Decrease in serum level of matrix metalloproteinases is predictive of the disease-modifying effect of osteoarthritis drugs assessed by quantitative MRI in patients with knee osteoarthritis

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ABSTRACT

Objectives To explore the impact of disease-modifying osteoarthritis drug (DMOAD) treatment on biomarker levels and their correlation with cartilage volume loss and disease symptoms in a 2-year phase III clinical trial in patients with knee OA.

Methods 161 patients with knee OA (according-to-protocol population) were selected from a 2-year DMOAD trial studying the effect of licofelone (200 mg twice daily) versus naproxen (500 mg twice daily). Clinical evaluation of patients was carried out using the Western Ontario and McMaster Universities (WOMAC) questionnaire. Biomarker measurements of matrix metalloproteinase (MMP)-1, MMP-3, interleukin (IL)-6, C reactive protein (CRP), cartilage oligomeric matrix protein (COMP) and type I collagen C-terminal telopeptide (CTX-I) in serum, type II collagen C-terminal telopeptide (CTX-II) in urine, and knee MRI were performed at baseline and 2 years.

Results Over time an increase occurred in all biomarker levels with the exception of IL-6, CRP and CTX-II which decreased. The increase in MMP-1 and MMP-3 was significantly less (p=0.05; p<0.01, respectively) in the licofelone group. The baseline MMP-1 level was significantly but inversely predictive of cartilage volume loss for the medial compartment in both univariate (p=0.04) and multivariate (p≤0.04) regression analyses, and COMP, a predictor for the lateral compartment, in both univariate and multivariate models (p<0.01). Baseline levels of IL-6 and CRP also showed a significant relationship with volume loss for the medial compartment (univariate analysis, p=0.04 and p=0.01, respectively; multivariate analysis, p=0.03, p=0.01). A significant association (univariate) was observed between the change in the levels of MMP-1 (p=0.03) and MMP-3 (p=0.02) and cartilage volume loss (lateral compartment) over 2 years. Baseline levels of CTX-I correlated (p=0.02) with an increase in the size of the bone marrow lesion in the medial compartment. The baseline CRP levels correlated with worsening of symptoms: WOMAC total index (p<0.01), pain (p<0.01) and function (p<0.01).

Conclusion Higher baseline values of IL-6, CRP and COMP are predictive of greater risk of cartilage loss in OA. However, over time a reduction in MMP-1 and MMP-3 levels correlated best with reduction in cartilage volume loss and the effect of drug treatment. Baseline CRP was found to be a good predictor of the symptomatic response to treatment.

INTRODUCTION

In osteoarthritis (OA) the severity and/or progression of structural alterations can be assessed by several means including imaging and to some extent the use of biomarkers.[1–6] Measurement of the serum and urine levels of biological substances derived from the cartilage, the synovium and the bone to assess the progression of joint structural changes has been of considerable interest.[1 3 6] The potential of such markers for predicting the disease progression and/or response to disease-modifying OA drugs (DMOADs) has prompted the conduct of a number of clinical studies that have produced a large body of information on the usefulness of such tests.[1]

Several biomarkers of cartilage degradation have been identified including type II collagen C-terminal telopeptide (CTX-II), which measures the C-terminal neopeptide generated by collagenase-mediated cleavage of the type II collagen triple helix (the 3/4 fragment),[3 4] and the cartilage oligomeric matrix protein (serum COMP), a member of the thrombospondin family of glycoproteins.[6] As a marker of bone resorption, type I collagen degradation can be assessed using a number of assays including type I collagen C-terminal telopeptide (CTX-I) which measures the epitopes located in the C-terminal crosslinked telopeptides of type I collagen.[3] The levels of some of the markers—CTX-I, CTX-II, COMP, matrix metalloproteinases (MMP)-3, MMP-1 and tissue inhibitor of MMP (TIMP1) as well as C reactive protein (CRP)—were found to be increased in patients with OA.[1 3 4 6] Among the prognostic markers, the urinary level of CTX-I[7] and serum level of COMP[8] were increased in patients with knee OA showing radiological progression of the disease. Similarly, urinary CTX-II levels were associated with the progression of radiologically detectable knee and hip OA,[1 6 7] and with cartilage loss in patients with knee OA in one of two studies using MRI.[9] However, these have not been demonstrated to be useful as diagnostic tests in individual patients.[10]

Assessing the efficacy of therapeutic interventions in patients with OA with the use of biomarkers was found to be extremely unsatisfactory. Only a small number of DMOAD trials have been successfully completed, and in none of these trials has any clear relationship between the levels of the biomarkers studied and the effects of

the medication been established. For instance, the DMOAD effect of glucosamine sulfate treatment in patients with knee OA[11][12] showed no significant effect on the levels of type II collagen neopeptides C2C or C12C.[13] Similarly, treatment with risedronate, a bisphosphonate, in patients with knee OA induced a dose-dependent decrease in the urinary CTX-I level and the CTX-II level,[14][15] but was found ineffective at reducing OA progression measured by x-rays. In another DMOAD clinical study using doxycycline in obese women with knee OA, markers of type II collagen synthesis/degradation and of proteoglycan aggrecan turnover were not predictive of joint space narrowing (JSN) as evaluated by x-rays.[16] Licofelone, a dual inhibitor of 5-lipoxygenase (LOX) and cyclo-oxygenase (COX), has demonstrated DMOAD activity both in vitro and in vivo in preclinical studies.[17–20] In a recent clinical trial in patients with knee OA it was shown to significantly reduce the loss of cartilage assessed by quantitative MRI and to improve disease symptoms.[21]

