Joint Biomarkers in Idiopathic Femoral Head Osteonecrosis: Comparison with Hip Osteoarthritis

SHINICHI KATO, HARUMOTO YAMADA, NOBUKI TERADA, KOICHI MASUDA, MARY ELLEN LENZ, MITSUHIRO MORITA, YASUO YOSHIHARA, and OSAMU HENMI

ABSTRACT. ***Objective.*** To compare concentrations of joint biomarkers in synovial fluid (SF) between idiopathic osteonecrosis of the femoral head (ION) and osteoarthritis (OA) of the hip joint.

Methods. Levels of the joint biomarkers cartilage oligomeric matrix protein (COMP), antigenic keratan sulfate (AgKS), and hyaluronan (HA) in SF samples from 21 cases of ION and their relationship to disease stage and history of steroid use were assessed and compared to the result of 29 cases of hip OA.

Results. In both the ION and hip OA groups, levels of COMP and AgKS in SF showed a significant positive correlation. The ION group had significantly higher levels of AgKS in SF than the hip OA group. In the ION group, stage II patients had significantly higher SF levels of both COMP and AgKS than those in stage III patients. No difference in level of HA in hip joint SF was found between steroid and non-steroid treated ION patients or between the stage II and III subgroups.

Conclusion. SF levels of COMP and AgKS may serve as useful joint biomarkers that reflect cartilage metabolism not only in hip OA but also in ION. (J Rheumatol 2005;32:1518–23)

Key Indexing Terms:
JOINT BIOMARKERS CARTILAGE OLIGOMERIC MATRIX PROTEIN
OSTEONECROSIS OSTEOARTHRITIS SYNOVIAL FLUID ELISA

The pathologic process involved in idiopathic osteonecrosis of the femoral head (ION) begins with a necrotic change in bone tissue that is thought be a result of disturbance in the blood circulation to the femoral head[1]. Once bone tissue necrosis leads to the depression of the femoral head that is characteristic of ION, pain with relatively abrupt onset is elicited. There is an abrupt mechanical stress that quickly destroys the cartilage; however, cartilage damage in this early disease stage of ION will not cause any clinical symptom or dysfunction and is not considered the essential pathogenesis of ION[2]. The degenerated cartilage on the femoral head side caused by femoral head depression results in destroyed cartilage on the acetabular side, and ION progresses to secondary osteoarthritis (OA).

On the other hand, the pathologic process of primary OA begins with unbalanced remodeling in cartilage that is initiated by multiple factors including genetic, developmental, and metabolic components. OA is defined as a gradual loss of cartilage, progressing through debilitation of joint structure and function and finally leading to an incongruous joint surface.

The destruction of hip joint tissues that takes place in ION occurs quite rapidly when compared with that of primary hip OA, thus the pathology of hip joint tissue degeneration and destruction is likely to differ. Thus, even though ION starts with femoral head bone tissue necrosis, the pathophysiologic evaluation of other hip joint tissues, e.g., cartilage and synovium, in ION would be informative.

Pathophysiological evaluation in OA and ION is essential for correct diagnosis and treatment of disease. Imaging techniques including radiography and magnetic resonance imaging (MRI) have provided information on arthritic morphological changes; however, they are not useful for obtaining earlier diagnosis or assessing the effectiveness of treatment; therefore more sensitive methods are required. Recently, biomarkers reflecting the metabolism of cartilage and synovium have been clarified.

A biomarker, which is a molecule that indicates an alteration in physiology from normal, should also specifically and sensitively reflect a disease state and be of use for diagnosis as well as for disease monitoring during and after ther-

From the Department of Orthopaedic Surgery, Fujita Health University, Second Hospital, Nagoya; Department of Orthopaedic Surgery, Fujita Health University College, Toyoake, Japan; Department of Orthopedic Surgery and Department of Biochemistry, Rush Medical College, Rush University Medical Center, Chicago, Illinois, USA; and Department of Orthopaedic Surgery, National Defense Medical College, Saitama, Japan.

