Defining the Role of Molecular Markers to Monitor Disease, Intervention, and Cartilage Breakdown in Osteoarthritis

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ABSTRACT. Osteoarthritis (OA) is associated with a loss of the normal balance between synthesis and degradation of the macromolecules that provide articular cartilage with its biomechanical and functional properties. The destruction of joint cartilage involves the degradation of matrix molecules which are released as fragments to joint fluid, blood, and urine, where they may be detected, for example, by immunoassay. It has been suggested that such molecular markers of cartilage matrix metabolism could be used as markers to determine diagnosis, prognosis, and severity, to predict response to therapy and monitor response to therapy, and to identify disease mechanisms on the molecular level. Since markers reflect ongoing dynamic changes in joints, they are perhaps most likely to serve as measures of prognosis and measures of response to treatment. Some markers may serve multiple functions. To function as adequate tests, they should meet a set of standards. It is only when markers have met such criteria that they will be accepted in the research and clinical community and will become widely used. (*J Rheumatol 1997;*24:782–5)

Key Indexing Terms:
OSTEOARTHRITIS CARTILAGE DIAGNOSIS REPRODUCIBILITY OF RESULTS

OUTCOME MEASURES OF OA AND MOLECULAR MARKERS

Osteoarthritis (OA) is associated with a loss of the normal balance between synthesis and degradation of the macromolecules that provide articular cartilage with its biomechanical and functional properties. Concomitantly, changes occur in the structure and metabolism of the synovium and subchondral bone of the joint. These processes result in the destruction of joint cartilage and changes in the function of the affected joints, which cause pain and physical disability.

Current therapy of OA is largely symptomatic, and is focused on decreasing pain and improving function with analgesics, nonsteroidal antiinflammatory drugs, or arthroplasty. However, new interventions are being proposed for treatment of joint disease[1,2] that may decrease the rate of joint destruction in OA. The ability to reproducibly and sensitively monitor disease progression and outcome for both joint and patient in intervention trials is critical to the development of new disease modifying treatment strategies in OA. There are 3 general ways by which OA can be assessed: (1) patient related measures of joint pain and disability (algofunctional scores[3,4]); (2) measurements of the structural (anatomical) changes in the affected joints (plain radiographs[5], magnetic resonance imaging[6], arthroscopy[7]); (3) measurements of the disease process exemplified by changes in metabolism or functional properties of the articular cartilage, subchondral bone, or other joint tissues (body fluid markers of cartilage and bone metabolism[8], bone scintigraphy[9], measurement of cartilage compression resistance by indentation or streaming potentials[10]).

Algofunctional scores, plain radiographs, and arthroscopy are in use to assess OA trials. Of these methods, only algofunctional scores have yet been fully validated as outcome instruments. Standardized imaging of joints by plain radiographic examination is proposed as the current gold standard to detect changes in joint structure in clinical trials of disease modifying drugs in OA[11]. However, even with improved techniques for patient positioning and image measurements and a future validation, plain radiographic examination remains only an indirect means to measure the disease process in OA, a surrogate marker. Magnetic resonance imaging (MRI) will find increasing use as a measure of joint changes in OA. Until methods to monitor joint cartilage quality or composition by MRI appear, the technique suffers from the same weakness as radiographic examination: it is at best an indirect measure of the current disease process that documents the consequences of disease. Methods that provide rapid information on function, composition, and

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Supported by the Swedish Medical Research Council, Lund University Medical Faculty and Hospital, the King Gustaf V 80-Year Fund, Zoega Foundation, Kock Foundation, Merck Research Laboratories, and Astra Läkemedel (to LSL), and NIH Grants AR20613 and AG09300 (to DTF).

Abstracted in part from Lohmander LS, Roos H, Dahlberg L, Lark MW: The role of molecular markers to monitor disease, intervention and cartilage breakdown in osteoarthritis. Acta Orthop Scand 1995;(suppl 266)66:84–7, *Felson D: Validating markers on osteoarthritis. Acta Orthop Scand 1995;*(suppl 266)66:205–7, *and Lohmander LS, Felson DT: Defining and validating the clinical role of molecular markers in osteoarthritis. In: Brandt KD, Lohmander LS, Doherty M, eds. Textbook of Osteoarthritis.* Oxford: Oxford University Press, 1997 (in press).

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metabolic processes in arthritic joint cartilage would help evaluate the role of new and old interventions in OA[12].

The destruction of joint cartilage in OA involves the degradation of matrix molecules, which are released as fragments to joint fluid, blood, and urine, where they may be detected, for example, by immunoassay. It has been suggested that such molecular markers of cartilage matrix metabolism could be used to diagnose, prognosticate, and monitor joint diseases such as rheumatoid arthritis and OA, and to identify disease mechanisms on the molecular level as reviewed[8,13-16]. Although many publications have described the increased release of markers of cartilage, bone, or synovial metabolism into joint fluid, serum, and urine in arthritis, several of these goals remain elusive.

The demands on a marker may differ, depending on its application as a diagnostic test, prognostic test, or evaluative test[17]. The diagnostic test focuses on the ability to detect differences between affected and unaffected individuals, often expressed in terms of sensitivity and specificity of the test. The evaluative test, on the other hand, focuses on the ability of the marker to monitor change over time in the individual patient, often expressed as sensitivity to change or effect size.