To date, very little information is available on the value of biomarkers in observational studies and none in DMOAD clinical trials in patients with OA assessing disease progression and cartilage volume loss using quantitative MRI. The aims of the present study were to extend the previous observations from a phase III DMOAD clinical trial[21] by studying the effect of drug treatment on the levels of a number of relevant OA biomarkers and their correlation with cartilage volume loss and disease symptoms.

PATIENTS AND METHODS

Patient selection

This study was a subanalysis of a recently published study in patients with knee OA comparing the oral intake of licofelone, a LOX-COX inhibitor, with naproxen.[21] Briefly, patients with primary symptomatic knee OA of the medial femorotibial compartment diagnosed according to the criteria of the American College of Rheumatology[22] were recruited from outpatient rheumatology clinics. The patients were required to have at least one of the following three risk factors for increased risk of radiographic progression: body mass index (BMI) >30 kg/m^2, presence of Heberden's nodes or female gender. The study was approved by the local ethics committees and all patients gave their oral and written informed consent to participate, including permission for the use of serum/urine to be collected throughout the study for biomarker studies. Subjects were randomly assigned to receive either therapeutic doses of licofelone (200 mg twice daily) or naproxen (500 mg twice daily) for 2 years. Only the patients who had taken all the study medication provided throughout the 2-year period were considered for this according-to-protocol population biomarker subanalysis.

Outcome measures

ELISAs

After overnight fasting, urine and blood samples were obtained. The blood samples were allowed to coagulate and then centrifuged at 2800 rpm/min for 10 min. The samples were stored at −80°C until analysed. Levels of MMP-1, MMP-3, interleukin (IL)-6, CRP, COMP and CTX-I in serum and CTX-II in urine were assessed for each sample collected at baseline and upon completion of the 2-year study.

All the markers were determined with specific assays. Fluorokine MAP human kits were used for the determination of MMP-1 (sensitivity 4.4 pg/ml), MMP-3 (sensitivity 1.3 pg/ml),

CRP (sensitivity 1.4 pg/ml) and IL-6 (sensitivity 0.36 pg/ml) (all from R&D Systems, Minneapolis, Minnesota, USA). COMP was measured using a specific ELISA (sensitivity 0.5 ng/ml; BioVendor, Candler, North Carolina, USA). CTX-I was determined by the serum CrossLaps ELISA (sensitivity 0.02 ng/ml) and the degradation products of CTX-II in urine were measured using CartiLaps ELISA (sensitivity 0.2 ng/ml); the values were corrected with creatinine concentration as indicated by the manufacturer (both assays were from ImmunoDiagnostic Systems Ltd, Boldon, UK). Urine creatinine was measured as previously described[23] (sensitivity 800 µmol/l). All measurements were carried out in duplicate and in accordance with the manufacturers' instructions.

Knee MRI acquisition

High-resolution three-dimensional (3-D) MR images were obtained using 1.5 T with an integrated knee coil. These examinations are optimised 3-D FISP acquisitions with water excitation (Siemens, Erlangen, Germany) or 3-D SPGR acquisitions with fat suppression (General Electric, Milwaukee, Wisconsin, USA), as previously described.[24]

Cartilage volume measurement

Knee joint cartilage volume was measured by two trained readers using a specially developed computer program (Cartiscope; ArthroVision Inc, Montreal, Quebec, Canada).[25] The readers were blinded to treatment and to MRI examination time point other than baseline. The cartilage volume at 6, 12 and 24 months was evaluated at the time of the initial study.[21] The change in knee cartilage volume was obtained by subtracting the follow-up volume from the baseline volume. The change in cartilage volume over time was calculated for the medial and lateral compartments of the knee. The reproducibility of the method has previously been shown to be excellent.[21][24][25]

Bone marrow lesion score

Bone marrow lesions (BMLs) were also assessed for extent and severity in the medial and lateral tibiofemoral compartments using a scale of 0–3 as previously described[26] (where 0=absence of oedema and 3=a large BML). Reliability of the individual grading system (0–3) for BML changes was found to be excellent.[26]

Clinical evaluation

Patients underwent clinical evaluation at baseline and at 24 months based on the Western Ontario and McMaster Universities (WOMAC) OA index for pain, stiffness, function and total score and the visual analogue scale (where 0=normal state and 100=most severe). There was a 24 h washout of analgesic medications prior to the clinical evaluation.

