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Hyaluronic acid levels may have predictive value for the progression of knee osteoarthritis

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Summary

Study objectives: To study prognostic value of different biochemical markers for morphological progression of early knee osteoarthritis.

Design: A total of 89 patients with knee osteoarthritis (OA) were enroled into the study. The follow-up period was 2 years. Radiological OA progression was evaluated by measuring joint space width. Pentosidine was detected using the HPLC method described earlier, cartilage oligomeric matrix protein (COMP) using the method published by our team. MMP-9, tissue inhibitors of metalloproteinases (TIMP), YKL-40 and hyaluronic acid were detected using commercially available kits.

Results: In the group of patients suffering from knee OA, higher serum levels of pentosidine ($P=0.04$), MMP-9 ($P=0.02$), TIMP ($P=0.04$) and COMP ($P=0.05$) were detected compared with healthy control subjects. Using a correlation analysis method, it has been found that the patients with higher basic serum levels of hyaluronic acid had a faster radiological progression ($r=0.56$, $P<0.005$), as well as the patients with higher basic serum pentosidine levels ($r=0.30$, $P<0.005$). Other biochemical markers had no statistically significant prognostic value.

Conclusions: In our study, serum levels of hyaluronic acid and pentosidine had a predictive value for further development of knee OA in that further joint space narrowing was detected in the patients with knee OA in the next 2 years.
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Key words: Osteoarthritis, progression, biochemical markers, hyaluronic acid, pentosidine.

Introduction

Osteoarthritis (OA) is the most common joint condition affecting up to 12% of the population[1]. Its prevalence in the population of 65 years of age and over is almost 50%. OA often causes disability resulting in enormous financial costs of the therapy, including final surgical treatment.

The American College of Rheumatology (ACR) has published the criteria for diagnosis and classification of OA. However, these are not convenient for the detection of early OA stages[2–4].

Also, the evaluation of OA progression has not been successfully addressed despite different organizations such as OARSI (Osteoarthritis Research Society International)[5] or OMERACT (Outcome Measures In Rheumatoid Arthritis Clinical Trials)[6] publishing their 'Recommendations'. These outcomes may be classified as indicators evaluating the symptoms (pain and functions), and indicators of the morphological seriousness and progression of the disease[7]. There has been a common view that in order to be able to evaluate the effectiveness of chondroprotective therapy, or 'structurally modifying drugs' as they are also called[5], it is necessary to identify a

morphological criterion. Although such methods as MRI scan[8] and ultrasound are being tested at present, the only validated indicator is still a flat X-ray image.

Recently a debate has been stirred concerning the most advantageous radiological equipment and techniques for OA progression evaluation[9]. The debate mainly concerns knee joints. There have been reports that most investigators now prefer imaging in semiflexion[10], both assisted and unassisted, with the help of fluoroscopy, and a digital computer assessment of the joint space width[11]. However, no clearly defined X-ray protocol has yet been published.

Biochemical OA markers represent a relatively new and rapidly developing research field. The possibility for using these markers for early OA diagnosis and prognostic evaluation as well as the methods of OA progression were proposed more than 15 years ago. The international GREES group has proposed the following points of interest which should be addressed by future trials studying biological markers[12]:

a use them as surrogate indicators for diagnosis, in addition to X-ray,
b identify healthy persons with a possible risk of OA,
c predict the development of early stages (both treated and untreated),
d evaluate OA progression.

The most complicated task is to identify and select suitable biochemical markers. OA is a structurally complex

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disease manifesting itself by the destruction of joint carti-lage, subchondral bone, sclerosis and cysts, osteophytes and synovial inflammation. Cartilage destruction is consid-ered to be the most important and primary event and therefore cartilage attracts most of the attention. However, it is also important to take other joint tissues into account.

Biochemical markers may be classified according to several aspects. According to their origin, it is possible to classify the markers as originating from cartilage, bone, or as markers reflecting synovial inflammation. The advan-tage of the marker would be its specificity to a certain tissue. However, this is mostly not the case. The markers may further be specified as indicators of degradation, indicators of synthesis and pure indicators of inflammation. Another classification of the markers is according to the compartment in which they may be detected: serum, urine and synovial fluid. During the last year, pentosidine a new potential indicator has been identified[13].

