

## **Expression of cathepsin K and MMP-1 indicate persistent osteodestructive activity in longstanding ankylosing spondylitis**

\*Michel Neidhart<sup>1</sup>, \*Xenofon Baraliakos<sup>2</sup>, Christian Seemayer<sup>1</sup>, Caren Zelder<sup>3</sup>, Renate E. Gay<sup>1</sup>, Beat A. Michel<sup>1</sup>, Heinrich Boehm<sup>3</sup>, Steffen Gay<sup>1</sup>, Jürgen Braun<sup>2</sup>

<sup>1</sup>WHO Collaboratory Center for Molecular Biology, University Hospital Zürich, Switzerland

<sup>2</sup>Rheumazentrum Ruhrgebiet Herne, Ruhr-University Bochum, Germany

<sup>3</sup>Clinic for Orthopaedics Spinal Surgery and Paraplegiology, Bad Berka, Germany

### **Address for correspondence:**

Prof. Dr. J. Braun  
Rheumazentrum Ruhrgebiet Herne  
Ruhr-University Bochum  
Landgrafenstr.15  
44652 Herne  
Germany  
Email: [J.Braun@Rheumazentrum-Ruhrgebiet.de](mailto:J.Braun@Rheumazentrum-Ruhrgebiet.de)

*\*The first two authors have equally contributed to this paper*

## **Abstract**

**Background.** Ankylosing spondylitis (AS) is a frequent largely genetically determined rheumatic disease that is characterised by spinal inflammation and new bone formation. However, the exact pathogenesis including pathology is still not clear.

**Objective.** To analyse tissue obtained at spinal surgery by immunohistochemistry and compare the specimen of patients with AS to those with degenerative disc disease (DDD).

**Methods.** Bony and soft tissue specimens of 30 AS and 20 DDD patients were obtained during spinal osteotomy from different anatomic regions including articular and spinous processes, interspinous ligaments and intervertebral disks. Immunohistochemistry was performed with established markers for cathepsin-K, MMP-1, MMP-3 and RANK-ligand.

**Results.** Cathepsin-K and MMP-1-positive cells were only observed in AS specimen. Cathepsin-K-positive multinucleated cells were detected at articular processes adjacent to fibrous tissues. MMP-1 was expressed in smaller mononuclear cells attached to bone, Invasion of bone by MMP-1 cells was seen at enthesal sites. In the intervertebral disks, most mononuclear cells were cathepsin-K-positive. Isolated cells expressing these matrix degrading enzymes found in DDD never showed signs of invasion. No differences were found for MMP-3 between AS and DDD. Clear expression of RANK ligand was only detected in one AS patient.

**Conclusion.** Cathepsin-K is strongly expressed in different regions of the spine in AS. Mainly mononuclear cells, fibroblast-like cells, and cells attached to bone and at sites of bone remodelling expressed cathepsin K suggestive of high osteoclastic activity. This supports the role of persistent inflammation in the pathogenesis of AS. How these changes relate to osteoproliferation remains to be determined.

## Introduction

Ankylosing spondylitis (AS), a chronic inflammatory rheumatic disease that affects the axial skeleton and other joint and organ structures, causes inflammatory back pain in the majority of affected patients as the most characteristic clinical symptom. AS leads to structural and functional impairments and a decrease in quality of life in many patients (1). Inflammation usually occurs at sites where ligaments and/or tendons attach to bone, and this may be followed by bone erosions and/or new bone formation at sites of attachment. This is clinically relevant because spinal movement of such patients becomes restricted when bone replaces elastic tissues of ligaments and tendons. A hallmark of AS are syndesmophytes and ankylosis going along with the fusion of vertebrae, apophyseal joints and intervertebral spaces. Syndesmophyte formation and progressive ankylosis have been described as a feature of abnormal bone remodelling (2), and uncoupling of inflammation and new bone formation have been found in an animal model (3).

