Association of markers of bone- and cartilage-degradation with radiological changes at baseline and after 2 years follow-up in patients with ankylosing spondylitis

D. Vosse¹,², R. Landewe¹,², P. Garnero³,⁴, D. van der Heijde¹,²,⁵, S. van der Linden¹,² and P. Geusens¹,²,⁶

Objective. There is a lack of knowledge on factors that reliably can predict radiological changes in patients with AS. We have investigated whether urinary C-terminal cross-linking telopeptide of type I (CTX-I) and type II (CTX-II) collagen, as specific biochemical markers of bone and cartilage degradation, respectively, are associated with radiological damage and progression, and with BMD in patients with AS.

Methods. Eighty-three patients with AS [mean (s.d.) age: 50.4 (12) yrs, 65% male, mean (s.d.) disease duration after diagnosis: 16.7 (10) yrs] who participate in an ongoing cohort study of patients with AS [Outcome in AS International Study (OASIS) cohort] were assessed for urinary CTX-I and -II. Results of both biochemical markers were compared with baseline scores for radiological damage (modified modified Stoke Ankylosing Spondylitis Spine Score, primarily reflecting syndesmophyte-formation and -growth), and with scores for radiological progression after 2 yrs follow-up. Markers were also associated with disease activity parameters and BMD.

Results. Mean duration of complaints was 28.6 yrs. At that time, 54% of patients had signs of radiological damage, and 35% of them showed radiological progression after 2 yrs. Baseline radiological damage (ρ = 0.24; P = 0.05) correlated with CTX-II, but not with CTX-I. CTX-II correlated with serological markers of inflammation (ESR ρ = 0.29 and CRP ρ = 0.30; P ≤ 0.01), but not with baseline BASDAI or BMD. There was a negative correlation between CTX-I and BMD of the trochanter (ρ = −0.31; P ≤ 0.01). In multivariate analyses, CTX-II significantly and independently contributed to explaining variation in radiological damage (standardized β = 0.27; P = 0.03) and progression (standardized β = −0.27; P = 0.05).

Conclusion. In AS, cartilage degradation plays a role in explaining radiological damage and progression in the spine.

KEY WORDS: Ankylosing spondylitis, Biochemical markers, Bone degradation, Cartilage degradation, Radiological damage, Radiological progression.

Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease mainly affecting the axial skeleton and is characterized by ossification of the spinal joints and ligaments. Bone loss in diseases such as AS and RA is related to inflammation [1–4]. Since inflammation in AS resides primarily in the spine, inflammation-induced bone failure may lead to vertebral fractures and deformities, and consequently to hyperkyphosis of the upper part of the spine. Nowadays, it is increasingly recognized that osteoporosis plays a role in vertebral deformities in patients with AS [5–8]. In RA, radiological damage includes cartilage destruction and bone erosion, which is considered to be the result of chronic inflammation. It has been shown that specific biochemical markers of type I collagen degradation (CTX-I) (reflecting bone) and type II collagen degradation (CTX-II) (reflecting cartilage) could predict radiographic progression in RA [9–11]. In AS, the pathophysiological processes underlying radiologic progression are unclear. Whilst excessive bone formation (syndesmophytes) is most characteristic of AS, erosions and destruction of ‘vertebral units’ (vertebral bone plus intervertebral disc) may occur. A few cross-sectional studies have analysed biochemical markers of bone turnover and have reported conflicting data, but there seems to be increased bone resorption [12, 13].

have addressed markers of cartilage turnover, but the association with radiological damage or BMD was not investigated in these studies [14, 15].

The aim of the present study was to investigate among AS patients the relationships between CTX-I and -II with disease activity, radiological damage and 2-yr radiological progression.

Patients and methods

Eighty-three AS patients, participating in the Outcome in AS International Study (OASIS) cohort, were participating in this analysis. OASIS is an international longitudinal, observational study on the outcome of AS [16]. The patients are followed at regular intervals according to a standardized protocol. This study was approved by the hospital ethics committee and informed consent was obtained from all subjects. The 48-month assessment was used for the baseline evaluation of the present analysis, and the 72-month assessment for the evaluation of radiological progression. Data that were collected include ESR, CRP, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) [17] and Bath Ankylosing Spondylitis Functional Index (BASFI) [18]. The 44-joint count was used to assess the number of swollen joints. Lateral radiographs of the cervical and lumbar spine obtained at 48 and 72 months were used to measure radiographic progression. At the baseline visit, second morning void urine samples were collected. The samples were stored at −20°C and analysed simultaneously.