Increased biochemical marker levels have been ident-ified e.g. in the serum or urine of OA patients compared with healthy control subjects in proteoglycan monomer fragments[14], keratansulphate[15], cartilage oligomeric matrix protein (COMP)[16], C telopeptide collagen II[17], hyaluronic acid[18], CRP[19], YKL 40[20], metalloproteinases[21] and pyridi-noline[22]. Predictive biochemical marker values for OA progression have been published for COMP[23], hyaluronic acid, CRP and TIMP[24,25].

The main objectives of our study were to: (a) perform a longitudinal clinical and morphological study of OA progres-sion in early/moderate OA and look for a correlation be-tween clinical and different morphological indicators (RTG, MRI, ultrasound and scintigraphy), (b) evaluate a battery of potential biochemical OA markers and to express a stand-point with regard to their value as predictive morphological progression indicators. In this study, the results will be presented which correlate biochemical markers with struc-tural damage. The correlation with clinical parameters will be the subject of the next publication.

Methods

In the longitudinal study, patients were followed for 2 years. The patients were examined at the beginning and at the end of the evaluated time period.

PATIENTS

Patients with knee OA according to the ACR criteria were enroled into the study[2,3] all had baseline radiographs and have proven osteophytes. These were symptomatic patients with a reported pain during the last month. The disease duration (from its diagnosing) did not exceed 3 years. Each patient has provided his/her written consent with the participation in the study. In the control group we used 20 volunteers employed in the Institute whose mean age was the same as that of the studied group and who did not suffer from clinically manifest osteoarthritis of the knee or other joints. Blood sera from healthy volunteers were only taken at baseline for ethical reasons.

RADIOLOGICAL EVALUATION

For X-ray imaging, a technique with knee in extension was used in order to achieve maximum standardization, as we described earlier[27].

The joint space width was measured at the narrowest point in the medial compartment of the femorotibial joint. For measuring, a 0.1 mm caliper and magnifying glasses was used. Each image was read by two experienced radiologists who did not know which of the images was first or last. This method, described by Lequesne[28], has been modified at our Institute[29]. When using this method, a so-called intraobserver error was 3.6% and interobserver error was 6.5%. In addition to the measurement of the joint space when evaluating OA progression, we also used the atlas published by R. Altman[30]. For bilateral affection, the knee which was in a worse clinical state was selected for examination.

BIOCHEMICAL MARKERS

After the collection, the blood samples were centrifuged and the serums were frozen at −70°C until their final processing.

COMP was determined using a sandwich ELISA mono-clonal antibodies 17-C10 and 16-F12[31]. Intra and inter essay variance of the method has been established as 8% and 10% respectively.

ELISA MMP-9, MMP-13 AND TIMP-1

The levels of matrix metalloproteinases MMP-9 and MMP-13 and of their tissue inhibitor TIMP-1 were detected by the ELISA method, using kits produced by the Biotrak/ Amersham Biosciences Company. To detect MMP-9 and TIMP-1, ELISA plates were covered with the serums diluted at 1:20, to detect MMP-13 undiluted serums were used. Each sample was applied into two pits. The concentrations were evaluated using a computer program of the ELISA reader Sun Rise supplied by the Schoeller Instruments company.

PENTOSIDINE

Serum pentosidine was detected by a method published by us earlier[13,40] which is based on high-pressure liquid chromatography (HPLC) in reversion phase. Pentosidine (PEN) monitoring was performed using a fluorescent de-tector at excitation and emission wavelength $\lambda_{EX}/\lambda_{EM}$=335/385 nm, column temperature was 40°C, mobile phase flow rate was 0.5 ml/min. A brief method description: 1 ml of the sample (serum, urine) was mixed with 1 ml of concentrated HCl (35%) and hydrolyzed at 105°C over the period of 16 h. Preliminary hydrolyzate refining (0.5 ml) was performed with spheric cellulose CC31 by selective sorption in 15 ml of n-butylalcohol – acetic acid – water mixture (8-1-1) and by desorption using 3 ml 0.05 M HCl. The hydrolyzate was evaporated and after being dissolved in 250 µl of the mobile phase, was applied into the HPLC column in the amount of 10 µl. The reproducibility of the HPLC evaluation was 1%, reproducibility of the entire method (i.e. including hydrolyzation and refining) 4.44%, recovery was 77±3.5% and sensitiveness limit was 17.6 femtomols.

