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Concise report

Serum biomarkers in prednisolone-treated hand osteoarthritis patients

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Abstract

Objectives. To investigate whether biomarkers are modulated by prednisolone treatment in patients with hand OA and whether they can predict response to prednisolone.

Methods. Biomarkers reflecting tissue turnover and inflammation [aggrecanase-derived neoepitope of arggecan (ARGS), MMP-derived neoepitope of type I collagen (C1M), MMP-derived neoepitope of type III collagen (C3M), marker of true type V collagen formation (PROC5), MMP-derived neoepitope of CRP (CRPM), citrullinated vimentin fragment (VICM), high-sensitivity (hsCRP)] were measured in sera from 78 patients with painful inflammatory hand OA, who were randomized between prednisolone or placebo treatment. Association of baseline biomarker levels with disease characteristics [visual analogue scale (VAS) pain, synovial thickening ultrasonography sum score and erosive OA] and OMERACT-Osteoarthritis Research Society International (OARSI) response after 6 weeks were analysed with linear or logistic regression and adjusted for age, BMI and sex. Change in biomarker levels after 6 weeks was assessed with linear regression adjusted for baseline biomarker levels, age, BMI and sex.

Results. For all patients (mean age 64 years, 79% female), there were no associations between biomarker levels and VAS finger pain or synovial thickening score at baseline. Patients with erosive hand OA had higher levels of C1M and hsCRP [adjusted geometric mean ratio 1.24 (95% CI 1.03, 1.49) and 1.91 (1.19, 3.06), respectively]. Biomarker levels did not decrease over time. There was no association between baseline biomarkers levels and OARSI response, except for CRPM [geometric mean ratio of 0.88 (0.77, 1.00)].

Conclusion. Erosive disease was associated with higher levels of C1M and hsCRP. Biomarker levels were not influenced by treatment with prednisolone. Current biomarkers were not associated with response to prednisolone in hand OA.

Key words: hand osteoarthritis, biomarkers, prednisolone, RCT

BASIC SCIENCE

Rheumatology key messages

- hsCRP and C1M were associated with erosive hand OA.
- Biomarker levels were not influenced by prednisolone treatment in hand OA.
- Biomarker levels were not associated with response to prednisolone treatment in hand OA.

Introduction

Hand OA is a common disease with considerable burden, but with a pathogenesis that is not fully elucidated and therapeutic options that are confined to symptomatic treatment or surgical intervention. The disease process involves joint tissue destruction, which encompasses degradation of extracellular matrix proteins in cartilage, bone and surrounding structures. The destruction of tissue, but also the subsequent remodelling, results in fragments in the SF. When fragments originating from the SF reach the circulation, these could potentially be used as biomarkers to reflect presence of disease, as well as disease severity and/or reaction to therapy, depending on the fragment's progenitor [1]. Since there is an unmet

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need for positive clinical trials in hand OA, research may benefit from tools, such as biomarkers, to select patients more likely to respond to therapy and thereby increase the likelihood of a successful outcome in drug testing trials. Biomarkers may also provide insight into the mode of action of a drug and ultimately be used for monitoring response to therapy. Serum biomarkers have previously been applied to identify subgroups of responders in RA clinical trials where treatment response is variable [2] and also in patients with knee OA [3]. However, in hand OA, there are limited data available about the use of biomarkers, partly because most biomarker studies in OA have been performed in patients with knee and hip OA.

The Hand Osteoarthritis Prednisolone Efficacy (HOPE) study is a randomized controlled trial in which inflammatory hand OA patients were treated with either prednisolone or placebo for 6 weeks [4]. After 6 weeks, pain (primary endpoint) was significantly reduced in patients receiving prednisolone as compared with patients receiving placebo. Synovial thickening measured using US, a clinical marker of inflammation, was also reduced, reflecting a direct influence of prednisolone on inflammation. Also, an effect of prednisolone on bone marrow lesions on MRI was observed in this study. Furthermore, prednisolone has a well-known effect on bone turnover.

The HOPE study is thus ideally suited to test whether biomarkers of connective tissue turnover and inflammation can be used to monitor disease severity after treatment in hand OA and furthermore whether baseline levels of biomarkers are associated with clinical response to treatment with prednisolone.