At 48 months, BMD was assessed by DXA (Hologic QDR 4500, NHANES-III reference group, Bedford, MA, USA).

Radiological assessment

To assess structural damage, the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) was used [19]. This method scores the anterior site of the lumbar (lower border T12 to the upper of S1) and cervical (lower border C2 to the upper border
of T1) spine at a lateral view. The anterior corners of each vertebra are examined and scored 1 for erosion, sclerosis and/or squaring, 2 for a syndesmophyte and 3 for total bony bridging, giving a maximum possible score of 72. In a previous experiment, we have determined that intra-observer and inter-observer reliability on mSASSS status scores of this method were excellent [20].

**Markers**

Urinary CTX-I was measured by the CrossLaps ELISA (Nordic Biosciences, Herlev, Denmark) [21]. This assay uses a polyclonal anti-serum raised against the β-isomerized EKAPβDGGR sequence of the C-telopeptide of α1 chains of human type I collagen. Intra- and inter-assay coefficients of variation were lower than 6 and 9%, respectively. Urinary CTX-II was measured by a competitive ELISA (CartiLaps; Nordic Biosciences, Herlev, Denmark) based on a mouse monoclonal antibody raised against the EKGPDP sequence of human type II collagen C-telopeptide [22]. This sequence is found exclusively in type II collagen and not in the other collagens, including type I collagen, or other structural proteins. The antibody used in this assay is absolutely specific for peptides containing a free C-terminal proline. Intra- and inter-assay coefficients of variation were less than 8 and 10%, respectively. Urinary CTX-I and -II levels were corrected by the urinary creatinine concentration. Urinary creatinine was measured by a standard colorimetric method. All measurements were performed in duplicate in a specialized central laboratory (Synarc, Lyon, France) and intra- and inter-assay coefficients of variation are <10%.

**Statistical analysis**

Spearman’s correlation coefficients were calculated to investigate univariate associations. Partial correlations with adjustment for disease duration, were calculated for CTX-I or -II levels vs ESR, CRP, mSASSS at baseline, BMD and 2-yr change in mSASSS. Linear regression analysis was performed to investigate which variables independently contributed to explaining radiological damage or progression. Independent variables entered in the model were: CTX-I,-II, CRP, BASDAI and mSASSS. Not normally distributed variables underwent a normalization procedure using the Van der Waerden technique.

**Results**

Table 1 shows the baseline characteristics of the 83 patients. Mean BASDAI was compatible with low to moderate disease activity Mean (s.d.) levels of CTX-I and -II were 190 (114) μg/mmol creatinine and 298 (260) ng/mmol creatinine, respectively. Elevated levels of CTX-I were found in 44 (53%) patients, elevated levels of CTX-II in 60 (72%) patients with a range from 74.1 to 1717.0 μg/mmol creatinine for CTX-I and from 11.5 to 628.1 ng/mmol creatinine for CTX-II. As a reference, CTX-I and -II levels of age- and sex-matched controls were 168 μg/mmol creatinine and 158 ng/mmol creatinine, respectively, in our laboratory. A significant percentage (54%) of patients had an increased mSASSS at baseline, 35% of patients showed some radiological progression over 2 yrs, ranging from 1 to 12 mSASSS units. Table 2 shows that CTX-II, but not CTX-I, significantly correlated with radiological damage at baseline, as well as with 2-yr radiological progression, and that these correlations remained significant after adjustment for disease duration. CTX-II also correlated with ESR and CRP and this correlation also remained significant after adjustment for disease duration. A negative correlation between CTX-I and BMD was found, especially for the area of the trochanter, which mainly consists of trabecular bone. Adjustment for disease duration did not influence this observation. Patients with increased levels of CTX-I or -II did not differ significantly in mSASSS scores or change compared with those with normal levels.

In a multivariate analysis, with baseline mSASSS as the dependent variable, CTX-II significantly and independently contributed in explaining radiological damage (Table 3). The total amount of variance explained, however, was small (5.6%). In this analysis, serological markers of inflammation or clinical disease activity at baseline did not contribute to explaining radiological progression. Expectedly, disease duration was significantly associated with mSASSS at baseline. A separate multivariate analysis with 2-yr change in mSASSS as the dependent variable yielded exactly similar results, with CTX-II weakly but significantly contributing to explaining variation in radiographic progression (standardized β: 0.27; P = 0.05). The significant contribution of CTX-II disappeared when mSASSS at baseline was added to the model.

