nificantly elevated compared to healthy controls and correlated to biochemical but not to clinical markers of disease activity. However, serum PIIINP was correlated to the inflamed synovial mass involved using a joint index score ($r = 0.55$; $p < 0.05$) and to the state of destructive changes ($r = 0.64$; $p < 0.05$). Serum PICP did neither differ from controls nor correlate to other markers of disease activity, but was related to duration of disease ($r = 0.84$; $p < 0.01$).

The serum/synovial fluid -ratio was for PIIINP 1:200, for PICP 1:6 and for ICTP 1:3. The synovial concentration of ICTP was correlated to degree of joint damage and to the circulating levels of ICTP.

Six patients have had recurrent synovitis within the first month. However, fluctuations in the collagen markers in serum or intraarticularly, did not differ between groups neither in pre-treatment values nor after 30 days.

Conclusion

The very high local concentrations of PIIINP may reflect a significant type III collagen formation in the synovial membrane. This is supported by the relation between the circulating levels and the synovial mass involved. The value of ICTP as a marker of bone degradation is supported by the correlation between the degree of destructive changes of the joint and the local concentrations of ICTP.

Circulating markers of collagen type I degradation and type III formation reflect the inflammatory state of joint disease.

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An examination of some molecular markers in blood and urine for discriminating patients with osteoarthritis from healthy individuals

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The identification of molecular markers (MM) in blood or urine, which reflect disease changes in osteoarthritis (OA), would greatly facilitate clinical studies (Lohmander et al. 1992). One application for molecular marker measurements is for diagnostic purposes. Thus one criterion for a good molecular marker is that it should discriminate between OA patients and normal individuals. A number of candidate molecular markers have been recently identified (Poole et al. 1994). The purpose of this study was to examine the ability of those markers to discriminate OA patients and normal individuals.

Methods

Sera and 24 hr urines were collected from 398 patients with a diagnosis of idiopathic OA with radiological grade 1–3 and symptoms. All patients had involvement of at least one large joint (knee, n=32, or hip, n=6). Each patient was removed from prior NSAID therapy for one week prior to the baseline visit. A cohort of 20 healthy individuals without joint pain were sampled twice at a one month interval. Samples were stored at –72 °C until assayed.

Keratan sulfate (KS) was measured using antibody AN9P1 in a competitive ELISA (Poole et al. 1989). C-propeptide of type II collagen (CP-II) was measured by RIA (Månsson et al. 1995); bone sialoprotein (BSP) was measured by ELISA (Saxne et al. 1995); cartilage oligomeric matrix protein (COMP) was measured using rabbit polyclonal antibody (Saxne and Heinegård 1992); the chondroitin sulfate epitope 846 of aggrecan was measured as described (Poole et al. 1994). Commercial ELISAs were used to measure C-reactive protein (CRP), Hemagen CRP Kit, Hemagen Diagnostics, Inc., 34 Bear Hill Road, Waltham, MA 02154; TNF-receptor type I

Table 1. Molecular markers—a comparison between healthy individuals and patients with osteoarthritis

Molecular marker[a]	Median value			Ratio of medians	Significance OA vs. normal	Significance in discriminant model	
	Normal 0	OA 1	units[b]	OA / normal	(Wilcoxon)	Full model[c]	Reduced model[d]
Age	49	58	years	1.18		*	**
846	0.08	0.039	µg/mL	0.49	***	*	**
KS	2.03	1.59	µg/mL	0.79	***	NS	
COMP	10.5	8.7	µg/mL	0.83	**	NS	**
TGFß1	48531	41209	pg/mL	0.85	NS	NS	
BSP	108.0	98.9	ng/mL	0.92	NS	NS	
CPII	23.35	22.84	ng/mL	0.98	NS	NS	
LP/CRE	0.93	1.06	nM/cg	1.14	**	NS	
HP/CRE	3.85	4.90	nM/cg	1.27	***	NS	
HA	43.8	57.5	ng/mL	1.31	NS	NS	
ECP	8.9	12.2	ng/mL	1.37	**	NS	
TNFRII	1629.0	2262.5	pg/mL	1.39	***	*	***
TNFRI	998.8	1420.5	pg/mL	1.42	***	NS	
IL-6	1.73	3.22	pg/mL	1.86	***	NS	
CRP	1.08	5.38	µg/mL	4.99	***	*	

[a]For definition of abreviations for the molecular markers, see the Methods.
[b]nM/cg=nM/centigram of creatinine.
[c]Discriminant analysis with all variables left in. P values are for the significance of the variable in the discriminant analysis.
[d]Discriminant analysis with four variables left in. P values are for the significance of the variable in the discriminant analysis.
NS = nonsignificant, * $p<0.05$, ** $p < 0.01$, *** $p < 0.001$.