The etiology of AS is far from being clear. The genes known to be associated with AS such as HLA-B27, ARTS-1, IL-23R and IL-1 account for more than 50% of the total risk (4, 5). However, fewer than 10% of HLA-B27-positive individuals in the general population become affected (6). Despite the possible triggering of reactive arthritis by preceding infections and the association of the spondyloarthritides with HLA-B27 there is no proven relationship between AS and an inciting infections, and antibiotics are ineffective in treating AS (7). The expression of matrix metalloproteinases (MMPs) in the synovium and bone of patients with rheumatoid arthritis (RA) and osteoarthritis (OA) has been an active area of investigation in the last years (8). In AS there are a few recent studies in which these factors have been studied on the serum level (9). In two separate cohorts serum levels of MMP-3 were found to correlate with disease activity (10, 11). A very recent finding was that MMP-3 levels may even predict new bone formation in AS (12). MMP-1 has a predominant role in RA and OA because it is rate limiting in the process of collagen degradation (8). Expression of both, MMP-1 and MMP-3, has been described in association with inflammatory cells present in granulation tissues found in herniated vertebral discs (13).

Osteoclasts which belong to the monocyte/macrophage family have been identified as key players in the osteodestructive process that is frequently observed in RA (2). The development of osteoclasts is largely influenced by the action of RANK ligand and M-CSF (14). In response to pro-inflammatory cytokines, cathepsin K has been made responsible for cartilage destruction mediated by fibroblasts and bone resorption by osteoclasts (15).

Corrective surgery may be indicated when a severe hyperkyphosis of AS leads to an unfavourable posture with loss of sagittal balance and loss of horizontal vision. Taking advantage of the profound experience of a specialized spine center, we analysed biopsy specimens obtained during spinal surgery in AS patients and controls concentrating on persistence of spinal inflammation. In this study we show in this study that spinal inflammation and osteodestruction are ongoing persistent processes in patients with established AS.

## Material and methods

**Patients and samples.** Bony and soft tissue specimens from 30 patients with AS who underwent surgical intervention of the spine because of hyperkyphosis, with or without vertebral “fusion” or spinal instability (“Andersson lesion”) were obtained during the surgery from the following sites: 1) articular processes, 2) spinous processes with interspinous ligaments and 3) intervertebral disks (Figure 1). The histological results were compared with specimens from a group of 20 patients with degenerative disk disease (DDD) undergoing spinal surgery for spinal and leg pain. Clinical and imaging parameters before and after surgery were analyzed for the correlation with the histopathology of the specimens.

The study was approved by the Ethical Committee of the “Landesärztekammer Thüringen” Patients gave informed consent prior to the intervention. They were informed that no extra bone material was removed during surgery, only organ tissue that was removed anyway because of the intervention was analysed.

**Imaging.** Conventional standardized radiographs of the thoracic and lumbar spine on one film were obtained in the standing position in posterior-anterior and lateral projection. In addition detailed views of the cervical, thoracic or lumbar spine region of interest were performed in the lateral view and were scored by using the modified Stokes AS spinal score (mSASSS, (16)). All images were obtained < 1 month before surgery.

Magnetic resonance imaging sequences were performed according to a standard protocol using a spine coil and/or a body-array coil. The MRI techniques administered to investigate sacroiliac and spinal inflammation in AS patients have been recently described (17). The sagittal view was assessed in the T2-weighted spin-echo (SE)/Turbo-SE-Mode sequence (repetition time (TR)/echo time (TE) 4000/108 ms, slice thickness 3-4 mm. All MRIs were evaluated by using the ASSpiMRI-a Score (18).

**Immunohistochemistry.** The immunohistological analysis included cathepsin K, MMP-1, MMP-3 and RANK ligand as molecules involved in osteodestruction. The tissues were fixed in 4% formaldehyde, decalcified using EDTA and NaOH, embedded into paraffin, and cut to 0.5 µm sections. Before use, the sections were deparaffined with xylol and ethanol. The labelling was performed with the following primary antibodies: murine monoclonal antibodies against human cathepsin K (clone 182-12G5, Chemicon), murine monoclonal antibodies against pro- and active MMP-1 (clone 36665, R&D), goat polyclonal antibodies against anti human pro- and active MMP-3 (AF513, R&D) and murine monoclonal antibodies against RANK ligand (TranceODF, clone 12A668, Imgenex). Either purified murine IgG or goat IgG were used as negative controls. Secondary antibodies were rabbit anti-mouse IgG HRP-conjugated or rabbit anti-goat IgG HRP-conjugated (Dako). Staining was performed using the EnVision+ System and the Vector VIP substrate kit (Dako).