**Discussion**

The first observation of this study is that both CTX-I and -II were increased in AS as compared with the age- and sex-matched controls.

**Table 2.** Correlation matrix for the relation between CTX-I and CTX-II vs measures of disease activity and radiographic damage or progression in 83 patients with AS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CTX-I</th>
<th>After adjustment for disease duration</th>
<th>CTX-II</th>
<th>After adjustment for disease duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>mSASSS at baseline</td>
<td>0.03</td>
<td>0.24**</td>
<td>0.28*</td>
<td></td>
</tr>
<tr>
<td>2-yr change in mSASSS</td>
<td>-0.06</td>
<td>0.26*</td>
<td>0.28*</td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>-0.03</td>
<td>0.30**</td>
<td>0.31**</td>
<td></td>
</tr>
<tr>
<td>ESR</td>
<td>-0.03</td>
<td>0.29**</td>
<td>0.30**</td>
<td></td>
</tr>
<tr>
<td>BASDAI</td>
<td>-0.18</td>
<td>0.01</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>BASFI</td>
<td>-0.21</td>
<td>0.02</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Swollen joint count</td>
<td>-0.10</td>
<td>-0.08</td>
<td>-0.10</td>
<td></td>
</tr>
<tr>
<td>BMD, femoral neck</td>
<td>-0.24</td>
<td>0.16</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>BMD, trochanter</td>
<td>-0.31*</td>
<td>-0.15</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

*p ≤ 0.05; **p ≤ 0.01. Figures are (partial) correlation coefficients.

**Table 3.** Multivariate analysis of variables contributing to spinal damage at baseline or progression after 2 yrs follow-up (by mSASSS) in 83 patients with AS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline damage</th>
<th>Progression 0-2yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>Standardized β</td>
<td>P-value</td>
</tr>
<tr>
<td>CTX-II (ng/mmol creatinine)</td>
<td>0.27</td>
<td>0.03</td>
</tr>
<tr>
<td>Disease duration (yrs)</td>
<td>-0.04</td>
<td>0.76</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0.33</td>
<td>0.004</td>
</tr>
<tr>
<td>BASDAI</td>
<td>0.11</td>
<td>0.37</td>
</tr>
<tr>
<td>Full model R²</td>
<td>0.056</td>
<td>0.061</td>
</tr>
</tbody>
</table>

All variables except BASDAI were normalized using a van der Waerden normalization procedure.
Bone- and cartilage-degradation in AS

unaffected population. Second, CTX-I and -II were moderately correlated, suggesting that in AS cartilage- and bone-degradation occur to some extent simultaneously as the result of a mutual trigger. Third, CTX-II was correlated with serological measures of disease activity (inflammation). In their relationship with radiological damage and progression (mSASSS), both markers also acted differently: CTX-II was to some degree associated with radiological-damage and -progression in AS—which points to a role for cartilage in explaining characteristic structural abnormalities in AS—whilst CTX-I, which reflects bone degradation, appeared not to be associated with radiological-damage or -progression at all.

In view of the evidence in RA that points to a tight relation between inflammation and bone- and cartilage-degradation [9–11], it is not surprising to find elevated CTX-I and -II levels in an inflammatory disease like AS. The elevated CTX-I levels, for example, confirm that inflammation results in loss of bone density, and the correlation we found between CTX-I and BMD is consistent with this observation. The elevated levels of CTX-II, and the association of CTX-II with markers of inflammation, also raise the suggestion that cartilage degradation in AS is up-regulated by inflammatory stimuli. These observations are consistent with observations in the literature that the suppression of inflammation by TNF-blocking drugs improves BMD [23] and reduces type II collagen degradation [15].

