

Osteoarthritis Initiative

NIH White Paper: Biomarkers, the Osteoarthritis Initiative

A Basis for Discussion

(Draft: February 3rd, 2000)

**Robin Poole, Ph.D., D.Sc. Director, Joint Diseases Laboratory
Shriners Hospitals for Children, Canadian Hospital Professor, Department
of Surgery, McGill University Associate Program Director, Canadian
Arthritis Network**

INDEX

[Introduction](#)

[Biomarkers: Definitions and Opportunities](#)

[Goals for The Study of BM Usage In OA](#)

[The Development and Use of BM In Clinical Studies In Osteoporosis \(OP\) - A
Paradigm for OA](#)

[Clinical and Other End Points and Body Fluids In Studies Involving BM](#)

End points

Which body fluids should be analysed: the whys and wherefores.

[Factors That May Influence BM Levels Other Than Disease](#)

Circadian rhythms

Peristalsis

Physical activity

Hepatic and renal disease

Age and sex

Surgery

[Collection, Preparation and Storage of Samples](#)

[The Sensitivity, Accuracy and Reproducibility of Assays, and Variations Over](#)

Time

Which Molecular Events Should Be Measured, In Which Tissues and For What Indications?

General

The types of measurements that BM may represent and the patient populations that we should examine.

Analyses of Results

The Need For Positive Controls In The Assessment of BM: A Further Case For RA Studies.

Specific Assays of BM Tissue and molecular specificities.

Cartilage: type II collagen degradation (indication: cartilage degradation)

Cartilage: type II collagen synthesis: c-propeptide (indication: cartilage synthesis)

Cartilage proteoglycan aggrecan: core protein (indication: cartilage turnover)

Cartilage proteoglycan aggrecan: core protein epitope (indication: cartilage turnover)

Cartilage proteoglycan aggrecan: keratan sulfate (KS) epitopes (indication: cartilage turnover)

Cartilage proteoglycan aggrecan 846 and 3B3 epitopes and other chondroitin sulfate epitopes (indication: cartilage turnover/synthesis)

Cartilage proteoglycan aggrecan: in core protein cleavage neoepitopes (indication: cartilage degradation)

Cartilage oligomeric protein (COMP) (indication: abnormal cartilage turnover/or cartilage/synovial cell activation)

Cartilage matrix protein (matrilin-1) (indication: cartilage turnover)

GP-39/YKL-40 (indication: cartilage turnover and synovitis)

Pentosidine (indication: general collagen breakdown)

Synovium: type III collagen amino-(N)-propeptide (indication: synovitis and soft tissue repair)

Bone: type I collagen N- and C- (carboxy) propeptides (bone and soft tissue synthesis repair)

Bone: type I collagen c-telopeptide (indication: bone and soft tissue resorption)

Bone: type I collagen cross-links (indication: bone resorption)

Bone: osteocalcin (indication: bone assembly/turnover)

Bone: sialoprotein (BSP) (indication: bone assembly/turnover)

C-reactive protein (CRP) (indication: systemic inflammation)

Hyaluronic acid (HA) (indication: synovitis)

Cytokines (indication: inflammation)

Matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) (indication: cartilage damage and/or synovitis)

Assays for Pain

[Concluding Remarks](#)

[Acknowledgements](#)

[References](#)

(Abbreviations: OA, osteoarthritis; RA, rheumatoid arthritis; OP, osteoporosis; SF, synovial fluid; BM, biomarker)

1. Introduction

In its present draft form this paper represents 'work in progress' that represents the author's viewpoint and a consensus of the responses (solicited by the author) from a number of investigators (see Acknowledgements) who share a common and critical interest in this topic. The opinions of these authors have been acknowledged (names shown in brackets in text) where appropriate. This is a paper and not a review. It should be considered as "work in progress" and, at this stage, as a draft document.

2. Biomarkers: definitions and opportunities

We should speak of biomarkers (BM) rather than markers since they reflect a biological entity. That these BM may, for example, indicate or reflect a specific biological or pathological process, or an identifiable consequence of a process, or a pharmacologic response to therapeutic intervention. A surrogate biomarker would be one that is intended to substitute for a clinical endpoint. A clinical endpoint is a characteristic or variable that measures how a patient feels, functions or survives. These definitions were formulated at the April 1999 Conference on Markers at National Institutes of Health (information supplied by S. Lohmander).

The Roche Group suggests that we classify BM. For example, 'direct' BM are those that specifically identify a known molecular process within a given tissue (such as cartilage or bone or synovium) and 'indirect' BM reflect a more general change which is not so clearly definable and which may represent contributions from several events and tissues. The latter may be represented by matrix metalloproteinases (MMPs), cytokines, growth factors, and inflammation markers, such as c-reactive protein (CRP). These indirect BM may thus represent cell to cell or cell to matrix signalling (through cytokines, growth factors or degradative mechanisms (MMPs)). Where possible it would help to identify prognostic BM that may predict the rate of disease progression, define susceptibility or response to therapy, or define specific catabolic or anabolic processes. Those BM that have value in identifying drug dosage and a response to therapy (response to therapy BM) may prove to be surrogates for a clinical endpoint(s). Side-effect or toxicity BM could help identify changes that are drug-related and identify compound or biologic specific organ toxicity or toxicity which may involve the immune response.

We must decide whether BM can be diagnostic of a disease such as osteoarthritis (OA) or of osteoporosis (OP). The consensus is that there does not appear to be any evidence for this. Can anybody convince us otherwise? BM are more likely to be of prognostic value with regard to disease progression or of response to therapy or of toxicity. Since there will probably not be a disease specific BM, they are therefore likely to be of value in different types of arthritis such as OA and rheumatoid arthritis (RA) where joint pathology is the common denominator. Detection of systemic effects of cytokines on skeletal turnover may extend their value to other inflammatory diseases.

BM are likely to be of value in helping identify and classify genetically different patients in clinical trials to ensure uniformity of populations that can then be used to better define change and responses to therapy in relationship to clinical endpoints-such as rate of disease progression. Combined with genetic typing, the use of BM may offer the potential to identify patient subsets in a manner not possible clinically, except perhaps retrospectively based upon a clinical response. Thus screening of patient populations with BM could provide improved opportunities for the definition of more homogeneous patient groups and the use of smaller trial populations. This would also have the advantage of offering more value in the use and assessment of BM as potential surrogates.

The identification of populations 'at risk' for OA (and RA) may be possible. An important aspect of identifying disease progression with BM is that they could provide a more rational molecular basis for the justification of patient care and

therapy (Vignon).

BM offer opportunities to more critically define the disease process: for example, is it continuous or phasic. Does it involve a significant contribution of cytokines and how does this relate to skeletal turnover and synovitis? Is synovitis a contributory factor in accelerated progression of idiopathic disease or posttraumatic disease? How do turnover and pathobiology, as defined by BM, compare in those with primarily large joint OA to those with small joint OA, compared to those with multiple large and small joint disease. Are these genetically identifiable distinctions and which can be complemented with BM? I think that the integration of BM analyses with genetic analyses should be included in future studies.

For maximal interpretation of the results of studies involving BM it is essential that each BM accurately reflects an identifiable and defined molecular process within a specific tissue or tissues—such as the synthesis or degradation of a matrix molecule: that the part or parts of the molecule that are measured or the cleavage site be known (Eyre). It would be important to see if we can identify BM that represent the amelioration of OA, be it symptomatologic or the regression of lesions. BM of pain (e.g., serotonin) should be included in our studies (Sowers).

The value of BM for specific indications may be greatest at a specific stage in disease development and/or its progression. Thus marked changes in cartilage degradation may more clearly be detected during a critical phase of the disease (Schnitzer). Irreversible events that contribute to disease progression should be identifiable and quantified using BM.

3. Goals for the study of BM usage in OA

(Some of these notes are based on OA BM Subcommittee 6/24/99 meeting at The Homestead, Va.)

In the long term BM should be used to expedite OA clinical trials enabling more rapid and less costly identification of novel, disability-preventing (or lessening) treatments. In the medium term it is important to establish whether BM can act as surrogates for OA status, improvement or slowing of progression, such as in response to therapy. In the short term we should establish a comprehensive understanding of available and candidate BM and the criteria we should use to select and prioritize BM. They must be assessed primarily in longitudinal clinical studies over a period where clinical change can be clearly defined e.g., by joint space narrowing. BM assays must be identified, established, and validated with appropriate reference standards. Suitable clinical material (from archival sources,

NIH, industry, academia, National Arthritis Networks, as in Canada) must be identified which has been sufficiently well characterized clinically (this must be very clearly defined). Patient populations of adequate size (to ensure power for statistical analyses of data), their homogeneity (identified in part by prior BM classification - see above) are considered essential. To date these issues have often been ignored in work with BM. If disease load (joint involvement) is not well-defined it would probably not be possible to accurately assess these BM (Moskowitz). How should this be described? Poorly defined patient populations can negate clear outcomes from clinical trials. One cannot assume that all patients suffer from the same disease process, although they may share clinical characteristics and have a common end point, represented by the loss of articular cartilage in a signal joint. The use of BM to first stratify, classify and define patient populations-both normal (for pharmacodynamic studies) and diseased, may be essential prior to subsequent effective assessment of these BM in clinical studies and therapeutic trials. Based on experience with OP, placebo controlled trials of disease modifying drugs in OA are most likely to be of the greatest value in assessment of BM usage in determining clinical outcome following therapy (Delmas).

The careful pre-clinical study of experimental animal models of OA, such as cruciate ligament section in the guinea pig, rabbit and dog, offers an opportunity for proof of concept of a BM by using uniform 'patient' populations and more carefully controlled conditions leading to the programmed development of OA pathology: such studies also offer staging of treatment. In view of the perceived importance of such preclinical studies in drug development we should consider placing part of our initial emphasis on animal studies whilst we define, identify and organize, as suggested above, appropriate human study populations for longitudinal investigations. This would have the added advantage of helping to facilitate drug discovery by better defining the preclinical strategy.

Similarly, in human studies the study of genetically-defined familial OA patient populations (Moskowitz) offers an opportunity to examine precocious OA during and immediately after onset of OA. We know fairly well when the disease is initiated and that it then progresses (Moskowitz).

4. The development and use of BM in clinical studies in osteoporosis (OP) -- a paradigm for OA?

Bone markers for OP are now being used as validated secondary end points in Phase II clinical trials for OP (Delmas). They have demonstrated value to predict rates of bone loss (Garnero et al., 1999), to predict fractures and thereby identify

patients at-risk (Garnero et al., 1996) and to monitor treatment efficacy (Garnero et al., 1994). This group has shown that in prospective studies (of 2 and 5 years duration) increased levels of some BM are associated with a 2-fold increased fracture risk independently of bone mineral density. When the diagnostic tests were combined, it was possible to identify a subgroup of women with low bone mass and high bone resorption, with a very high risk of fracture. For monitoring antiresorptive treatment, the decrease of bone markers after 3 to 6 months (which is dose dependent) is correlated with a gain of bone mass observed by dual x-ray absorptiometry at 2-3 years. By using measurements at baseline and within 3-6 months, BM have proved very powerful instruments with which to identify individual responders to treatment and non-responders when change in bone mineral density at 2-3 years is taken as a reference. This has accelerated drug development, identification and effective dosage in Phase 2 (Delmas). We should ask how useful were preclinical animal studies in OP in BM development and assessment. Would these OP researchers change the way in which BM were developed for OP? They should identify a process that we could follow, based on their experiences.

5. Clinical and other end points and body fluids in studies involving BM.

a) End points

These should be carefully defined, especially for OA. The end points must be relevant and appropriate. Are pain, disability, function, joint replacement and maintenance of joint structure all definable? The integration of use and development of BM with imaging (MRI and X-ray) is considered essential. The two approaches should complement each other as new knowledge and understanding of the significance and value of BM and of MRI at the level of cartilage, bone and synovitis are acquired. How these relate to X-ray change (in bone and cartilage) and synovitis needs to be described. With the general lack of evidence for 'joint repair' in OA, the maintenance of structure (prevention of progression of degenerative change) should be the first priority, both therapeutically and for a BM.

b) Which body fluids should be analysed: the whys and wherefores

The easiest and most practical body fluid to analyse is urine since it does not involve an invasive procedure. Then the question that must be asked is what type of urine sample. Twenty four hour collections are perhaps ideal but they are not practical. The first or second morning void appear of equal value with respect to their relationship to clinical change in studies of OP (Garnero, Delmas, Eyre).

However, since BM may exhibit circadian rhythms and in bone these BM can peak at night, (although at different times) should their measurement involve the first morning void? The recommendation is that we use either the first or second void. At present we have no reported evidence for diurnal rhythms for soft tissue BM, such as those from cartilage. Analyses of circadian rhythms for all BM should be made before final decisions are taken (see below).

