TISSUE-DERIVED MACROMOLECULES AND MARKERS OF INFLAMMATION IN SERUM IN EARLY RHEUMATOID ARTHRITIS: RELATIONSHIP TO DEVELOPMENT OF JOINT DESTRUCTION IN HANDS AND FEET

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SUMMARY

We have previously shown that serum concentrations of cartilage oligomeric matrix protein (COMP) are increased early in rheumatoid arthritis (RA) patients who subsequently develop advanced large-joint destruction. A prognostic value for joint damage of serum concentrations of hyaluronan (HA) is also suggested by previous studies. In contrast, serum concentrations of bone sialoprotein (BSP) have not been useful for identifying patients with progressive large-joint destruction. In the present study, we have examined the hypothesis that serum concentrations of these tissue-derived markers are of prognostic value in RA for the development of radiographically detectable joint damage in hands and feet. Serum concentrations of COMP, HA and BSP were quantified in samples obtained from 62 patients within the first year after onset of RA and were related to the development of radiographically detectable damage in these joints after 5 yr. Neither the serum concentrations of COMP nor of BSP at inclusion predicted joint damage in hands and feet after 5 yr, and the concentration of these proteins did not change over the 5 yr period. However, the serum concentration of HA at inclusion correlated with the radiographic score at the 5 yr follow-up (r = 0.425, P < 0.01), but was not a better predictor in this respect than the erythrocyte sedimentation rate or C-reactive protein levels at inclusion. Thus, serum concentrations of the three studied tissue-derived macromolecules were in this study not useful for identifying patients prone to small-joint destruction.

KEY WORDS: Rheumatoid arthritis, Cartilage oligomeric matrix protein, Bone sialoprotein, Hyaluronan, Joint destruction.

RHEUMATOID arthritis (RA) is a chronic inflammatory disorder which may lead to pronounced damage of the joints. However, the progression of joint destruction varies considerably between patients and the search for instruments that predict the degree of future joint damage in the very early stages of the disease is of major interest in clinical rheumatology [1–3]. Novel therapeutic principles with the potential of retarding the destructive process are currently being developed and thus the need for clinically feasible means for assessing the prognosis in the individual patient is now urgent [4–6].

Sociodemographic factors and clinical and laboratory variables have been tested for use as predictive factors for future joint damage. Potential prognostic laboratory markers include measures of generalized inflammation, genetic markers, autoantibodies and tissue-derived antigens [2, 3]. In most studies, the acute-phase response, as measured by the C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR), correlates with the radiological progression of joint damage in groups of patients [1, 7–12]. In the individual patient, however, the prognostic value of these variables for the development of joint destruction seems limited [1, 7]. A possible way to overcome this shortcoming could be to use time-integrated values, as has been suggested [13, 14].

The quantification of tissue-derived macromolecules or their fragments in serum is another possible way of assessing the destructive potential. The rationale for this is the hypothesis that increased or changing serum levels of a tissue-derived ‘marker’ reflect changes in the tissue turnover. If sustained for a certain period of time, such changes may lead to uncoupling of the normal balance between matrix degradation and repair, and permanent joint damage could ensue [15]. Examples of such tissue-derived macromolecules are cartilage oligomeric matrix protein (COMP) [16, 17], bone sialoprotein (BSP) [16, 18] and hyaluronan (HA) [19], putative markers for cartilage, bone and synovial tissue, respectively.

COMP is a pentameric protein consisting of subunits, each with a Mr of ~100 kDa. The protein shares extensive homologies with the thrombospondin family of proteins, but is a unique gene product. It is enriched in the superficial zone of adult cartilage. COMP is readily quantifiable in synovial fluid and serum by immunoassay [16, 17]. Increased serum concentrations of COMP early after disease onset have in two separate longitudinal studies been found in RA patients who developed severe large-joint (hip or knee) destruction leading to joint replacement ([20]; Månsson et al., unpublished observations). Interestingly, in one study sequential measurements were performed, which showed decreasing serum concentrations of COMP over time with concentrations reaching those found in patients with no large-joint involvement 2–3 yr after disease onset [20].