**Statistics.** The Wilcoxon signed rank test used for comparing two dependent samples. The Mann-Whitney U-test was used to compare the frequency of positive staining between AS and DDD considering each site of specimen separately. Statistics were performed by dichotomous between patients with positive (score of ‘1’) or negative (‘0’) staining to each site of the vertebral body examined. Mann-Whitney U-test compared the frequency of positive staining between AS and DDD.

## Results

**Patient's demographics.** Overall, 27/30 (90%) AS patients and 7/20 (35%) patients from the control group were male. The mean age in the AS group was  $62.4 \pm 11.6$  years (range 31 – 73 years) and in the control group it was  $49.3 \pm 10.5$  years (range 44 – 82 years). Finally, the mean disease duration since diagnosis was  $5.6 \pm 5.7$  years (range 1-20 years) in the AS group.

**Imaging.** There was only limited spinal inflammation present in the MRI (mean ASSpiMRI-a score: 0,3 units), and clearly less when compared to previous studies (18), but more radiographic damage (mean mSASSS: 48,5 units), as compared to recent cohorts (19). The spinal segment that was most often selected for osteotomy was T12/L1.

### Immunohistochemistry.

#### ***Cathepsin K-positive multinucleated cells at sites of bone destruction.***

In the sections of tissues from patients with AS, isolated or clustered large, mono- or multinucleated cells, were detected to be positive for cathepsin K. Based on their morphology, the great majority of these cells could be classified as osteoclasts. In the articular processes, numerous cathepsin K positive multinucleated cells were found adjacent to fibrous tissues (Figure 2a). In the spinous processes, most cathepsin K-positive multinucleated cells were accompanied by unstained mononucleated cells, possibly fibroblasts or osteoclast precursors (Figure 2b). In the intervertebral disks, a large number of mononuclear fibroblast-like cells were found to be positive for cathepsin K and to invade into bone (Figure 2c).

Positive cells for cathepsin K could also be observed in patients with DDD, but they were lower frequent (Figures 2d and 3). However, the occurrence of cathepsin K positive mononuclear and fibroblast-like cells were highly significantly increased in AS, compared to DDD ( $p < 0.001$ , Figures 3a-b). Cathepsin K-positive multinucleated cells were detected in 40-60% of the patients with AS or DDD (Figure 3c), but as shown in Figure 2, they were less frequent and active in DDD. These observations were reflected by the number of cells attached to bone, which was highly significantly increased in AS, compared to DDD ( $p < 0.001$ , Figure 3d). Taken these data together, cathepsin K positive multinuclear and mononuclear cells appeared actively involved in the destruction of bone.

#### ***MMP-1 positive mononuclear cells invading bone.***

In the articular processes and intervertebral disks, numerous small mononuclear cells attached to bone expressed MMP-1 (Figure 4a, c). Some of these cells appeared to be involved in osteodestruction. In the spinous processes with interspinous ligaments, such MMP-1 positive cells were identified to invade bone (Figure 4b). Cells expressing MMP-1 were observed also in DDD; but they were either isolated or clustered and only attached to bone (Figure 4d), but never invading (0/20 patients, 0%), as it was the case in AS (20/30 patients, 67%,  $p < 0.001$ ). Moreover, the occurrence of MMP-1 positive mononuclear cells was also significantly increased in the articular processes of patients with AS, compared to DDD ( $p < 0.01$ , Figure 5a).

The cells expressing MMP-1 invading bone appeared morphologically different from those expressing cathepsin K. MMP-1 cannot resorb mineralized bone, but presumably the bone had been partially degraded previously by osteoclasts, either directly or by releasing molecules capable of breaking down mineralized bone.