Urine can only be used for BM which survive and are detectable therein. These are known to include collagen cross-links and telopeptides and neoepitopes of collagen triple helical cleavage by collagenase (I. Otterness, T. Woodworth et al.; L. Atley, D. Eyre et al.; A.R. Poole, R.C. Billinghamurst, M. Ionescu, et al., unpublished). BM content should always be related to creatinine using a standard commercial assay. It should be remembered that what is measured in urine reflects what is not removed by tissues such as the liver and kidneys. Therefore we must ask whether this is always truly representative of that which is released into body fluids. Urine may contain a sub-set of breakdown products which more clearly, less clearly or as clearly reflects a molecular process (usually degradation) that has taken place within a tissue. The demonstrated value of analyses of bone cross-links in urine clearly establishes urine as an essential body fluid in the analyses of at least some BM.

Blood is the body fluid that is routinely examined in clinical chemistry. It is logical therefore that standard analyses of blood chemistry be accompanied by selected analyses of BM, particularly since some of these may already be routinely assayed, such as an acute phase protein. Whether serum or plasma should be analysed must be established for each BM. So far there are no published indications for skeletal BM that analyses of plasma give results that are different to those from serum analyses: this may be an issue in the study of proteinases. The majority of investigators prefer serum. Sampling sites should be standardized. For example, if some patients have joint disease in their hands then this could influence BM concentrations in the venous drainage. This conclusion is based on experimental work in which remote and draining blood vessels from damaged joints were analyzed (Rayan et al., 1998).

The body fluid that bathes and is closest to the pathology and which should best reflect disease activity in OA is unquestionably synovial fluid (SF). Blood and urine measurements probably reflect more systemic metabolism. Data is therefore not so easy to obtain since in the adult the synovial joints may represent only about 10 percent of the total mass of cartilage including intervertebral discs (Attencia et al., 1989) (Thonar). Thus blood and urine are likely to be more reflective of systemic change unless the 'OA load' is sufficient to

be detectable and recognizable (Moskowitz). SF should more clearly reveal what is happening within that joint. This is often indicated when a joint-derived BM is analyzed. It may be much more concentrated in SF than in serum. Examples are: the 846 epitope of aggrecan (mean 40 fold; Poole et al., 1994); cartilage oligomeric protein (mean 10 fold; Saxne and Heinegård, 1992). The degradation products in SF more clearly reflect those that are released from cartilage. Drainage of molecules from SF via regional lymphatics can result in a reduction in size (Fraser and Laurent, 1989) and removal of content (D. Heinegard, R.Fraser, A.R. Poole, unpublished) of BM. Therefore, comparable analyses of SF, serum and urine (where appropriate) should be made in initial investigations to clearly establish the relative concentrations and presence (in urine) of BM in these different compartments, and whether clinical changes with time may be more clearly reflected in one or more of these compartments (Eyre).

Synovial fluid is more difficult to obtain but its great potential value in BM evaluations is obvious. There is general agreement on this. To ensure recovery of sufficient joint fluid from a knee it is possible to lavage the joint with a fixed volume under defined conditions to ensure recovery of diluted SF. A standardized regimen involving injection of 20 ml sterile saline into a knee with immediate flexion/extension ten times followed by rapid aspiration of joint fluid has proved to be of practical value in longitudinal studies of the knee in OA. This avoided problems with 'dry' joints (A.R. Poole, J. Shiroky, unpublished).

The issue of SF dilution can be addressed by examining marker ratios until we have an acceptable dilution marker. The latter should be identified: it must be measurable with a convenient assay. Lavaging a joint may influence joint metabolism (Thonar) although we have no convincing evidence for this in longitudinal equine studies where repeated lavages were used (Robion et al., 2000). How long this effect (if any) has on subsequent measurements must depend upon when these are made. Thus the effect of lavaging should be investigated for each BM, starting perhaps with preclinical studies in animals.

The same rationale for sampling of body fluids should be used in pre-clinical studies in animals. These are more feasible in larger animals such as the guinea pig, rabbit, and dog where SF can be obtained. Consideration of SF volume has proved very important in studies in the dog (J. Matyas, A.R. Poole unpublished). Catheterization of animals can be used to obtain urine samples at defined times. Again, the same issues concerning diurnal rhythms apply to animal work. Animal studies offer the opportunity to obtain and analyse some BM in articular cartilages from the same joints that are analyzed for SF. This permits a careful comparison of changes in articular cartilage and an assessment of whether these

are reflected in SF, serum or urine. Studies of this kind have been made in patients who are already scheduled for arthroplasty of the knee, whereby undiluted SF and fresh cartilage are both obtained at surgery (R. Leff, I. Elias, A.R. Poole et al., in preparation).

In any studies of this kind it is essential to determine half-lives of BM in SF and peripheral blood. Otherwise data interpretation is difficult. Usually half lives in blood are expected to be shorter because there usually exists a very efficient hepatic and/or renal clearance route. The tissues through which these BM are cleared should be identified. The amino propeptide of type III procollagen is cleared by receptor-mediated endocytosis in the liver endothelial cells (Smedsrod, 1988). In the case of nonglycosylated collagen fragments this usually involves the kidney, as is the case for osteocalcin (Price et al., 1981). For proteoglycans and hyaluronan (hyaluronic acid) the liver is involved where receptors for glycosaminoglycans reside on sinusoidal endothelial cells. In blood the half life may be as short as 2-5 min for hyaluronic acid (Fraser and Laurent, 1989). The presence of synovitis may accelerate clearance rates of BM from the joint (Myers et al., 1995, 1996) although there was no evidence for differences in proteoglycan aggrecan clearance in rabbits with and without synovitis (Page-Thomas et al., 1987). Little is known of the effects of inflammation on other markers but these should be investigated.

The basement membranes of the kidney can lead to selective filtration of markers, as do the lymphatics. Thus glycosaminoglycans with their high negative charge may not readily penetrate these membranes (Thonar) although glycosaminoglycans such as chondroitin 4- or 6-sulfate are detectable in urine (Lee and Tieckelmann, 1981). Keratan sulfate can lose its antigenicity relatively quickly for reasons that remain unclear (Thonar).

6. Factors that may influence BM levels other than disease.

a) Circadian rhythms. These have been examined for only some BM. In the case of bone there is a circadian rhythm with a nocturnal peak for osteocalcin (Gundberg et al., 1985) and at about 8:00 a.m. for collagen cross-links (Gertz et al., 1998). In patients with RA the cytokine interleukin-6 (IL-6) reaches a nocturnal peak earlier than osteocalcin (Arvidson et al., 1994). This is of interest in view of the involvement of IL-6 in inflammation and in bone physiology. In contrast, TNF α shows no evidence for a circadian rhythm (Arvidson et al., 1994). But what about its receptors? We should ask if there is evidence for circadian rhythms for cartilage markers. We have no evidence for this in RA (A.R. Poole, R. Hallgren, unpublished).

b) Peristalsis. Hyaluronic acid is synthesized by synovial cells (and many other cells) and is potential marker of synovitis in OA as in RA. However, it is most concentrated in the draining lymphatics of the intestine (Engstrom-Laurent, 1989). Not surprisingly circulating levels are increased following a meal (Pharmacia, unpublished). Therefore it is important to avoid sampling of peripheral blood within a period of about 3 hrs following a meal. Does peristalsis influence other markers? We should ask these questions.

c) Physical activity. Early morning activity following overnight bed rest leads to an increase in circulating hyaluronan (Engstrom-Laurent and Hällgren, 1985b) and MMP-3 and keratan sulfate epitope (Manicourt, et al., 1999) in healthy persons. This is much more pronounced in those with RA. This increase correlates with RA clinical status and represents a valuable research tool but is a less practical method of analysis.

d) Hepatic and renal disease. Since the clearance of BM occurs predominantly via the liver and/or kidneys, any disease involving these tissues will influence BM levels in serum and urine. Hepatic disease (e.g., cirrhosis) causes marked elevations in serum hyaluronan (Engstrom-Laurent, 1989) and would likely also influence the removal of glycosaminoglycan-rich molecules such as proteoglycans. Renal disease would influence osteocalcin levels. Thus there should be awareness of these issues in patient studies.

e) Age and sex. Growth is accompanied by elevated serum skeletal BM as a consequence of growth plate activity. This is clearly reflected in the peripheral circulation and in urine for aggrecan and type II collagen (Thonar, et al., 1988; Carey et al., 1997). Thus analyses of BM in growing children in studies of arthritis is complicated by the growth component. Since fracture repair usually occurs via the same process of endochondral ossification, (the basis of skeletal growth) this must be taken into consideration in studies of patients with OA.

Age-related increases are commonly seen in BM. For example, significantly higher values for cartilage (Poole et al., 1990) and bone (del Pino et al., 1991) BM are found in adult males compared to females. Changes may also occur at menopause and in post-menopausal populations such as in the case of bone markers. BM often increase with ageing in adult populations possibly reflecting preclinical degeneration in OA. Hence populations must be carefully matched and defined with respect to age and sex.

f) Surgery. This can also influence BM levels. These effects can last several weeks (Thonar et al., A.R. Poole et al., unpublished) (Eugene Thonar to

complete reference).

7. Collection, preparation and storage of samples.

Some molecules, such as osteocalcin, are labile at -20°C but stable at -70°C . They may, however, also be labile on freezing and thawing. Hence it is recommended that all samples be aliquoted; rapidly frozen and stored at -70°C following centrifugation (at least 600 g 3000 rpm for 10 min) to remove particulate material and any clots. Samples must be labelled in a standardized fashion and in such a way that labels do not detach. There should be a central storage repository for body fluids. All methods of collection, processing and storage must be standardized. It is perhaps appropriate that NIH be responsible for this 'library' of samples. As in the case of the assay laboratory (preferably the same for all assays) this storage site must also be a GLP facility.

Sample volume and management is of very great importance since these collections will represent extremely valuable archival material for future studies. It should be carefully identified based on assay sensitivities and sample volume requirements. Since some existing assays may require up to 100 ml per sample per assay, assays in triplicate therefore represent a requirement for about 325 ml. Clearly collections of 20 ml blood providing at least 10 ml serum will provide opportunities for only a limited number of assays (1 ml for 3 different assays x 10, represents about 30 different assays). Thus we must ask ourselves whether 20 ml clotted blood is sufficient. Twice this volume is probably more appropriate for studies of this kind. Plasma will provide larger volumes but poor mixing of anticoagulants at the level of sample collection can often result in problems of clot formation following frozen storage and sample thawing.

The recovery of SF does not ordinarily require anticoagulants. Clots can be permitted to form over 1 hr at room temperature, prior to centrifugation. However, in view of the high viscosity (due to presence of hyaluronic acid and difficulties therefore in accurately pipetting such samples) it is usually necessary and recommended that these samples be treated with hyaluronidase (e.g., Streptomyces, protease-free) prior to assay. A convenient protocol is to use the hyaluronidase at pH 5.0 and incubate overnight at 37°C with proteinase inhibitors to prevent any epitope degradation by proteinases. This should be standardized and performed in a central laboratory prior to aliquoting samples (but this will represent two cycles of freeze and thaw i.e., collection, freezing, shipping to central laboratory, thawing, digestion, aliquoting, freezing, possible shipping to another assay laboratory, thawing, assay). This requires careful discussion. Urines and sera/plasma should be shipped frozen on dry ice for

storage. For unstable molecules, such as osteocalcin, aliquots should be created at the collection site to avoid another cycle of freezing/thawing.

The regularity with which samples should be taken, and which body fluids are required (and this depends upon the assay and study) also needs careful attention. It is suggested that we debate a single versus two baseline samples (one only for joint fluid?) and at what intervals apart, and then the regularity of sampling according to the study (indication) or the clinical trial. Decisions for baselines should be based upon exploratory studies of BM variability over time in patients with OA. In shorter term 3-6 month Phase II studies, samples should perhaps be taken at baseline and then at monthly intervals. Synovial fluid samples taken only at the end of the study would avoid any possible effects caused by prior joint penetration on sampling. However, this would prevent an opportunity for individual longitudinal studies of prognostic value which may be more valuable in BM assessment and validation than cross-sectional group analyses. Personally I would suggest that joint fluid analyses be performed at the start and conclusion of a study for maximal data retrieval and simplicity of incorporation of joint analyses in a clinical trial. The indication for this would be to address change in individual patients as opposed to differences between patient populations. To determine whether early BM changes are prognostic of outcome following therapy we also need to take samples after 1 month from commencement of treatment to permit assessment of early response to therapy.

In longer term Phase III studies, where the principal end point is joint space narrowing (x-ray), samples should perhaps be taken after 1 month (for prognostic indications) and then at 3 and 6 months and every 6 months thereafter until the conclusion of the study. This would address issues related to prognosis, therapeutic effects and disease progression.