S. Kato, MD, Department of Orthopaedic Surgery, Fujita Health University, Second Hospital; H. Yamada, MD, PhD, Professor and Chairman, Department of Orthopaedic Surgery, Fujita Health University College; N. Terada, MD, Associate Professor, Department of Orthopaedic Surgery, Fujita Health University, Second Hospital; K. Masuda, MD, Associate Professor, Departments of Orthopaedic Surgery and Biochemistry; M.E. Lenz, MS, Instructor, Department of Biochemistry, Rush Medical College; M. Morita, MD, PhD; Y. Yoshihara, MD, PhD; O. Henmi, MD, Department of Orthopaedic Surgery, National Defense Medical College.

Address reprint requests to Dr. S. Kato, Department of Orthopaedic Surgery, Fujita Health University, Second Hospital, 3-6-10 Otobashi, Nakagawaku, Nagoya, 454-8509, Japan.

Accepted for publication March 30, 2005.

apy[3]. Joint biomarkers[4], otherwise referred to as metabolic markers[5], molecular markers[6], or biochemical markers[7], are molecules whose concentration in synovial fluid (SF), blood, or urine provides information about the *in vivo* metabolic status of cartilage, synovium, or other joint tissue[8]. To date, the use of joint biomarkers has mainly been reported in OA[4-6] and rheumatoid arthritis (RA)[9-11]. The joint biomarkers selected for this study were cartilage oligomeric matrix protein (COMP), antigenic keratan sulfate (AgKS), and hyaluronan (HA). All 3 biomarkers are measurable in serum or SF[5]. COMP and AgKS in synovial joints were reported to be elevated in OA and traumatized joints as a result of cartilage destruction during the disease process[12].

Our purpose was to investigate the changes in the joint biomarkers COMP, AgKS, and HA in hip joint SF of ION and OA patients and compare levels of these 3 joint biomarkers.

MATERIALS AND METHODS

Patients and samples. SF samples were obtained by direct joint capsule needle puncture while the patients (21 cases of ION and 29 cases of hip joint OA) were undergoing femoral endoprosthesis replacement, total hip replacement, or femoral osteotomy. The samples were centrifuged at 1000 g for 20 min at 4°C and the supernatants were stored at –80°C until analyzed.

The diagnostic criteria and radiological staging system for idiopathic femoral head necrosis proposed by the Japanese Ministry of Health and Welfare was used to classify the ION patients, as follows: Stage 1: No specific findings of osteonecrosis on simple radiographic images; however, specific findings are observed on MRI, bone scintigram, or histology from core decompression. Stage 2: Demarcating sclerosis is seen on simple radiograph, without collapse of the femoral head. Stage 3: Collapse of the femoral head, including crescent sign, is seen without joint space narrowing. Mild osteophyte formation of the femoral head or acetabulum may be seen. Stage 4: Osteoarthritic changes are seen[13].

Standard projection radiography was performed, taking anteroposterior (AP) radiographs of the pelvis in a standing position with 20° of internal rotation of the hip joint. The lateral view of the femoral head was taken in the AP direction while the patient was positioned supine with the hip in 90° of flexion, 45° of abduction, and neutral rotation. The focus to film distance was 90 cm. Radiographic staging sorted the ION patients into 7 stage II (early stage) and 14 stage III (advanced stage) cases. Of these ION cases, 7 had been induced by steroid use for the treatment of systemic lupus erythematosus (SLE). These patients with steroid induced ION had a history of high dose steroid treatment (> 30 mg/day); however, most of the patients were administered only low dose steroids at the time of surgery.

The diagnosis of hip OA was made according to the criteria recommended by the American College of Rheumatology[14]. Hip OA patients were subdivided based on the radiological staging system of the Japanese Orthopaedic Association, as follows: Advanced stage: moderate diminution of the joint space, and partial contact with subchondral bone accompanied by sclerotic changes in the acetabulum. Terminal stage: joint space is greatly impaired, and acetabulum has been destroyed[15]. As radiographically staged, there were 2 cases of advanced stage and 27 terminal stage hip OA patients. Patient demographics are shown in Table 1.