Several of the requirements of a new marker, be it diagnostic, evaluative, or prognostic, are general and may be expressed in terms of validity[18,19]. Does the test have face validity (is it credible?), construct validity (are the results expected, do they change in proportion to clinical change?), content validity (is it relevant across multiple domains change in the disease?), criterion validity (does it correlate with a gold standard?), or discriminant validity (does it detect the smallest clinically relevant change?).

THE MEANING OF MARKERS

Markers can serve as diagnostic tests that help to distinguish joints with OA from unaffected joints or other joint diseases. The concentration of keratan sulfate in serum was originally suggested to serve as a diagnostic test for generalized OA[20]. Subsequent experience has, however, not fulfilled this promise[21,22], although this serum marker may yet serve to reflect cartilage proteoglycan degradation in some situations[23]. Considerable overlap exists between affected and unaffected individuals, and serum concentrations are influenced by age and sex[24]. Other studies have shown differences in knee joint fluid concentrations of aggrecan fragments, COMP (cartilage oligomeric matrix protein) fragments, bone sialoprotein fragments, and matrix metalloproteinases and their inhibitors between OA, knee-healthy reference groups, rheumatoid arthritis (RA), and reactive arthritis[25-29]. While these investigations show significant differences of mean values between the study groups with only moderate overlap, interpretation is confounded because comparisons between groups are cross sectional[17,29]. These studies should therefore be regarded only as hypothesis generating, and will require confirmation in prospective studies.

The term "marker" might also be used for a test evaluating disease severity rather than its presence or absence. In OA, disease severity (or stage) is measured by Kellgren and Lawrence grade of radiological changes and by the amount of cartilage loss on arthroscopy or by the patient's degree of functional impairment, among other methods. Several reports have suggested that assay of molecular markers of cartilage metabolism may provide complementary information on joint disease stage[30,31]. While further experience in this area is needed, molecular markers clearly have the potential to provide unique information on joint cartilage quality not currently available by other staging methods.

Biochemical assays of molecular markers developed to evaluate OA have also been promoted as prognostic markers and tested to see whether they predict the later occurrence or worsening of OA. For example, it was shown that levels of serum hyaluronan (but not keratan sulfate) in patients with diagnosed knee OA at study entry predicted subsequent progression of knee OA at 5 year followup[32]. In the same study population, an increase in serum COMP during the first year after study entry was associated with radiographic progression of OA at 5 year followup[33]. Studies on rheumatoid patient groups have indicated that serum levels of COMP and the chondroitin sulfate epitope 846 are associated with rapid disease progression in that condition[34]. These reports describe results obtained in patient groups of limited size, and often do not specify the strength of the relationship between marker levels and disease progression. However, they suggest that further progress in the area of prognostic markers is very likely with prospective, longitudinal studies on larger patient cohorts.

Measures of disease dynamics evaluate the ongoing repair and degradative processes occurring within a joint, and might be classified as prognostic measures. Concentrations in joint fluid of molecular markers for both degradation and synthesis are consistent with the changes in metabolic rate observed for these molecules in animal models *in vivo* and in human osteoarthritic cartilage *in vitro*[8].

Markers may also be used to predict response to therapy. Structure analysis of the fragments released from or remaining in the cartilage matrix may yield important information on the character of the metabolic process or protease responsible. Results obtained on aggrecan fragments may here serve as an example. The structures of the fragments released into joint fluid, and of those remaining in the matrix, are consistent with 2 different proteolytic activities in cartilage matrix in OA[35-37]. One of these proteases generates fragments consistent with the action of "classic" matrix metalloprotease such as stromelysin, while the other, as yet unidentified, protease generates fragments consistent with the action of an unidentified protease, "aggrecanase." Similar and ongoing structure analysis of fragments of cartilage collagens and matrix proteins may yield information

on the role of different proteases in different phases of disease development, critical for our understanding of cartilage metabolism. This information may in turn be used to predict responsiveness to treatment specific for a proteolytic activity such as a collagenase or aggrecanase. The usefulness of this concept relies on the identification of disease mechanisms with at least a relative specificity for a condition or disease stage, and on the availability of agents specific for these processes.

Molecular markers have also been suggested as being useful to monitor response to therapy in OA, to be used as sensitive surrogate outcome measures in clinical trials of new disease modifying treatments. Here, advances in our understanding of disease mechanisms assisted by structure analysis of molecular fragments released from human joint cartilage, as outlined in the preceding paragraph, will be critical. With the current absence of disease modifying treatments in OA, the role of molecular markers in this area remains speculative[38]. Experience from the treatment of patients with RA is also limited, but suggests that cartilage molecular markers are responsive to treatment[28,39]. Randomized, controlled clinical trials of new disease modifying treatments of OA will represent a precious opportunity to validate OA markers as outcome measures.

CONCLUSION

Molecular markers for OA could serve different purposes. Since markers reflect ongoing dynamic changes in joints, they are perhaps most likely to serve as measures of prognosis and measures of response to treatment. Some markers may serve multiple functions. To function as adequate tests, they should meet a set of standards. It is only when markers have met such criteria that they will be accepted in the research and clinical community and will become widely used.

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