8. The sensitivity, accuracy and reproducibility of assays, and variations over time

Requirements for assay sensitivity are determined by the concentration of the antibody-reactive material epitope(s) to be measured in the sample. For convenience sensitivity should be set so that samples can be measured in as small a volume as possible which can be accurately pipetted. This conserves precious samples and expands the number of assays that can be performed in BM selection studies. Defined conditions of sample dilution should be identified as sample volume in total assay volume. Approximately half-maximal inhibition should be attempted for assays to ensure good reproducibility. Parallelity of immunoreactivity (of sample inhibition) with that of standard inhibition curves should be demonstrated to reflect reactivity that is related to concentration of the

epitope(s) and is not influenced by other molecules present in the sample. To confirm the latter, 'spiking studies' are necessary demonstrating accurate recovery of added epitope(s) or standard comparable in concentration to that present in the sample being assayed. The coefficient of variation of an assay should be no more than 5% intraassay and 10% interassay.

Are these coefficients of variation acceptable, based on power analyses of group sizes? Clearly the more accurate the assay the greater the opportunity to minimize patients population sizes. Other such opportunities are offered by stratification and selection of populations by prescreening with BM, ideally with genetic analyses to help rationalize differences in BM on healthy populations.

Understanding BM variability in healthy persons and in patients in longitudinal studies is important, if we are to be able to address changes in disease activity revealed by BM. An example of such a study is that by Lohmander et al (1998).

9. Which molecular events should be measured, in which tissues and for what indications?

a) General.

Before we examine the measures let us first consider the indications. What do we want to measure? Perhaps a BM may offer insights at an early stage of the disease or provide evidence of secondary changes in established disease (e.g., osteophytic formation or development of a synovitis). Some BM may have more power to detect changes in disease activity caused by therapy or as part of a phasic disease process whereby disease activity may vary. Other BMs could be of value prognostically. Therefore these issues should be carefully considered since it is likely that various BM will provide very different indications. Hence we should classify them according to the indications which we identify (Witter). The Roche group has suggested that we identify

(a) "Stratification" BM that mark disease progression and susceptibility to therapy, (b) "Response" BM that indicate changes in the rate of disease progression in response to medication (c) "Side-effect" BM. Here animal studies would again be of value. (d) BM may prove useful for screening for patient BM homogeneity and for establishing dosages (Witter).

We must identify any markers that can identify or predict structural damage. We should be able to measure well characterized molecular and tissue changes that are reflected clinically and by imaging. A better understanding of the significance of MRI and BM should result from a comparative study of BM with MRI by

analyses of SF. Cartilage volume, loss (joint space narrowing), subchondral bone changes and synovitis are parameters that can be addressed by imaging. We should determine how much correlation we can expect between a BM or a group of markers and a structural endpoint (Witter) such as that reflected by x-ray or imaging. This may be a transient correlation because of a critical change in disease activity (Schnitzer) at a specific period of time following commencement of therapy. Or it may be a lasting change (Witter). Again, the general consensus is that we should learn our lessons from work on OP. Let's avoid mistakes of the past and develop testable hypotheses for BM evaluation and validation. But first, what were these mistakes-if any? Generation of data from analyses of potential surrogate BM may at this stage be of greatest value so we can start to see if there are correlations with specific endpoints, such as in a longitudinal study of the knee.

b) The types of measurements that BM may represent and the patient populations that we should examine.

Osteoarthritis is characterized by the degeneration of articular cartilage. This results from a direct attack on matrix molecules resulting in their cleavage, damage to these molecules and their loss: it is accompanied by a response of the tissue to this damage which involves enhanced matrix synthesis and turnover. The most direct evidence of pathology is cartilage degradation. A secondary more indirect indication would therefore be cartilage matrix synthesis. The amount of synthesis in relationship to degradation may prove of great importance in determining disease progression. Thus biosynthesis and its relationship to damage at the level of a single molecule may offer valuable indications. If we are studying cartilage-related events it is important that these BM be cartilage-specific or as specific as possible. Thus type II collagen and the cartilage proteoglycan aggrecan, which are principally found in hyaline cartilages, (type II collagen is also present in the vitreous of the eye), are perhaps ideal candidate molecules for the specific study of cartilage pathology, just as osteocalcin and bone specific cross-links of type I collagen are used successfully as BM of bone turnover.

The turnover of matrix molecules in OA involves not only accelerated processes but also the initiation of events that are not ordinarily encountered in healthy articular cartilage, perhaps not even as part of an ageing process. Thus molecules that are principally synthesized in fetal tissues (such as tenascin and fibronectin, and probably cartilage oligomeric protein), make their reappearance in increased amounts in diseased cartilage. Some of these are, of course, not tissue specific and may therefore be of more limited value.

The cleavages of major cartilage matrix molecules have been more clearly defined, in part as a result of the development and use of anti-neoepitope antibodies that recognize carboxytermini and aminotermi generated by proteinase cleavages. This has proved possible for type II collagen (Billinghurst et al., 1997; Song et al., 1999; Huebner et al., 1998; L. Atley and D. Eyre, unpublished) and for aggrecan core protein (Lark et al., 1995a, 1997; Hughes et al., 1995). The use of such cleavage markers should be of much value in assessment of disease progression and of responsiveness to therapy designed to arrest such degradative processes. Likewise since the excessive matrix synthesis and the presence of degradative proteinases, such as metalloproteinases (MMPs), is closely associated with this damage we have other potential although less direct BM of tissue damage.

The use of assays that measure the synthesis and the appearance or reappearance of cartilage matrix molecules will probably reflect more the development of OA and the progression of the disease rather than the response to therapy designed to slow or arrest damage. Depending upon how cleavage and synthesis are coupled, so one might also see responses to therapy at the biosynthetic level. We still know little of how these different processes are interrelated. So we should keep an open mind until we can make evidence-based interpretations of these analyses.

Early changes in OA have been shown to involve significant changes in bone turnover. This has been recorded by scintigraphy (Dieppe et al., 1993; McCarthy et al., 1994; Hutton et al., 1986). Osteocalcin is increased in patients with OA (Campion et al., 1989; A.R. Poole, P. Dieppe et al., unpublished). Early changes in bone turnover in the onset of OA are also indicated by a reduction in serum osteocalcin in ageing female populations (Sowers et al., 1999). Bone and cartilage BM in SF correlate with scintigraphic scans in OA (Sharif et al., 1995c). It remains to be seen how useful measurements of bone type I collagen turnover at the level of cross-link (resorption) or propeptide (synthesis) analyses prove to be in OA. The contrasting increases in osteocalcin observed in early osteoporosis (del Pino et al., 1991) and in established OA clearly signify different types of bone changes in early disease to those seen in early OA. Reductions in osteocalcin may result from a subchondral inflammatory process causative of the increased bone turnover, since reductions in serum osteocalcin are seen in RA (Kröger et al., 1993).

Whether changes that may clinically involve only one or two large joints are really detectable systemically (urine and serum) remains to be established. With a small disease 'load' (Moskowitz) it may be difficult to detect such changes in

these body fluids. Systemic changes in serum or urine BM may in fact reflect differences in skeletal turnover that are either more generalized or reflect preclinical OA. Thus we must recognize the fact that BM may reflect changes that are not clinically identifiable at this time. Moreover, alterations in knee joints can be rapidly identified by SF analyses of BM following injury (Lohmander). But are changes in a single knee reflected in blood or urine? Do we know this? A systematic analysis may help us resolve the issue of whether individual joint changes are reflected systemically. When there is no other identifiable pathology. We can better detect systemic changes where multiple joints are involved (clinically and sub-clinically) in the case of familial (genetically determined) OA (Moskowitz; Bleasel et al., 1999). Such populations provide us with a reference point for more systemic changes in cartilage metabolism.

But are these changes that we measure only restricted to joints? How do they influence bone metabolism since the defect is in the cartilage? Do these changes also involve non-articular changes? All this needs to be established. We could examine cartilage matrix protein (matrilin-1) which is absent from healthy adult articular cartilages (Paulsson and Heinegard, 1982) although it reappears in articular cartilage in OA (Okimura et al., 1997).

BM outliers, observed in a subset of persons in 'healthy' control populations, such as seen for a keratan sulfate epitope (Thonar et al., 1985), may reflect patients 'at risk' or 'resistant' to the development of OA. Since these cases were first identified in the mid-80's perhaps we can soon ask whether these differences correlate with any clinical outcomes. Those people in 'healthy' populations who share BM features seen in OA may be those at risk for OA. An example of this is the reduction in c-propeptide of type II collagen in OA. Some healthy persons also exhibit a low serum c-propeptide comparable to that in OA (Nelson, et al., 1998).

Since patients with OP do not usually develop OA, (Dequeker et al., 1985; Hamerman, 1997) it would be helpful to examine these patients to identify their BM profiles which may then reflect characteristics of patients less likely to develop OA.

Synovitis is commonly observed in established OA but it is usually limited (Goldenberg et al., 1982; Gordon et al., 1984). This is an inflammatory process that involves upregulation of hyaluronic acid synthesis by synovial cells caused by increased production of cytokines such as interleukin-1a, b, tumor necrosis factor-a and transforming growth factor-b (Hamerman and Wood, 1984; Butler et al., 1988; Haubeck et al., 1994). There is evidence for a general upregulation of

synthesis of some of these and other cytokines in OA cartilage, (Melchiorri et al., 1998) as well as of their receptors (Poole and Howell, 2000; Webb et al., 1997). In RA serum hyaluronic acid upregulation correlates with synovitis (Keyszer et al., 1999; Poole, et al., 1990; Poole and Dieppe, 1994). Serum hyaluronic acid may therefore offer opportunities to study synovitis in OA.

Matrix metalloproteinases (MMP) and their inhibitors (TIMPs) are upregulated in SF and serum RA (Ishiguro et al., 1996; Manicourt et al., 1995; Yoshihara et al., 1995) and in OA (Lohmander et al., 1993). They probably originate from synovium and cartilage. This probably also results from cytokine or growth factor activation (Poole et al., 1995). Cartilage oligomeric protein (COMP) is synthesized by synovial cells, as well as by chondrocytes, especially on activation with transforming growth factor b (Recklies et al., 1998; Dodge et al., 1998). Thus COMP, MMPs, and hyaluronic acid may provide insights into synovitis as well as cartilage pathology. There is also upregulation of synthesis of type III collagen (as amino propeptide) in inflamed synovia in RA and OA (Sharif et al., 1996). This increased synthesis of type III collagen almost certainly reflects synovitis, whereas type I collagen synthesis can also be upregulated when bone matrix turnover is increased (Christenson, 1997) and may more clearly reflect bone changes.

Markers of systemic inflammation, such as the acute phase c-reactive protein, have value in studies of OA (Sharif et al., 1997). Biomarkers of pain such as serotonin (Sowers) and muscle activity (for mobility indications) should be included to link with clinically defined function. It is clearly important wherever possible to rigorously rationalize the selection of known BM at a tissue and molecular basis, and to do the same in the selection, identification and search for new BM, identifying the specific indication(s) for which each BM may be best suited and the requirements where BM are not presently available for a specific indication.

10. Analyses of results

The analysis of data comparing two or more BM offers advantages, especially with respect to the analyses of synthesis versus degradation of molecules such as type II collagen or aggrecan, and for comparing changes in bone and cartilage (Eyre). The balance between two key events in matrix turnover may have more predictive or indicative value than analysis of a single event alone. It may be possible to establish a predictive equation of a process in OA using a combination of markers (Witter). This is being done for RA using linear multiple regression analyses (A. Fraser, P. Emery, A.R. Poole, D. Veale et al, in preparation)

Analyses of results employing discriminant analyses using backward elimination of non-informative variables provides opportunities to identify markers that can discriminate patients with OA (Otterness et al., 1995). Analyses of this kind can help understand how a proteinase marker may associate with a disease and relate to a specific tissue or event or the sort of indication(s) a new BM may reflect. These analyses are of real value in selecting and classifying markers. It would be helpful for example, to see if an MMP, such as stromelysin (MMP-3), derived from synovium and/or cartilage, relates to events such as synovitis (how does it compare to hyaluronic acid) or cartilage degradation (collagen II or aggrecan cleavage).

Integration of time-related changes (A.R. Poole, T. Saxne, D. Heinegård, R. Eastell et al., unpublished) can provide valuable insights into changes over time. Correlations between BM can provide valuable investigative insights into pathology and will help in prioritizing and classifying BM for specific indications.

Logarithmic conversion of data can be an effective way of dealing with a non-uniform distribution of data thereby permitting analyses with more standard statistical tests (Dieppe, Otterness). Otterness identified standard statistical formula for defining the number of patients that would be required to reject a null hypothesis (i.e., hypothesis of no difference) with given alpha (p value, typically 0.05) and beta (type II error toleration, typically 0.8 or 0.9). The formula is:

$$N = 9(s_1^2 + s_2^2)(Z_{1-\alpha/2} + Z_{1-\beta})^2 / (m_1 - m_2)^2$$

where s_1 and s_2 are the standard deviations and m_1 and m_2 are the mean values of the control and the OA populations, and Z is the normal probability distribution function for $1-\alpha/2$ and $1-\beta$, respectively. The formula is multiplied by 9 to determine the population size that would be required to detect a 33% improvement in the marker. (That value is chosen somewhat arbitrarily, but is based on the concept that most treatments will not bring the marker back to baseline (Otterness).

