Osteoarthritic patients with high cartilage turnover show increased responsiveness to the cartilage protecting effects of glucosamine sulphate

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Abstract

Objective

Glucosamine sulphate has been shown in a large double-blind, placebo-controlled clinical trial to prevent structural damage and improve clinical symptoms of osteoarthritis (OA). We investigated whether early response in a newly developed biochemical marker of collagen type II degradation (CTX-II, CartiLaps ELISA) could reflect the long-term preservation of hyaline cartilage.

Methods

Study subjects comprised 212 knee OA patients participating in a clinical trial of the effects of glucosamine sulphate. Disease symptoms were assessed quarterly by WOMAC scoring and X-ray analysis was performed at baseline and after 3 years. Urine samples were obtained at baseline and after 1, 2 and 3 years for measurement in the CartiLaps assay. The measurements were corrected for creatinine.

Results

At baseline the patients had an average concentration of urinary CTX-II of 222.4±159.5 ng/mmol creatinine. This was significantly above the CTX-II levels measured in urine samples from 415 healthy controls (169.1±92.3 ng/mmol, $p<0.0001$). There was no significant difference in the CTX-II response in the placebo group and the glucosamine treated group. However, those with high cartilage turnover presented a significant decrease in CTX-II after 12-month glucosamine treatment. Thus, thee group with CTX II concentrations above normal average + 1SD decreased 15.5 % after 12-month therapy. The 12 months change in CTX-II in OA patients with elevated CTX-II at baseline correlated with the change in average joint space width observed after 36 months ($R=0.43$, $p<0.05$). Increased baseline levels of CTX-II were associated with a worsening of the WOMAC index ($p<0.01$).

Conclusion

The data indicate that measurement of urinary collagen type II C-telopeptide fragments enables the identification of OA patients with high cartilage turnover who at the same time are most responsive to therapy with structure modifying drugs.

Key words

Osteoarthritis, glucosamine sulphate, collagen type II, biochemical marker.

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Introduction

A central element of osteoarthritis (OA) is the non-reversible degradation of cartilage, which starts prior to clinical diagnosis of OA and persists until the end stage of the disease where most articular cartilage of affected joints is lost. At this stage the mobility of the joint is severely compromised, and joint replacement surgery remains the only treatment option.

The majority of therapies in OA act by relieving disease symptom (i.e. non-steroidal-anti-inflammatory drugs (NSAID) and the newer COX-2 inhibitors). In fact, a recent review of the literature indicates that different classes of NSAIDs may have effects on chondrocytes ranging from deleterious to beneficial with regard to glycosaminoglycan synthesis (1). Very few, if any, therapies are available that have a convincing effect of slowing or halting the underlying cartilage degradation, which is the prime culprit causing the progressive joint destruction accompanying the disease. Thus, there is an unmet therapeutic need for compounds which can act on the cells and enzyme systems mediating the cartilage degradation in OA.

Recently it was reported from a placebo-controlled clinical trial that glucosamine sulphate had beneficial effects on both the symptoms and underlying cartilage degradation process in OA (2). Glucosamine sulphate is the sulphate salt of the naturally occurring aminosaccharide glucosamine, which is an essential component of glucosaminoglycans and proteoglycans in cartilage. The pharmacological actions of glucosamine on cartilage and joint tissues have not been fully elucidated. Derivatives of the compound is suggested to act as a free radical scavenger and inhibitor of nitric oxide synthesis (3), but glucosamine or its metabolites may also have long term effects as modulator of cartilage metabolism and stimulation of anabolic activities concomitant with a down regulation of matrix metalloproteinase activity in cartilage (4-6).

