CONCISE REPORTS

Relations between synovial fluid and serum concentrations of osteocalcin and other markers of joint tissue turnover in the knee joint compared with peripheral blood

C Salisbury, M Sharif

Abstract

Objective—To determine if osteocalcin (OC) is locally produced in the joint and to study the relation between markers of bone, cartilage, and synovial tissue turnover.

Methods—The concentrations of OC, keratan sulphate epitope (5D4), and hyaluronate (HA) were measured in paired serum and synovial fluid in 10 healthy volunteers and 15 patients with osteoarthritis (OA) and 16 with rheumatoid arthritis (RA). OC was measured with a commercial immunoradiometric assay and concentrations of 5D4 and HA were measured using enzyme linked immunosorbent inhibition assays.

Results—Synovial fluid OC was found to be significantly lower than serum ($p < 0.001$) in all patients and controls. Synovial fluid OC concentrations were directly correlated with serum concentrations ($r = 0.63$, $p < 0.001$) and with age ($r = 0.48$, $p < 0.01$). There were also some relations between OC, HA, and 5D4 in patients with OA and RA. The OC concentrations were directly correlated with HA ($r = 0.68$, $p < 0.01$) in OA serum and there was a similar correlation in RA synovial fluid ($r = 0.69$, $p < 0.01$). A weak negative correlation was found between OC and 5D4 in OA serum ($r = -0.55$, $p = 0.035$) while a weak positive correlation was found in RA serum ($r = 0.53$, $p = 0.034$). **Conclusions**—These results show that more OC is present in the circulation than in knee joint fluids suggesting that synovial fluid OC may be derived from the blood.

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Currently available clinical and radiographic methods used to assess osteoarthritis (OA) and rheumatoid arthritis (RA), such as anatomical changes in an affected joint, can only provide crude, insensitive measures of disease activity and outcome. Biochemical marker is a promising new tool for the investigation of joint disease. Measurement of markers in synovial

fluid probably provide the most accurate reflection of the current metabolic status of arthritis in any one joint,¹ whereas serum concentrations provide information on the systemic turnover of metabolites in a person.

Osteocalcin (OC), a proposed marker of bone formation,¹⁻³ has been studied widely in serum and synovial fluid from patients with joint disease,³⁻⁵ however it remains unclear whether OC is produced locally in the joint or derived from the blood. Therefore, the main objective of this study was to determine the source of OC production in both arthritis and normal condition. In addition, serum and synovial fluid concentrations of OC were correlated with values of putative markers of cartilage damage and synovial inflammation to test the hypothesis that measurement of different matrix component markers in body fluids may provide an insight into the relation between different tissues within the joint organ.

Methods

PATIENTS

Local ethical committee approval was obtained for the study. The study included 15 patients with OA whose diagnosis was based on the combination of use related pain and radiographic changes of definite OA (Kellgren and Lawrence grade 2 or above)⁶ and 16 with diagnosis of RA (Larsen index 3-5) using the American College of Rheumatology criteria.⁷ Ten healthy people with no knee pain and no history or signs of joint disease (controls) were used in the study. The control subjects were not matched for age or sex with either the OA or RA groups. Both the patients and the control group have been described in more detail previously.⁸

STUDY DESIGN

Synovial fluid and serum were collected, processed, and stored at -70°C until analysis as reported previously.⁸ Serum and synovial fluid OC were measured using a solid phase 'sandwich' immunoradiometric assay (ELSA-OSTEO (CIS bio international, ORIS Group, Gif-sur-Yvette, France)), which uses a monoclonal antihuman OC antibody specific

Rheumatology Unit,
University of Bristol
Division of Medicine,
Bristol Royal
Infirmary, Bristol
C Salisbury
M Sharif

Correspondence to:
Dr M Sharif, Rheumatology
Unit, University of Bristol
Division of Medicine, Bristol
Royal Infirmary,
Marlborough Street, Bristol
BS2 8HW.

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Figure 1 Concentrations of osteocalcin (OC) in paired synovial fluid and serum from patients with OA and RA and healthy controls. Horizontal bars show the mean value in each group.

for the whole molecule (carboxylated or decarboxylated) and OC peptide 1-43 (the antibody does not recognise human OC peptides 7-19, 25-37 and 37-49; CIS bio international). The assay has a detection limit of 0.4 ng/ml with intra-assay and inter-assay variations of less than 1%. Measurement was on previously unfrosted paired serum and synovial fluid and all samples were assayed in duplicate.

Serum and synovial fluid keratan sulphate epitope (5D4) and hyaluronate (HA) were measured using ELISAs^{9,10} with some minor modifications.¹¹

STATISTICAL ANALYSIS

The HA and KS values were logarithmically transformed (natural log; Ln) before analysis. Correlations were calculated using Pearson product-moment coefficient and for non-parametric data (untransformed) Spearman's correlation coefficient was used. Student's two tailed unpaired *t* tests were used to compare between group differences. Statistical significance was set at the 5% level.

Results

There was no significant difference between the OA and RA patients with respect to age (mean 66.5, range 53–81; and 60.3, 29–78 years respectively) or sex (male:female 6:9 and 4:12 respectively). The normal controls however were significantly younger (mean 49.9, range 40–52 years; $p < 0.01$) than the OA and RA patients and had higher male:female ratio (7:3).

Synovial fluid and serum OC increased linearly with age ($r = 0.48$, $p < 0.01$ in both cases) and synovial fluid OC was significantly lower than serum ($p < 0.001$) in all patients and controls. Moreover, synovial fluid OC concentrations (mean (SD)) were higher in men compared with women (14.03 (4.71) *v* 11.06 (5.81); $p = 0.082$). Figure 1 shows the relation between paired serum and synovial fluid OC concentrations in each patient and controls. To allow for known variations in serum OC with age and other possible between patient differences in serum OC concentrations, the synovial fluid/serum OC ratios was calculated for each subject and then compared between groups. The ratio of synovial fluid/serum OC was similar in OA and controls (mean (SD), 0.69 (0.21) *v* 0.63 (0.06)). How-

Figure 2 Correlation between serum and synovial fluid OC concentrations.