The services of statisticians in planning and interpreting these studies are essential.

11. The need for positive controls in the assessment of BM: a further case for RA studies.

It is obvious that we need to perform experiments with therapeutic agents that can knowingly reduce disease activity in arthritis and skeletal damage. Arguably, therefore, it may be wise to consider that some BM assessments be made in

patients with RA where there is more effective therapy available which can be used to reduce disease activity and potentially provide measurable changes in skeletal damage and synovitis. Low dose methotrexate treatment has been shown to be effective as well as TNF α inactivation. Therefore longitudinal studies in RA which would be shorter may help in identifying and selecting BM of inflammation and skeletal change if analyses are made of body fluids from patients where a reduction in joint space narrowing and/or synovitis has been demonstrated. The bone crosslink deoxypyridinoline has been shown to be reduced following treatment of RA with methotrexate (Yasser et al., 1998).

12. Specific assays of BM Tissue and molecular specificities:

Cartilage: type II collagen degradation (indication: cartilage degradation)

There is a general consensus that the measurement of the degradation of cartilage macromolecules, such as of type II collagen, offer clear opportunities to detect and measure cartilage degradation, especially since assays for type II collagen degradation are now available. Type II collagen cleavage by collagenase and its denaturation is upregulated in OA (Hollander et al., 1994; Billinghamurst et al., 1987; Dahlberg et al., 2000). None of these assays have yet been described in detail in full peer-reviewed publications.

David Eyre has developed a urine assay that employs a monoclonal antibody to measure a cross-link epitope in the c-telopeptide that includes a carboxyterminus generated by cleavage of this telopeptide (2B4 antibody). The telopeptide sequence provides specificity for this type II molecule. Other related assays are in development for serum and SF (7H3 antibody). The 2B4 assay has been used to study cartilage damage in patients with OA and after joint injury (Moskowitz et al., 1999; Atley et al., 1998a, b; Lohmander et al., 200a; Atley et al., 2000).

A monoclonal antibody to a c-telopeptide epitope has been used in a urine assay developed by the group of C. Christiansen (Christgau et al., submitted). Ivan Otterness has produced a monoclonal antibody to the primary collagenase cleavage site in type II collagen of the kind described by Billinghamurst et al (1997). This recognizes the c-terminal neopeptide of the α chain TCA piece. In combination with an antibody to a type II collagen specific intrachain epitope N-terminal to this cleavage site, an ELISA sandwich assay (TIINE) has been established for urine (Saltarelli et al., 1999; Woodworth et al., 1999). These assays have reported the increased presence of epitope in urine in OA.

Poole and colleagues have developed an ELISA assay (C2C or COL2-3/4CLong mono) for the primary collagenase cleavage site in type II collagen employing a

monoclonal antibody that recognizes a collagenase-generated conformational epitope that has sequence specificity for type II collagen. The serum assay has been used to study cartilage erosions in experimental inflammatory arthritis and their treatment (Song et al., 1999). In experimental OA the cleavage of type II collagen by collagenase is markedly increased as revealed by analyses of SF (J. Matyas, A.R. Poole, et al., unpublished; Q. Chu, M. Markel, A.R. Poole et al., unpublished) in dogs and serum (J. Matyas, A.R. Poole et al., unpublished) and in SF in rabbits (L. Killar, A.R. Poole et al., unpublished). Significant increases in urine have been detected by these assays in arthritis: serum elevations in RA have been observed which are related to disease activity (A.R. Poole, M. Fitzcharles, R.C. Billinghamurst, E. Keystone, unpublished; A. Fraser, D. Veale, P. Emery, unpublished). Similar results have been obtained for early RA in SF analyses showing a relationship between articular cartilage damage and synovitis (A.R. Poole, unpublished).

Cartilage: type II collagen synthesis: c-propeptide (indication: cartilage synthesis)

This molecule is synthesized as a procollagen which contains amino and carboxy propeptides. These are removed extracellularly by single cleavages of amino- and carboxy (C) proteinases as collagen is incorporated into the fibril. Their half life is about 18 hr (Nelson et al., 1998). The c-propeptide content and release from the cartilage is directly correlated with collagen synthesis (Nelson et al., 1998). Serum assays were described, first by Hinek et al, (1987, 1988) and then by Shinmei et al (1993). The latter is a bead-based assay (Teijin Co., Japan) whereas the other was originally a radioimmunoassay (Mansson et al 1995). These assays were used to demonstrate increases in c-propeptide content in SF following knee injury and in primary OA (Lohmander, et al., 1996), a decrease in serum in OA (Nelson et al., 1998) and an increase in serum in RA (Nelson et al., 1998; Mansson et al., 1995). How these assays compare is not known. An ELISA assay is presently under commercial development employing monoclonal antibodies (E. Diamendes, unpublished).

Cartilage proteoglycan aggrecan: core protein (indication: cartilage turnover)

This was one of the first molecules to be studied as a BM following the earlier work of Ziff et al (1956) on elevated urinary hydroxyproline in children and in RA. Heinegard et al (1985) used polyclonal antibodies to aggrecan to detect its elevation in serum in an animal model of OA. These polyclonal antibodies have since been used extensively by Heinegård and Saxne et al and by Lohmander et al.

Cartilage proteoglycan aggrecan: core protein epitope (indication: cartilage turnover)

An I-F21 monoclonal based ELISA assay detects an epitope outside the G1 domain (Møller et al., 1994). It is increased in SF in post-traumatic knee injury and primary OA (Lohmander et al., 1999). It is valuable when used in combination with the chondroitin sulfate 846 epitope assay of aggrecan to establish ratios of these epitopes.

Cartilage proteoglycan aggrecan: keratan sulfate (KS) epitopes (indication: cartilage turnover)

The elevation of a KS epitope in sera of patients with OA (Thonar et al., 1985; Sweet et al., 1988) was observed using a monoclonal antibody (Caterston et al., 1983). These elevations have not always been observed using the same or other antibodies, presumably because of assay and epitope differences (Poole et al., 1994, Spector et al., 1992). Serum KS epitope is elevated during developing of experimental OA in the dog (Manicourt et al., 1991). Proteoglycan aggrecan fragments detected in SF are also elevated in OA (Thonar et al., 1993; Saxne, Heinegård, 1995). They are generally larger in size in OA than RA (Witter et al., 1987) and contain mostly both non-functional G1 domain (cannot bind to hyaluronic acid to form aggregates) and the KS epitope rich region. The changes observed in serum epitope content can vary. Maximally they are of the order of about 30-40%.

KS epitope content is increased in SF in experimental OA (Campion et al., 1991). Most recent work on KS epitopes has been by Thonar et al., who have shown in serum studies in RA that KS epitope content is inversely related to TNF α content (Manicourt et al., 1993). These epitopes also show inverse relationships to serum acute phase protein in RA (Poole et al., 1990). In OA joint fluid the KS epitope is inversely related to polymorph content (Poole et al., 1994). These observations may reflect the inhibitory effect of inflammation on aggrecan synthesis. For example, if such epitopes reside on newly synthesized molecules, the synthesis of which can be inhibited by upregulation of cytokines such as interleukin-1 and TNF α , in inflammation, then this could account for the inverse relationship to inflammation related BM.

Cartilage proteoglycan aggrecan: 846 and 3B3 and other chondroitin sulfate epitopes (indication: cartilage turnover/synthesis)

The 846 epitope was originally identified in human fetal cartilages using an IgM monoclonal antibody isolated by Glant et al (1986). Almost absent in healthy

adult articular cartilage, it reappears in OA (Rizkalla et al., 1992). Recent work has revealed that the release of this epitope from cartilage in culture is correlated with proteoglycan synthesis (H. Jugessur, A.R. Poole unpublished). The epitope is present on the largest molecules that in healthy cartilage show complete aggregation with hyaluronic acid (Rizkalla et al., 1992). This suggested that it is present on intact molecules in normal and OA cartilages. Confirmation of this has come from rotary shadowing analyses of antibody binding to aggrecan (M. Morgelin, A.R. Poole, B. Månsson, D. Heinegard, unpublished) which reveal that it is detected in healthy fetal human aggrecan it is present in close proximity to the G3 domain as originally suggested by Rizkalla et al (1992). This epitope is increased in content in SF following injury (Lohmander et al., 1999) and in SF in primary OA (Poole et al., 1994) where its mean ratio to serum represents an approximately 40 fold increase in content in SF reflecting its origin from OA cartilage where it is concentrated. It is more elevated earlier in the disease (Ishiguro et al., 1999). It is also increased in serum in chronic RA (Poole et al., 1994) but suppressed in content in early rapid progressive RA (Månsson et al., 1995) possibly reflecting impaired aggrecan synthesis in this acute inflammatory condition as discussed above for the KS epitope. It is elevated in SF in experimental OA in rabbits (L. Killar, A.R. Poole et al., unpublished) and exhibits an inverse correlation to KS epitope content in OA SF (Poole et al., 1994). In serum analyses, this epitope, together with COMP and the TNF α p-75 receptor, can discriminate OA patients from normals (Otterness et al., 1995). This assay has recently been converted to an ELISA assay and will be available commercially (HDM Diagnostics, Toronto).

Another native chondroitin sulfate epitope (3B3) is elevated in SF in early experimental OA, reflecting the upregulation of this epitope in degenerate cartilage (Ratcliffe et al., 1993). 3B3 is also elevated following knee injury together with another chondroitin sulfate epitope 7D4 (Hazell et al., 1995). Both 3B3 and 7D4 monoclonals were isolated by B. Caterson et al. One of the concerns when measuring these epitopes is there the influence of epitope density on antibody reactivity. This can be largely overcome in the KS assay by use of monovalent antibody fragments (Fab) (Poole et al., 1989; Poole et al., 1994).

Cartilage proteoglycan aggrecan core protein cleavage neoepitopes (indication: cartilage degradation)

There are two principle cleavage sites in the G1-G2 interglobular domain of aggrecan: the MMP and the aggrecanase cleavage sites (Poole et al., 1995). Anti-neoepitope antibodies have been prepared to both the carboxy and amino

terminal neoepitopes that are generated in these two cleavage sites (Hughes et al., 1992; Hughes et al., 1995; Lark et al., 1995; Fosang et al., 1995). Assays are being developed to these and other aggrecan cleavage epitopes for commercial use (HDM, Toronto). These assays should be of comparable value to the type II collagen degradation assays, especially since aggrecan is actively degraded and synthesized in diseased cartilage to replace that which is lost.

Immunohistochemical studies of OA and RA cartilages have revealed the presence of both cleavage sites (Lark et al., 1997) as in the case of experimental models of arthritis (Van Meurs et al., 1999).

Cartilage oligomeric protein (COMP) (indication: abnormal cartilage turnover and/or cartilage/synovial cell activation?)

This thrombospondin is increased in content and degraded in degenerate OA cartilage (DiCesare et al., 1996) and levels are increased in SF following joint injury and in primary OA (Lohmander et al., 1994). There is a significant increase in serum COMP in OA patients with higher Kellgren-Lawrence grades and more joint involvement (Clark et al., 1999). An increase in serum COMP was also seen in two OA studies of patients exhibiting more rapid disease progression (Sharif et al., 1995b; Petersson et al., 1998a). Such patients also show an elevated serum hyaluronic acid content (Sharif et al., 1995a). Serum COMP also correlates with joint space width at entry and with yearly mean joint space narrowing (Conrozier et al., 1998). Increased serum COMP is one of a number of BM changes that are seen in patients with a type II collagen mutation in type II collagen that develops a familial OA (Bleasel et al., 1999).

Whether the majority of the COMP detected in body fluids originates from cartilage has not as yet been clearly established. Since activated synovial cells, especially those exposed to TGF β , upregulate COMP gene expression and synthesis (Recklies et al., 1998; Dodge, et al., 1998) and COMP serum levels are elevated in rats with experimental inflammatory arthritis with synovitis (Vingsbo-Lundberg et al., 1998; Larsson et al., 1997), elevated COMP may also reflect a synovitis. This remains to be clearly determined. Polyclonal and monoclonal assays are available in these research laboratories.

Cartilage matrix protein (matrilin-1) (indication: cartilage turnover.)

This molecule is not ordinarily detected in healthy adult articular cartilages although it is present in non-articular cartilages (Paulsson et al., 1982). It reappears, however, in articular cartilage in OA (Okimura et al., 1997). It can be detected by immunoassay and is increased in content in serum in inflammatory

arthritis (Saxne and Heinegård, 1989). Further studies in OA are needed.

GP-39/YKL-40 (indication: cartilage turnover and synovitis?)