Measurement of biomarkers

Measurement of COMP. Levels of COMP in hip joint SF were measured by the competitive indirect inhibition ELISA[16] using an anti-COMP monoclonal antibody (17-C10, kindly provided by Dr. V. Vilim, Institute of Rheumatology, Praha, Czech Republic).

Table 1. Patient demographics. The stage of ION was assessed by radiological findings according to the criteria of the Japanese Ministry of Health and Welfare. The stage of hip joint OA was assessed by radiological findings according to the criteria of the Japanese Orthopaedic Association. Values are mean ± SD.

Disease	N	Age, yrs	Female/male
ION stage II	7	31.2 ± 7.4	0/7
ION stage III	14	52.0 ± 13.0	4/11
Total ION	21	46.3 ± 15.0	4/17
OA advanced	2	42.0 ± 4.2	2/0
OA terminal	27	64.1 ± 11.7	24/3
Total OA	29	55.5 ± 15.8	26/3

Measurement of AgKS. AgKS in hip joint SF was quantified by the well characterized ELISA with an inhibition step using an anti-KS monoclonal antibody, 1/20/5-D-4, specific for the highly sulfated epitope on KS chains[17]. Concentrations are reported as equivalents of an international standard of KS purified from human costal cartilage.

Measurement of HA. HA in hip joint SF was quantified by an ELISA as described[18]. This ELISA takes advantage of an anti-KS monoclonal antibody (1/20/5-D-4) to differentiate between the coated aggregating rat chondrosarcoma proteoglycan that captures the HA and the KS-bearing aggregating proteoglycan added subsequently. HA of 4 MDa molecular weight (Healon, Pharmacia, Uppsala, Sweden) was used as a standard.

Statistical analysis. Intragroup correlations of biomarkers were analyzed by the Spearman rank correlation coefficient. The Mann-Whitney U-test was employed to analyze intergroup differences of biomarker levels and to test effects of steroid use and radiographic stage in ION. The level of significance was established at $p < 0.05$.

RESULTS

Correlation among joint biomarkers within ION and OA groups.

We separately analyzed the data to determine if there were any correlations among COMP, AgKS, and HA SF levels within the ION patients and the OA patients. A significant positive correlation was found between SF levels of COMP and AgKS in both groups ($p < 0.05$), but not between COMP and HA, nor between AgKS and HA concentrations (Table 2, Figures 1 and 2).

Comparison of COMP, AgKS, and HA concentrations in ION and OA.

The hip joint SF concentration of COMP was 80.0 ± 47.0 µg/ml (mean ± SD) in hip OA and 78.2 ± 46.3 µg/ml in ION,

Table 2. Correlations between hip joint SF levels of COMP, AgKS, and HA in ION and OA patients.

	AgKS	HA
ION		
COMP	0.538*	0.059
AgKS		0.03
OA		
COMP	0.445*	0.166
AgKS		0.285

* $p < 0.05$.

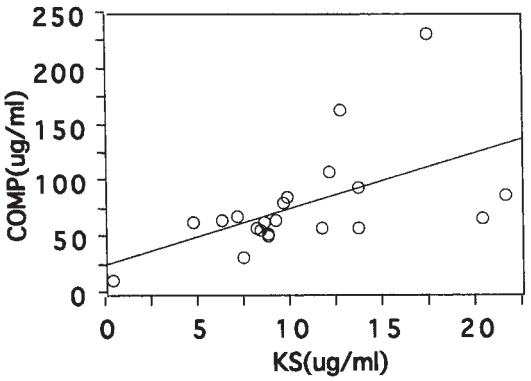

Figure 1. A significant positive correlation between hip joint SF levels of COMP and AgKS was found in patients with ION ($p < 0.05$).