Table 1 Serum and synovial fluid concentrations of osteocalcin (OC), keratan sulphate epitope 5D4, and hyaluronate (HA) in patients with osteoarthritis, rheumatoid arthritis, and healthy controls

Marker	OA	RA	Controls	p Values		
				OA v RA	OA v controls	RA v controls
SF OC (ng/ml)	13.71 (5.83)	10.67 (6.58)	12.47 (1.97)	0.183	0.457	0.317
Serum OC	20.68 (7.76)	25.68 (10.17)	19.80 (3.31)	0.091	0.698	0.054
Ln sf HA	11.53 (0.62)	11.32 (0.43)	12.23 (0.13)	0.303	<0.001	<0.001
LN serum HA	4.93 (0.56)	5.03 (0.74)	4.14 (0.33)	0.649	<0.001	<0.001
Ln SF 5D4	9.23 (0.48)	8.78 (0.89)	10.70 (0.21)	0.087	<0.001	<0.001
Ln serum 5D4	6.38 (0.13)	6.33 (0.18)	6.44 (0.14)	0.412	0.246	0.079

Values expressed as mean (SD). SF = synovial fluid; Ln = natural log, p values are from unpaired two tailed Student's *t* test.

ever, the ratio was significantly lower in RA than OA and controls (0.39 (0.14); $p < 0.001$ and $p < 0.01$ respectively). Figure 2 shows the overall correlation ($r=0.63$) between serum and synovial fluid OC concentrations in all subjects. The correlation was stronger in the controls ($r=0.87$, $p<0.001$) and RA ($r=0.82$, $p<0.001$), than in the OA group ($r=0.65$, $p<0.01$).

Table 1 shows the concentrations of OC and other markers. There were no significant differences in serum OC and 5D4 between groups but serum HA was significantly higher in the OA and RA groups. There were no significant differences in synovial fluid OC concentrations between groups. However, synovial fluid concentrations of 5D4 and HA were significantly decreased in OA and RA (see table 1). There were some significant ($p<0.05$) associations between OC, HA, and 5D4 in the patients and controls. In serum the correlation coefficient r between between OC and 5D4 was -0.55 in OA and 0.53 in RA. A positive correlation ($r=0.68$) between concentrations of serum OC and HA was also found in OA serum. In synovial fluid correlations were found in RA patients between concentrations of OC and HA ($r=0.69$) and in OA patients between HA and 5D4 concentrations ($r=0.57$).

Discussion

This study is one of a number of investigations undertaken to explore the relation between local synovial fluid OC and circulating serum OC in human joint disease.³⁻⁵ Synovial fluid from healthy controls is not readily available for comparison, and to our knowledge, this is the first report that includes OC measurement of paired synovial fluid and serum from healthy controls. Comparisons were made between OC concentrations, proposed markers of cartilage aggrecan degradation, and synovial inflammation in different body fluid compartments in OA, RA, and healthy controls.

The concentrations of synovial fluid OC was significantly lower than serum concentrations in the patients and controls. Similar results were found in other studies³⁻⁵ suggesting that most of the synovial fluid OC is derived from the blood. In healthy controls and RA patients there was a clear relation between the concentration of OC in serum and synovial fluid. The correlation was weaker in patients with OA perhaps because of increased local bone turnover.

Serum OC is known to increase with age and is generally higher in women than men.^{12,13} Moreover we have reported here that synovial fluid OC concentrations also increase with age but seem to be higher in men than women. Accordingly, patient's OC concentrations ought to be compared with age and sex matched healthy controls. In this study because of the difficulty of obtaining synovial fluid from normal controls, an age and sex matched range was not available for comparison. However we have used the ratio of synovial fluid/serum OC for comparison between groups, which is unlikely to be influenced by demographic difference (age and sex) and therefore may represent a more accurate measurement of changes in the subchondral bone.

RA patients showed significantly lower synovial fluid/serum OC ratios than OA patients and healthy controls, whereas the ratio values of OA patients were similar to the controls. Studies of serum OC concentrations in RA patients have shown discrepant findings with reports of increased,¹⁴ reduced,¹⁵ or normal¹⁶ concentrations of serum OC compared with healthy controls. These different results may reflect heterogeneity of bone involvement in RA, the influence of different variables on serum OC concentration, or methodological differences in the OC assay.

HA and 5D4 results contrasted with OC in that synovial fluid concentrations were very much higher than serum in all patients and controls (on average HA was 10 000 times and 5D4, 20 times higher in synovial fluid compared with serum), suggesting local production of HA and KS in the joint, a finding that has been previously reported.^{9,17} No correlation between synovial fluid and serum concentrations of HA were found in patients with OA, RA or controls and the same was true for 5D4. This result is not surprising, as serum concentrations are probably influenced by the release of HA and 5D4 from many arthritic joints as well as metabolism in the whole skeleton, thus only a small fraction of serum HA and 5D4 is derived from the knee joint where the synovial fluid was aspirated. Finally, our finding that there are some associations between the putative markers of bone turnover, cartilage aggrecan degradation, and synovial inflammation in both OA and RA are rather intriguing and support the generally held views that the joint functions as an organ and in arthritis metabolic changes in the tissues of the joint are interrelated.

In conclusion, the results show that in all cases serum concentrations of OC were higher than synovial fluid concentrations indicating that most synovial fluid OC is derived from the circulating blood.

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