This is a glycoprotein which is a member of the chitinase family. It is a secretory product of activated chondrocytes and synovial cells (Hakala et al., 1993). It is upregulated in OA cartilage (Volck et al., 1999). Elevated serum levels have been reported in OA using a commercial assay (Chondrex, Novadex, Inc., San Diego; Harvey et al., 1998).

Pentosidine (indication: general collagen breakdown)

This is an age-related advanced glycation end product found on a number of molecules, in particular collagens. It can be detected by HPLC assays (Miyata et al., 1998). Pentosidine content is elevated in blood in RA (Miyata et al., 1998; Rodriguez-Garcia et al., 1998) but not in OA (Miyata et al., 1998). Its content correlates with inflammation markers such as c-reactive protein (Miyata et al., 1998) and may therefore reflect more systemic changes in collagen degradation induced by inflammation

Synovium: type III collagen amino (N) propeptide (indication: synovitis and soft tissue repair)

This assay is used to study type III collagen synthesis. The propeptide is upregulated in inflamed synovium. Thus the increase seen in OA (Sharif et al., 1996) suggests the presence of synovitis since studies in RA of elevated serum N-propeptide of type III reveal an association with progression of bone erosions (Hørslev-Petersen et al., 1986) as in the case of hyaluronic acid (see Poole and Dieppe, 1994 for review). Commercial assays for the N-propeptide of type III procollagen (inflammation/synovitis) as RIAs are available from Orion Diagnostica, Oulunsalo, Finland and Farnos Diagnostica, Turku, Finland (see papers by L. Risteli and J. Risteli).

Bone: type I collagen N- and C (Carboxy) propeptides (indication: bone and soft tissue collagen repair)

Serum assays for type I collagen N- (ELISA) and C-propeptides have been used to study bone formation. They seem to be inferior to serum osteocalcin and bone alkaline phosphatase in studies of metabolic bone disease (Ebeling et al., 1992). These collagen assays are not, however, specific for bone although they have been used to study bone turnover. Commercial assays are available from Orion and Farnos

Bone: type I collagen C-telopeptide (indication: bone and soft tissue resorption)

The serum and urine Crosslaps assay (Osteometer, Herlev, Denmark) employs two monoclonal antibodies in an ELISA sandwich which detects a linear epitope in the C-telopeptide of type I collagen $\alpha 1$ chain (Christagau et al., 1998). The assays show good correlations between serum and urine and reflect bone resorption.

Bone: type I collagen cross-links (indication: bone resorption).

This area was pioneered by Robins et al (1986, 1994) using HPLC and by Eyre and his group. A number of urine immunoassays for type I collagen cross-links have been marketed. These assays are usually restricted to urine. There is an ELISA cross-link (XL) assay for the C-telopeptide $\alpha 1$ (I) (Crosslaps, Osteometer, Denmark); an RIA for 3 cross-linked fragments of the $\alpha 1$ (I) C-telopeptide (Orion, Finland/Incstar Stillwater, Mn., USA); an NTX ELISA assay for $\alpha 2(1)$ and $\alpha 1$ (1) amino telopeptide cross-links (Ostex, Seattle); an ELISA for deoxypyridinoline cross-links (Metra Biosystems, Los Angeles). These assays have been compared in RA (St. Clair et al., 1998) and in OP (Delmas and Garnero, 1998). These assays have predictive value for therapeutic outcome in the treatment of OP (Greenspan et al., 1998), predicting hip fracture (Garnero et al., 1996) and bone loss (Garnero et al., 1999). Comparisons have been made between assays (Garnero et al., 1994).

In view of the marked changes in bone turnover that have been observed in OA and (as in RA) are accompanied by increases in urinary deoxypyridinoline cross-links (Seibel et al., 1989) assays of this kind should have value in the study of bone turnover in OA. Already cross-links have provided indications of disease development and progression (Thompson et al., 1992). They may help in identifying and stratifying/classifying patient populations in view of the marked changes in bone turnover in OA (MacDonald et al., 1994; Otterness et al., 1995).

Bone: osteocalcin (indication: bone assembly/turnover)

This is also used to study bone turnover in OP. In established OA serum osteocalcin is increased (Campion, et al., 1989; A.R. Poole, P. Dieppe, unpublished) whereas a decrease is associated with OA onset in an ageing female population (Sowers et al., 1999). The indications for osteocalcin for studying bone turnover would be expected to be similar to these for type I collagen cross links. Both may be of value in detecting early changes and alterations in the progression of OA. The formation of osteophytes involves endochondral ossification and new bone formation which should be reflected by

osteocalcin measurement. It may be helpful to ask of the OP studies whether it is osteocalcin or cross-links, which are of most value for any specific indications and whether ratios of these BM may be of value.

Bone: sialoprotein (BSP) (indication: bone assembly/turnover)

BSP is another molecule which is predominantly found in bone although it can be synthesized as a splice variant in some tumors. An ELISA assay can detect elevations in BSP in patients who previously presented with chronic knee pain and with abnormal bone scans (Petersson et al., 1998a). Serum BSP is increased in OA patients with bone scan abnormalities (Petersson et al., 1998b) but at baseline serum BSP is not related to OA progression. It does, however, correlate inversely with osteophyte grade and sclerosis (Conrozier et al., 1998). This protein should be evaluated with other specific bone proteins or components thereof. The literature to date mainly concerns elevations of BSP in RA where bone erosions are very much a feature of the pathology (Saxne et al., 1995).

C-reactive protein (CRP) (indication: systemic inflammation)

That limited systemic inflammation is a feature of OA is demonstrated by the small but significant elevation of serum CRP (Sharif et al., 1997; Otterness et al., 1995). The value of this assay in relationship to disease activity and progression is unclear. It may be of more value in classifying patients.

Hyaluronic acid (HA) (indication: synovitis)

This is synthesized by many cells. In RA elevations in serum HA, can be detected by different assays including those that employ the G1 domain of aggrecan and link protein (that bind specifically to HA) (Lindqvist et al., 1992). Serum HA shows significant correlations with joint counts, and joint inflammation (pain, tenderness and swelling), (for review, see Poole and Dieppe, 1994). It correlates with disease progression in RA (Paimela et al., 1991). Persistently elevated HA is seen in OA patients with more rapid disease progression (Sharif et al., 1995a). Elevations are often seen in OA (Goldberg et al., 1991) and in experimental OA (Myers and Brandt, 1987; Manicourt et al., 1995). As in RA they probably reflect a synovitis. Their current availability of commercial assays remains unclear.

Cytokines (indication: inflammation)

The normal and pathological turnover of skeletal tissues is regulated by autocrine and paracrine signalling which involves cytokines such as interleukin-1a1, b,

interleukin-6 and tumor necrosis factor- α . In OA there is upregulation of expression of these cytokines in chondrocytes and of their receptors (see review Poole and Howell, 2000). Moreover, synovitis results in further metalloproteinase, cytokine and growth factor generation (Firestein et al., 1991; Ritchlin et al., 1994; Miyosaka et al., 1988; Okamoto et al., 1997). These cytokines are regulated in part by natural soluble scavenger receptors. Therefore analyses of these molecules (ligand and especially the receptor) can provide valuable insights into disease activity. Stimulation of c-reactive protein synthesis in the liver results from IL-6 activation. In RA IL-6 has been shown to correlate with changes in aggrecan levels (Manincourt et al., 1993). TNF α p55 and p75 receptor levels also correlate with disease activity and inflammation, including IL-6, in JRA (Gattorno et al., 1996). Serum TNF α p75 (type II) receptor is capable of discriminating OA patients from normals (Otterness et al., 1995). Thus it would make sense to include these extracellular signalling molecules and their regulators in studies of BM in OA.

Matrix Metalloproteinases (MMPs) and their inhibitors (TIMPs) (indication: cartilage damage and/or synovitis)

These MMPs, which are involved in the degradation of cartilage, include collagenase-1 (MMP-1) and stromelysin-1 (MMP-3) are upregulated in OA (Poole et al., 1995; Poole and Howell, 2000). Tissue inhibitors of MMPs (TIMPs) are also upregulated. These originate from fibroblasts, synovial cells and chondrocytes exposed to cytokines such as IL-1 or TNF α (Poole et al., 1995; Poole 1997). Thus upregulation of an MMP is indicative of cytokine activation. MMP-3 is upregulated in SF of OA patients, averaging 15-45 times that of the healthy reference group (Lohmander et al., 1993). This elevation is also seen following joint injury. Elevations of TIMP-1, one of the inhibitors of MMPs, are also observed but to a lesser degree MMP-1 is increased but less than MMP-3. The assays used to detect these proteinases can influence the result. Whereas Lohmander et al (1993) used a combined monoclonal/polyclonal sandwich ELISA to recombinant enzyme (pro or active), others have used polyclonal antisera to detect MMP-3 (pro or active?) and show higher elevations in RA and more MMP-3 in serum than synovial fluid in the inflammatory arthritis (Taylor et al., 1994).

Serum MMP-1 is elevated in OA as much as RA whereas MMP-3 is only elevated in RA (Keyszer et al., 1999). Changes in TIMP-1 were not observed in either RA or OA. The complex of MMP-1 and TIMP-1 is increased more in RA than OA. Levels of these BM in SF are independent of the stage of OA (Ishiguro et al., 1999).

Assays for Pain

These should be considered. Serotonin is an example (Sowers).

Concluding Remarks

This document is intended to serve as a basis for discussion and guide for the assessment of the value of BM in the study and treatment of OA: in particular their value in clinical trials as surrogates. It seeks to address the issues that we must deal with, to identify the criteria which we should be looking for in a BM by addressing the pathology of OA, the processes that we need to track and treat. The BM may provide a surrogate for future clinical trials and studies in this disease. But don't let us reinvent the wheel. Our colleagues in OP research have done so much to bring BM to a point of respectability and value. They have carefully demonstrated what they can do in BM development. We should draw from their experience. Let's make sure that we know what we are measuring and how this may be influenced, for example, by circadian rhythms, clearance rates of BM from body fluids, sex, age and therapy. In OA we have arguably the greatest challenge - much greater than RA or osteoporosis-because of the generally slow progression of the disease, clinical diversity and often very much less skeletal disease "load" than in RA and OP. Clinical heterogeneity is a real challenge. We should use BM to address this in pretrial assessments. We must link imaging to BM development to clinical trials. Remember that academics, industry, the Food and Drug Administration and the National Institutes of Health all have much to offer. But we need each other to make it possible. That's why progress has been very slow, spotty and somewhat disorganized to date in OA and RA BM research and development. So often the academic lacks the access to or awareness of clinical material and vice versa. So if we do this well, get the right people involved, and think longitudinally then we should indeed have much to offer the management of OA by developing new tools to aid and accelerate drug development and eventually control this crippling disease.

Acknowledgements

The author would like to thank the following for their invaluable perspectives and contributions which greatly assisted in the writing of this white paper: James Witter, Eugene Thonar, Pierre Delmas, Patrick Garnerro, Thasia Woodworth, Gayle Lester, Joan McGowan, Dick Heinegard, Stefan Lohmander, Thomas Schnitzer, David Eyre, Gregory Downing, Mary Fran Sowers, Eric Vignon, Rollie Moskowitz, Markus Hosang, Randall Stevens, Rudolf Reiter, Hal Van Wart, and other members of the Roche Group. If I have left someone out, then please

forgive me and add your name to the list. If I have omitted reference to a key study, please realize that this is not intended to be a major review. I have kept away from too much assay detail. This can come later. I would like to thank Joan McGowan and Gayle Lester for their kind invitation to scribble these notes (called a white paper) although the timing for me was horrendously difficult and the paper is a 'work in progress'. Let's use this as a foundation and build from here.

The authors' research is presented funded by Shriners Hospitals for Children, National Institute of Ageing, National Institutes of Health, the Medical Research Council of Canada, the Riva Foundation, Roche Biosciences and Wyeth Ayerst.