STATISTICAL ANALYSIS

The comparison of OA patients with healthy control subjects was performed using the *t*-test.

The correlation of biochemical markers with joint space narrowing was performed using a correlation coefficient in linear regression.

Table I

	Demographic characteristic of the OA patients with knee joints (N=89)	
	Mean±SD	Controls
Males/females	30/59	8/12
Age (year)	56.7±7.2	51.7±6.4
Disease duration (years)	2.9±1.8	–
BMI	28.6±4.6	27.8±5.2
Joint space at the beginning of the study	4.95±1.46	–
Joint space narrowing over 2 years (mm)	–0.40±0.79	–

Table II
Biochemical markers in patients with knee OA

Indicator	Study beginning		Study end		Controls
	Value	P 1	Value	P 2	
Hyaluronic acid (ng/ml)	30.2±19.6	NS	27.1±16.6	0.1	32.5±20.6
Pentosidine (nmol/l)	143.4±99.0	0.04	125.6±76.9	NS	115.3±34.0
YKL-40 (ng/ml)	59.0±34.9	NS	64.0±41.7	NS	65.8±35.3
MMP-9 (ng/ml)	147.1±101.4	0.02	150.7±131.9	0.05	97.9±67.0
TIMP (ng/ml)	872.7±155.1	0.04	833.8±161.8	NS	781.8±145.7
COMP (ng/ml)	1063.2±626.4	0.05	1235.5±647.5	0.04	1022.9±646.0

*N=89. MMP-9: Metalloproteinase 9; MMP-13: metalloproteinase 13; TIMP: tissue inhibiters of metalloproteinases; COMP: cartilage oligomeric matrix protein; P 1: significance, *t*-test of the original value compared with control values; P 2: final values compared with control values.

Results

A total of 100 patients were enroled into the study of which 89 patients presented themselves for the final examination after 2 years. There were 59 females and 30 males (Table I). The mean age was approximately 57 years and the mean disease duration was 2.9 years.

RADIOLOGICAL PROGRESSION EVALUATION

The joint space width was 4.95±1.46 mm at the beginning of the study and 4.55±1.58 mm at the end of the study. The mean joint space narrowing was 0.4±0.79 mm over the period of 2 years, which corresponds with a mean yearly progression of 0.2 mm.

BIOCHEMICAL MARKERS

Table II shows the comparison of biochemical marker values at the beginning and end of the study between the entire group of patients with osteoarthritis and the healthy control subjects. At the beginning of the study, higher values of pentosidine (143.4±99.0 nmol/l vs 115.3±34.3 nmol/l, *P*=0.04), MMP-9 (147.1±101.4 mg/ml vs 97.9±67.0, *P*=0.02), TIMP (872.7±155.1 ng/ml vs 781.8± 145.7 ng/ml, *P*=0.04) and of COMP (1063.2±626.4 ng/ml vs 1022.9±6460 ng/mol, *P*=0.05) were detected. At the end of the study, only the values of the following markers were significant compared with control subjects: MMP-9 (150.7±131.9 vs 97.9±67 ng/ml, *P*=0.05) and COMP (1235.5±647.5 vs 1022.9±646.0 ng/ml, *P*=0.04). No significant changes were registered during the 2-year period.

We have also performed a correlation of individual biochemical marker values in relation to the narrowing of the joint space. A significant relation has been detected for the correlation between the initial values of hyaluronic acid and joint space narrowing during the next 2 years (*r*=0.56) (*P*<0.005) but not for hyaluronic acid values at the end of the study (*r*=0.06) (Fig. 1). Basic pentosidine levels (*r*=0.30) (*P*<0.005) had a significant relation to further joint space narrowing and a positive relation was also detected at the end of the study (*r*=0. 34, *P*=0.005) (Fig. 2). No correlation has been found between initial and final COMP levels and joint space narrowing (Fig. 3).