Methods

Study population and intervention

All patients with symptomatic hand OA according to the ACR criteria [5] of the HOPE trial [4] with available stored serum ($n = 78$ of the original 92) were used for the present analysis. In short; patients had signs of inflammation and finger pain of $\geq$30 mm on a 100 mm visual analogue scale (VAS) with a flare-up after washout of NSAIDs. Patients with pain located predominantly in the thumb base instead of the fingers were excluded, as were patients suffering from comorbidities including chronic inflammatory rheumatic diseases such as psoriasis or FM, and patients that used systemic or local immunomodulating drugs or received IA hyaluronic acid injections up to 90 days before the start of the trial [4]. The study was approved by medical ethics committees at the LUMC and Zuyderland medical centre and conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. All patients provided written informed consent. This study was registered with the Netherlands Trial Registry, number NTR5263.

Patients received either 10 mg placebo ($n = 38$) or prednisolone ($n = 40$) daily to which they were, in double-blind fashion, randomly assigned. After 6 weeks medication was tapered to cessation in 2 weeks. Patients were followed 14 weeks. Paracetamol as rescue medication and a stable dosage of chondroitin sulphate/glucosamine/bisphosphonate/tetracycline/estrogens were allowed. NSAIDs and injections with glucocorticoids or hyaluronic acid were not.

Radiographic damage was assessed with the Kellgren–Lawrence system (0–4 per joint, 30 joints) [6]. Erosive OA was defined as having at least one finger joint in the Verbruggen–Veys erosive or remodelling phase [7]. Synovial thickening was scored semi-quantitatively using ultrasonography (0–3 per joint, 30 joints) and sum scores were calculated [8, 9]. The primary endpoint of was VAS finger pain (0–100) at week 6. OMERACT-Osteoarthritis Research Society International (OARSI) responder criteria were used to determine response on prednisolone [10].

Biomarkers

Serum samples were collected at baseline, 6 weeks and 14 weeks and stored at –80°C. High-sensitivity (hsCRP) was measured at LUMC using the Roche Cobas c502. CRPM, ARGS, C1M, C3M, PROC5 and VICM were analysed at Nordic Biosciences lab using validated hand-held ELISAs. Analysis was performed following standard operating procedures adhering to regulatory guidelines. Variation coefficients were <10%. In sera of 19 healthy controls (mean age 55 years, S.D. 3.3, 52% female), MMP-derived neoepitope of CRP (CRPM), aggrecanase-derived neoepitope of arggecan (ARGS), MMP-derived neoepitope of type I collagen (C1M), MMP-derived neoepitope of type III collagen (C3M), marker of true type V collagen formation (PROC5), citrullinated vimentin fragment (VICM) were measured and showed geometric mean (95% CI) of 7.22 (6.34, 8.25) ng/ml, 211 (164, 273) pM, 16.9 (11.4, 25.2) ng/ml, 10.0 (8.84, 11.4) ng/ml, 409 (342, 488) ng/ml and 1.83 (1.11, 2.99) ng/ml, respectively. For hsCRP normal reference was <0.3 ng/l.

Statistics

Data were analysed using Stata version 16.1.

All biomarker data were logarithmically transformed to reach normal distribution. Geometric mean with corresponding 95% CI were used to report results.

Logarithmically transformed baseline biomarker levels were compared between prednisolone- and placebo-treated patients with Student's *t*-test. Association between baseline biomarker levels and baseline disease characteristics were analysed using linear regression, adjusted for age, BMI and sex, with disease characteristic as the determinant and biomarker levels as the outcome. Change in biomarker levels between baseline and week 6 was analysed using linear regression, crude as well as adjusted for baseline biomarker levels, age, BMI

and sex with logarithmically transformed biomarker levels as dependent and visit as independent. This analysis was also performed stratified for treatment.

Results

Sera of 78 patients with a mean age of 64 years (S.D. 9.1), 79% female and a mean BMI of 27 kg/m^2 (S.D. 4.6) were available. They had a median disease duration of 3.8 years (interquartile range 0.2–7.9) and 71% had erosive disease. Mean VAS score of pain in the fingers was 52 mm (S.D. 20), mean Kellgren–Lawrence sum score was 36 (S.D. 16) on a 0–120 range, and mean US synovial thickening score 17 (S.D. 6.3) on a 0–90 range (supplementary Table S1, available at *Rheumatology* online). Patients from whom serum was available were comparable in both patient and disease characteristics to patients from whom no serum was available (data not shown). Baseline patient and disease characteristics were well balanced between prednisolone- and placebo-treated patients (data not shown).