The potential beneficial actions of glucosamine on joint tissue metabolism have prompted us to investigate whether this compound is able to down regulate cartilage degradation in OA patients. This process was monitored with an immunoassay specific for fragments derived from the C-telopeptide domain of collagen type II (CTX-II, CartiLaps ELISA) (7). This assay has been demonstrated to reflect situations with elevated cartilage degradation such as OA and RA, and it has been used in longitudinal studies for monitoring the effect of steroid treatment in RA (7,8). In a cross sectional evaluation of biochemical markers of bone, cartilage and synovial tissue metabolism in patients with knee OA, increased levels of the CTX-II marker was shown to correlate with radiological damage (9) and similar data has also been obtained in a study of recently diagnosed RApatients (10). The assay has also been shown to reflect a potential structure modifying effect of bisphosphonate treatment, suggesting that the marker may be useful to monitor therapies acting on cartilage metabolism (11).

In the present study we set out to obtain answers to the following questions: (1) Do CTX II levels differentiate patients with and without manifest OA ? (2) Can we by the use of the biological marker isolate high risk (i.e., high turnover) patients who presumably gain more therapeutic benefits by the use of structure modifying agents? (3) Can early responses in the biological marker find a potential role for reassuring patients to continue long-term therapy with the structure modifying drug with the hope of preserving cartilage? These questions were addressed in a cohort of 212 knee OA patients treated with oral glucosamine sulphate 1500 mg daily or placebo for 3 years. Agroup of 415 men and women without clinically manifest OA was also examined to obtain reference values of the biomarker for comparative purposes.

Materials and methods

Study protocol and patients

The study design and patient inclusion criteria as well as baseline demographic data have previously been published (2). Briefly described, the study cohort consisted of patients above 50 years of age (mean 66 years) with OA of the medial femoro-tibial compartment diagnosed according to the clinical and

radiological criteria of the American College of Rheumatology. The 212 patients (162 female) were recruited at the Bone and Cartilage Metabolism Research Unit of the University Hospital Centre in Liege, Belgium. The severity of their disease corresponded to grade 2 or 3 on the Kellgreen and Lawrence scoring scale, with an average disease duration of 7.8 years. They were divided in two groups equally sized (n = 106) treated with either 1500 mg glucosamine sulphate daily or placebo for three years. The glucosamine sulphate used in the study was a defined pure substance produced from chitin according to GLP practice by Rotta Research Group and approved by the regulatory European authorities. In the preparation given to the patients, glucosamine, sulphate, chloride and sodium ions are present in of stochiometric ratios 2:1:2:2. Urine samples were obtained at baseline and after 12 months as second morning void samples without dietary restrictions.

The primary outcome measures in the trial were disease symptoms as assessed by the Western Ontario and McMasters University osteoarthritis index (WOMAC, VA 3.0 version) performed quarterly, and apparent structural damage as the mean joint space width assessed by X-ray analysis of the medial compartment of the tibio-femoral joint. The radiographs were digitized and analyzed automatically using an automated system (11). Additionally the minimum joint space width was determined visually on the radiograph using a 0.1 mm graduated magnifying lens. All radiographs were obtained at a single radiological unit in Liege and quantified in London by a single reader blinded to the treatment assignment of the patient. The mean (SD) short- and long-term coefficients of variation of this measurement method were calculated to 1.82% (1.29) and 1.62% (1.31) respectively for the medial compartment (2).

The control cohort consisted of 133 men and 282 women above 50 years of age (average age 61.4±7.6 years, range 50 – 79 years) without clinically manifest arthritis or other musculoskeletal-skeletal disorders. None of these patients had taken any medication with a documented effect on bone or cartilage metabolism. The local Ethics Committee in the county of Northern Jutland approved the study. All participants gave their written informed consent prior to inclusion. Urine samples from the control subjects were obtained as second morning void and were measured in the CTX-II assay to provide a reference range for comparison to the knee OA patients of the glucosamine study.

