The role of molecular markers to monitor disease, intervention and cartilage breakdown in osteoarthritis

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Osteoarthritis (OA) involves the loss of the physiological balance between degradation and replenishment of intercellular matrix in the cartilage and other tissues of the joint. This uncoupling eventually results in changes in the structure of the affected joints, which may cause pain and physical disability. OA evolves slowly and is a heterogeneous condition. Concurrent with the slow natural progression of OA, the patient and the joint tissues age, inducing additional and confounding functional changes in the musculoskeletal system. The molecular signals regulating either the breakdown of the matrix or its replenishment are unknown.

To treat OA today we are still limited to moderating the pain and discomfort by e.g analgesics, or by replacing the destroyed joint surfaces with plastic and metal. Even with the remarkable success of arthroplasty as a treatment for joint destruction in OA, we still need the means to prevent the articular cartilage degradation in joint disease. This need is especially evident for patients at risk for development of OA at young age after e.g. joint injury. For this group, joint replacement is not a solution due to the high risk for loosening and revision (Knutson et al. 1994).

New interventions are now being proposed for treatment of joint disease (Vincenti et al. 1994, Campion 1994), which may have the ability to alter the rate of joint destruction in OA. Unless improved techniques to assess the disease process are developed, standardized and validated, it will be impossible to properly evaluate the role of either new or old interventions (Dieppe et al. 1995).

Monitoring of osteoarthritis

There are currently three general methods used (or proposed) to assess OA:

- *patient-related measures of joint pain, disability or endpoint* [algofunctional scores such as WOMAC (Bellamy et al. 1988), index of severity of knee or hip disease (Lequèsne et al. 1987) or frequency of total joint replacement (Vingård et al. 1991)]
- *clinical or imaging measurements of the anatomical changes in the affected joints* [plain radiographs (Spector and Cooper 1993), magnetic resonance imaging (Chan et al. 1991), arthroscopy (Dougados et al. 1994), high frequency ultrasound (Myers et al. 1994)]
- *measurements of the disease process* such as changes in metabolism or functional properties of the articular cartilage, subchondral bone or other joint tissues [body fluid markers of cartilage and bone metabolism (Lohmander 1994, Poole 1994, Saxne 1995), bone scintigraphy (Dieppe et al. 1993), measurement of cartilage compression resistance by indentation (Kiviranta et al. 1995) or streaming potentials (Bonassar et al. 1995)].

Of these techniques, algofunctional scores, plain radiographs and arthroscopy are currently in use to assess drug efficacy in OA trials. It might be argued that of these methods, only the algofunctional scores have been formally validated as outcome instruments. The frequency of joint replacement might be seen as an attractive and unambiguous end-point. However, it is markedly influenced by regional variations in health-care availability and similarly marked temporal changes induced by socioeconomic realities, which makes it difficult to use in prospective studies. Techniques of measurement of anatomical changes in the affected joint are in a phase of rapid development and standardized imaging of joints by x-rays and magnetic resonance will no doubt find increasing use in OA trials. Concurrently, biophysical readouts such as measurement of cartilage matrix streaming potentials are also being proposed.

Molecular markers of osteoarthritis

The destruction of joint cartilage in OA involves the proteolytic degradation of matrix molecules, which are then released into joint fluid, blood and urine where they may be detected by biochemical or immunochemical assays. It has been hypothesized that such molecular 'markers' of cartilage matrix metabolism could be used to diagnose, prognosticate and monitor joint diseases such as rheumatoid arthritis and OA (Lohmander 1994, Poole 1994, Saxne 1995). These goals have not yet been reached. However, as we gain a better understanding of these markers, we learn more about the molecular pathogenic mechanisms and the dynamic and fragile balance between matrix degradation and synthesis in cartilage (Lohmander et al. 1993a, Lohmander 1994). For example, it was shown that the structure of aggrecan fragments in human joint fluid and cartilage in both osteoarthritis and rheumatoid arthritis is consistent with the action of both a classic matrix metalloproteinase such as stromelysin and another putative protease, 'aggrecanase' (Lohmander 1994, Bayne et al. 1994, Lark et al. 1995). A more detailed understanding of these processes is critical to the development of new therapeutic modalities for the treatment of joint disease. At the same time, these findings may lead to the development of new and specific methods to monitor intervention and outcome of treatment aimed at preventing the destruction of joint cartilage in arthritis (Caterson et al. 1995).

Validation of molecular markers

The assignment of, as an example, a molecular fragment of a cartilage matrix molecule released into joint fluid, serum or urine as an 'OA marker' requires several criteria to be met (Tugwell and Bombardier 1982; Felson 1995). These criteria are often expressed in terms of validity: *face validity* (is the marker sensible?), *construct validity* (are the results expected, do they change in proportion to clinical change?), *content validity* (does the marker reflect multiple domains of improvement or deterioration in OA?), *criterion validity* (does the marker measure what you think it is measuring, does the marker predict or correlate with "gold standard" measures of OA outcome such as pain-function score or radiography?), *discriminant validity* (does the marker detect the smallest clinically important change?).

In addition, an understanding of the relationship between changes in marker concentrations in the body fluid compartment in question and changes in cartilage matrix metabolism is also required.

The future use of markers as outcome measures in e.g. clinical trials of new treatments for OA will further require the general availability of reproducible assays and data on the 'normal' or base-line range of concentrations of these markers in reference populations. We shall also require knowledge of the variability both over time in the individual and between individuals in representative cohorts in order to calculate the needed number of patients and the required response to treatment in a clinical trial setting. On the basis of such data we may also assess the sensitivity and specificity of the marker for e.g. diagnostic purposes.