Positive controls for MMP-1, MMP-3 and cathepsin K were tissue sections of rheumatoid arthritis synovial tissues. Strong expressions of MMP-1 and MMP-3 were detected, whereas cathepsin K was found in single cells at sites of invasion. In contrast to MMP-1 and cathepsin K, no differences in the expression of MMP-3 between AS and DDD were found. In those patients, MMP-3 was not negative, but expressed in mononuclear cells to the same extent in both patient groups (data not shown).

While all patients expressed MMP-3, expression of RANK ligand could only be detected in AS patients (Fig. 6, representative example).

## Discussion

This study shows that there is persistence of osteodestruction in patients with longstanding AS. Cathepsin K, one of the main collagenolytic proteases produced by osteoclasts, is strongly expressed in AS, much more than in DDD examined for comparison. In our immunohistochemical analysis, mainly mononuclear cells, fibroblast-like cells and cells attached to bone especially at sites of bone remodelling showed expression of cathepsin K and MMP-1, suggesting increased occurrence of osteoclasts. This phenomenon found in spinal biopsy specimens of AS patients appeared similar to activated osteoclasts at sites of bone destruction in patients with RA (14, 15). In specimen of AS patients obtained at hip replacement recent immunohistological data showed that osteoclastic foci and angiogenesis in the subchondral bone marrow were increased at sites with remaining cartilage (20).

However, in AS, new bone formation is much more characteristic for the disease process, and it is unclear how inflammation and new bone formation relate. Some recent data have even indicated an uncoupling of these processes (2, 3) and anti-TNF therapy, despite strong clinical efficacy (21), does not seem to have a major influence on radiographic progression (22-24). However, the methodology (the mSASSS only evaluates the cervical and the lumbar spine, and has limited sensitivity to change as indicated by a mean change of 1 scoring unit within 2 years), the study design (historical cohort) and the power of the trials performed so far have been debated. Our data obtained from patients without recent anti-TNF therapy indicate that inflammation and osteodestruction persist in AS patients with much radiographic damage. This is also in line with the recent observation that MMP-3 levels of patients with AS predict radiographic damage (12). This may indicate a role for these features also for new bone formation. It is, however, still not clear how these processes are linked.

Possibly, systemic triggers are involved in the activation and/or differentiation of osteoclasts in AS. We detected RANK ligand in the spinous process of at least one AS patient. This is consistent with recent data showing that serum levels of RANK ligand are upregulated in patients with AS – in possible relationship with bone mineral density and radiological changes (25).

Taken together, these data suggest that *de novo* generation of osteoclasts is involved in the ongoing pathological process in AS.

In the early stage of inflammatory rheumatic diseases, it is believed that matrix degrading enzymes play an important role (26). Our data demonstrate numerous foci of active bone destruction at the site of the surgical intervention which is chosen for static reasons. These data are in line with a previous study (27), based on

biochemical markers, which showed that bone turnover in patients with AS reflects normal osteoblastic activity, but high osteoclastic activity. An interesting observation of our study is that MMP-3 was almost not detected in the biopsy specimen examined. Since our stainings included both pro- and active MMPs and since all patients expressed MMP-3, this was not a problem of sensitivity, but could be due to the fact that the intensity of expression in immunohistochemistry is more qualitative than quantitative. In contrast, ELISAs of biological fluids such as serum are better to provide quantitative data. Indeed, recent data have suggested that MMP-3 serum levels correlate with disease activity (10, 11). Furthermore, elevated MMP-3 levels in the periphery could potentially well derive from other sources such as circulating monocytes.

There is no straightforward explanation for this discrepancy, it, however, seems possible that the study populations were different in terms of disease duration (almost 30 years in our patients).

It seems likely that persistent inflammation and osteodestruction is responsible for the continuous pain reported by patients with AS (28) but this has not been proven to date. In a recent study (20) there was some correlation between inflammatory changes seen by MRI and cellular inflammation detected by immunohistology in specimen that were also obtained during osteotomy of AS patients. However, there is also some but limited evidence for a dissociation between spinal inflammation as detected by MRI and clinical symptoms in some AS patients (Braun J, personal communication).