References

- Arvidson, N., B. Gudbjornsson, L. Elfman, A.-C. Rydén, T.H. Tötterman, R. Hällgren, 1994. Circadian rhythm of serum interleukin-6 in rheumatoid arthritis. *Ann Rheum Dis* 53:521-524.
- Atley LM, Shao P, Ochs V, Shaffer K, Eyre DR. Matrix metalloproteinase-mediated release of immunoreactive telopeptides from cartilage type II collagen. *Trans. Orthop. Res. Soc., New Orleans*, 23(2): 850, 1998.
- Atley LM, Sharma L, Clemens JD, Shaffer K, Pietka TA, Riggins JA, Eyre DR. 2000b. The collagen II CTx degradation marker is generated by collagenase 3 and in urine reflects disease burden in knee OA patients. 46th Annual Meeting of the Orthopædic Research Society, Orlando, FL, March 12-15. Attencia, L.J., C.A. McDevitt, W.B. Nile, L. Sokoloff. 1989. Cartilage content of an immature dog. *Connect Tiss Res* 18:235-242.
- Billinghurst, R.C., L. Dahlberg, M. Ionescu, A. Reiner, C. Bourne, P. Rorabeck, P. Mitchell, J. Hambor, O. Diekmann, H. Tschesche, J. Chen, H. Van Wart, A.R. Poole. 1997. Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage. *J.Clin.Invest.* 99:1534-1545.
- Bleasel, J., A.R. Poole, D. Heinegård, T. Saxne, D. Holderbau, M. Ionescu, P. Jones, and R.W. Moskowitz. 1999. Changes in serum cartilage marker levels indicate altered cartilage metabolism in families with osteoarthritis-related type II collagen gene. *Arth.Rheum.* In Press:
- Butler, D.M., G.F. Vitti, J. Leizer et al. 1988. Stimulation of the hyaluronic acid levels of human synovial fibroblasts by recombinant human tumor necrosis factor a, tumor necrosis factor b (lymphotoxin), interleukin-1a and interleukin-1b.

Arthritis Rheum 31:1281-1289.

Campion, G.W., P.D. Delmas, P.A. Dieppe. 1989. Serum and synovial fluid osteocalcin (bone GLA protein) levels in joint disease. Br J Rheumatol 28:393-398.

Campion, G.V., F. McCrae, T.J. Schnitzer, M.E. Lenz, P.A. Dieppe, and E.J.M.A. Thonar. 1991. Levels of keratan sulfate in the serum and synovial fluid of patients with osteoarthritis of the knee. Arthritis Rheum 34:1254-1259.

Carey, D.E., M. Alini, M. Ionescu, J.S. Hyams, J.C. Rowe, L.C. Rosenberg, and A.R. Poole. 1997. Serum content of the c-propeptide of cartilage molecule type II collagen in children. Clin Exp Rheum 15:325-328.

Caterson, B., J.E. Christner, J.R. Baker. 1983. Identification of a monoclonal antibody that specifically recognizes corneal and skeletal keratan sulfate. J Bio Chem 258:8848-8854.

Christagau, S., C. Rosenquist, P. Alekanderson, N.H. Bjarnason, P. Ravn, C. Fledelins, C. Herling, P. Qvist, C. Christiansen. 1998. Clinical evaluation of the serum crosslaps one step ELISA, a new assay measuring the serum concentration of bone-derived degradation products of type I collagen c-telopeptides. Clin Chem 44:2290-2300.

Christenson, R.H. 1997. Biochemical markers of bone metabolism: an overview. Clin Biochem 30:573-593.

Clark, A.G., J.M. Jordan, V. Vilim, J.B. Renner, A.D. Dragomir, G. Luta, V.B. Kraus. 1999. Serum cartilage oligomeric matrix protein reflects osteoarthritis presence and severity. Arthritis Rheum 42:2356-2364.

Conrozier, T., T. Saxne, C.S.S. Fan, P. Mathieu, A.M. Tran, D. Heinegård, E. Vignon. 1998. Serum concentrations of cartilage oligomeric matrix protein and bone sialoprotein in hip osteoarthritis: a one year prospective study. Ann.Rheum.Dis. 57:527-532.

Dahlberg, L., C. Billingham, P. Manner, M. Ionescu, A. Reiner, M. Tanzer, D. Zukor, J. Chen, H. Van Wart, A.R. Poole. 2000. Collagenase-mediated cleavage of type II collagen is selectively enhanced in osteoarthritis cartilage and can be arrested with a synthetic inhibitor which spares collagenase-1 (MMP-1). Arthritis Rheum In press:

Delmas, P.D., P. Garnero. 1998. Biological markers of bone turnover in

osteoporosis. In Osteoporosis, J.C. Stevenson, and R. Lindsay (Eds.), Chapman and Hall, London, pp. 117-136. Del Pino, J., E. Martin-Gomez, M. Martin-Rodriguez, C. Lopez-Sosa, M. Cordero, J.L. Lanchares, J.R. Garcia-Talavera. 1991. Influence of sex, age, and menopause in serum of osteocalcin (BGP) levels. *Klin Wochenschr* 69:1135-1138.

Dequeker, J. 1985. The relationship between osteoporosis and osteoarthritis. *Clin Rheum Dis* 11:271-296.

DiCesare, P.E., C.S. Carlson, E.S. Stolerian, N. Hauser, H. Tulli, M. Paulsson. 1996. Increased degradation and altered tissue distribution of cartilage oligomeric matrix protein in human rheumatoid and osteoarthritic cartilage. *J Orthop Res* 14:946-955.

Dieppe, P.A., J. Cushnaghan, P. Young et al. 1993. Prediction of the progression of joint space narrowing in osteoarthritis of the knee by bone scintigraphy. *Ann Rheum Dis* 52:557-563.

Dodge, G.R., D. Hawkins, E. Boesler, L. Sakai, S.A. Jimenez. 1998. Production of cartilage oligomeric matrix protein (COMP) by cultured human dermal and synovial fibroblasts. *Osteoarthritic Cart* 6:435-440.

Ebeling, P.R., J.M. Peterson, B.L. Riggs. 1992. Utility of type I procollagen propeptide assays for assessing abnormalities in metabolic bone diseases. *J.Bone Min.Res.* 7:1243-1250.

Engström-Laurent, A. 1989. Changes in hyaluronan concentration in tissues and body fluids in disease states. In: *The Biology of Hyaluronan*. D. Evered, J. Whelan (Eds.), John Wiley and Sons, Chichester, U.K., pp. 233-247.

Engström-Laurent, A., R. Hällgren. 1985a. Circulating hyaluronate in rheumatoid arthritis: Relationship to inflammatory activity and the effect of corticosteroid therapy. *Ann.Rheum.Dis.* 44:83-88.

Engström-Laurent, A., R. Hällgren. 1985b. Circulating hyaluronic acid levels vary with physical activity in healthy subjects and in rheumatoid arthritis patients: Relationship to synovitis mass and morning stiffness. *Arthritis Rheum* 30:1333-1338.

Firestein, G.S., M.M. Paine, B.H. Littman. 1991. Gene Expression (collagenase, tissue inhibitor of metalloproteinases, complement, and HLA-DR) in rheumatoid arthritis and osteoarthritis synovium: quantitative analysis and effect of

intraarticular corticosteroids. *Arthritis and Rheum* 34:1094-1105.

Fosang, A.J., K. Last, P. Gardiner, D.C. Jackson, L. Brown. 1995. Development of a cleavage-site-specific monoclonal antibody for detecting metalloproteinase-derived aggrecan fragments: detection of fragments in human synovial fluids. *Biochem J* 310:2337-343.

Fraser, J.R.E., T.C. Laurent. 1989. Turnover and metabolism of hyaluronan. In: *The Biology of Hyaluronan*. D. Evered, J. Whelan (Eds.), Ciba Foundation Symposium 143., John Wiley and Sons, Chichester, pp. 41-59.

Garnero, P., E. Hausherr, M.C. Chapy, C. Marcelli, H. Grandjean, C. Muller, C. Cormier, G. Breart, P.J. Meunier, P.D. Delmas. 1996. Markers of bone resorption predict hip fracture in elderly women. The EPIDOS prospective study. *J.Bone Min.Res.* 11:1531-1538.

Garnero, P., W.J. Shih, E. Gineyts, D.B. Karpf, P.D. Delmas. 1994. Comparison of new biochemical markers of bone turnover in late postmenopausal osteoporotic women in response to alendronate treatment. *J Clin Endocrinol Metab* 26:1693-1699.

Garnero, P., E. Sornay-Rendy, F. Duboeuf, and P.D. Delmas. 1999. Markers of bone turnover predict postmenopausal forearm bone loss over 4 years: the OFELY study. *J.Bone Min.Res.* 14:1614-1621.

Gattorno, M., P. Picco, A. Buoncompagni, F. Stalla, P. Facchetti, M.P. Sormani, V. Pistoia. 1996. Serum p55 and p75 tumour necrosis factor receptors as markers of disease activity in juvenile chronic arthritis. *Ann.Rheum.Dis.* 55:243-247.

Gertz, B.J., J.D. Clemens, S.D. Holland, S. Greenspan. 1998. Application of a new serum assay for type I collagen cross-linked N-telopeptides: assessment of diurnal changes in bone turnover with and without alendronate treatment. *Calc Tiss Int* 63:102-106.

Glant, T., K. Mikecz, P.J. Roughley, E. Buzas, A.R. Poole. 1986. Age-related changes in protein-related epitopes of human articular-cartilage proteoglycans. *Biochem.J.* 236:71-75.

Goldberg, R.L., J.P. Huff, M.E. Lenz, P. Glickman, R. Katz, E.J.-M.A. Thonar. 1991. Elevated plasma levels of hyaluronate in patients with osteoarthritis and rheumatoid arthritis. *Arth.Rheum.* 34:799-807.

Goldenberg, D.L., M.S. Egan, A.S. Cohen. 1982. Inflammatory synovitis in

degenerative joint disease. *J Rheumatol* 9:204-209.

Gordon, G.V., C. Villaneuva, H.R. Schumacher, and V. Gohel. 1984. Autopsy study correlating degree of osteoarthritis, synovitis and evidence of articular calcification. *J Rheumatol* 11:681-686.

Greenspan, S.L., R.A. Parker, L. Ferguson, H.N. Rosen, L. Maitland-Ramsey, D.B. Karpf. 1998. Early changes in biochemical markers of bone turnover predict the long-term response to alendronate therapy in representative elderly women: A randomized clinical trial. *J. Bone Min. Res.* 13:1431-1438.

Gundberg, C.M., M.E. Markowitz, M. Mizruchi, J.F. Rosen. 1985. Osteocalcin in human serum: a circadian rhythm. *J Clin Endocrinol Met* 60:736-739.

Hakala, B.E., C. White, A.D. Recklies. 1993. Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. *J. Biol. Chem.* 268:25803-25810.

Hamerman, D. 1997. The aging skeleton. Osteoarthritis and osteoporosis. In: *Osteoarthritis: Public Health Implications for an Aging Population*. D. Hamerman (Ed.), Johns Hopkins University Press: Baltimore, pp. 99-121.

Hamerman, D., D.D. Wood 1984. Interleukin-1 enhances synovial cell hyaluronate synthesis. *Proc Soc Exp Biol Med* 177:205-210.

Harvey, S., M. Weisman, J. O'Dell, T. Scott, M. Krusemeier, J. Visor, C. Swindlehurst. 1998. Chondrex: A new marker of joint disease. *Clin Chem* 44:509-516.

Haubeck, H.-D., R. Kock, D.-C. Fischer, E. van de Leur, K. Hoffmeister, H. Greiling. 1995. Transforming growth factor b1, a major stimulator of hyaluronan synthesis in human synovial lining cells. *Arthritis Rheum* 38:669-677.

Hazell, P.K., C. Dent, J.A. Fairclough, M.T. Bayliss, T.E. Hardingham. 1995. Changes in glycosaminoglycan epitope levels in knee joint fluid following injury. *Arthritis Rheum* 38:953-959.

Heinegård, D., S. Inerot, J. Wieslander, G. Lindblad. 1985. A method for the quantification of cartilage proteoglycan structures liberated to the synovial fluid during developing degenerative joint disease. *Scand J Clin Lab Invest* 45:421-427.

Hinek, A., A. Reiner, A.R. Poole. 1987. The calcification of cartilage matrix in

chondrocyte culture: studies of the C-propeptide of type II collagen (chondrocalcin). *J Cell Biol* 104:1435-1441.

Hinek, A., A.R. Poole. 1988. The influence of vitamin D metabolites on the calcification of cartilage matrix and the C-propeptide of type II collagen (chondrocalcin). *J. Bone Min. Res.* 3:421-429.

Hollander, A.P., T.F. Heathfield, C. Webber, Y. Iwata, R. Bourne, C. Rorabeck, A.R. Poole. 1994. Increased damage to type II collagen in osteoarthritic articular cartilage detected by a new immunoassay. *J Clin Invest* 93:1722-1732.

Hørslev-Petersen, K., K.D. Bentsen, P. Junker, I.B. Lorenzen. 1986. Serum amino-terminal type III procollagen peptide in rheumatoid arthritis. Relationship to disease activity, treatment and development of joint erosions. *Arthritis Rheum* 29:592-599.

Huebner, J.L., I.G. Otterness, E.M. Freund, B. Caterson, V. Kraus. 1998. Collagenase-1 and collagenase-3 expression in a guinea pig model of osteoarthritis. *Arthritis Rheum* 41:877-890.

Hughes, C.E., B. Caterson, A.J. Fosang, P.J. Roughley, J.S. Mort. 1995. Monoclonal antibodies that specifically recognize neopeptide sequences generated by "aggrecanase" and matrix metalloproteinase cleavage of aggrecan: Application to catabolism in situ and in vitro. *Biochem J* 305:799-804.