What is the evidence that any of these OA marker 'promises' will be fulfilled?

Perhaps the most promising data so far to suggest the applicability of the joint disease marker concept pertain to rheumatoid arthritis. It was thus shown that patients with early-stage rheumatoid arthritis and who had high concentrations of aggrecan fragments in joint fluid progressed more rapidly towards joint destruction than patients with low concentrations (Saxne and Heinegård 1995). In serum in the early stages of the disease, rapidly progressive joint destruction was associated with high cartilage oligomeric matrix protein concentrations, but low concentrations of the aggrecan-related epitope 846 (Månsson et al. 1995).

Reference data on molecular markers

Only a very limited amount of data on reference concentrations and longitudinal and cross-sectional marker variations have been published (Roos et al. 1995, Dahlberg et al. 1994). We have examined a homogenous group of some 50 patients with knee complaints and arthroscopic cartilage changes, but no radiographic evidence of OA (unpublished data). We obtained 8 joint fluid samples from each patient over a one year period. The group-average 'cross-sectional' coefficient of variation (standard deviation/mean x 100) was about 45 % for aggrecan fragment concentrations at a single sampling occasion, while the average 'longitudinal' coefficient of variation for a single patient over one year and 8 samples was 18 % for this particular marker (unpublished). This suggests that within-patient variability for this marker is considerably less than between-patient variability, and that marker data in the trial setting should be compared against a carefully determined baseline level for each individual patient.

A review of our own published data on reference groups and different cohorts with joint pathology yields some preliminary information on the potential power of body fluid markers of OA to discriminate between a normal joint and a joint with pathology (Table 1). Calculations give specificity levels around

Table 1. Concentrations of molecular markers of cartilage matrix turnover assayed in joint fluid and serum, with calculations of specificity and sensitivity for discrimination between presence and absence of knee joint pathology. Joint pathology in this context includes diagnosed injury to cruciate ligament and/or meniscus in the presence or absence of osteoarthritic joint changes detected by arthroscopy or radiography. Data compiled from (Lohmander et al. 1993b, Lohmander and Shinmei 1994, Lohmander and Thonar 1994, Lohmander et al. 1995a, 1995b, Roos et al. 1995).

	VOL[a]	SFAGN[b]	SF846[c]	SFSLN[d]	SFCLN[e]	SFTIMP[f]	SFPCIIC[g]	SKS[h]
Joint pathology (n)	2352	2119	385	1037	614	1028	428	758
Median	5	66	0.6	21	0.6	15	3.4	293
10th percentile	0.5	30	0.4	2.7	0	5	0.9	196
90th percentile	50	204	1.0	137	8.1	55	10.1	426
Reference (n)	118	88	9	77	26	77	49	137
Median	1	70	0.3	4.7	0.1	5	1.7	277
10th percentile	0.2	32	0.2	0.4	0	1.9	0.8	196
90th percentile	1.9	102	0.4	23.4	0.4	12	6.1	422
Specificity %[i]	83	83	82	83	84	84	83	84
Sensitivity %[i]	75	59	91	69	76	78	59	57

[a] Total volume of joint fluid aspirated (mL)
[b] Synovial fluid aggrecan fragments detected by Alcian blue precipitation (ug/mL)
[c] Synovial fluid 846 epitope in aggrecan detected by immunoassay (ug/mL)
[d] Synovial fluid stromelysin-1 (MMP-3) protein detected by immunoassay (nM)
[e] Synovial fluid collagenase (MMP-1) protein detected by immunoassay (nM)
[f] Synovial fluid tissue inhibitor of metalloproteinase (TIMP-1) protein detected by immunoassay (nM)
[g] Synovial fluid procollagen II C-propeptide detected by immunoassay (ng/mL)
[h] Serum keratan sulfate (5D4-epitope) detected by immunoassay (ng/mL)
[i] Specificity and sensitivity are calculated as sensitivity = (a/(a+c)) and specificity = d/(b+d)), where the arbitrary cut-off point was set equal to the 80th percentile of the values for the reference group and where

	Disease present	Disease absent
Test positive	a	b
Test negative	c	d

80 % at the chosen arbitrary cut-off for reference concentrations, and sensitivities varied between 56 % for keratan sulfate in serum to 91 % for the aggrecan-associated 846 epitope in joint fluid. A combination of assays of two or more markers in the same sample improves both specificity and sensitivity, so that for example the combination of assays of stromelysin, collagenase and TIMP in joint fluid results in a specificity of 93 % and a sensitivity of 90 %. These results were calculated retrospectively and on a 'pooled' group of patients with joint pathology, which included diagnosed injury to cruciate ligament and/or meniscus in the presence or absence of osteoarthritic joint changes as detected by arthroscopy and radiography. In addition, only few reference samples were assayed for some of the markers. These results should therefore be taken as mere indicators of future diagnostic possibilities. However, calculations based on cohorts with more specific knee joint pathology such as primary OA, posttrauma OA or injury to menisci or ligaments, gave similar results.

Conclusions

These examples suggest that biochemical markers of cartilage matrix turnover may in the future indeed fulfill their promise as useful tools in the care and investigation of arthritis patients. It is, however, also quite clear that no marker has yet been formally validated as an outcome instrument for OA. Validation will require access to substantial, well-characterized patient cohorts for randomized and prospective intervention studies, in addition to sensitive and specific assays. We should also recognize the heterogeneity of OA; a given marker may need to be separately validated for OA in different joints and caused by different etiologies.

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