies and applied in clinical trial cohorts also demonstrating a significant association with disease activity and response to therapy [67]. In addition, the B cell lineage marker CD20 has been identified in synovium as a potential cellular biomarker, that may complement circulating autoantibodies in predicting a patients response to therapy with Rituximab [68]. There is at least one study that suggests CD68 may not be a useful biomarker in PsA [69] while in the same study there is some evidence to suggest that CD3 positive T cells may be a useful biomarker of disease activity and response to therapy in the synovial tissue of PsA patients. In the synovia of individuals with arthralgia, the presence of CD3 positive cells was weakly positively associated with future development of RA . Surprisingly in the same study the authors noted an association between the presence of CD8 positive cells and ACPA positivity [70].

New methods for biomarker discovery

The general utility of synovial tissue biomarkers is questioned as it does involve an invasive procedure to obtain tissue by either needle biopsy, increasingly undertaken with ultrasound guidance or under direct visualisation at arthroscopy [71]. It is currently hoped that using patient stratification models it may be possible to discover an association between a relevant synovial tissue biomarker and one that can be easily measured in peripheral blood samples. There have been rapid advances in technology in the last number of years, not least of all, an '-omic' approach to biomarker discovery including genomics, proteomics and transcriptomics. In a recent publication Villanova and colleagues have suggested a 'new approach' that will employ multiple 'omic' technologies to discover new biomarkers, that can subsequently be validated, at an analytical and a clinical level, and only then qualify and be commercialized into a standardized assay for clinical usage [72]. These techniques have also been applied to synovial tissue biopsies and to serum samples from RA and PsA patients yielding some preliminary but interesting findings. There does appear to be increased expression of metalloproteinase enzymes in synovium around the cartilage-pannus junction [73]. Furthermore, the circulating levels of MMP, in particular MMP-3, does appear to predict the prognosis with respect to joint damage in early RA [74,75]. Circulating MMP3 levels have also been shown to be independently associated with the response to TNFi therapy in PsA patients [76].

At least one study has also shown that changes in circulating cartilage biomarkers in patients with RA and PsA correlate with disease activity, radiological progression and the response to therapy with TNF inhibitors [77].

There is a large international consortium effort underway to analyse circulating biomarkers in over 1000 PsA patients, as part of an Omeract/GRAPPA programme, examining C-reactive protein, serum amyloid-A; the collagen biomarkers C2C, C1,2C and CPII; MMP3, as mentioned above; markers of bone turnover including Dickkopf-1, sclerostin, bone alkaline phosphatase, C-telopeptide fragments of type II collagen (CTX-II), CTX-1, receptor activator of nuclear factor-κB ligand, and osteoprotegerin [78]. Independently, a group from New Zealand has recently published evidence of biomarkers of bone remodelling such as Dkk-1 and M-CSE are associated with bone erosion and underlying osteoclastogenesis in PsA patients [79].

Genetic markers may be useful in PsA patients, however presently do not have a role to play as biomarkers of diagnosis – HLAB27 is present in up to 70% of cases, and there are associations with HLACw6, PSORS1 and the IL23 receptor among others [72]. The utility of genetic markers of PsA in clinical practice is limited and to date is confined to the research agenda. As outlined above they may be incorporated into a multi-'omic' approach to biomarker development. However, it should be noted that the detailed studies in RA patients have demonstrated a clinical utility of combined biomarkers including genotype, circulating autoantibodies with environmental risk factors, especially cigarette smoking to identify prognosis [80], such studies have not yet been performed in PsA patients.

Conclusion

When comparing the biomarkers between RA and PsA, a clear difference is the presence of autoantibodies, which are observed in RA patients, but not in PsA patients. In RA patients, ACPA and RF are currently in use for clinical diagnosis. It seems that additional autoantibodies identified in RA patients, as yet, may not result in additional value as diagnostic biomarkers, however they may add prognostic information. As many patients are positive for several autoantibodies it is interesting to combine different autoantibodies to investigate whether combining this information will provide more insight regarding the diagnosis, prognosis and prediction of response to therapy. Many apparently healthy people present with one or more autoantibodies, even years before disease onset, and it remains a focus of current research whether healthy subjects in the general population with circulating antibodies are predetermined to develop RA.

Interestingly, even though PsA and RA have many clinical and pathological similarities, regarding biomarkers there do appear to be significant differences, suggesting differences in disease process and mechanisms. In PsA patients the key biomarkers , especially in early phase disease appear to be related to the vascular factors of the inflammatory response.

Biomarkers in the skin and the synovium including angiogenic growth factors do appear to be relevant. It remains to be seen if the measurement of these factors in the peripheral circulation are useful either in the diagnosis or the determination of prognosis in patients with PsA. In addition, the new approach of biomarker discovery in psoriasis and PsA using gene signature data to identify key molecules and their links with the clinical phenotype may yield interesting results in the near future.

Many different biomarkers have been identified in RA and PsA some shared, although most differ between diseases. In many studies, only one biomarker is investigated at a specific timepoint. In order to acquire a clear overview with regards to the usefulness of all different biomarkers, an important aspect of future research would be to measure large numbers of biomarkers longitudinally in the same cohort. In this manner, data can be analysed using a systems biology approach to discover the important associations with disease. This would also allow the identification of the best set of biomarkers to predict prognosis and possibly the response to specific treatments. The first major challenge for the future of biomarker research in RA and PsA is to identify specific circulating biomarkers for the diagnosis of PsA and prognostic markers useful for both RA and PsA. The next major challenge is to develop specific biomarkers to identify RA and PsA individuals at high risk of progressive damage and to predict response to specific therapies.

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