CTX-II measurements

Urinary levels of collagen type II C-telopeptide fragments were measured by the CartiLaps ELISA assay. The assay uses a highly specific monoclonal antibody MAbF46 specific for a 6-amino acid epitope (EKGPDP) derived from the collagen type II C-telopeptide (7). Briefly described, the assay was developed as a competitive immunoassay, where streptavidine-coated plates (Micro Coat, Munich, Germany) are incubated first with a biotinylated peptide representing the EKGPDP collagen type II derived epitope, and then with sample or calibrator in combination with the MAbF46 antibody diluted in a phosphate buffered saline (PBS) derived assay buffer. After an overnight incubation at 4°C, a peroxidase-conjugated rabbit anti-mouse antibody is added (Jackson Laboratories, USA), and the amount of bound antibody is visualized by the use of a chromogenic peroxidase substrate. The concentration of unknown samples is determined by constructing a standard curve from measurement of the calibrators with known concentrations of EKGPDP peptide. All samples were measured in duplicate. All samples from one individual were measured in the same ELISA plate and two control samples were included on each ELISA plate. The average intra- and inter- assay CV was 7.1% and 8.4%, respectively (7). Three genuine control samples were included on each microtitre plate and if measurements deviated by more than ±20% from the predetermined values the plate was re-measured.

The concentration of the CTX-II ELISA (ng/l) was standardized to the total urine creatinine (mmol/l): concentration/creatinine =ng/mmol. Creatinine concentration was measured using a Cobas MIRAanalyzed according to the manufactures instructions (Roche Diagnostics, Basel, Switzerland).

Statistical methods

Measurement results obtained with the CTX-II biomarker were not normally distributed and consequently the mean values and standard deviations were calculated by non-parametric statistics. Differences between groups were assessed by non-parametric Man-Whitney tests and Kruskal-Wallis one-way analysis of variance (ANOVA). Subgroup analysis for assessment of high-turnover patients was performed using pre-defined cut-off value of 1 standard deviation above the mean CTX-II level in the healthy reference cohort corresponding to 261.3 ng/mmol, and a further subgroup defined by significantly elevated baseline CTX-II above the 95% percentile of the control cohort (350.4 ng/mmol). Subsequent statistical analysis was done for both cut-off values comprising 61 (+1 SD cut-off) and 28 (95% percentile cut-off) study subjects. Similar results were obtained for the two pre-defined high turnover sub-populations. However, the smaller population size in the 95% percentile group compromised the statistical analysis and thus only results for the larger high turnover sub-group with CTX-II levels 1 SD or more above the controls are reported. Spearman's correlation tests were used to assess the association between the CTX-II marker and parameters of disease symptoms and radiological assessments of structural damage. A p value <0.05 was considered significant for the differences and correlations.

Results

Cartilage turnover at baseline and identification of high and low turnover patients

The mean CTX-II level in the baseline samples from the OAcohort with clinically manifest OA was significantly elevated compared to a matched control cohort (222.4 ± 159.5 vs. 169.1 ± 92.3 ng/mmol, $p < 0.001$). No signifi-

Table I. CTX-II levels, total WOMAC score and X-ray parameters in the study cohort of the glucosamine sulphate study grouped according to cartilage 'turnover status' assessed with the CartiLaps ELISAspecific for collagen type II C-telopeptide fragments (CTX-II).

	'High Turnover'group	'Low Turnover'group
CTX-II (ng/mmol)	Above 261.3	Below 261.3
Number of patients	61 (14 male)	151 (36 male)
WOMAC (Total index)	1030 ± 421	971.5 ± 501
Total joint space width (mm)	5.10 ± 1.34	5.45 ± 1.31
Minimum joint space width (mm)	3.67 ± 1.35	4.00 ± 1.26

cant differences were found in age or sex distribution between the control and the OA cohorts. Although the OA population had higher levels of CTX-II compared to controls, a considerable 'overlap'was apparent between the two groups.

In order to analyze disease parameters in OApatients with an elevated CTX-II concentration, two groups of patients with elevated cartilage turnover were analyzed separately from the total patient cohort. One group constituted 'elevated turnover' OA patients having a pronounced elevation in baseline CTX-II level above the normal average + 1 SD (261.3 ng/mmol). This group was analyzed separately as a potential 'high risk' group of patients (Table I). OA patients with a high turnover rate defined as baseline CTX-II levels 1 SD above the normal average comprised approximately one-third of the study population (29 glucosamine, and 32 placebo treated patients). Data on disease symptoms and X-ray parameters of structural damage from the entire patient cohort has previously been published (2).