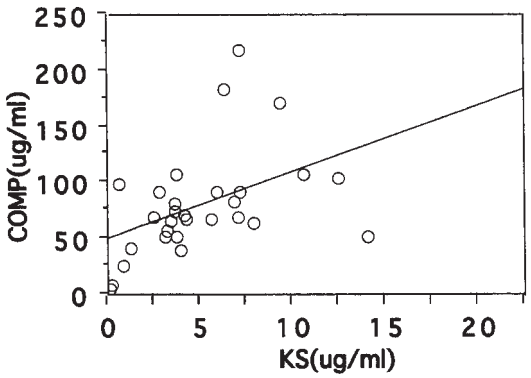

Figure 2. A significant positive correlation between hip joint SF levels of COMP and AgKS was found in patients with hip OA ($p < 0.05$).

with no significant difference between the 2 disease groups ($p = 0.62$). The ION patients had a significantly higher SF AgKS concentration than OA patients [ION 9.2 µg/ml (median), 10.5 ± 4.97 µg/ml (mean ± SD); hip OA 4.0 µg/ml (median), 5.03 ± 3.52 µg/ml (mean ± SD); $p < 0.001$; Figure 3]. There was no difference in the hip joint SF HA concentration between OA (77.8 ± 41.8 µg/ml) and ION (72.2 ± 34.0 µg/ml) patients ($p = 0.48$).

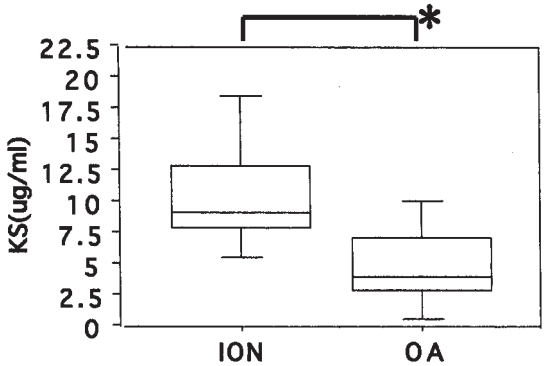

Figure 3. ION patients had a significantly higher level of hip joint SF AgKS than hip OA patients ($p < 0.001$). Horizontal bar represents the median value of the data.

Effect of steroid use and disease stage on joint biomarkers in ION patients.

COMP. There was no significant difference in the COMP hip joint SF concentration between the steroid treated patients and non-steroid ION patients (steroid 53.3 ± 23.5 µg/ml; non-steroid 90.6 ± 50.5 µg/ml; $p = 0.062$). However, the stage II ION subgroup by radiological examination showed a higher COMP value than the stage III ION subgroup [stage II 82.4 µg/ml (median), 108.6 ± 65.5 µg/ml (mean ± SD); stage III 61.7 µg/ml (median), 62.9 ± 23.9 µg/ml (mean ± SD); $p < 0.05$; Figure 4].

AgKS. There was no significant difference in the hip joint SF levels of AgKS between steroid and non-steroid treated ION patients (steroid 9.8 ± 6.1 µg/ml; non-steroid 10.8 ± 4.5 µg/ml; $p = 0.77$). The stage II ION subgroup had a significantly higher hip joint SF level of AgKS than the stage III ION subgroup [stage II 12.7 µg/ml (median), 14.2 ± 5.7 µg/ml (mean ± SD); stage III 8.65 µg/ml (median), 8.6 ± 3.4 µg/ml (mean ± SD); $p < 0.05$; Figure 5].

Hyaluronan. No difference in HA hip joint SF levels was found between steroid and non-steroid treated ION patients [steroid 72.8 ± 58.1 µg/ml (mean ± SD); non-steroid 72.0 ±

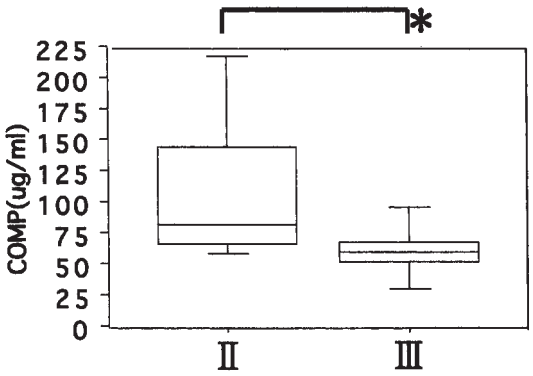

Figure 4. Patients with stage II ION, by radiological examination, had a higher hip joint SF level of COMP than patients with stage III ION ($p < 0.05$). Horizontal bar represents the median value of the data.