It is more difficult to find an appropriate explanation for the different relationships of CTX-I and -II with radiological-damage and -progression. Radiographic-damage and -progression, as measured by the mSASSS, mainly reflects the formation and growth of syndesmophytes, which is a proliferative—rather than a destructive—process. Usually, bone homeostasis is maintained by a tight coupling of bone formation and bone degradation, implying that a shift in one of both is followed by a concurrent shift in the other. The lack of association between CTX-I and mSASSS suggests that bone degradation (CTX-I) and bone proliferation (syndesmophyte measured by mSASSS) are not increased concurrently. It looks as if bone proliferation and bone degradation are uncoupled in AS. Further evidence supporting this uncoupling can be found in recent studies in the literature: states of bone degradation (low BMD, osteoporosis) and bone proliferation (syndesmophyte formation and growth) occur simultaneously in AS [24], and profound and sustained suppression of inflammation, for example, by TNF-blocking drugs, which reverses low BMD, does not result in an inhibition of radiological progression as measured by mSASSS (syndesmophytes) [25, 26]. Bone turnover has also been studied in detail in AS, supporting the picture of uncoupling [27, 28]. In these studies, markers of bone formation [osteocalcin and type I procollagen carboxy-terminal propeptide (P1CP)] and bone resorption [pyridinium cross-links pyridoline (PYD) and deoxy-pyridinoline (D-PYD)] were investigated in conjunction with inflammatory markers (acute-phase response). In both studies, increased levels of markers of bone resorption were found without a concomitant increase in levels of markers of bone formation.

Somewhat surprisingly, CTX-II was to some degree correlated with radiological damage and progression, and this association remained after the adjustment for markers of inflammation, with which CTX-II was also correlated. One may conclude from this observation that cartilage degradation is somehow related to the process of syndesmophyte formation. The type of analysis that we have done does not allow any conclusion about causality in the relationship between CTX-II release and radiological progression, which implies that cartilage degradation either precedes or follows syndesmophyte formation or growth. The absence of any relationship between inflammatory markers and radiological progression, and the presence of such a relationship between CTX-II and inflammatory markers, may add to the suggestion that cartilage degradation is a secondary effect of syndesmophyte-formation and -growth in the spine. One could imagine, for example, that ossification of the facet joints, the intervertebral discs and the ligaments goes along with the destruction of the existing cartilage. In a different context, Garnero et al. [29] have described an association between CTX-II and spinal disc degeneration. They found that post-menopausal women with lumbar spine disc degeneration (radiological intervertebral space narrowing) had an increase in urinary CTX-II levels. Interesting in this regard is the recent observation, in biopsies of patients with knee OA, that CTX-II is primarily localized at the interface of bone and cartilage. Accordingly, urinary CTX-II may reflect at least in part degradation of calcified cartilage, which may imply that the source of CTX-II in this study could be the vertebral bone–intervertebral disc interface, a locus of pathology in AS [30].

A number of limitations of this study can be mentioned. A first limitation may be that urine sampling in OASIS was not systematically performed, so that in only 83 patients’ urine samples were ultimately available. However, the selection of patients was completely random, and this should not have had any impact on the results. Another consequence of a small sample size is that the power to detect subtle relationships was not extremely high. However, we were able to statistically support weak associations (correlation coefficients of ~0.20) and smaller correlations, even if they are statistically significant, are hardly, if ever, clinically informative. A methodological aspect in studies exploring associations that is more important than sample size is the presence of a broad spectrum of the phenotype of the disease under study. Our study meets this criterion, since we have included patients with zero radiological progression as well as with major progression (12 mSASSS units in 2 yrs) and all values in between. A third limitation is that we were not able to longitudinally collect urine samples in this cohort, in order to check for variability in levels and correlations. Finally, the contribution of CTX-II in explaining radiological damage and progression, though statistically significant, is rather small, implying that other and unknown processes different from cartilage degradation contribute to syndesmophyte-growth and -formation in AS.

In conclusion, AS is characterized by bone and cartilage degradation. The former reflects the systemic inflammatory effects on bone density and can be influenced by TNF-α blocking agents, while the latter is somehow associated with syndesmophyte formation, which is not influenced by anti-inflammatory treatment modalities. This underlines the suggestion that bone degradation and new bone formation are separate processes in AS. Our findings suggest that bone resorption, reflected by CTX-I, and extra-osseous new bone formation, reflected by mSASSS and associated with cartilage damage (CTX-II), are different aspects of structural changes in AS. Future treatment strategies should take these differences into account.

**Rheumatology key messages**

- Both CTX-I and -II were increased in patients with AS.
- CTX-II is associated with radiological-damage and -progression in AS.
- New bone formation and cartilage damage, may represent different aspects of structural changes in AS.

**Disclosure statement:** The authors have declared no conflicts of interest.

**References**