Compared to the patients with CTX-II levels below normal average+1 SD, the patients in the 'high-turnover' group with baseline CTX-II levels 1SD above the average of the control cohort were characterized by higher WOMAC scores (total score of 1030 vs. 971.5, $p > 0.1$) and a lower average joint space width (5.1vs. 5.45 mm, $p = 0.09$) and lower minimum joint space width (3.67vs 4.00, $p < 0.05$) (Table I). There was no difference in sex distribution or average age or BMI in the two subcohorts of the OA patient population grouped according to CTX-II levels.

The 'high-turnover' patients treated with glucosamine had an average gain in joint space width (JSW) of 0.083 mm over 3 years, whereas the placebo patients in this group had a decrease in JSW of 0.44 mm ($p = 0.07$) (Fig.1). In the total cohort, the glucosamine treated patients had a decrease in joint space width of 0.06 mm compared to 0.31 mm in the placebo treated group (2).

The glucosamine treatment resulted in a significant improvement in disease symptoms compared to the placebo group, as previously described (2). Among the high-turnover group the global WOMAC score at 12 months showed a 21.5% decrease in the glucosamine treated group compared to a 5.9% decrease in the placebo-treated patients. At the end of the study (after 36 months) the high-turnover glucosamine patients had a 24.5% decrease in the global WOMAC score, which was indicative of clinical improvement, whereas the placebo treated high-turnover patients had a 4.5% increase, indicative of an unchanged or mildly worsened clinical status (Fig. 1).

Effect of glucosamine treatment on CTX-II levels

When taking the entire study population into account, the 12-month value for CTX II was lower in the glucosamine treated compared to the placebo treated group, but differences did not reach statistical significance (Fig. 2). Thus, absolute levels CTX-II in the glucosamine treated and placebo groups were 235 ± 8 and 276.5 ± 14 ng/mmol, respectively (mean ±SEM). Baseline levels were comparable; 216.5 ± 9 ng/mmol and 219.5 ± 9 for the treated and

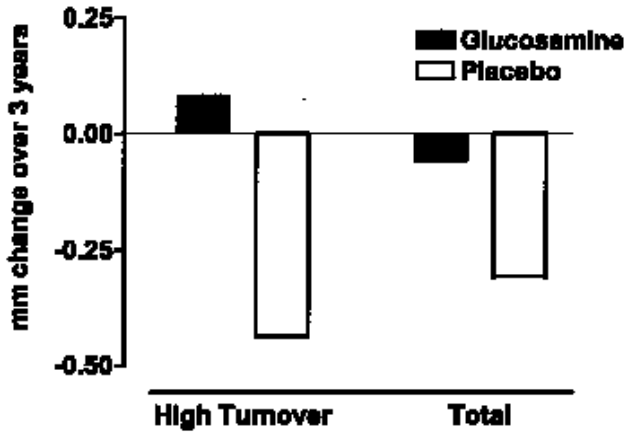

Fig. 1. Progression in radiologically assessed structural joint damage parameters over the 3-year study period in knee OApatients with baseline CTX-II levels 1 SD above the mean of the reference population ('high-turnover' group) and of the total study cohort. The patients were divided into treatment (■) and placebo (❒) groups.