Statistical analysis

Data were entered into a computerised database using a blinded double-entry procedure. Results were reported only as according-to-protocol for patients who had taken all the study medication and had all the outcome (clinical, structural and biochemical) evaluations collected. Absolute changes in biomarker levels at 2 years compared with baseline between the treatment groups were assessed using an analysis of covariance where the baseline value of the biomarker and the treatment were the independent variables and the value at 2 years of the biomarker was the dependent variable. Regressions (univariate and multivariate) were used to assess whether biomarker levels

at baseline predicted cartilage change over time. In the univariate analysis, the dependent variable was the cartilage change over time and the independent variable was the biomarker level at baseline. A first multivariate analysis was done for each biomarker adjusted for age, gender and treatment (licofelone vs naproxen), and a second one adjusted for the same covariates plus all biomarkers in the model simultaneously. Similar regressions were applied to assess the relationship between changes in biomarker levels at 2 years and change in cartilage volume also at 2 years, controlling for the aforementioned confounding variables. All statistical analyses were done using SAS software Version 9.1 (SAS Institute Inc, Cary, North Carolina, USA). All tests were two-sided and a p value ≤0.05 was considered statistically significant. Analyses were not adjusted for multiple comparisons.

RESULTS

Patient characteristics and outcomes

One hundred and sixty-one patients (82 in the licofelone group and 79 in the naproxen group) took all the study medication and had baseline and 2-year MRI evaluations. At study entry the patients in both treatment groups had similar mean age, gender, mean BMI, WOMAC pain score, cartilage volume and biomarker levels (table 1). Of note, the mean±SD baseline values of MMP-3 were greater for men than for women (18.3±7.3 vs 11.1±5.0; p<0.01, two-sample t test).

In the original study[21] a statistically significant difference was found in cartilage volume loss (global knee) between the licofelone and naproxen groups (7.4±3.3% cartilage loss at 2 years for the naproxen group vs 5.9±3.2% for the licofelone group; p<0.01, two-sample t test). There were similar and significant improvements from baseline in all WOMAC scores at 2 years for both treatment groups with no significant difference between the groups.[21]

Stepwise multilinear regression was also used to assess the role of covariates such as baseline mean age, gender, BMI and treatment (licofelone vs naproxen) effect on cartilage loss. The data showed that the treatment group was a strong predictor of lateral compartment cartilage volume loss (β estimate at 110.0 for the licofelone group, p<0.01, data not shown).

Biomarkers

Change in absolute biomarker levels between the two treatment groups at 2 years

Data on the change in biomarker levels at 2 years compared with baseline levels were available for almost all patients (81 for the licofelone group and 74 for the naproxen group). In both treatment groups there was an increase in biomarker levels at 2 years, with the exception of IL-6, CRP and urinary CTX-II in which a decrease was observed (table 2). When comparing the two treatment groups, the increase in MMP-3 and, to a lesser extent, the MMP-1 levels was significantly less in the licofelone group than in the naproxen group. For IL-6, CRP, COMP, CTX-I and urinary CTX-II, no significant differences were seen between the treatment groups.

Relationship between baseline absolute biomarker levels and percentage of cartilage volume loss at 2 years

Unexpectedly lower levels of MMP-1 at baseline were significantly associated with cartilage volume loss at 2 years in the medial compartment in both univariate (p=0.04) and multivariate regression analyses adjusted for age, sex, treatment (p=0.03) and all biomarkers (p=0.04) (table 3). This association was not found for the lateral compartment. Conversely, higher levels at baseline of IL-6 and CRP were associated with cartilage volume loss observed in univariate analysis (p=0.04, p=0.01, respectively) and in the multivariate analysis (age, sex and treatment, p=0.03, p=0.01) for the medial compartment. Similarly, higher levels of the COMP biomarker at baseline were significantly associated with cartilage volume loss in the lateral compartment in both univariate (p<0.01) and multivariate (p<0.01) models.

Relationship between change in absolute biomarker levels and percentage of cartilage volume loss at 2 years

An increase in cartilage volume loss in the lateral compartment was significantly associated with MMP-1 (p=0.03) and MMP-3 (p=0.02) levels at 2 years in univariate analyses (table 4). A decrease in the CRP level at 2 years was significantly associated with cartilage volume loss at 2 years for the medial compartment in both univariate (p=0.01) and multivariate regression analyses adjusted for age, sex, treatment (p=0.001) and all biomarkers (p=0.04). A decrease in the IL-6 level at 2 years was also significantly associated with cartilage volume loss in the medial compartment in the univariate model (p=0.05).