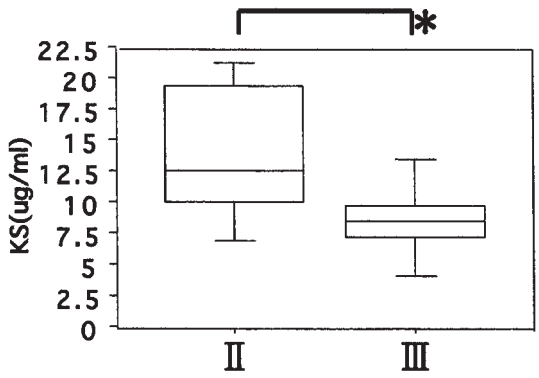

Figure 5. Patients with stage II ION, by radiological examination, had a higher hip joint SF level of AgKS than patients with stage III ION ($p < 0.05$). Horizontal bar represents the median value of the data.

19.8 µg/ml (mean ± SD); p = 0.51] or between the stage II and III subgroups [81.8 ± 45.3 µg/ml (mean ± SD) vs 67.1 ± 26.8 µg/ml (mean ± SD), respectively; p = 0.61].

DISCUSSION

We measured and compared hip joint synovial fluid biomarker concentrations (COMP, AgKS, and HA) in 2 distinct hip joint diseases, ION and hip OA. We measured biomarkers in SF collected during hip surgery. SF from the hip joint is not easy to collect; however, we consider this to be the most informative sample compared with other body fluids, e.g., blood or urine, since it is directly exposed to cartilage and synovium. Biomarkers in SF provide information about changes taking place in a single joint[7], in this case the hip joint. Other advantages of SF are that the concentration of a specific marker is much higher than in blood or urine and the chance of identifying a high proportion of all fragments is much greater. SF COMP concentration increases after trauma, such as ligament or meniscus injury, and levels follow a similar pattern to those of aggrecan, KS, or chondroitin 6-sulfate[12]. COMP is generally high in OA SF, is maximal in early OA, and declines as disease stage progresses[12]. It has also been verified that there are fragments as well as intact forms of COMP in cartilage, and a chronic joint destructive disease such as OA has a higher proportion of degraded fragments than normal cartilage[7]. Because the mechanism of degradation of COMP is thought to involve matrix metalloproteinase (MMP) and serine protease, degradation enzymes of the extracellular matrix in joint cartilage, COMP is regarded as one of the markers of the turnover of cartilage[7,8]. The ELISA method used in our study utilized an antibody that was capable of recognizing fragmented as well as intact forms of COMP[16]. It is notable that serum COMP has been proposed to be a prognostic marker for joint destruction in RA and OA[7,8], and that increased baseline serum COMP levels were associated with hip destruction in advanced hip OA[19].

The glycosaminoglycan AgKS, a molecule found almost exclusively in aggrecan, is an example of a "direct" biomarker of cartilage metabolism, one that originates principally from cartilage[17]. It is generally accepted that an increase in the SF level of AgKS-bearing fragments provides a measure of the degradation of the cartilage matrix[20-23]. It has been reported that SF AgKS concentration is higher than normal in the initial stages of OA and declines inversely as disease stage advances[20-22]. However, the serum level of AgKS may also represent an increase in aggrecan synthesis, because it is not clear whether this marker reflects degradation of mature resident aggrecan or newly synthesized molecules[7].

SF HA in OA decreases in proportion to progression of the radiographic stage, and thus is considered an index of synovial membrane inflammation[23]. Interestingly, the serum level of HA has prognostic value in OA of the knee[24].