(Quantikine™ Human sTNF RI Immunoassay, R&D Systems, Minneapolis, MN 55431; TNF-receptor type II, Quantikine™ Human sTNF RII Immunoassay, R&D Systems, Interleukin 6 (IL-6), High Sensitivity Human IL-6 Immunoassay, R&D Systems; transforming growth factor ß1 (TGFß1), Quantikine™ Human TGFß1 Immunoassay, R&D Systems; eosinophil cationic protein (ECP), Pharmacia ECP RIA, Pharmacia Diagnostics AB, S-751 82 Uppsala, Sweden; hyaluronic acid (HA), Pharmacia Hyaluronic Acid Test , Pharmacia Diagnostics AB.

The collagen crosslinks hydroxylysyl pyridinoline (HP) and lysyl pyridinoline (LP) were analyzed after hydrolysis of urinary protein and separation of the cross-links on HPLC and detection using a flourescent detector (Eyre et al. 1984). Creatinine was determined on each urine and the values for HP and LP were divided by the creatinine concentration.

Statistical analyses were made with software from SAS (SAS Institute, Cary, NC 27512) and Statistica (StatSoft Inc., 2325 E. 13th St., Tulsa, OK 74104). The normal and osteoarthritis groups were compared at baseline by discriminant analysis using backward elimination of non-informative variables, by Wilcoxon rank sum statistics, and by calculation of medians. For all statistical tests which require normal distributions, the use of logarithms of all independent variables except age was necessary.

Results and Discussion

In order to determine whether any of the molecular markers were able to distinguish the OA population from the healthy population, we first examined which of the variables demonstrated statistically significant differences between the OA patients and controls. Both visits of the healthy individuals were compared with the baseline visit of the OA patients. Because the values, particularly within the normal group, were in many cases not normally distributed, the log distribution was used and geometric means were determined. The values were then examined using Wilcoxon Rank Sum Scores. For each of the 14 molecular markers (MM), the data are summarized in the Table. Bone sialoprotein, hyaluronic acid, C-propeptide of type II collagen, and TGFß1 all failed to show a significant difference between the OA group and the control group. The ten other MM (TNF-RI, TNF-RII, CRP, ECP, IL-6, KS, HP/Cre, LP/Cre, COMP, 846) all showed highly significant differences between normals and OA patients. In all cases, the 95% range of the normal and the OA populations overlapped.

Discriminant analysis was used to determine which linear combinations of MMs are most efficient for distinguishing OA patients from normal individuals. The method of stepwise backward elimination was used to remove non-significant ($P > 0.10$) variables from the statistical model. At each step, the model was evaluated by cross-validation, in which discriminant coefficients are computed without using values from a particular subject, that subject is reclassified, and the process is repeated for each subject. Rates of errors in classifying normal and OA subjects are

shown in the Table. One near-optimal model contains age plus three MMs (COMP, 846, and TNF type II receptor), and places approximately 90% of both OAs and the normals into the correct diagnosis. The simplest effective model is the one containing age plus TNF type II receptor, which places 90% of normals and 86% of OA patients into the correct diagnostic category.

Conclusion

Based on the analysis of 14 MMs, reliable discrimination of knee OA with better than 90% discrimination of OA and normals could be obtained. It is interesting that the best discrimination in this analysis came from the use of one marker from each of three groups: the inflammatory markers (TNF type II receptor), markers of cartilage synthesis (846) and markers of cartilage turnover (COMP).

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Evolution of a matrix metalloproteinase assay to assess inhibitor potency and selectivity

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Matrix metalloproteinases (MMPs) are a class of zinc-containing extracellular proteinases involved in extracellular matrix turnover and remodeling. As such, these enzymes are critical in maintaining the proper biochemical composition and physical characteristics of all tissues, but in particular those tissues whose composition is largely extracellular matrix, such as cartilage. Under normal physiological conditions, constitutive expression of the MMPs is low, as is their catalytic activity which is held in check by naturally occurring inhibitors termed TIMPs (tissue inhibitor of metalloproteinases). However, under pathologic conditions such as rheumatoid and osteoarthritis, MMP expression in cartilage is disregulated. MMP levels are high with enzymic activity exceeding the level of the natural inhibitors. This condition leads to a loss of proteoglycan and collagen from articular cartilage culminating in the ultimate destruction which characterizes the pathology of arthritic diseases. Based upon this scenario, intensive efforts are focused on the identification of inhibitors which will block the action of MMPs in arthritis. Predictive, high through-put enzymic assays are essential for inhibitor identification. Ideally, such assays must be convenient, kinetically predictive, and flexible to accommodate the numerous candidate MMPs potentially involved in cartilage degradation. Thus, we have evaluated several MMP assays for inhibitor assessment and these are detailed below.

Our initial assay was based upon proteolysis of ^{3}H-transferrin (H Nagase, personal communication). This assay employed detection of protease cleavage products based upon the extent of solubilization of TCA precipitable radioactivity initially present in the transferrin substrate. The advantages of this assay were its broad utility toward several proteases and ease of detection of enzymic activity present in cartilage extracts or culture medium. Although this assay gave linear kinetics with respect to time and enzyme, its linearity with respect to substrate was anomolus.