Association of baseline biomarkers levels with baseline diseases characteristics are depicted in Fig. 1. There were no associations between biomarker levels and pain (VAS pain fingers) or inflammation as seen on ultrasonography (synovial thickening score). Patients with erosive hand OA had higher levels of C1M and hsCRP with a geometric mean ratio of 1.24 (95% CI 1.03, 1.49) and 1.91 (95% CI 1.19, 3.06), respectively, as compared with patients with non-erosive hand OA (Fig. 1).

There were no significant differences between baseline biomarker levels of prednisolone- and placebo-treated patients (supplementary Table S2, available at *Rheumatology* online). After 6 weeks of treatment there were no significant changes over time in concentrations of biomarkers (Fig. 2). Adjustment for baseline biomarker levels, sex, age and BMI did not change these results. Stratified for treatment, VICM levels increased slightly in the prednisolone-treated group with geometric mean ratio (between week 6 and baseline) of 1.22 (95% CI 1.01, 1.48), but not in the placebo-treated group (mean ratio 0.96; 95% CI 0.82, 1.14).

Responders to treatment (placebo and prednisolone) had lower baseline levels of CRPM [geometric mean ratio 0.88 (95% CI 0.77, 1.00)]. For responders to prednisolone treatment, baseline CRPM levels were lower than those without a response [mean ratio 0.82 (95% CI 0.68, 0.98)]. For the other biomarkers there was no association between baseline levels and OMERACT-OARSI response (supplementary Fig. S1, available at *Rheumatology* online).

Discussion

In this *post hoc* analysis of data from the HOPE study, in which patients were treated with prednisolone or placebo for 6 weeks, we investigated the levels of a set of biomarkers associated with cartilage (ARGS), bone (C1M) and connective tissue turnover (PRO-C5, C3M), and inflammation (CRPM, hsCRP, VICM). We found an association between baseline levels of hsCRP and C1M and the presence of erosive disease, but not with other disease characteristics. Treatment did not affect biomarker levels.