Our results showed that hip joint SF COMP and AgKS levels significantly and positively correlated in both ION and in hip OA. These 2 diseases differ pathophysiologically in that a rapid destructive process follows the disruption of the femoral head in ION, whereas hip OA is characterized by a slower destructive process. Little information has been reported on SF COMP levels, furthermore, correlation between SF COMP and AgKS levels in ION and OA has not been reported. However, because these 2 markers correlated in these 2 distinct joint diseases, our results indicate that COMP and AgKS might reflect the same phase of cartilage destruction, and that COMP and AgKS might be useful joint biomarkers of cartilage degradation. In our study, there is a difference in the mean age of patients between the OA and ION groups. Also, most of the OA patients were in the terminal disease stage. We have reported that KS concentration in SF from hip OA patients measured using high performance liquid chromatography decreases with the progression of OA stage[25]. The mean age difference between the 2 disease groups and bias in the stage of OA patients would affect the levels of AgKS and COMP.

In addition to fetal and adult articular chondrocytes and fetal tendon and ligament fibroblasts, COMP is also produced by synovial fibroblasts from normal and OA synovial tissues[26]. There is also evidence that COMP mRNA is expressed in the synovial membrane tissue of RA and OA patients[26]. As noted, HA is considered to be a marker of synovial membrane inflammation[27]. Because we found no correlation between COMP and HA levels in hip joint SF in either ION or hip OA, the SF COMP level was assumed to be less affected by synovial membrane inflammation.

It has been reported that in SF, cartilage degradation markers such as chondroitin 4-sulfate and chondroitin 6-sulfate did not differ between ION and OA[28]. However, levels of MMP-3 and type II procollagen C-peptide (pCOL II-C), a marker of type II collagen synthesis in chondrocytes, were reported to be higher in ION than in OA[29]. In our study, hip joint SF levels of AgKS were significantly higher in ION than in hip OA. In ION, the cartilage tissue remains in the normal state until it is destroyed, over a very short time, by pressure imposed by bone-head compression, thereby liberating a large amount of fragments of cartilage matrix aggrecan. In contrast, in hip OA, the cartilage tissue remaining is diminished by longterm destruction; therefore, the total amount of aggrecan fragment liberated into the joint would be much less than in ION. Interestingly, we found no significant differences in the SF COMP levels between ION and hip OA. This discrepancy between SF COMP and AgKS levels, which were positively correlated in each disease state, may be ascribed to differences in the mechanism of cartilage destruction in both diseases. Further, there is a report that COMP differs from aggrecan in the mechanism by which it is released from cartilage tissue into the SF and in the manner it is cleared from the joint cavity[12]. These dif-

ferences might have to be considered in interpreting our results of SF COMP.

Iwase, *et al* reported that the SF HA level was significantly higher in early stage ION compared to early stage hip OA[28]; however, we found no such difference. This discrepancy may be explained by the fact that there were more cases of ION and hip OA at relatively advanced stages in our study. Early stage ION involves synovial membrane inflammation caused by the more rapid release of cartilage debris than that found in hip OA, but as the disease stage of ION progresses, those differences between ION and hip OA may no longer exist.

Jingushi, *et al* compared hip joint SF markers between stage IV and stage III or II ION patients and found a significantly higher level of aggrecan fragments in stage IV subjects[29]. In contrast, in our study, the stage II ION subgroup showed significantly higher COMP and AgKS levels than the stage III ION subgroup. Because our study did not include stage IV cases of ION, we cannot compare our results with those of Jingushi, *et al*, but our results possibly suggest that a greater amount of matrix degradation products are released from cartilage of stage II ION, which had been normal just before depression of the femoral head, than from cartilage of stage III ION.

There were 7 cases of ION that had possibly been induced by steroids. Steroid use is well known to suppress production of cartilage matrix including aggrecan[30,31]. These patients with steroid induced ION had a history of high-dose steroid treatment (> 30 mg/day); however, most of the patients were administered only low-dose steroid at the time of collection of SF. We expect that longterm steroid treatment, even at a low dose, would affect cartilage metabolism. Others have also studied effects of steroids on joint markers in ION, but no significant effects have been reported. We found no difference in steroid effects on SF AgKS levels in our study. On the other hand, there was a tendency toward lower concentrations of SF COMP in steroid users, although this was not statistically significant ($p = 0.062$). COMP is located in the superficial matrix of the cartilage, especially in the territorial matrix around chondrocytes, and its appearance differs from other cartilage matrix constituents, e.g., type II collagen and aggrecans, during chondrogenesis[32]. The mechanism of COMP release from cartilage into SF has not been sufficiently analyzed; however, COMP present in the superficial layer of cartilage would reflect cartilage metabolism more directly than AgKS. Therefore, we hypothesize that there is a possibility that lower levels of COMP might reflect the suppression of cartilage metabolism by steroids. Further study with a larger number of cases is required.