BML changes and correlation with CTX-I levels

The mean BML score increased significantly over 2 years (baseline 3.67±3.57 vs 4.44±3.58 at 2 years; p<0.01). A significant

Table 1 Baseline characteristics by treatment group

	Licofelone (n=82)	Naproxen (n=79)
Age (years)	60.0 ± 8.7	60.4 ± 7.7
Gender (% female)	70.7	62.0
BMI (kg/m^2)	32.5 ± 5.9	31.1 ± 5.5
WOMAC		
Pain	58.2 ± 11.9	57.4 ± 11.7
Stiffness	66.1 ± 17.2	61.1 ± 18.0
Function	57.1 ± 16.0	54.6 ± 17.1
Total	58.1 ± 13.6	55.7 ± 14.8
MRI cartilage volume (mm^3)		
Medial compartment	5.653 ± 1.664	5.645 ± 1.581
Lateral compartment	6.173 ± 1.730	6.341 ± 1.759
Biomarkers		
MMP-1 (ng/ml)	3.9 ± 3.2	3.4 ± 2.0
MMP-3 (ng/ml)	12.8 ± 6.1	14.2 ± 7.4
IL-6 (pg/ml)	2.8 ± 2.8	2.2 ± 2.3
CRP (mg/l)	7.1 ± 9.2	7.6 ± 12.2
COMP (ng/ml)	835.7 ± 349.6	809.9 ± 354.4
CTX-I (ng/ml)	0.3 ± 0.2	0.4 ± 0.2
U-CTX-II (ng/mmol creatinine)	342.2 ± 228.1	217.2 ± 135.3

Results are shown as mean±SD.
BMI, body mass index; COMP, cartilage oligomeric matrix protein; CRP, C reactive protein; CTX-I, type I collagen C-terminal telopeptide; IL-6, interleukin-6; MMP, matrix metalloproteinase; MRI, magnetic resonance imaging; U-CTX-II, urinary type II collagen C-terminal telopeptide; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index (100 = worst score).

Table 2 Absolute value change from baseline in biomarker levels at 2 years

	Licofelone (n=81)	Naproxen (n=74)	p Value*
MMP-1	0.3 ± 1.1	0.6 ± 1.1	0.05
MMP-3	0.9 ± 3.3	4.4 ± 6.1	<0.01
IL-6	−0.4 ± 2.3	−0.4 ± 2.0	0.73
CRP	−1.7 ± 8.3	−3.7 ± 10.7	0.07
COMP	59.4 ± 222.3	33.9 ± 180.2	0.36
CTX-I	0.07 ± 0.20	0.02 ± 0.18	0.18
U-CTX-II	−15.1 ± 159.7	−2.1 ± 138.3	0.11

Results are shown as mean±SD. A minus value sign indicates a decrease in biomarker level.
*Analysis of covariance (two-tailed) adjusted for the baseline value of the biomarker.
COMP, cartilage oligomeric matrix protein; CRP, C reactive protein; CTX-I, type I collagen C-terminal telopeptide; IL-6, interleukin-6; MMP, matrix metalloproteinase; U-CTX-II, urinary type II collagen C-terminal telopeptide.

Table 3 Relation between baseline biomarker values and percentage change in cartilage volume at 2 years

	β (estimate)	Medial 95% CI	p Value	β (estimate)	Lateral 95% CI	p Value
Univariate biomarker analysis (n=142)						
MMP-1 (ng/ml)	0.0035	(0.0001 to 0.0068)	0.04	−0.0004	(−0.0029 to 0.0022)	0.79
MMP-3 (ng/ml)	−0.0004	(−0.0018 to 0.0010)	0.59	−0.0003	(−0.0014 to 0.0007)	0.54
IL-6 (pg/ml)	−0.0044	(−0.0085 to −0.0003)	0.04	−0.002	(−0.005 to 0.001)	0.28
CRP (mg/l)	−0.0012	(−0.0020 to −0.0003)	0.01	−0.0003	(−0.0010 to 0.0003)	0.29
COMP (ng/ml)	1.3×10^{-6}	$(-24.8 \times 10^{-6}$ to $27.4 \times 10^{-6})$	0.92	-4.1×10^{-5}	$(-5.9 \times 10^{-5}$ to $-2.2 \times 10^{-5})$	<0.01
CTX-I (ng/ml)	0.01	(−0.04 to 0.06)	0.65	−0.03	(−0.07 to 0.01)	0.10
U-CTX-II (ng/mmol creatinine)	-2.3×10^{-7}	$(-4.8 \times 10^{-5}$ to $4.7 \times 10^{-5})$	0.99	-1.4×10^{-5}	$(-5.1 \times 10^{-5}$ to $2.2 \times 10^{-5})$	0.44
Multivariate biomarker analysis (n=125)*						
MMP-1 (ng/ml)	0.0037	(0.0003 to 0.0071)	0.03	−0.0002	(−0.0028 to 0.0023)	0.85
MMP-3 (ng/ml)	−0.0008	(−0.001 to 0.002)	0.55	−0.0006	(−0.0018 to 0.0006)	0.33
IL-6 (pg/ml)	−0.005	(−0.0088 to −0.0005)	0.03	−0.002	(−0.005 to 0.001)	0.31
CRP (mg/l)	−0.0012	(−0.0020 to −0.0004)	0.01	−0.0002	(−0.0008 to 0.0005)	0.59
COMP (ng/ml)	1.0×10^{-5}	$(-17.3 \times 10^{-6}$ to $38.1 \times 10^{-6})$	0.46	-3.8×10^{-5}	$(-5.7 \times 10^{-5}$ to $-1.9 \times 10^{-5})$	<0.01
CTX-I (ng/ml)	0.02	(−0.03 to 0.07)	0.40	−0.03	(−0.07 to 0.01)	0.11
U-CTX-II (ng/mmol creatinine)	-1.2×10^{-5}	$(-6.3 \times 10^{-5}$ to $3.8 \times 10^{-5})$	0.63	-2.8×10^{-5}	$(-6.5 \times 10^{-5}$ to $8.9 \times 10^{-5})$	0.14
Multivariate biomarker analysis (n=125)†						
MMP-1 (ng/ml)	0.0037	(0.0002 to 0.0072)	0.04	−0.0009	(−0.0035 to 0.0016)	0.46
MMP-3 (ng/ml)	-8.3×10^{-5}	(−0.002 to 0.002)	0.93	0.0004	(−0.0010 to 0.0018)	0.58
IL-6 (pg/ml)	−0.003	(−0.008 to 0.001)	0.17	−0.002	(−0.005 to 0.002)	0.33
CRP (mg/l)	−0.0009	(−0.0020 to 0.0002)	0.10	−0.0002	(−0.0010 to 0.0006)	0.69
COMP (ng/ml)	3.5×10^{-6}	$(-28.4 \times 10^{-6}$ to $35.3 \times 10^{-6})$	0.83	-3.7×10^{-5}	$(-6.0 \times 10^{-5}$ to $-1.4 \times 10^{-5})$	<0.01
CTX-I (ng/ml)	0.04	(−0.02 to 0.10)	0.18	−0.02	(−0.06 to 0.02)	0.32
U-CTX-II (ng/mmol creatinine)	-2.9×10^{-5}	$(-8.1 \times 10^{-5}$ to $2.3 \times 10^{-5})$	0.27	-1.5×10^{-5}	$(-5.3 \times 10^{-5}$ to $2.3 \times 10^{-5})$	0.43