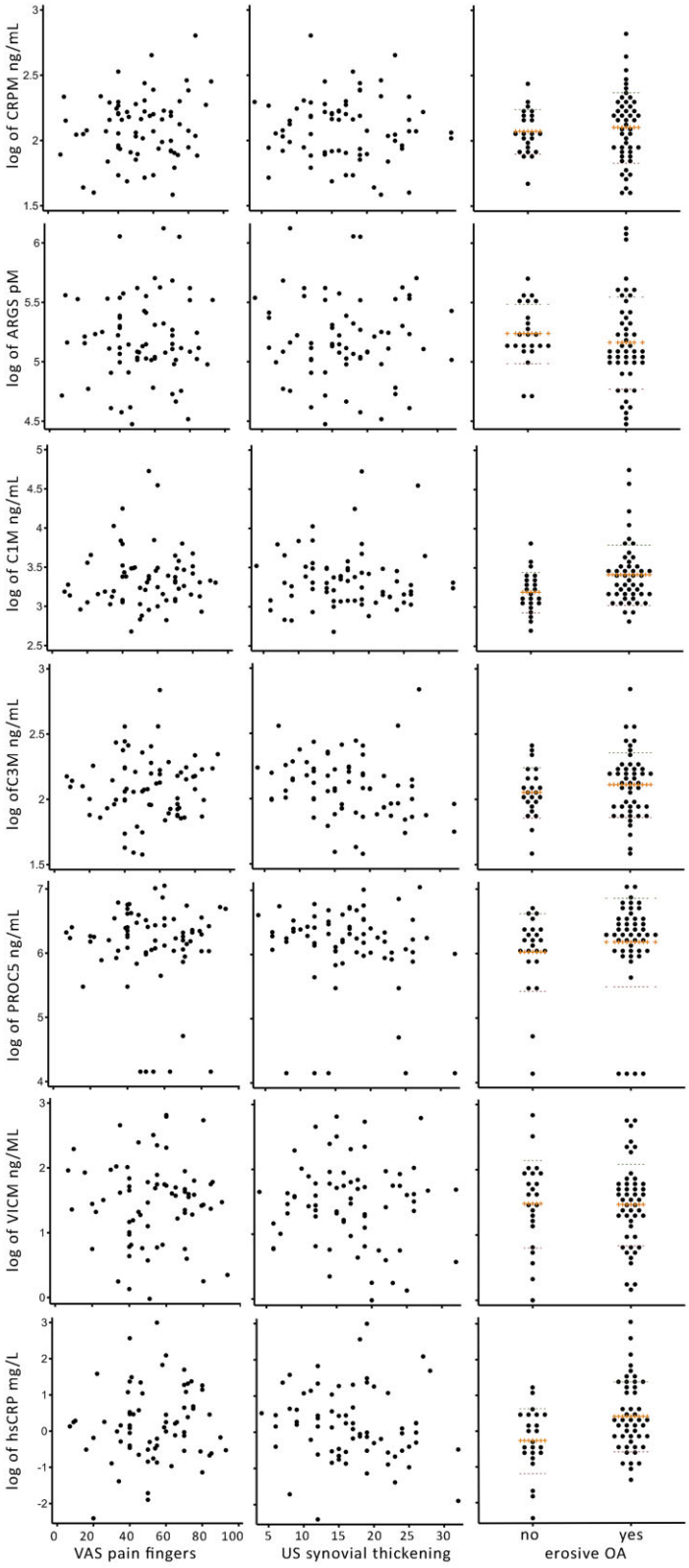
Higher hsCRP levels were associated with the presence of erosive hand OA. This is in line with the study of Punzi *et al.* who found higher hsCRP levels in patients with erosive hand OA than in non-erosive patients [11]. This confirms the association of inflammation with the erosive hand OA subset, as shown in previous studies [12–14], although we did not find an association of hsCRP levels with US signs of inflammation in this study.

C1M levels were also associated with the presence of erosive hand OA. C1M is a marker for extracellular matrix turnover of type I collagen, a major component of bone and connective tissue and the most abundant collagen in the body, and this turnover is likely more pronounced in erosive disease. Higher levels of C1M have also been found in knee OA patients scheduled for total knee replacement [15]. This might indicate that erosive OA could be viewed as endstage OA.

Biomarker levels did not decrease after treatment with prednisolone. These findings are in contrast to some previous studies concerning anti-inflammatory treatment and these biomarkers. In a study with lutikizumab given to patients with erosive hand OA, hsCRP, C1M, C3M and CRPM levels decreased after treatment with lutikizumab as compared with placebo [16]. Lutikizumab is, however, an IL-1 antagonist with mode of action different from that of prednisolone as it acts more directly on downstream cytokines. It therefore may have a different influence on these biomarkers. Moreover, decrease in biomarker levels was not associated with clinical response and therefore this study could not show usefulness of these biomarkers in hand OA for monitoring disease activity. We did detect a slight increase in VICM levels after treatment with prednisolone. This is counterintuitive since VICM is a marker of inflammation and clinical signs of inflammation were decreased in these patients. It should be replicated in further studies to deduce whether this finding has clinical relevance.

Biomarker levels at baseline were not associated with response to prednisolone, except for CRPM levels which were lower in patients responding to prednisolone. Previous findings show that changes in C1M levels were able to predict response to tocilizumab in RA patients. We could not replicate these findings, because we did not observe change in C1M levels. It is possible that we did not find an association of treatment response with biomarker levels, because these levels were already low at baseline, more in the range of healthy individuals. This in contrast to patients with RA, where levels of the selected biomarkers were significantly higher than in healthy individuals. OA patients suffer from low-grade

Fig. 1 Biomarker levels association with pain, inflammation of erosive disease



Logarithmic transformed levels of CRPM, ARGS, C1M, C3M, PROC5, VICM and hsCRP are shown (y-axes) for different levels of VAS pain fingers (left panels), synovial thickening score on US (middle panels) or erosive disease [right

(continued)

inflammation, as compared with other rheumatic diseases. Although this population of hand OA patients was particularly selected on the presence of inflammatory OA, the total inflammatory load is likely still low. This may be explained by a relatively low number of inflamed joints and the small size of the affected joints. It would be interesting to see whether cartilage and synovial markers measured in the SF are a better reflection of disease state and treatment response, but harvesting SF from small joints is a challenge.