We carried out a comparative study of joint biomarkers including COMP, which has recently attracted attention as a marker of cartilage destruction. We observed positive correlation of synovial fluid COMP level to AgKS in hip OA and ION, but no correlation to synovial fluid HA. Our results suggest that COMP is a useful joint biomarker for cartilage degradation not only in hip OA, but also in ION, which is characterized by the rapid destruction of cartilage.

REFERENCES

1. Ficat RP. Idiopathic bone necrosis of the femoral head. Early diagnosis and treatment. J Bone Joint Surg Br 1985;67:3-9.
2. Assouline-Dayan Y, Chang C, Greenspan A, Shoenfeld Y, Gershwin ME. Pathogenesis and natural history of osteonecrosis. Semin Arthritis Rheum 2002;32:94-124.
3. Fung ET, Wright GL Jr, Dalmasso EA. Proteomic strategies for biomarker identification: progress and challenges. Curr Opin Mol Ther 2000;2:643-50.
4. Poole AR. Can serum biomarker assays measure the progression of cartilage degeneration in osteoarthritis? Arthritis Rheum 2002;46:2549-52.
5. Thonar E, Manicourt D. Noninvasive markers in osteoarthritis. In: Moskowitz R, Goldberg V, Howell DS, Altman RD, Buckwalter J, editors. Osteoarthritis: diagnosis and medical/surgical management. Philadelphia: W.B. Saunders; 2001:293-313.
6. Garnero P, Ayral X, Rousseau JC, et al. Uncoupling of type II collagen synthesis and degradation predicts progression of joint damage in patients with knee osteoarthritis. Arthritis Rheum 2002;46:2613-24.
7. Garnero P, Rousseau JC, Delmas PD. Molecular basis and clinical use of biochemical markers of bone, cartilage, and synovium in joint diseases. Arthritis Rheum 2000;43:953-68.
8. Thonar EJ-MA, Lenz ME, Masuda K, Manicourt D-H. Body fluid markers of cartilage metabolism. In: Seibel MJ, Robins SP, Bilezikian JP, editors. Dynamics of bone and cartilage metabolism. San Diego: Academic Press; 1999:453-64.
9. Paimela L, Heiskanen A, Kurki P, Helve T, Leirisalo-Repo M. Serum hyaluronate level as a predictor of radiologic progression in early rheumatoid arthritis. Arthritis Rheum 1991;34:815-21.
10. Crnkic M, Mansson B, Larsson L, Geborek P, Heinegard D, Saxne T. Serum cartilage oligomeric matrix protein decreases in rheumatoid arthritis patients treated with infliximab or etanercept. Arthritis Res Ther 2003;5:R181-5.
11. Forslind K, Eberhardt K, Jonsson A, Saxne T. Increased serum concentrations of cartilage oligomeric matrix protein. A prognostic marker in early rheumatoid arthritis. Br J Rheumatol 1992;31:593-8.
12. Lohmander LS, Saxne T, Heinegard DK. Release of cartilage oligomeric matrix protein into joint fluid after knee injury and in osteoarthritis. Ann Rheum Dis 1994;53:8-13.
13. Sugano N, Atsumi T, Ohzono K, Kubo T, Hotokebuchi T, Takaoka K. The 2001 revised criteria for diagnosis, classification, and staging of idiopathic osteonecrosis of the femoral head. J Orthop Sci 2002;7:601-5.
14. Altman R, Alarcon G, Appelrouth D, et al. The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hip. Arthritis Rheum 1991;34:505-14.
15. Shima Y. Present status of surgical treatment of arthrosis deformans of the hip and summary of therapeutic evaluation. Nippon Seikeigeka Gakkai Zasshi 1971;45:828-31.
16. Vilim V, Lenz ME, Vytasek R, et al. Characterization of monoclonal antibodies recognizing different fragments of cartilage oligomeric matrix protein in human body fluids. Arch Biochem Biophys 1997;341:8-16.
17. Thonar EJ, Lenz ME, Klintworth GK, et al. Quantification of keratan sulfate in blood as a marker of cartilage catabolism. Arthritis Rheum 1985;28:1367-76.
18. Li XQ, Thonar EJ, Knudson W. Accumulation of hyaluronate in