*Adjusted for age, sex and treatment.
†Adjusted for age, sex, treatment and all biomarkers.
COMP, cartilage oligomeric matrix protein; CRP, C reactive protein; CTX-I, type I collagen C-terminal telopeptide; IL-6, interleukin-6; MMP, matrix metalloproteinase; U-CTX-II, urinary type II collagen C-terminal telopeptide.

Table 4 Relation between change in biomarker in absolute value and percentage change in cartilage volume at 2 years

	β (estimate)	Medial 95% CI	p Value	β (estimate)	Lateral 95% CI	p Value
Univariate biomarker analysis (n=142)						
MMP-1 (ng/ml)	−0.004	(−0.01 to 0.005)	0.37	−0.01	(−0.01 to −0.001)	0.03
MMP-3 (ng/ml)	−0.001	(−0.003 to 0.001)	0.25	−0.002	(−0.003 to −0.0003)	0.02
IL-6 (pg/ml)	0.005	(0.00004 to 0.009)	0.05	0.002	(−0.001 to 0.006)	0.23
CRP (mg/l)	0.001	(0.0004 to 0.002)	0.01	0.0005	(−0.0002 to 0.001)	0.14
COMP (ng/ml)	-1.7×10^{-5}	$(-6.4 \times 10^{-5}$ to $3.0 \times 10^{-5})$	0.48	-3.3×10^{-6}	$(-3.9 \times 10^{-5}$ to $3.3 \times 10^{-5})$	0.86
CTX-I (ng/ml)	−0.004	(−0.05 to 0.04)	0.87	−0.02	(−0.06 to 0.02)	0.30
U-CTX-II (ng/mmol creatinine)	-9.0×10^{-6}	$(-7.4 \times 10^{-5}$ to $5.6 \times 10^{-5})$	0.78	-8.5×10^{-6}	$(-5.9 \times 10^{-5}$ to $4.2 \times 10^{-5})$	0.74
Multivariate biomarker analysis (n=125)*						
MMP-1 (ng/ml)	−0.004	(−0.012 to 0.005)	0.37	−0.01	(−0.01 to 0.001)	0.09
MMP-3 (ng/ml)	−0.0008	(−0.003 to 0.001)	0.42	−0.001	(−0.002 to 0.0005)	0.19
IL-6 (pg/ml)	0.004	(−0.0002 to 0.009)	0.06	0.001	(−0.002 to 0.005)	0.40
CRP (mg/l)	0.001	(0.0003 to 0.002)	0.01	0.0003	(−0.0004 to 0.001)	0.44
COMP (ng/ml)	2.4×10^{-5}	$(-6.9 \times 10^{-5}$ to $2.5 \times 10^{-5})$	0.36	-4.8×10^{-6}	$(-3.9 \times 10^{-5}$ to $3.0 \times 10^{-5})$	0.78
CTX-I (ng/ml)	−0.007	(−0.06 to 0.04)	0.77	−0.03	(−0.06 to 0.01)	0.16
U-CTX-II (ng/mmol creatinine)	-2.3×10^{-6}	$(-6.8 \times 10^{-5}$ to $6.3 \times 10^{-5})$	0.94	-2.1×10^{-6}	$(-5.0 \times 10^{-5}$ to $4.6 \times 10^{-5})$	0.93
Multivariate biomarker analysis (n=125)†						
MMP-1 (ng/ml)	−0.004	(−0.01 to 0.01)	0.50	−0.01	(−0.01 to 0.002)	0.14
MMP-3 (ng/ml)	−0.0002	(−0.003 to 0.002)	0.89	−0.001	(−0.003 to 0.0008)	0.25
IL-6 (pg/ml)	0.003	(−0.003 to 0.008)	0.33	0.002	(−0.002 to 0.006)	0.26
CRP (mg/l)	0.001	$(4.83 \times 10^{-5}$ to 0.003)	0.04	0.0002	(−0.0008 to 0.001)	0.64
COMP (ng/ml)	-5.1×10^{-6}	$(-5.7 \times 10^{-5}$ to $4.6 \times 10^{-5})$	0.85	-1.84×10^{-6}	$(-4.0 \times 10^{-5}$ to $3.7 \times 10^{-5})$	0.92
CTX-I (ng/ml)	0.006	(−0.05 to 0.06)	0.82	−0.02	(−0.06 to 0.02)	0.23
U-CTX-II (ng/mmol creatinine)	1.5×10^{-6}	$(-7.1 \times 10^{-5}$ to $7.4 \times 10^{-5})$	0.97	1.46×10^{-6}	$(-5.3 \times 10^{-5}$ to $5.5 \times 10^{-5})$	0.96