Our observations may have implications for the treatment of AS patients. On the one hand, it has been shown that the bisphosphonate pamidronate which has anti-osteoclast activity, improves the disease activity of patients with AS (29). It is unknown whether there is an influence of bisphosphonates on radiographic damage but it seems likely that the osteoporosis and osteopenia found in 20-50% of AS patients (30) will improve by this treatment.. On the other hand, TNF- $\alpha$  blockers have been clearly shown to improve the clinical symptoms (21) and the spinal inflammation as detected by MRI (31, 32) of AS patients. However, their effect on new bone formation may be limited (see above). This may be different for continuous therapy with NSAIDs (33). In consequence, studies should be performed to combine such approaches. For ethical reasons, it will be difficult to prove the effects of therapy by immunohistology.

In conclusion, unlike other proteases, cathepsin K - one of the main collagenolytic protease in osteoclasts - is significantly stronger expressed in patients with AS, as compared to DDD. Mainly mononuclear cells, fibroblast-like cells and cells attached to bone and at sites of bone destruction express cathepsin K, reflecting high osteoclastic activity in those regions. RANK ligands could be involved in the generation of osteoclasts. Our data support the view that cells expressing cathepsin K or MMP-1 play an important role in the pathogenesis of AS, which is not only characterized by osteoproliferation, but also by inflammation and ongoing osteodestruction of bone.

*The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non-exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if accepted) to be published in Annals of the Rheumatic Diseases editions and any other BMJ PGL products to exploit all subsidiary rights, as set out in licence <http://ard.bmjournals.com/ifora/licence.pdf>*

**Acknowledgement.**

We thank Mrs. Maria Comazzi-Fornallaz (center for experimental rheumatology, Zürich) for excellent technical support.



## References

1. Braun J, Sieper J. Ankylosing spondylitis. *Lancet* 2007;369(9570):1379-90.
2. Schett G, Landewe R, van der Heijde D. Tumour necrosis factor blockers and structural remodelling in ankylosing spondylitis: what is reality and what is fiction? *Ann Rheum Dis* 2007;66(6):709-11.
3. Lories RJ, Derese I, de Bari C, Luyten FP. Evidence for uncoupling of inflammation and joint remodeling in a mouse model of spondylarthritis. *Arthritis Rheum* 2007;56(2):489-97.
4. Brown MA. Non-major-histocompatibility-complex genetics of ankylosing spondylitis. *Best Pract Res Clin Rheumatol* 2006;20(3):611-21.
5. Brown MA. Human leucocyte antigen-B27 and ankylosing spondylitis. *Intern Med J* 2007;37(11):739-40.
6. Reveille JD. The genetic basis of ankylosing spondylitis. *Curr Opin Rheumatol* 2006;18(4):332-41.
7. Inman RD. Mechanisms of disease: infection and spondyloarthritis. *Nat Clin Pract Rheumatol* 2006;2(3):163-9.
8. Burrage PS, Mix KS, Brinckerhoff CE. Matrix metalloproteinases: role in arthritis. *Front Biosci* 2006;11:529-43.
9. Zhu J, Yu DT. Matrix metalloproteinase expression in the spondyloarthropathies. *Curr Opin Rheumatol* 2006;18(4):364-8.
10. Yang C, Gu J, Rihl M, Baeten D, Huang F, Zhao M, et al. Serum levels of matrix metalloproteinase 3 and macrophage colony-stimulating factor 1 correlate with disease activity in ankylosing spondylitis. *Arthritis Rheum* 2004;51(5):691-9.
11. Chen CH, Lin KC, Yu DT, Yang C, Huang F, Chen HA, et al. Serum matrix metalloproteinases and tissue inhibitors of metalloproteinases in ankylosing spondylitis: MMP-3 is a reproducibly sensitive and specific biomarker of disease activity. *Rheumatology (Oxford)* 2006;45(4):414-20.
12. Maksymowych WP, Landewe R, Conner-Spady B, Dougados M, Mielants H, van der Tempel H, et al. Serum matrix metalloproteinase 3 is an independent predictor of structural damage progression in patients with ankylosing spondylitis. *Arthritis Rheum* 2007;56(6):1846-53.
13. Matsui Y, Maeda M, Nakagami W, Iwata H. The involvement of matrix metalloproteinases and inflammation in lumbar disc herniation. *Spine* 1998;23(8):863-8; discussion 868-9.
14. Teitelbaum SL. Osteoclasts: what do they do and how do they do it? *Am J Pathol* 2007;170(2):427-35.
15. Hummel KM, Petrow PK, Franz JK, Muller-Ladner U, Aicher WK, Gay RE, et al. Cysteine proteinase cathepsin K mRNA is expressed in synovium of patients with rheumatoid arthritis and is detected at sites of synovial bone destruction. *J Rheumatol* 1998;25(10):1887-94.
16. Creemers MC, Franssen MJ, van't Hof MA, Gribnau FW, van de Putte LB, van Riel PL. Assessment of outcome in ankylosing spondylitis: an extended radiographic scoring system. *Ann Rheum Dis* 2005;64(1):127-9.
17. Braun J, Bollow M, Sieper J. Radiologic diagnosis and pathology of the spondyloarthropathies. *Rheum Dis Clin North Am* 1998;24(4):697-735.
18. Braun J, Baraliakos X, Golder W, Brandt J, Rudwaleit M, Listing J, et al. Magnetic resonance imaging examinations of the spine in patients with ankylosing spondylitis, before and after successful therapy with infliximab: evaluation of a new scoring system. *Arthritis and Rheumatism* 2003;48(4):1126-36.
19. Wanders AJ, Landewe RB, Spoorenberg A, Dougados M, van der Linden S, Mielants H, et al. What is the most appropriate radiologic scoring method for