human lung carcinoma as measured by a new hyaluronate ELISA. Connect Tissue Res 1989;19:243-53.
19. Conrozier T, Saxne T, Fan CS, et al. Serum concentrations of cartilage oligomeric matrix protein and bone sialoprotein in hip osteoarthritis: a one year prospective study. Ann Rheum Dis 1998;57:527-32.
20. Lohmander LS, Neame PJ, Sandy JD. The structure of aggrecan fragments in human synovial fluid. Evidence that aggrecanase mediates cartilage degradation in inflammatory joint disease, joint injury, and osteoarthritis. Arthritis Rheum 1993;36:1214-22.
21. Lohmander LS, Roos H, Dahlberg L, Hoerrner LA, Lark MW. Temporal patterns of stromelysin-1, tissue inhibitor, and proteoglycan fragments in human knee joint fluid after injury to the cruciate ligament or meniscus. J Orthop Res 1994;12:21-8.
22. Roos H, Dahlberg L, Hoerrner LA, et al. Markers of cartilage matrix metabolism in human joint fluid and serum: the effect of exercise. Osteoarthritis Cartilage 1995;3:7-14.
23. Dahl LB, Dahl IM, Engstrom-Laurent A, et al. Concentration and molecular weight of sodium hyaluronate in synovial fluid from patients with rheumatoid arthritis and other arthropathies. Ann Rheum Dis 1985;44:817-22.
24. Sharif M, George E, Shepstone L, et al. Serum hyaluronic acid level as a predictor of disease progression in osteoarthritis of the knee. Arthritis Rheum 1995;38:760-7.
25. Yamada H, Miyauchi S, Morita M, et al. Content and sulfation pattern of keratan sulfate in hip osteoarthritis using high performance liquid chromatography. J Rheumatol 2000;27:1721-4.
26. Recklies AD, Baillargeon L, White C. Regulation of cartilage oligomeric matrix protein synthesis in human synovial cells and articular chondrocytes. Arthritis Rheum 1998;41:997-1006.
27. Wells AF, Klareskog L, Lindblad S, Laurent TC. Correlation between increased hyaluronan localized in arthritic synovium and the presence of proliferating cells. A role for macrophage-derived factors. Arthritis Rheum 1992;35:391-6.
28. Iwase T, Hasegawa Y, Ishiguro N, et al. Synovial fluid cartilage metabolism marker concentrations in osteonecrosis of the femoral head compared with osteoarthrosis of the hip. J Rheumatol 1998;25:527-31.
29. Jingushi S, Lohmander LS, Shinmei M, et al. Markers of joint tissue turnover in joint fluids from hips with osteonecrosis of the femoral head. J Orthop Res 2000;18:728-33.
30. Robion FC, Doize B, Boure L, et al. Use of synovial fluid markers of cartilage synthesis and turnover to study effects of repeated intra-articular administration of methylprednisolone acetate on articular cartilage in vivo. J Orthop Res 2001;19:250-8.
31. Richardson DW, Dodge GR. Dose-dependent effects of corticosteroids on the expression of matrix-related genes in normal and cytokine-treated articular chondrocytes. Inflamm Res 2003;52:39-49.
32. Hedbom E, Antonsson P, Hjerpe A, et al. Cartilage matrix proteins. An acidic oligomeric protein (COMP) detected only in cartilage. J Biol Chem 1992;267:6132-6.