Hughes, C.E., B. Caterson, R.J. White, P.J. Roughley, J.S. Mort. 1992. Monoclonal antibodies recognizing protease-generated neopeptides from cartilage proteoglycan degradation. *J Biol Chem* 267:16011-16014.

Hutton, C.W., E.R. Higgs, P.C. Jackson et al. 1986. ^{99m}TcHMDP bone scanning in generalized nodal osteoarthritis. II. The four hour bone scan image predicts radiographic change. *Ann Rheum Dis* 45: 622-626.

Ishiguro, N., T. Ito, H. Ito, H. Iwata, H. Jugessur, M. Ionescu, A.R. Poole. 1999. Relationship of matrix metalloproteinases and their inhibitors to cartilage proteoglycan and collagen turnover. *Arthritis Rheum* 42:129-136.

Ishiguro, N., T. Ito, K.-I. Obata, N. Fujimoto, H. Iwata. 1996. Determination of stromelysin-1, 72 and 92 KDa type IV collagenases, tissue inhibitor of metalloproteinase-1 (TIMP-1) and TIMP-2 in synovial fluid and serum from patients with rheumatoid arthritis. *J. Rheumatol* 23:1599-1604.

Keyszer, G., I. Lambiri, R. Nagel, C. Keusser, M. Keyszer, E. Gromnica-Ihle, J.

Fraz, G.R. Burmester, K. Jung. 1999. Circulating levels of matrix metalloproteinases MMP-3 and MMP-1, tissue inhibitor of metalloproteinases 1 (TIMP-1) and MMP-1/TIMP-1 complex in rheumatic disease. Correlation with clinical activity of rheumatoid arthritis. *J Rheumatol* 26:251-258.

Kröger, H., J. Risteli, L. Risteli, I. Penttila, E. Alhava. 1993. Serum osteocalcin and carboxyterminal propeptide of type I procollagen in rheumatoid arthritis. *Ann Rheum Dis* 52:338-342.

Lark, M.W., E.K. Bayne, J. Flanagan, C.F. Harper, L.A. Hoerrner, N.I. Hutchinson, I.I. Singer, S.A. Donatelli, J.R. Weidner, H.R. Williams, R.A. Mumford, L.S. Lohmander. 1997. Aggrecan degradation in human cartilage. Evidence for both matrix metalloproteinase and aggrecanase activity in normal, osteoarthritic, and rheumatoid joints. *J Clin Invest* 100:93-106.

Lark, M.W., H. Williams, L.A. Hoerrner, J. Weidner, J.M. Ayala, c.F. Harper, A. Christen, J. Olszewski, Z. Konteatis, R. Webber, R.A. Mumford. 1995. Quantification of a metalloproteinases-generated aggrecan G1 fragment using monospecific anti-peptide serum. *Biochem.J.* 307:245-252.

Larsson, E., A. Müssener, D. Heinegård, L. Klareskog, T. Saxne. 1997. Increased serum levels of cartilage oligomeric protein and bone sialoprotein in rats with collagen arthritis. *Br J Rheumatol* 36:1258-1261.

Lee, G.J.-L., H. Tieckelmann. 1981. The application of high-performance liquid chromatography in enzymatic assays of chondroitin sulfate isomers in normal human urine. *J. Chromatography* 222:23-31.

Lindqvist, U., K. Chichibu, B. Delpech, B., R.L. Goldberg, W. Knudson, A.R. Poole, T.C. Laurent 1992. Seven different assays of hyaluronan compared for clinical utility. *Clin Chem* 38:127-132.

Lohmander LS, Atley LM, Pietka TA, Eyre DR. 2000. The release of cross-linked peptides from type II collagen into joint fluid and serum is increased in osteoarthritis and after joint injury. 46th Annual Meeting of the Orthopædic Research Society, Orlando, FL, March 12-15.

Lohmander, L.S., L. Dahlberg, D. Eyre, M. Lark, E.J.-M.A. Thonar, L. Ryd. 1998. Longitudinal and cross-sectional variability in markers of joint metabolism in patients with knee pain and articular cartilage abnormalities. *Osteoarth Cart* 6:351-361.

Lohmander, L.S., L. Dahlberg, L. Ryd, D. Heinegard. 1989. Increased levels of proteoglycan fragments in knee joint fluid after injury. *Arthritis Rheum* 32:1442

Lohmander, L.S., L.A. Hoerrner, M.W. Lark. 1993. Metalloproteinases, tissue inhibitor, and proteoglycan fragments in knee synovial fluid in human osteoarthritis. *Arthritis Rheumatism* 36:181-189.

Lohmander, L.S., M. Ionescu, H. Jugessur, A.R. Poole, 1999 Changes in joint cartilage aggrecan metabolism after knee injury and in osteoarthritis. *Arthritis Rheum* 42: 534-544.

Lohmander, L.S., T. Saxne, D. Heinegård. 1994. Release of cartilage oligomeric matrix protein (COMP) into joint fluid after knee injury and in osteoarthritis. *Ann Rheum Dis* 53:8-13.

Lohmander, L.S., Y. Yoshihara, H. Roos, T. Kobayashi, H. Yamada, M. Shinmei. 1996. Procollagen II c-propeptide in joint fluid: Changes in concentration with age, time after knee injury and osteoarthritis. *J Rheumatol* 23:1765-1769.

MacDonald, A.G., P. McHenry, S.P. Robins, D.M. Reid. 1994. Relationship of urinary pyridinium crosslinks to disease extent and activity in osteoarthritis. *Br J Rheum* 33:16-19.

Manicourt, D.-H., O. Cornu, M.E. Lenz, A. Druetz-Van Egeren, E.J.-M.A. Thonar. 1995. Rapid and sustained rise in the serum levels of hyaluronan for anterior cruciate ligament transection in the dog knee joint. *J Rheumatol* 22:262-269.

Manicourt, D.-H., N. Fujimoto, M. Obata, E.J.-M.A. Thonar. 1995. Levels of circulating collagenase, stromelysin-1 and tissue inhibitor of matrix metalloproteinase 1 in patients with rheumatoid arthritis. Relationship to serum levels of antigenic keratan sulfate and systemic parameters of inflammation. *Arthritis Rheum* 38:1031-1039.

Manicourt, D.H., M.-E. Lenz, E.J.-M.A. Thonar. 1991. Levels of serum keratan sulfate rise rapidly and remain elevated following anterior cruciate ligament transection in the dog. *J Rheumatol* 18:1872-1876.

Manicourt, D.H., P. Poilvache, A. Nzeusseu, A. van Egeren, J.P. Devogelaer, M.E. Lenz, E.J.-M.A. Thonar. 1999. Serum levels of hyaluronan, antigenic keratan sulfate, matrix metalloproteinase 3, and tissue inhibitor of metalloproteinase 1, change predictably in rheumatoid arthritis patients who have begun activity after a night of bed rest. *Arthritis Rheum* 42:1861-1869.

Manicourt, D.-H., R. Triki, K. Fukuda, J.-P. Devogelaer, C. Nagant de Deuxchaisnes, E.J.M.A. Thonar. 1993. Levels of circulating tumor necrosis factor α and interleukin-6 in patients with rheumatoid arthritis: Relationship to serum levels of hyaluronan and antigen in keratan sulfate. *Arthritis Rheum* 36:490-499.

Månsson, B., D. Carey, M. Alini, M. Ionescu, L.C. Rosenberg, A.R. Poole, D. Heinegård, T. Saxne. 1995. Cartilage and bone metabolism in rheumatoid arthritis: difference between rapid and slow progression of disease identified by serum markers of cartilage metabolism. *J.Clin.Invest.* 95:1071-1078.

McCarthy, C. J. Cushnaghan, P. Dieppe. 1994. The predictive role of scintigraphy in radiographic osteoarthritis of the hand. *Osteoarthritis Cart* 2:25-28.

Melchiorri, C., R. Melicino, L. Frizziero, T. Silvestri, L. Pulsatelli, I. Mazzetti, R.M. Borzi, M. Uguccioni, A. Facchini. 1998. Enhanced and coordinated in vivo expression of inflammatory cytokines and nitric oxide synthase by chondrocytes from patients with osteoarthritis. *Arthritis Rheum* 41:2165-2174.

Miyasaka, N., K. Sato, M. Goto, M. Sasano, M. Natasuyama, K. Inoue, and K. Nishioka. Augmented interleukin-1 production and HLA-DR expression in the synovium of rheumatoid arthritis patients. Possible involvement in joint destruction. *Arthritis and Rheum* 31:480-486.

Miyata, T., N. Ishiguro, Y. Yasuda, T. Ito, M. Nanagaku, H. Iwata, K. Kurokawa. 1998. Increased pentosidine, an advanced glycation end product, in plasma and synovial fluid from patients with rheumatoid arthritis and its relation with inflammatory markers. *Biochem Biophys Res Comm* 244:45-49.

Møller, H.J., F.S. Larsen, T. Ingemann-Hansen, J.H. Poulsen. 1994. ELISA for core protein of the large aggregating proteoglycan, aggrecan: comparison with the concentrations of immunogenic keratan sulphate in synovial fluid, serum and urine. *Clin Chem Acta* 225:43-55.

Moskowitz RW, Holderbaum D, Atley LM, Eyre DR. Type II collagen C-telopeptide 2B4 epitope is a marker for cartilage degradation in familial osteoarthritis. *Amer. Coll. Rheum.*, San Diego, CA, Nov. 8-12, 1998. Myers, S.L., K.D. Brandt, O. Eilam. 1995. Even low grade synovitis accelerates the clearance of protein from the canine knee. Implications for measurement of synovial fluid "markers" of osteoarthritis. *Arthritis Rheum* 38:1085-1091.

Myers, S.L., B.L. O'Connor, K.D. Brandt. 1996. Accelerated clearance of albumin from the osteoarthritic knee: implications for interpretation of concentrations of

"cartilage markers" in synovial fluid. *J Rheumatol* 23:1744-1748.

Myers, S.L. K.D. Brandt. 1987. Studies of synovial hyaluronic acid synthesis in canine osteoarthritis. *J Rheumatol* 14:1150-1155.

Nelson, F., L. Dahlberg, A. Reiner, I. Pidoux, G. Fraser, E. Brooks, M. Tanzer, E. Bogoch, L.C. Rosenberg, A.R. Poole. 1996. The content of C-propeptide of type II procollagen in cartilage reflects the synthesis of this molecule which is marked by an increase in osteoarthritis. (UnPub)

Okamoto, H., M. Yamamura, Y. Morita, S. Harada, H. Makino, Z. Ota. 1997. The synovial expression and serum levels of interleukin-6, interleukin-11, leukemia inhibitory factor, and oncostatin M in rheumatoid arthritis. *Arthritis Rheum* 40:1096-1105.

Okimura A., Y. Okada, Makihira et al. 1997. Enhancement of cartilage matrix protein synthesis in arthritic cartilage. *Arthritis Rheum* 40:1029-1036.

Otterness, I.G., R.O. Zimmerer, A.C. Swindell, A.R. Poole, T. Saxne, D. Heinegård, M. Ionescu, E. Weiner. 1995. An examination of some molecular markers in blood and urine for discriminating patients with osteoarthritis from healthy individuals. *Acta Orthop Scand (Suppl. 266)* 66:148-150.

Page-Thomas, D.P., D. Bard, B. King, J.T. Dingle. 1987. Clearance of proteoglycan from joint cavities. *Ann.Rheum.Dis.* 46:934-937.

Paimela, L., A. Heiskanen, P. Kurki, T. Helve, and M. Leirisalo-Repo. 1991. Serum hyaluronate levels as a predictor of radiologic progression in early rheumatoid arthritis. *Arthritis Rheum* 34:815-821.

Paulsson, M., and D. Heinegård. 1982. Radioimmunoassay of the 148-kilodalton cartilage protein: distribution of the protein among bovine tissues. *Biochem J* 207:207-213.

Petersson, I.F., T. Boegard, B. Svennsson, D. Heinegård, T. Saxne. 1998a. Changes in cartilage and bone metabolism identified by serum markers in early osteoarthritis of the knee joint. *Br J Rheum* 37:46-50.

Petersson, I.F., T. Boegard, J. Dahlstrom, B. Svensson, D. Heinegård, T. Saxne. 1998b. Bone scan and serum markers of bone and cartilage in patients with knee pain and osteoarthritis. *Osteoarth Cart* 6:33-39.

Poole, A.R. 1997. Cartilage in health and disease. In *Arthritis and Allied*

Conditions: A Textbook of Rheumatology, 13th Ed. D. McCarty and W. Koopman, (Eds.), Lea and Febiger, Philadelphia. 279-233. Poole, A.R. 1997. Can osteoarthritis as a disease be distinguished from ageing by skeletal and inflammation markers? Implications for 'early' diagnosis, monitoring skeletal changes and effects of therapy. In Osteoarthritis and the Ageing Population. D. Hamerman, editor. Johns Hopkins University Press, Baltimore, Maryland. 187-214. Poole, A.R., M. Alini, A.H. Hollander. 1995. Cellular biology of cartilage degradation. In Mechanisms and Models in Rheumatoid Arthritis. B. Henderson, J.C.W. Edwards, and E.R. Pettipher, editors. Academic Press, London, England. 163-204.

