

Factors influencing the development and effectiveness of biomarkers in rheumatoid arthritis and osteoarthritis

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Clinical unmet needs in diagnosis of rheumatoid arthritis & osteoarthritis

Musculoskeletal disease is the most common cause of chronic disability worldwide. Impaired musculoskeletal health accounts for approximately 2% of total global disability adjusted life years, of which osteoarthritis (OA) is 1.1% and rheumatoid arthritis (RA) is 0.3% [1]. If OA and RA could be identified in the early stages, currently available treatments for RA could be initiated earlier and treatments for early-stage OA developed could prevent pain and disability of advanced disease. OA is a progressive joint disease characterized by joint inflammation and reparative bone response; the latter eventually proving to be inadequate as insidious chronic pain and impairment of joint mobility develop. It affects approximately 100 million people globally and is the primary diagnosis in a majority of 2.9 million joint replacements. Joints replaced are predominantly hips (1.4 million) and knees (1.1 million), with increasing replacements of shoulders, elbows and ankles. This trend highlights the advances made in material science and implant design. The pain and physical disability during development and progression of OA and underlying pathogenesis remain unresolved. Currently, the care of patients with OA is management of progressive increase in symptoms by general practitioners until total joint replacement is judged appropriately. If detection and diagnosis of early-stage OA could be implemented clinically,

remedial interventions to decrease the joint damaging process and/or boost the bone reparative process in the early stages may prevent and reverse OA development. Early-stage detection of RA is available clinically and is based mainly on the anti-cyclic citrullinated peptide (CCP) antibody test which has sensitivity of 61%. This requires improvement and refinement for continued clinical use [2]. For RA, the understanding of underlying inflammatory disease mechanisms has led to the development of life-altering medications such that morbidity that can be controlled and limited for many patients. Moreover, it is considered that in early-stage RA there may be a possibility of remission or cure [3,4]. This could be implemented more effectively with improved early-stage diagnosis.

Finding an early-stage diagnostic marker

A biomarker is a diagnostic indicator of a clinical endpoint and often is a reporter of early-stage disease development. Concerted efforts are being made to develop biomarkers for early-stage detection of OA and RA, including biochemical markers and features in radiographic and MRI of joints. Several potential markers have been identified and further validation and refinement is in progress [5,6]. Further improvements are required to produce a diagnostic test of acceptably high sensitivity and specificity. Biomarker selection is likely to be most beneficial when

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focused on early-stage mechanisms and changes in the disease process – such as protein citrullination and related autoimmunity likely producing anti-CCP antibody positivity in early-stage RA [6]. We may also look again at some older markers of bone turnover and resorption, such as hydroxyproline (Hyp) [7], with improved detection and quantitation. From such considerations, we recently developed a diagnostic test for early-stage detection and typing of OA, RA and other inflammatory joint disease [8].

Early-stage arthritis diagnosis & typing by a diagnostic algorithm

There may be a single biomarker with diagnostic characteristic to improve early-stage detection of OA, RA and other inflammatory joint disease. It is more likely, however, that effective arthritis diagnosis will be achieved by a combination of clinical variables and biomarkers or ‘features’ in a diagnostic algorithm. Algorithms are developed by the process of machine learning – an application of computer science where the weighing of different features is optimized or trained on experimental clinical data. The algorithm is then validated on independent subject/patient datasets. The outcome is a probability that a person has or does not have a joint disease. A diagnostic algorithm was developed to incorporate added value of power Doppler sonography for early detection of inflammatory joint disease [9]. Further refinements are available: for example, our recent combination of subject’s age, gender, anti-CCP positivity, plasma citrullinated protein (CP) and Hyp provided both early-stage detection and type of arthritis (early OA, early RA and other inflammatory joint disease) [8]. Algorithms may be optimized for diagnosis and for progression and therapeutic monitoring. The strength of this approach is that it provides a data-driven discovery of complex combinations of features and their relative influence on the joint disease of interest (diagnosis of early-stage type of arthritis) by logical, step-wise analysis without preconception and bias. The limitations are: access to relevant data, implementation can only proceed when all data collection is completed and the expertise required for variable selection for inclusion in the analysis.