Discussion

The objective of our study was mainly to identify the importance of so-called biochemical markers for OA progression. A group of patients suffering from primary knee OA of a relatively early stage (<3 years) were selected for the study.

Joint space narrowing over a 2-year period was 0.4 mm, which corresponds with 0.2 mm a year. Literature data on a mean yearly joint space narrowing differ substantially (10 times) ranging from 0.06 to 0.6 mm/year[32]. In our previous studies, we detected a yearly joint space narrowing rate of only 0.1 mm/year[27,29]. Apart from the used technique, the yearly joint space narrowing rate also depends on patients selected for the study. In our study, the patients suffered from more severe symptoms than in previous studies. As to radiological technique, the patients' knees were in extension when making X-ray images, the reason for it being an uncertainty at the beginning of the study (and virtually to the present time) as to which semiflexion techniques is the most advantageous for longitudinal patient studies[33].

One of the possibly important features of biochemical OA markers is their potential as a diagnostic tool. In accordance with a published literature, we found higher COMP[16], TIMP[26] and MMP 9[34] values in patients with OA. The finding of higher pentosidine levels in OA patients compared to healthy control subjects is relatively new.
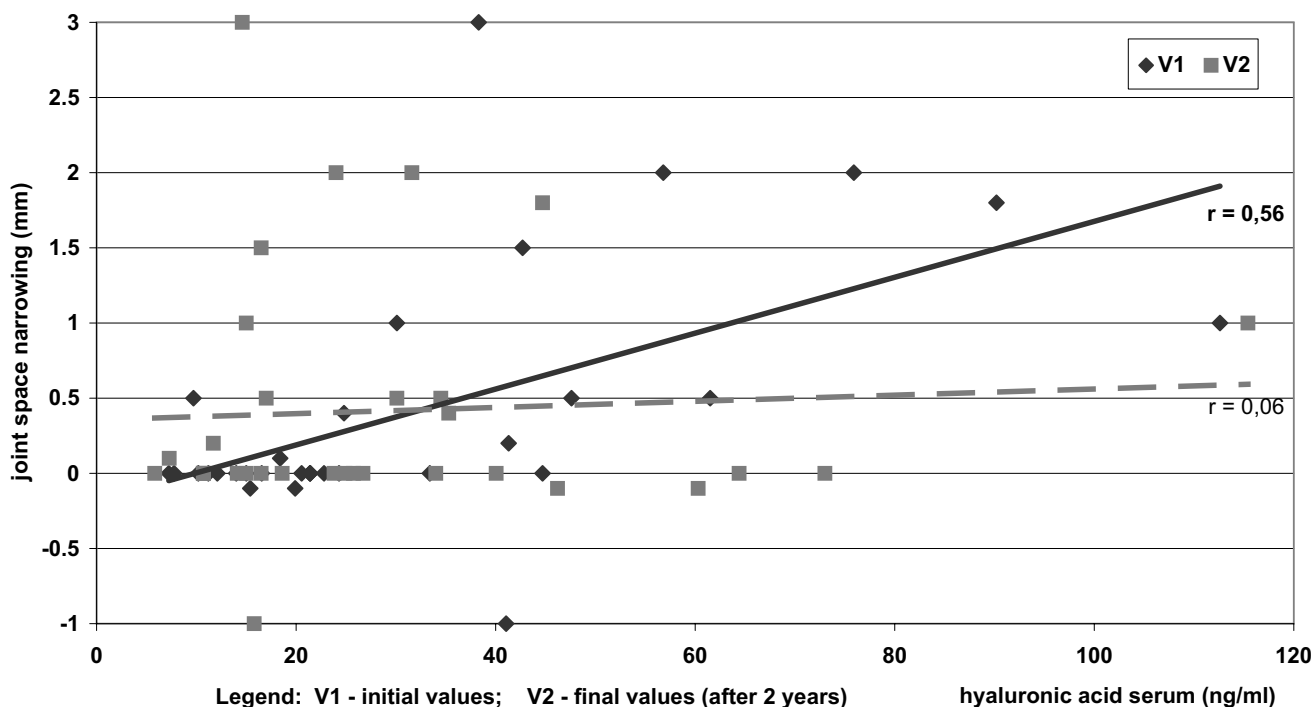
Fig. 1. Correlation between serum hyaluronic acid levels and knee joint space narrowing in patients with OA during 2 years.
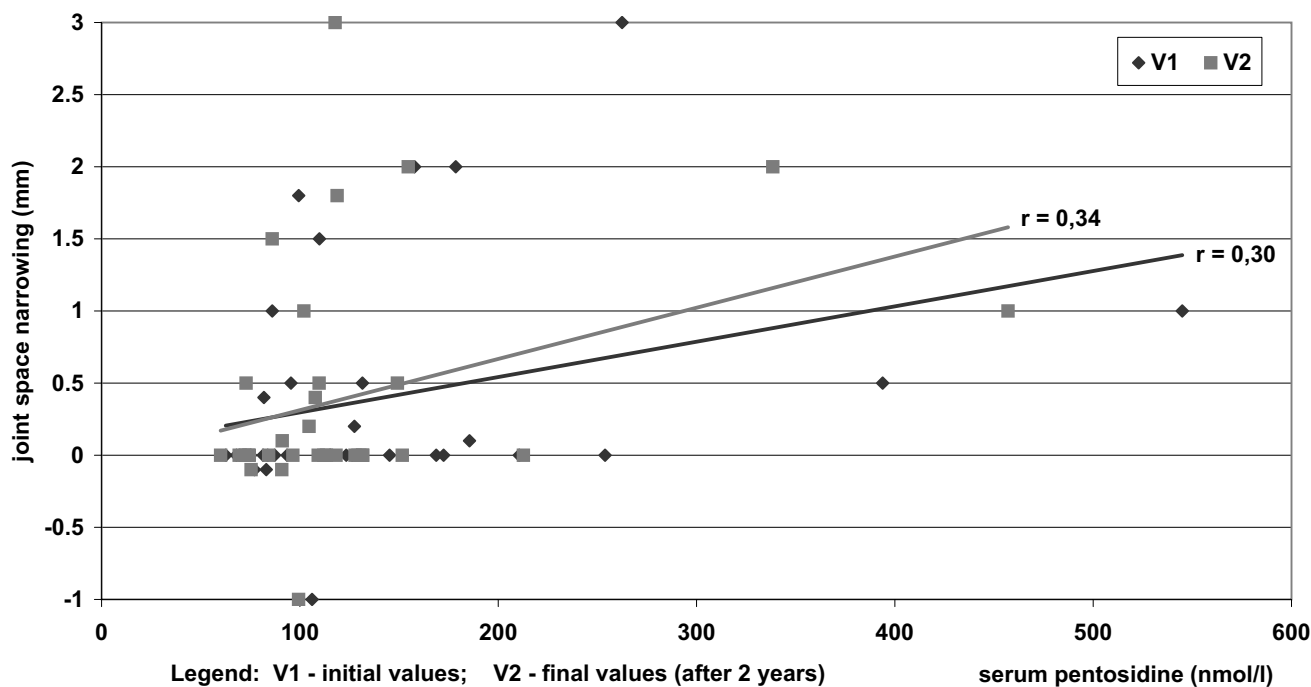

Fig. 2. Correlation between serum pentosidine levels and knee joint space narrowing in patients with OA during 2 years.

However, despite the fact that these values were significantly higher in the group with OA compared with the control group, the clinical importance of the observation is disputable. The differences are higher only to a certain extent and in so-called healthy control subjects there is such a variance in the values that their importance for an individual patient is rather low.

Apart from the diagnostic importance of biochemical markers also their prognostic value is being considered.