*Adjusted for age, sex and treatment.
†Adjusted for age, sex, treatment and all biomarkers.
COMP, cartilage oligomeric matrix protein; CRP, C reactive protein; CTX-I, type I collagen C-terminal telopeptide; IL-6, interleukin-6; MMP, matrix metalloproteinase; U-CTX-II, urinary type II collagen C-terminal telopeptide.

correlation was found between the baseline level of CTX-I and the increase in BML size in the medial compartment (r=0.193, p=0.02). However, changes in the CTX-I level over time did not correlate with BML (r=−0.14, p=0.10).

Biomarker levels and knee OA symptoms (WOMAC)

Baseline CRP levels were found to be associated with the changes over time in symptoms of knee OA (figure 1). The baseline levels were significantly correlated with a worsening WOMAC total index (r=0.27, p<0.01), pain index (r=0.24, p<0.01) and function index (r=0.27, p<0.01). No correlations were found for any of the other biomarkers studied (data not shown).

DISCUSSION

The biomarkers that are most commonly used in the field of OA clinical research are those that can measure the anabolism/catabolism of cartilage macromolecules, bone resorption, synovial inflammation and some of the periarticular soft tissues.[1–6] Longitudinal and clinical studies have so far explored the validity of biomarkers to predict or assess the progression of structural changes and response to DMOAD treatment. In most of these studies x-rays were used to assess disease severity and/or progression, whereas MRI was only employed in two observational longitudinal studies in patients with knee OA.[9] However, no data are yet available on the value of biomarkers in DMOAD
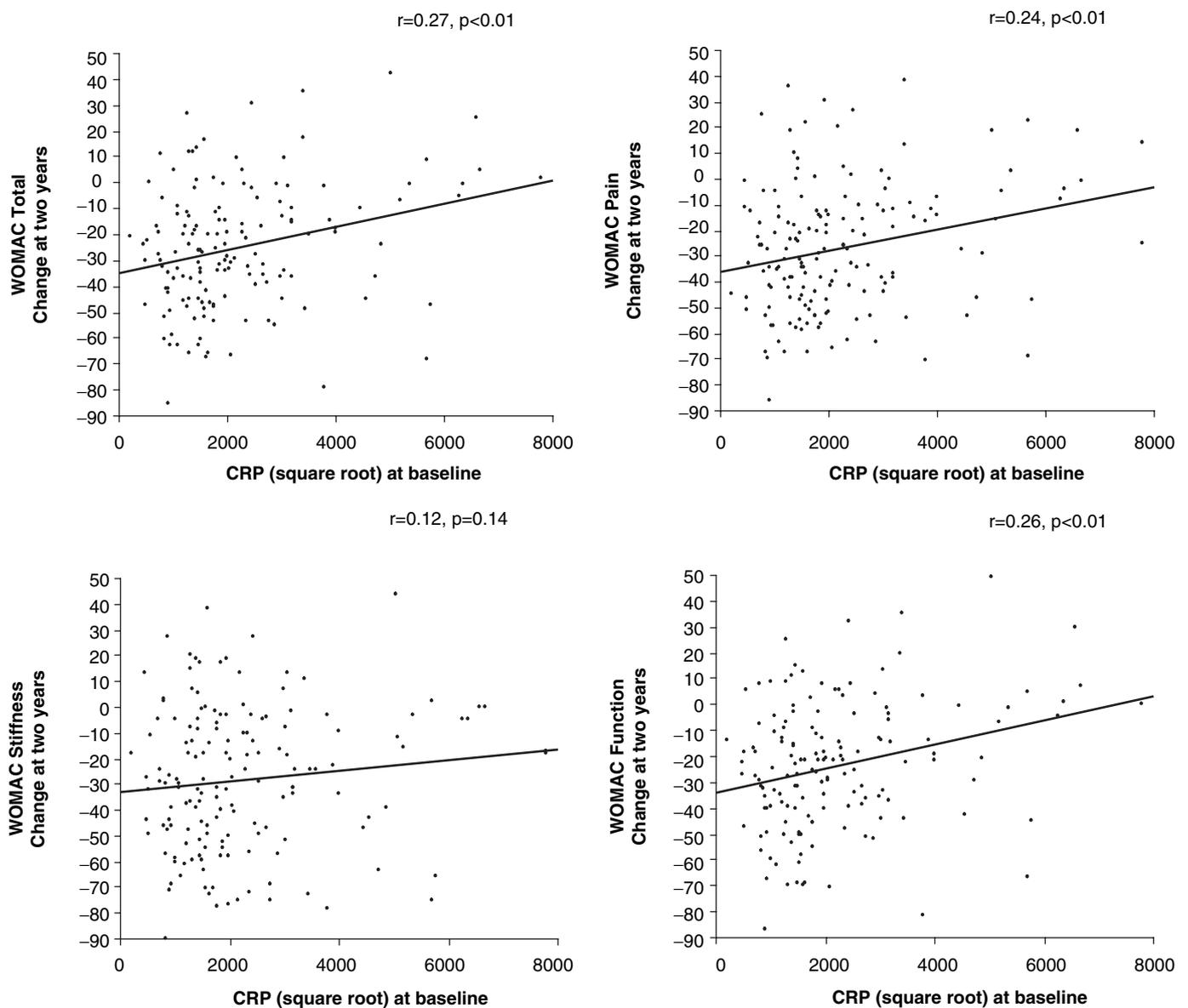
Figure 1 Univariate correlations between C reactive protein (CRP) at baseline and change in Western Ontario and McMasters Universities (WOMAC) scores (total, pain, stiffness and function) at 2 years (Pearson correlation coefficients).

clinical studies using MRI technology. The present study is therefore the first of this type and provides novel and useful information for the field of OA and drug development.