Analytic technology for biomarker measurement: immunoassay, quantitative MS or both targeted to mechanisms of decline in skeletal health

For development and implementation of clinical chemistry assays of proteins and metabolites, immunoassay remains an analytical method of choice based on its high-analytical specificity, sensitivity and sample throughput. For multiple protein biomarkers, mul-

tiplex immunoassays may be used at increased cost – with careful control of crossreactivity to antigens. Few protein multiplex immunoassays have been validated, however, for diagnostic application in the clinical setting [10]. The analytical method of choice for detection and quantitation of small molecule metabolites is stable isotopic dilution analysis LC–MS/MS, which has robust, crossreaction-free multiplexing for analytes at minimal additional cost. LC–MS/MS lacks high-sample throughput but operates at moderate sample throughputs – as good as or higher than conventional clinical chemistry chromatographic methods (e.g., glycated hemoglobin A1C). LC–MS/MS is also being developed for multiplexed protein analysis by quantitation of peptides in sample tryptic digests [11]. Targeted metabolomics and proteomics focused on early-stage pathogenic mechanisms of decline in skeletal health are likely to provide biomarker analytes with greatest diagnostic utility. In our recent research, we combined both immunoassay and focused LC–MS/MS metabolomics and proteomics for markers of protein citrullination and bone turnover: immunoassay of anti-CCP antibody positivity and LC–MS/MS analysis of CP and Hyp. LC–MS/MS analysis of Hyp had superior analytic performance compared with conventional chromophoric and immunoassay methods [8]; interferences in previous measurement may have obscured the diagnostic value of Hyp.

Uses of a biomarker diagnostic test

The age/gender/CP/anti-CCP antibody/Hyp biomarker test [8] requires further validation. Thereafter, it may be possible to reliably detect and type early-stage arthritic disease. This opens several further new areas of clinical investigation. First, it will be possible to assess the test response to progression to more advanced disease and hence use of the test or a refinement of it as a risk predictor of arthritis progression and a basis for implementing targeted care and treatment. Second, and following from this, there will be the opportunity to assess test response to treatment outcome and apply it for therapeutic monitoring. Third, once a link to diagnostic test response to disease progression and regression has been established, the test may be used to facilitate clinical assessments in the evaluation of new treatments.

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How will this impact on current clinical practice? Established or advanced OA is evaluated clinically and radiologically. Plain radiographs are the mainstay of

investigation and once the diagnosis is made – which is sensitive for only established or advanced OA – ongoing evaluation is based on the pain and joint function experienced by the patient. Current management of established OA tends to be a symptomatic treatment until it gets to a stage where joint replacement surgery is needed. At this stage, a biomarker may help the diagnosis but may not have an impact on management until effective treatments are developed. It may be applicable in ongoing evaluation of established OA – particularly during acute flare-ups where arthritic joints become hot, swollen and tender. Once the diagnosis of established OA is made, it is the extent of patient's symptoms that dictate timing of surgery. Radiographic image features correlate very poorly with symptoms and a biomarker test may assist with evaluation and selection of patients for joint replacement surgery. Patient evaluation using biomarkers, as well as imaging and questionnaires, may also bring an opportunity to refine the process by which patients are offered surgery to ensure that appropriate surgery is performed at the correct time. The biomarker response may also be of benefit in assessment of postimplantation responses. Persistent pain, functional impairment, infection and implant loosening are a few of the common effects of surgery, and a biomarker may be of use in determining the physiological aspects of these problems as opposed to purely mechanical ones.

The future

There is no routine clinical test to diagnose early-stage OA, and tests for early-stage RA require refinement. A blood-based laboratory test for early-stage diagnosis and typing of arthritis, such as our recent development [8], may greatly enhance the capability of clinical healthcare professionals to direct care and treatments to people who need and will benefit from it. It may thereby improve clinical outcomes and cost-effectiveness. It will also likely revitalize biomedical and phar-

maceutical research to advance understanding of disease mechanisms and development of new treatments, particularly for OA. For example, we noted that protein citrullination is higher in plasma than synovial fluid in early-stage OA, which may point to important systemic metabolic factors contributing to the development of OA. As the pathogenesis of OA becomes clearer, the scope for the development of new drugs increases. The role of biomarker in objectively testing drug efficacy will bring to bear medical therapies for the management of OA before joint replacement surgery is considered.

Conclusion

Further research is required to produce, validate and optimize diagnostic biomarkers for clinical implementation of early-stage diagnosis of arthritis. Diagnosis of arthritis at the early-stage, type of joint disease, risk of progression and monitoring of effect of treatment will be of great clinical value. Biomarkers are likely to make a key contribution: those linked mechanistically to mechanisms of early-stage decline in skeletal health and development of disease are expected to be the most useful. Our combination of CP/anti-CCP antibody positivity and Hyp in a diagnostic algorithm is a recent example [8]. Immunoassay and/or quantitative LC-MS/MS approaches are likely to be important biomarker assay platforms where high-level automation is crucial for clinical implementation.

Financial & competing interests disclosure

The authors are inventors on a related patent: Diagnostic for Determining Skeletal Health. WO2014016584 A2 (2014). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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