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BSP is a bone-specific protein, particularly enriched in the subchondral bone [16]. This protein is released from all bone into the circulation, where it can be quantified by immunoassay [18]. Serum levels of BSP are increased in patients with RA compared with both controls and patients with non-erosive reactive arthritis [18]. However, studies of the prognostic value of this protein for large-joint destruction in RA have as yet not shown any such features of the protein ([21]; Månsson et al., unpublished observations).

Serum HA in RA is believed to originate mainly from the inflamed synovial membrane [19]. Although serum HA often correlates markedly well with levels of CRP or with ESR, an additional prognostic value is suggested by a study where serum HA correlated with the radiological progression of joint lesions in hands and feet of patients with RA [22]. In our longitudinal studies of patients with different outcome regarding large-joint destruction, somewhat conflicting results have been obtained. In one of the studies, the patients who developed large-joint destruction had significantly higher initial serum HA levels than those not developing such destruction [21]. In the other study, the difference between the groups did not reach statistical significance, although a trend towards higher levels was found in the group developing large-joint destruction (Månsson et al., unpublished observations).

The studies reviewed suggest a prognostic potential for serum COMP, and possibly for serum HA, for the development of large-joint destruction, but currently very little information regarding the prognostic value of tissue-derived markers for small-joint destructiveness is available. We have therefore examined the prognostic potential of COMP, BSP and HA, measured in the first serum sample available, for the development of radiographically detectable joint damage in hands and feet over the first 5 yr of follow-up in patients with early RA. For comparison, serum levels of CRP and the ESR were also measured. In addition, we also intended to relate changes in the serum concentrations of COMP and BSP to the progression of small-joint damage over the 5 yr period.

MATERIALS AND METHODS

Patients
The patients were part of a prospective study at the department of rheumatology at Lund University Hospital. The patients were actively recruited from primary care units in the surrounding area during the years 1985–1987. The primary inclusion criterion was definite RA according to the American College of Rheumatology 1958 criteria [23]. Furthermore, a disease duration of <24 months and age ≥18 yr were required for inclusion. All patients were of Caucasian origin. As yet, 113 patients have been followed for 5 yr. The 62 patients included in the present study were those who had been monitored for 5 yr and in whom radiographic examinations of hands and feet had been performed at study entry, and after 1, 2 and 5 yr. In the remaining 51 patients, the series of radiographs were not complete and samples from these patients were therefore not included in the study. There were no statistically significant differences between the included or excluded patients concerning demographic, clinical, genetic, radiographic or laboratory characteristics at study start. Neither did the included or excluded groups differ regarding clinical or laboratory parameters at the 5 yr follow-up. The age of the 62 included patients was median 52 (42–59, interquartile range) yr. They had a disease duration at inclusion of median 9.0 (4.5–12.5) months. There were 39 females and 23 males and 35 patients were Waaler–Rose positive. The Larsen score at inclusion was median 6 (3–11). At study start, all but two patients were taking non-steroidal anti-inflammatory drugs. No patient was taking oral glucocorticoids or disease-modifying anti-rheumatic drugs (DMARDs). During the 5 yr follow-up, 41 (68%) patients were treated with DMARDs for 6 months or more. The most commonly used DMARDs were chloroquine (27), d-penicillamine (18), gold compounds (8); sodium aurothiomalate (2), auranofin (6), methotrexate (6) and sulphasalazine (4). Twenty-one patients received more than one drug and 10 patients more than two drugs during follow-up.

Radiographic assessment
Radiographs of hands and feet were obtained at study start, and after 1, 2 and 5 yr. We used standard film and an anteroposterior angle. The radiographs were evaluated according to Larsen et al. (24). Thirty-two joints were assessed: MCP I–V, IP I, PIP II–V, the wrist, IP I and MTP II–V in both hands and feet. Each joint was compared with a standard reference film. The changes were graded on a six-grade scale from 0 to 5. Grade 0 indicates a normal joint, grade 1 joint space narrowing, periartricular osteoporosis or soft-tissue swelling, grades 2–5 increasing erosions and destruction. The wrist score was multiplied by five and the scores for the individual joints were added, giving a theoretical score range of 0–200. All radiographs were read by one of the authors (EF). All radiographs from one patient were read at the same time, thus comparing the changes from year to year [25]. Clinical and laboratory information was not available at the time of radiological evaluation.