The identification of biomarkers that can predict disease progression and patient response to DMOAD treatment is crucial to support new drug development programmes. There has been no published DMOAD study in which biomarkers were identified and positively correlated with the protective effect of treatment. A recent DMOAD study in patients with knee OA of the effect of doxycycline demonstrated a positive correlation in the placebo group between baseline MMP-3 levels and JSN[27] and an inverse relationship between MMP-3 levels and the rate of JSN in the doxycycline group. Mazzuca et al[16] reported in the same study an association between JSN and markers of collagen and proteoglycan turnover in knee OA, but no correlation between the protective effect of doxycycline on JSN and the levels of these biomarkers.

In the present study the marked and significant reduction in the levels of both MMP-3 and MMP-1 in patients treated with licofelone compared with naproxen correlated somewhat with the significant reduction in cartilage volume loss found in the licofelone treatment group.[21] Since the blood samples were taken at a fairly uniform time of day from all patients during the trial and the treatment groups were balanced for age and gender, it is highly unlikely that these factors could have influenced the MMP measurements, which was found in earlier reports.[28 29] In this study, contrary to a previous report in a normal population,[28] gender did not have any significant impact on the response of the MMPs to treatment. The findings on MMPs from the present study are in line with previous preclinical studies, both in vitro and in vivo, in which licofelone reduced MMP expression and synthesis in OA joint tissues and cells.[17 19 20] Together these findings are of paramount importance in view of the predominant role that MMPs are believed to play in the pathophysiology of OA, particularly in the degradation of cartilage.[30]

The level of IL-6 was fairly stable with only a minimal decrease found during the course of the study. This finding could indicate that the non-steroidal anti-inflammatory drug (NSAID) treatments administered during the course of the study, and as previously demonstrated,[31] had little effect on the circulating level of

Extended report

this cytokine. An increase in circulating blood levels of IL-6 was previously shown to be an independent predictor of the appearance of knee OA,[32] and the present study does support IL-6 as a possible useful predictor of knee OA progression. However, caution should be exercised as only the analysis for the medial compartment was found to be significant, limiting the strength of our observation. Moreover, the cartilage volume loss found over time was associated with a decrease in the IL-6 level, which may seem counterintuitive.