This study was limited by a relatively small number of patients. However, it is unique in the sense that it is a placebo-controlled clinical trial in hand OA with a positive primary outcome and we therefore consider this population the best available to study the response of biomarkers to therapy. Furthermore, it may be argued that there are more possible biomarkers than we have measured. However, based on extensive previous experience we think our selection includes the most promising candidates at present [1, 2, 15, 17–20].

In conclusion, in hand OA markers of cartilage and bone turnover and inflammation levels vary. Erosive disease was associated with higher levels of C1M and hsCRP. None of the markers of cartilage, connective tissue or inflammation was affected by treatment with prednisolone; only VICM levels increased slightly. This may indicate that a short course of prednisolone has a limited direct effect on tissue remodelling, and acts solely as a symptom modifying drug. These results indicate that increased collagen turnover and inflammation could be a hallmark of certain subsets of patients with hand OA such as erosive hand OA, but more research is necessary to verify these results.

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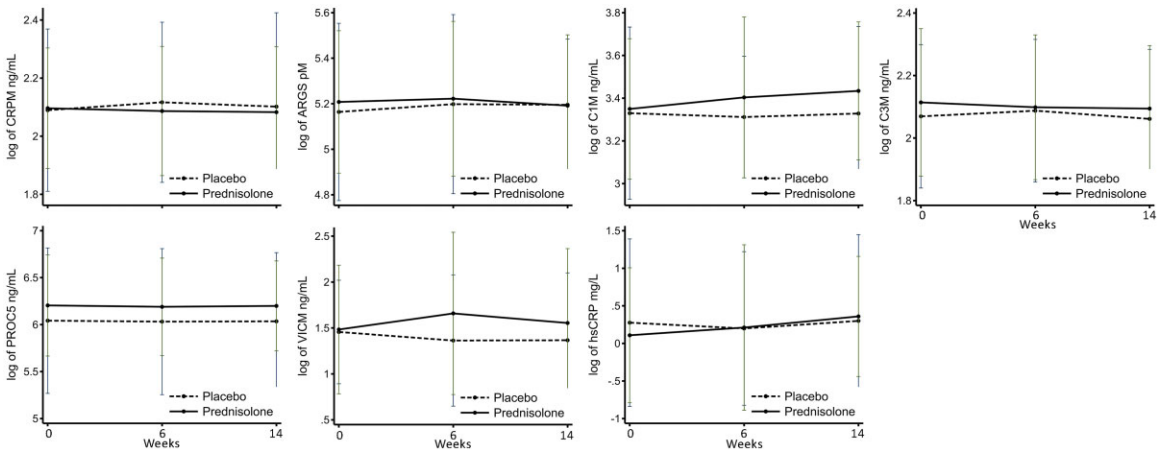
Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

Fig. 2 Biomarker levels over time



Logarithmic transformed levels [mean (S.D.)] of CRPM, ARGS, C1M, C3M, PROC5, VICM and hsCRP are shown (y-axes) over time (x-axes). Biomarkers were measured at baseline, week 6 and week 14. CRPM: MMP-derived neoepitope of CRP; ARGS: aggrecanase-derived neoepitope of arggecan; C1M: MMP-derived neoepitope of type I collagen; C3M: MMP-derived neoepitope III collagen; PROC5: marker of true type V collagen formation; VICM: citrullinated vimentin fragment, hsCRP: High sensitive CRP.

Fig. 1 Continued

panels; mean (S.D.)]. CRPM: MMP-derived neoepitope of CRP; ARGS: aggrecanase derived neoepiptope of arggecan; C1M: MMP-derived neoepitope of type I collagen; C3M: MMP-derived neoepitope of type III collagen; PROC5: marker of true type V collagen formation; VICM: citrullinated vimentin fragment, hsCRP: high-sensitivity CRP; VAS: visual analogue scale.

Supplementary data

Supplementary data are available at *Rheumatology* online.

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