Biochemical analyses
Analyses were performed on serum samples drawn from the patient at the first visit to the out-patient clinic. COMP and BSP were also measured on samples obtained after 2 and 5 yr. All samples were drawn in the morning between 09:00 and 11:00 a.m. after normal ambulatory activity. Serum was centrifuged and stored in aliquots at −80°C. ESR was measured according to Westergren. CRP was
TABLE I
Correlations between levels of the laboratory variables and the Larsen score at inclusion, after 1 yr, after 2 yr and after 5 yr. Values for erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), cartilage oligomeric matrix protein (COMP), hyaluronan (HA) and bone sialoprotein (BSP) refer to measurements in the first serum sample obtained from each patient, drawn at study inclusion.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Inclusion</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR</td>
<td>0.105</td>
<td>0.446***</td>
<td>0.611***</td>
<td>0.590***</td>
</tr>
<tr>
<td>Serum CRP</td>
<td>0.168</td>
<td>0.491***</td>
<td>0.558***</td>
<td>0.561***</td>
</tr>
<tr>
<td>Serum COMP</td>
<td>0.412**</td>
<td>0.258*</td>
<td>0.155</td>
<td>-0.022</td>
</tr>
<tr>
<td>Serum HA</td>
<td>0.209</td>
<td>0.371**</td>
<td>0.465***</td>
<td>0.425**</td>
</tr>
<tr>
<td>Serum BSP</td>
<td>0.276*</td>
<td>0.061</td>
<td>0.083</td>
<td>0.182</td>
</tr>
</tbody>
</table>

*p < 0.05; **p < 0.01; ***p < 0.001.

TABLE II
Correlations between levels of the different laboratory variables. Values for erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), cartilage oligomeric matrix protein (COMP), hyaluronan (HA) and bone sialoprotein (BSP) refer to measurements in the first serum sample obtained from each patient, drawn at study inclusion.

<table>
<thead>
<tr>
<th></th>
<th>Serum HA</th>
<th>Serum CRP</th>
<th>ESR</th>
<th>Serum BSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum COMP</td>
<td>0.388**</td>
<td>0.078</td>
<td>0.036</td>
<td>-0.081</td>
</tr>
<tr>
<td>Serum HA</td>
<td>0.510***</td>
<td>0.433**</td>
<td>0.837***</td>
<td>0.101</td>
</tr>
<tr>
<td>Serum CRP</td>
<td>0.837***</td>
<td>0.101</td>
<td>0.099</td>
<td></td>
</tr>
<tr>
<td>ESR</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*p < 0.05; **p < 0.01; ***p < 0.001.

Table I shows the correlation between the levels of the laboratory variables at study start and the Larsen score at inclusion, after 1 yr, after 2 yr and after 5 yr. Values for erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), cartilage oligomeric matrix protein (COMP), hyaluronan (HA) and bone sialoprotein (BSP) refer to measurements in the first serum sample obtained from each patient, drawn at study inclusion.

**Statistical calculations**

Differences between groups were tested by Mann–Whitney’s test for independent samples. The χ² test was used for discrete variables. Correlations were calculated using the Spearman correlation coefficient. A multiple regression analysis was performed to establish whether HA and the acute-phase reactants contained independent information regarding the joint damage process. P values of < 0.05 were considered significant.

**RESULTS**

Table I shows the correlation between the levels of the laboratory variables at study start and the Larsen score at inclusion, after 1 yr, after 2 yr and after 5 yr. Serum COMP at entry correlated with the radiographic changes at study start, but to a lesser extent with the radiographic findings after 1 yr, and not at all with the 2 and 5 yr Larsen scores. Serum BSP showed a weak, but significant correlation with the Larsen score at entry. None of the other variables correlated with the radiographic score at entry, but importantly ESR, CRP and HA at entry correlated with the 1, 2 and 5 yr radiographic score.

In the multiple regression analysis, with joint damage score after 5 yr as the dependent variable and HA, CRP and ESR as independent variables, only CRP was significant (data not shown).

The 26 patients with erosive changes in the hands and/or feet at inclusion did not differ from the 36 patients without such changes in their levels of any of the serum variables at inclusion.