The CRP level decreased over time in both treatment groups but was more pronounced in the naproxen group. Data from a previous cross-sectional study showed that the increased level of CRP found in patients with knee OA can predict disease progression.[33] In the present study the baseline level of CRP was also found to be predictive of cartilage volume loss in the medial compartment, a finding similar to that observed for IL-6. The predictive value of baseline IL-6 and CRP levels on cartilage volume loss could possibly be related to the fact that IL-6 and CRP are known biomarkers of synovial inflammation, a factor which has been correlated to a more rapid progression of cartilage lesions in the medial tibiofemoral compartment.[34] The reduction in CRP level over time, however, contrasts with the observations of the previous report,[33] and was somewhat surprising. Based on the protective effect of licofelone on cartilage volume loss, one would have expected a greater decrease in the CRP level in this treatment group than in the naproxen group. It may be that an independent effect of the drug treatment per se, such as that previously reported with the NSAID tenidap,[31] may have influenced the level of CRP. This would be supported by the fact that no significant change in the level of IL-6 was observed between the two groups, and this cytokine is known to be the main factor in the stimulation of CRP synthesis. The drug effect may also explain the inverse correlations found between the change in levels of IL-6 and CRP and the cartilage volume over time.

Further analysis of the data indicated that the factor with the highest predictive value (multivariate analysis) was the drug treatment (licofelone) received by patients. Moreover, the baseline MMP-1 level was found to be predictive (inverse correlation) of cartilage loss in the medial compartment on both univariate and multivariate analyses. The latter confirmed the independent predictability of the biomarkers. To our knowledge, no data exist on the predictive value of blood MMP-1 levels in patients with knee OA. There is, however, a study showing that synovium from patients with rapidly destructive hip OA produces a large amount of MMP-1.[35] In that context, the finding in the present study that a lower level of MMP-1 at baseline was associated with greater cartilage volume loss at 2 years was unexpected and requires explanation. Since the patients with knee OA included in the study had fairly severe disease and therefore end stage cartilage lesions could be expected in the medial compartment,[21] hypothetically, the increased level of MMP-1 produced by the OA cartilage may have been in a declining phase. This is supported by previous ex vivo experiments using cartilage explants from patients with knee OA.[36]

The findings of a correlation between the change in MMP-1 and MMP-3 levels over time and the cartilage volume loss seem far more promising. These data on MMP-3 are in line with recent studies on knee and hip OA.[27 35] The fact that this was limited to the lateral compartment on univariate analysis may be related to the exploratory nature of the study. Another plausible explanation could be that the progression of disease in the patient population studied was predominantly in the lateral compartment where a large amount of cartilage loss was

detected.[21] It is therefore not surprising that the strongest correlation was detected in the lateral compartment. The association of a decrease in MMP-1 and MMP-3 levels by licofelone with a reduction in cartilage loss adds additional evidence for the existence of a definite relationship between MMP levels and the progression of OA.

The baseline COMP level was found to be a very strong predictor of the loss of cartilage in the lateral compartment on both univariate and multivariate analyses. This concurs with the results of a cross-sectional study in which the serum COMP level correlated with the presence and severity of OA.[37] However, our findings conflict with a longitudinal study of knee OA in which the COMP level was predictive of radiographic progression (JSN) over a 5-year period,[38] while in our study the increase in COMP level over time was not correlated with disease progression. This could possibly be explained by the fact that our study was shorter in duration (2 years) and the sample size was relatively small for this purpose. Another explanation is that a positive correlation was found only with cartilage loss in the lateral compartment in our study, while the above study[38] used x-rays to measure disease progression and therefore the correlation was made with reference to cartilage loss in the medial compartment.

The baseline level of CTX-I was found to be predictive of an increase in BML score. This concurs with previous MRI studies in patients with knee OA which have shown a progression of these lesions over time.[26 39–40] No correlation was found between the baseline level of urinary CTX-II or its change over time and the loss of cartilage, in contrast to a previous study in patients with knee OA followed by x-ray which showed a positive correlation between the level of CTX-II and JSN.[2] This obviously needs further exploration.

This study has some limitations. Since the statistical significance was set without correcting for multiple testing, caution is advised in extrapolating these results without further testing in a formal study using biomarkers as primary outcomes. Moreover, we chose a subpopulation within a larger trial based on tight usage of the study medications such as licofelone and naproxen. We did not perform the analyses of the whole 355 intent-to-treat cohort to determine whether all the aforementioned results were robust.

To our knowledge, the findings of this study are novel and provide new information that will be of great value to the conduct of future DMOAD trials. They remain, however, to be validated in complementary studies. Moreover, it will be most interesting in the context of DMOAD trials to verify whether the decrease in MMPs can be detected in the early phase of a study and whether it would be predictive of the long-term outcomes which unfortunately could not be explored in the present study due to its design.

Patient consent Obtained.

Ethics approval This study was approved by the local ethics committees.

Provenance and peer review Not commissioned; externally peer reviewed.

Contributors All authors have read and approved the manuscript and contributed to the study design, data analysis, interpretation of data and drafting and revision of the manuscript. MD and J-PR were responsible for the accuracy of the data.

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