In this study, a higher progression risk of OA progression was posed by increased serum hyaluronic acid values, which is, however, predominantly a marker of synovial inflammation. A prognostic value of serum hyaluronic acid values has already been published[18].
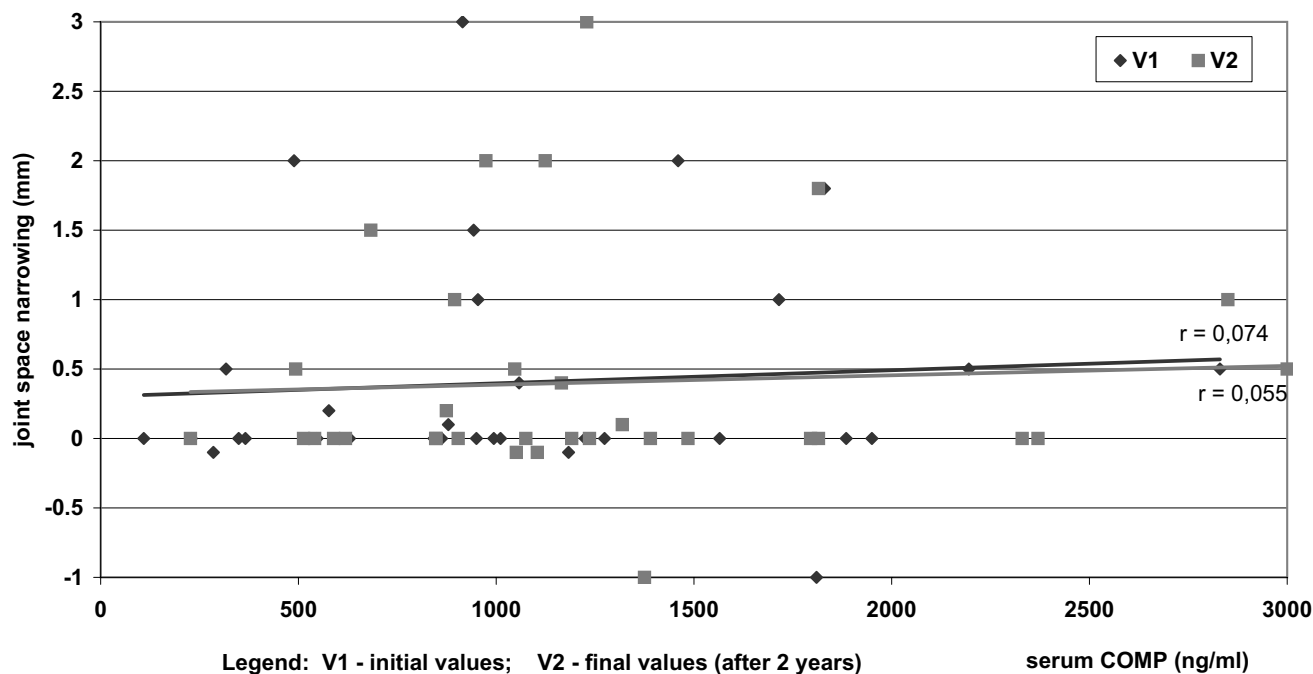
Fig. 3. Correlation between serum COMP levels and knee joint space narrowing in patients with OA during 2 years.

Final glycation products (advanced glycation end-products – AGE) have enjoyed the attention of researchers in the last 10 years[35]. Of the AGE group, one of the best-defined products is pentosidine detectable by the HPLC method. AGE have been studied in connection with aging[36] but have also been detected in a number of other diseases such as renal insufficiency[37] or rheumatoid arthritis[38]. Recently, De Grott *et al.*[39] has found AGE accumulation in joint cartilage which had a negative impact on the balance of synthesis and degradation of extra-cellular matrix proteoglycans. Recently, Špacek *et al.* has detected only insignificantly higher urine pentosidine levels in knee OA patients compared with healthy control subjects However, after the surgery of the joint affected by the arthritic process, a significant decrease in urine pentosidine levels was detected[13]. Pentosidine serum levels are not tissue specific, but were found in another study correlation of pentosidine and COMP in synovial fluid[41]. We have found in the same study, correlation of pentosidine in synovial fluid and serum (r^2=0.7, P<0.001) but no corre-lation in COMP levels in synovial fluid and serum (r^2=0.00092). We suggest that pentosidine may be a more suitable putative local marker of cartilage pathology than COMP.

To establish a potential prognostic value of pentosidine, which has been reported in this study, further trials will be required.

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