Table II shows the correlation between the different serum variables at study start. COMP correlated weakly with HA, but not at all with CRP, BSP or ESR. Both CRP and ESR correlated with HA. ESR and CRP showed a highly significant correlation, as expected. BSP did not correlate with any of the other variables.

The serum levels of BSP and COMP did not change significantly over the 5 yr period (Table III), and these levels did not correlate significantly with the changes in radiographic score over the 5 yr period. Neither did we find any significant correlation

TABLE III
Bone sialoprotein (BSP) and cartilage oligomeric matrix protein (COMP) concentrations at study entry, and after 2 and 5 yr. The figures denote the median (interquartile range). The concentrations did not change significantly over the 5 yr period.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Year 2</th>
<th>Year 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum BSP (ng/ml)</td>
<td>124.6 (100.9–143.0)</td>
<td>126.0 (102.0–148.7)</td>
</tr>
<tr>
<td>Serum COMP (µg/ml)</td>
<td>8.7 (7.8–9.7)</td>
<td>8.6 (7.5–9.3)</td>
</tr>
</tbody>
</table>
where increased concentrations early in disease contrasts with the findings in our previous studies occurring most frequently in patients who later developed large-joint destruction ([20]; Månsson et al., unpublished observations). The most likely explanation for this discrepancy could be that changes in the cartilage turnover in large joints involve a larger cartilage mass and, consequently, the contribution to the circulation of fragments from these joints is greater and more easily detectable. For BSP, the findings are in line with the results of the studies regarding large-joint destruction [21]. The significant correlation between serum COMP, and to a lesser extent serum BSP, and radiographic changes at inclusion suggests, however, that the levels of these markers reflect changes in the tissue turnover perhaps preferentially very early in the process. Another explanation for the lack of a significant relationship between early COMP and BSP values and radiographic outcome after 5 yr could be that the summarized Larsen score does not clearly distinguish between cartilage and bone changes. More sensitive imaging techniques or scoring systems may possibly reveal relationships between changes in a certain tissue and serum concentrations of fragments from that tissue.

The significant correlation between the acute-phase reactants at entry and radiographic changes after 1, 2 and 5 yr confirms the findings of previous studies [1,7,9,11,22]. In contrast to Paimela et al. [22], we could not prove any additional prognostic value in HA measurements. Serum COMP correlated weakly to serum HA, but not to any of the other variables, and serum BSP did not correlate with any of the other serum markers. This suggests that the inflammatory process per se does not influence the serum levels of COMP and BSP.

The absolute levels of COMP were in the range found in normal blood donors, which is in line with previous findings in patients with RA [17]. This could reduce the diagnostic potential of cross-sectional measurements of serum COMP, but might, on the other hand, increase the diagnostic specificity of increased serum concentrations in certain patient groups, e.g. patients prone to rapid hip destruction [20]. For BSP, the serum levels were of the same order as previously found in RA patients, i.e. significantly higher than in blood donors, which suggests generalized bone involvement early in the disease process [18].

The serum concentrations of both COMP and BSP were remarkably stable over time in individual patients (data not shown), also reflected by the unchanged median values (Table III). This finding is in line with other results both in RA patients and in healthy controls (T. Saxne, unpublished). Thus, there was no difference in serum levels of COMP or BSP over time between those patients who developed radiographic changes in the joints of hands and feet and those who did not. The stable serum levels indicate that changes in serum levels over time in individual patients might be a sensitive instrument for evaluating therapeutic interventions with the potential to modulate the tissue process.

In conclusion, this study has confirmed the prognostic value of measurement of the inflammatory markers ESR and CRP early in the disease course for the development of small-joint destruction in RA. However, the study clearly shows the limited usefulness of these variables in the individual patient, since at best correlation figures of 0.6 were found, i.e. a considerable number of individuals with low ESR or CRP will develop destruction. In an attempt to increase the prognostic power, we measured three tissue markers in this study, but in contrast to findings in studies of large joints, none of these provided additional prognostic information regarding the development of destruction in the joints of hands and feet. It is possible that other tissue markers or combinations of markers may prove more useful. Studies which address both large- and small-joint involvement in the same patient in a longitudinal fashion are needed to evaluate tissue markers further as prognostic instruments for the development of joint destruction in RA.

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