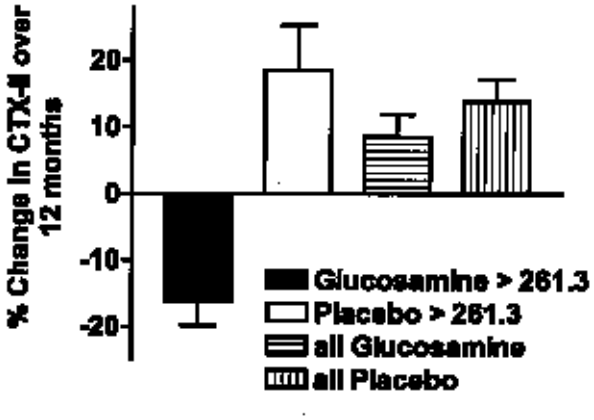

Fig. 2. Response over 12 months to glucosamine or placebo treatment. The response is expressed as the average % change from baseline within the high turnover group with baseline CTX-II levels above 261.3 ng/mmol and in the total study cohort.

the placebo group, respectively. Thus, in both groups a small increase from baseline had occurred over the first 12 months of the study period. However, when comparing patients with 'high-turnover', who were hypothesized to benefit the most from the therapy, we found a statistically significant decrease from baseline of 16.2% in the glucosamine treated group compared to the 18.6% increase in the placebo group during the first 12 month of the treatment (Fig. 2). Absolute concentrations the glucosamine treated high turnover group decreased from 413±28 to 336±26 ng/mmol whereas in the placebo group they increased from 375±33 to 411±52 ng/mmol during the 12 months.

Correlation between the change in CTX-II and structural damage assessed by X-ray

The change in CTX-II concentration observed over the first 12 months in patients with elevated baseline CTX-II concentrations 1SD above the average of the control cohort was correlated with the 3-year progression in structural damage as assessed by mean joint space width on X-ray analysis ($R = 0.43$, $p < 0.05$) (Fig.3). In the placebo group there was a weak correlation between baseline CTX-II and the change over 3 years in the joint space width ($R = 0.27$, $p = 0.03$).

Correlation between disease symptoms and CTX-II

The correlation between baseline CTX-II levels and the WOMAC scores was assessed. When the entire study cohort was included in this analysis, a relatively low correlation was seen between the baseline measurement of cartilage degradation and subsequent WOMAC scores. However, when high-turnover patients were analyzed separately, the placebo treated OA patients showed a significant correlation to subsequent WOMAC scores, peaking at 18 months (Fig. 4). This indicates that the current level of cartilage degradation as assessed by the CTX-II assay may be related to the subsequent development of disease symptoms.

Discussion

Glucosamine sulphate has in a large placebo-controlled study been demonstrated to provide significant relief of disease symptoms and to prevent structural joint damage as assessed by X-ray in knee OA patients (Fig.1) (2). A second study comprising a comparable cohort of patients have provided similar results (13) and thus there is mounting clinical evidence for a structure modifying effect of glucosamine in OA. In this analysis we assessed whether the CTX-II assay may provide a useful diagnostic tool for the evaluation of those patients who benefit the most from such treatment. Our results suggest that OA patients with elevated CTX-II at baseline (high-turnover patients) are more responsive to such treatment, as suggested by the observation that in these patients glucosamine sulphate evoked a significant decrease in the rate of cartilage degradation during the first 12 months of the treatment period. Furthermore, the change in CTX-II was indicative of the degree of structural damage, the final outcome of the treatment.

Cartilage degradation was estimated by measuring the urinary excretion of the C-telopeptide fragments of collagen type II. The CTX-II assay is highly specific for collagen type II, which is exclusively found in cartilage where it is a major structural component (14, 15). Specific proteolytic degradation of collagen type II is a key event in cartilage turnover both *in vitro* and *in vivo* (15) and thus metabolites derived from this protein hold a potential as biochemical markers of cartilage degradation (17,18). In two cross-sectional studies on knee and hip OA patients, CTX-II levels were significantly elevated and CTX-II levels correlated with the clinical severity of OAand the remaining cartilage surface area and joint space width assessed by radiographic analysis (9, 19). In studies of early RA patients, CTX-II levels were shown to predict subsequent progres-