Poole, A.R., P. Dieppe. 1994. Biological markers in rheumatoid arthritis. *Arthritis Rheumatism* 23:17-31.

Poole, A.R., Howell, D.S. (2000). Etiopathogenesis of osteoarthritis. In: *Osteoarthritis: Diagnosis, and Management*, 3rd ed. Ed. By R.W. Moskowitz, D.S. Howell, V.M. Goldberg and H.J. Mankin. WB Saunders Co. In press.

Poole, A.R., M. Ionescu, A. Swan, P. Dieppe. 1994. Changes in cartilage metabolism in arthritis are reflected by altered serum and synovial fluid levels of the cartilage proteoglycan aggrecan: Implications for pathogenesis. *J.Clin.Invest.* 94:25-33.

Poole, A.R., C. Webber, A. Reiner, and P.J. Roughley. 1989. Studies of a monoclonal antibody to skeletal keratan sulphate: Importance of antibody valency. *Biochem.J.* 260:849-856.

Poole, A.R., J. Witter, N. Roberts, F. Piccolo, R. Brandt, J. Panquin, and M. Baron. 1990. Inflammation and cartilage metabolism in rheumatoid arthritis. Studies of the blood markers hyaluronic acid, orosomucoid and keratan sulfate. *Arth.Rheum.* 33:790-799.

Price, P.A., M.K. Williamson, J.W. Lothringer. 1981. Origin of the vitamin K-dependent bone protein found in plasma and its clearance by kidney and bone. *J Biol Chem* 256:12760-12766.

Ratcliffe, A., W. Shurety, B. Caterson. 1993. The quantitation of a native chondroitin sulfate epitope in synovial fluid lavages and articular cartilage from canine experimental osteoarthritis and disuse atrophy. *Arthritis Rheum* 36:543-551.

Rayan, V., E.J.-M.A. Thonar, L.-M. Chen, M.E. Lenz, J.M. Williams. 1998.

Regional differences in the rise in blood vessels of antigenic keratan sulfate and hyaluronan after chymopapain-induced knee joint injury. *J Rheumatol* 25: 521-526.

Recklies, A.D., L. Baillargeon, C. White. 1998. Regulation of cartilage oligomeric matrix protein synthesis in human synovial cells and articular chondrocytes. *Arthritis Rheum* 41:997-1006.

Ritchlin, C., E. Dwyer, R. Bucala, R. Winchester. 1994. Sustained and distinctive patterns of gene activation in synovial fibroblasts and whole synovial tissue obtained from inflammatory synovitis. *Scand J Immunol* 40:292-298.

Rizkalla, G., A. Reiner, E. Bogoch, A.R. Poole. 1992. Studies of the articular cartilage proteoglycan aggrecan in health and osteoarthritis: evidence for molecular heterogeneity and extensive molecular changes in disease. *J.Clin.Invest.* 90:2268-2277.

Robins, S.P., P. Stewart, C. Astbury, H.A. Bird. 1986. Measurement of the cross-linking compound pyridinoline in urine as an index of collagen degradation in joint disease. *Ann.Rheum.Dis.* 45:969-973.

Robins, S.P., G. Woitge, R. Hesley, J. Ju, S. Seyedin, M.J. Seibel. 1994. Direct, enzyme-linked immunoassay for urinary deoxypyridinoline as a specific marker for measuring bone resorption. *J Bone Miner Res* 9:1643-1649.

Robion, F.C., B. Doizé, L. Boiré, M. Marcoux, M. Ionescu, A. Reiner, A.R. Poole, S. Laverty. Use of synovial fluid markers of cartilage synthesis and turnover to study effects of repeated intraarticular administration of methylprednisolone acetate on articular cartilage in vivo. *J Orthop Res*, In revision.

Rodríguez-García, J., J.R. Requena, S. Rodríguez-Segade. 1998. Increased concentrations of serum pentosidine in rheumatoid arthritis. *Clin Chem* 44:250-255.

Saltarelli, M.J., K.S. Johnson, E.H. Pickering, I.G. Otterness, M.D. Vazquez-Abad, and T.G. Woodworth. 1999. Measurement of urinary type II collagen neopeptide levels in rheumatoid arthritis patients. *Arthritis Rheum* 42 (Suppl. 9), S249.

Saxne, T., D. Heinegård. 1989. Involvement of nonarticular cartilage, as demonstrated by release of a cartilage-specific protein, in rheumatoid arthritis. *Arthritis Rheum* 32:1080-1086.

- Saxne, T., D. Heinegård. 1992. Cartilage oligomeric matrix protein: a novel markers of cartilage turnover detectable in synovial fluid and blood. *Br J Rheumatol* 31:583-591.
- Saxne, T., D. Heinegård. 1995. Matrix proteins: Potentials as body fluid markers of changes in the metabolism of cartilage and bone in arthritis. *J Rheumatol* 22:71-74.
- Saxne, T., L. Zunino, D. Heinegård. 1995. Increased release of bone sialoprotein into synovial fluid reflects tissue destruction in rheumatoid arthritis. *Arthritis Rheum* 38:82-90.
- Seibel, M., A. Duncan, S.P. Robins. 1989. Urinary hydroxy-pyridinium crosslinks provide indices of cartilage and bone involvement in arthritis diseases. *Arthritis Rheum* 16:964-970.
- Sharif, M., E. George, P.A. Dieppe. 1995c. Correlation between synovial fluid markers of cartilage and bone turnover and scintigraphic scan abnormalities in osteoarthritis of the knee. *Arthritis Rheum* 38:78-81.
- Sharif, M., C.J. Elson, P.A. Dieppe, J.R. Kirwan. 1997. Elevated serum c-reactive protein levels in osteoarthritis. *Br J Rheumatol* 36:140-141.
- Sharif, M., E. George, P.A. Dieppe. 1996. Synovial fluid and serum concentrations of aminoterminal propeptide of type III procollagen in healthy volunteers and patients with joint disease. *Ann.Rheum.Dis.* 55:47-51.
- Sharif, M., E. George, L. Shepstone, W. Knudson, E.J.M.A. Thonar, J. Cushnagan, P. Dieppe. 1995a. Serum hyaluronic acid level as a predictor of disease progression in osteoarthritis of the knee. *Arth.Rheum.* 38:760-767.
- Sharif, M., T. Saxne, L. Shepstone, J.R. Kirwan, C.J. Elson, D. Heinegård, P.A. Dieppe. 1995b. Relationship between serum cartilage oligomeric matrix protein levels and disease progression in osteoarthritis of the knee joint. *British Journal of Radiology* 34:306-310.
- Shinmei, M., K. Ito, S. Matsuyama, Y. Yoshihara, K. Matsuzawa. 1993. Joint fluid carboxy-terminal type II procollagen peptide as a marker of cartilage collagen biosynthesis. *Osteoarthritis Cartilage* 1:121-128.
- Smedsrod, B. 1988. Amino terminal propeptide of type III procollagen is cleaved from the circulation by receptor-mediated endocytosis in liver endothelial cells. *Collagen Rel Res* 8:375-388.

Song, X.-Y., L. Zeng, W. Jin, J. Thompson, D.E. Mizel, K.-J. Lei, R.C. Billinghamurst, A.R. Poole, S.M. Wahl. 1999. Secretory leukocyte protease inhibitor suppresses the inflammation and joint damage of bacterial cell wall-induced arthritis. *J.Exp.Med.* 190:535-542.

Sowers, M., L. Lachance, D. Jamadar, M.C. Hochberg, B. Hollis, M. Crutchfield, M.L. Jannausch. 1999. The associations of bone mineral density and bone turnover markers with osteoarthritis of the hand and knee in pre- and perimenopausal women. *Arthritis Rheum* 42:483-489.

Spector, T.D., L. Woodward, G.M. Hall, A. Hammond, A. Williams, M.G. Butler, I.T. James, D.J. Hart, P.W. Thompson, D.L. Scott. 1992. Keratan sulphate in rheumatoid arthritis, osteoarthritis, and inflammatory diseases. *Ann.Rheum.Dis.* 51:1134-1137.

St. Clair, E.W., S.A. Moak, W.E. Wilkinson, L. Sanders, T. Lang, R.A. Greenwald. 1998. A cross-sectional analysis of 5 different markers of collagen degradation in rheumatoid arthritis. *J Rheumatol* 25:1472-1479.

Stoop, R., P.M. van der Kraan, P. Buma, A.P. Hollander, C.R. Billinghamurst, A.R. Poole, W.B. van den Berg. 1999b. Type II collagen degradation in spontaneous osteoarthritis in C57BL/6 and BALB/C mice. *Arthritis and Rheum* 42:2381-2389.

Sweet, M.B.E., A. Coelho, C.M. Schnitzler, T.J. Schnitzer, M.E. Lenz, I. Jakim, K.E. Kuettner, E.J.-M.A. Thonar. 1988. Serum keratan sulfate levels in osteoarthritis patients. *Arthritis Rheum* 31:648-652.

Taylor, D.J., N.T. Cheung, P.T. Dawes. 1994. Increased serum pro MMP-3 in inflammatory arthritides: a potential indicator of synovial inflammatory monokine activity. *Ann Rheum Dis* 53:768-772.

Thompson, P.W., T.D. Spector, I.T. James, E. Henderson, D.J. Hart. 1992. Urinary collagen crosslinks reflect the radiographic severity of knee osteoarthritis. *Br J Rheumatol* 31:759-761.

Thonar, E.J.-M.A., M.E. Lenz, G.K. Klintworth, B. Caterson, L.M. Pachman, P. Glickman, R. Katz, J. Huff, K.E. Kuettner. 1985. Quantification of keratan sulfate in blood as a marker of cartilage catabolism. *Arthritis Rheum* 28: 1367-1376.

Thonar, E.J.-M.A., L.M. Pachman, M.E. Lenz, J. Hayford, P. Lynch, K.E. Kuettner. 1988. Age related changes in the concentration of serum keratan sulphate children. *J. Clin Chem Clin Biochem* 26:57-63.

- Thonar, E.J.M.A., M. Shinmei, L.S. Lohmander. 1993. Body fluid markers of cartilage changes in osteoarthritis. *Rheum Dis Clin North Am* 19:635-637.
- Van Meurs, J., P. van Lent, A., Holthuysen, I., Singer, E., Bayne, W. van den Berg. 1999. Kinetics of aggrecanase- and metalloproteinase-induced neoepitopes in various stages of cartilage destruction in murine arthritis. *Arthritis Rheum* 42: 1128-1139.
- Volck, B., K. Ostergaard, J.S. Johansen, C. Garbarsch, P.A. Price. 1999. The distribution of YKL-40 in osteoarthritic and normal human articular cartilage. *Scand J Rheum* 28:171-179.
- Vingsbo-Lundberg, C., T. Saxne, H. Olsson, R. Holmdahl. 1998. Increased serum levels of cartilage oligomeric matrix protein in chronic erosive arthritis in rats. *Arthritis Rheum* 41:544-550.
- Webb, G.R., C.I. Westacott, and C.J. Elson. 1997. Osteoarthritic synovial fluid and synovium supernatants upregulate tumor necrosis factor receptors on human articular chondrocytes. *Osteoarthritic Cart* 5:427-437.
- Witter, J., P.J. Roughley, C. Webber, N. Roberts, E. Keystone, A.R. Poole. 1987. The immunologic detection and characterization of cartilage proteoglycan degradation products in synovial fluids of patients with arthritis. *Arthritis Rheum* 30:519-529.
- Woodworth, T.G., I.G. Otterness, M.J. Saltarelli, K. Johnson, E.H. Pickering. 1999. Urinary collagen type II peptide (TIINE) in osteoarthritis (OA) patients is associated with disease severity. *Arthritis Rheum* 42: (Suppl. 9), S258.
- Yasser, M., E. Miedary, I.H. Abubakr, M.E. Baddini. 1998. Effect of low dose methotrexate on markers of bone metabolism in patients with rheumatoid arthritis. *J Rheumatol*, 25:2083-2087.
- Yoshihara, Y., K. Obata, N. Fujimoto, N., K. Yamashita, T. Hayakawa, M. Shinmei. 1995. Increased levels of stromelysin-1 and tissue inhibitor of metalloproteinases-1 in sera from patients with rheumatoid arthritis. *Arthritis Rheum* 38:969-975.
- Ziff, M., A. Kibrick, E. Dresner, H.J. Gribetz. 1956. Excretion of hydroxyproline in patients with rheumatic and non-rheumatic diseases. *J Clin Invest* 35:579-587