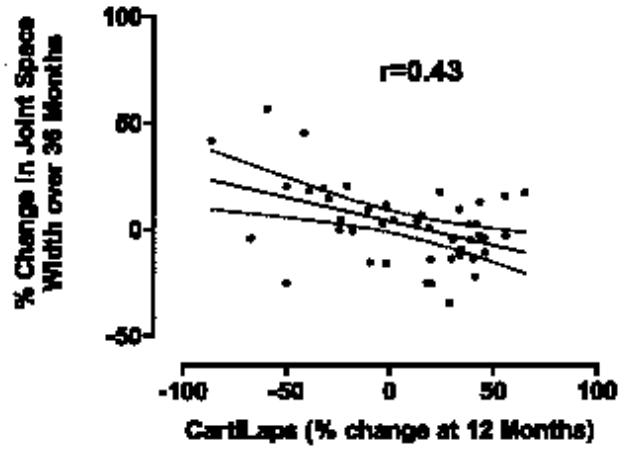

Fig. 3. Correlation between the 3-year change in average joint space width and the change in CTX-II observed over the first 12 months in the group of high turnover OA patients having baseline CTX-II levels 1 SD above the mean of the reference population (261.3 ng/mmol).

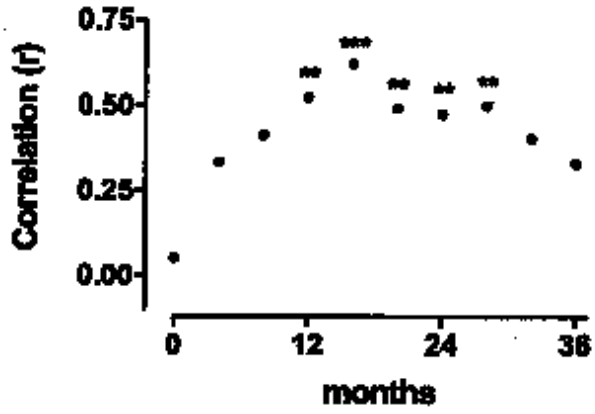

Fig. 4. Correlation between baseline CTX-II levels and WOMAC scores at different time points in the placebo-treated high turnover group with baseline CTX-II > 1 SD above the mean of the reference population (261.3 ng/mmol).

sion in radiologically assessed joint damage (10, 20). CTX-II levels can also be indicative of therapeutic effects as indicated by decreases in RA patients treated with corticosteroids, an agent with a well-documented structure-modifying effect (8).

In the cohort of knee OA patients examined in this study, urinary CTX-II levels at baseline were significantly elevated compared to a large group without clinically manifest arthritis or other diseases affecting cartilage metabolism. The considerable 'overlap' between the control cohort and the knee OA patients of the glucosamine study could be attributed to subclinical OA-like metabolic changes in articular cartilage in the reference cohort. Given the average age of these apparently healthy individuals, it is difficult to exclude that a substantial number of participants might have some form of OA such as changes in the load-bearing joints (hip, knee, vertebras). However, such subtle changes can be very difficult to capture with currently available conventional techniques. Bearing these limitations in mind, we defined high cartilage turnover as a urinary CTX-II concentration 1 SD above the mean of the asymptomatic reference cohort. At this level cartilage turnover is likely to be at a substantially high level. Indeed stratification of the OA cohort using this cut-off suggested the presence of more severe disease in these patients as assessed by radiographic and disease scoring measures (Table I), although only the difference in joint space width reached statistical significance. The approach of identifying high-risk patients by marker measurements is comparable to the stratification of subjects according to BMD measurements, where individuals with BMD 1 SD below the healthy reference cohort are categorized as 'osteopenic' and recognized to have a higher risk for the subsequent development of osteoporosis and skeletal fractures (21).

Studies in osteoporosis indicate that bone resorption markers are useful to identify high-risk patients and that their short-term responses to anti-resorptive agents are indicative for the long-term efficacy of the treatment assessed by bone mineral density measurements (21, 22). Applying this concept, we tested the hypothesis that OA patients with high cartilage turnover are more likely to benefit from structure modifying therapy in terms of preservation of cartilage. The results suggested that these 'high-risk' patients responded with more prominent decreases in CTX-II levels during the initial 12 months of glucosamine treatment and approached levels characterizing the symptom-free cohort. This observation implies that by measuring baseline levels of cartilage degradation it is possible to isolate high-risk patients who would benefit the most from therapy with structure modifying agents.

The effect of the glucosamine treatment on CTX-II levels such as that observed in this study is likely to represent a direct effect on systemic cartilage turnover. Glucosamine or metabolites of this compound have in a number of *in vitro* and *in vivo* studies demonstrated a direct effect on cartilage metabolism or metabolically active chondrocytes. These effects include a suppression of catabolic activity partly mediated by an inhibition of Matrix Metallo-Proteinases (MMP) and a stimulation of anabolic activity apparent as a stimulation of proteoglycan synthesis (6, 23, 24). Glucosamine and its metabolites are constituents in the formation of chondroitin sulphate and other essential oligosaccharide moieties of the extra-cellular matrix of cartilage (23,24). The exact relevance of these effects for the *in vivo* situation in OA patients is not clear at present, but *in vitro* studies strongly argue for a metabolic effect of glucosamine or its metabolites on chondrocytes.

The decrease in CTX-II concentration observed in the high turnover patient group was less pronounced than the decrease in CTX-II observed after prednisolone therapy in RA, or bisphosphonate treatment (8, 10, 11). This may suggest that the glucosamine treatment has a relatively mild effect on cartilage turnover, which is most pronounced in patients with the more aggressive form of OA reflected by elevated CTX-II levels at baseline. This decrease in cartilage degradation apparent as a decrease in CTX-II levels was also reflected in the halted progression of structural damage of the affected knee joints as assessed by X-ray analysis (Figs. 1 and 3). Furthermore, the change observed in the first 12 months in CTX-II levels in OA patients with elevated CTX-II levels at baseline showed a correlation with the progression of joint space narrowing over the 3-year study period. This suggests that CTX-II levels may not only predict the progression in structural damage accompanying the disease, but CTX-II responses to structure modifying agents may also be indicative of their long-term efficacy in preserving cartilage tissue in affected joints.

CTX-II levels at baseline were correlated with the subsequent emergence of disease symptoms as assessed by the total WOMAC score (Fig. 4). In high turnover patients who had not received treatment with glucosamine, the correlation between baseline CTX-II and the clinical severity of OA (WOMAC) at 12 months was particularly noteworthy. This result indicates that in untreated OA patients with a high cartilage turnover, the elevated cartilage degradation is first reflected by clinical symptoms after a certain lag period of 1 to 1 1/2 years. This may also indicate that although the symptoms of the disease are coupled to the underlying tissue destruction, this link is not direct. The apparent beneficial effects of glucosamine on joint pain and other disease symptoms may thus be mediated through other mechanisms than merely the deceleration of cartilage damage.

It must be pointed out that this study has several limitations. First of all, the observed effects of the glucosamine treatment were small and only seen in a sub-group of the OA patients with elevated CTX-II levels at baseline. Although the reference cohort included in the study was free of clinical symptoms of OA, it is likely that a considerable number of the subjects in this age-group had sub-clinical OA-like changes in their articular cartilage. Accordingly, the cut-off value defined in the present study is unlikely to represent a generally applicable measure indicative of a 'healthy' cartilage turnover.

Determination of such a 'healthy' mean turnover level would have required a thorough and systemic radiological evaluation using highly advanced imaging techniques. If patients with 'subclinical' OA were included in our control cohort they would be expected to have elevated CTX-II levels (7, 9, 10), thereby increasing the average CTX-II concentration and resulting in a higher cut-off value. Finally, the precision of the traditional radiological method used for assessing small changes in cartilage mass is poor, which somewhat hampered the correlation analysis between levels of CTX-II and structural changes (12).

In conclusion, the observations of the study suggest that urinary CTX-II can be used to isolate high-risk patients who are often the ones who will benefit most from treatment with structure modifying agents. However, the ultimate test of the clinical applicability of the assay requires further investigations with more potent cartilage specific drugs.

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