ankylosing spondylitis? A comparison of the available methods based on the Outcome Measures in Rheumatology Clinical Trials filter. *Arthritis Rheum* 2004;50(8):2622-32.

20. Appel H, Loddenkemper C, Grozdanovic Z, Ebhardt H, Dreimann M, Hempfing A, et al. Correlation of histopathological findings and magnetic resonance imaging in the spine of patients with ankylosing spondylitis. *Arthritis Res Ther* 2006;8(5):R143.

21. Braun J, Brandt J, Listing J, Zink A, Alten R, Golder W, et al. Treatment of active ankylosing spondylitis with infliximab: a randomised controlled multicentre trial. *Lancet* 2002;359(9313):1187-93.

22. Baraliakos X, Listing J, Brandt J, Haibel H, Rudwaleit M, Sieper J, et al. Radiographic progression in patients with ankylosing spondylitis after 4 yrs of treatment with the anti-TNF- $\alpha$  antibody infliximab. *Rheumatology (Oxford)* 2007;46(9):1450-3.

23. Van der Heide D, Landewe R, Deodhar A, Baker D, Han J, Xu W, et al. Radiographic progression in patients with ankylosing spondylitis after 2 years of treatment not inhibited with infliximab. *Ann Rheum Dis* 2007;66(Suppl II):85.

24. van der Heijde D, Landewe R, Ory P, Vosse D, Zhou L, Tsuji W, et al. Two-year etanercept therapy does not inhibit radiographic progression in patients with ankylosing spondylitis. *Ann Rheum Dis* 2006;65 (Suppl II):81.

25. Kim HR, Kim HY, Lee SH. Elevated serum levels of soluble receptor activator of nuclear factors-kappaB ligand (sRANKL) and reduced bone mineral density in patients with ankylosing spondylitis (AS). *Rheumatology (Oxford)* 2006;45(10):1197-200.

26. Cunnane G, FitzGerald O, Hummel KM, Gay RE, Gay S, Bresnihan B. Collagenase, cathepsin B and cathepsin L gene expression in the synovial membrane of patients with early inflammatory arthritis. *Rheumatology (Oxford)* 1999;38(1):34-42.

27. Yilmaz N, Ozaslan J. Biochemical bone turnover markers in patients with ankylosing spondylitis. *Clin Rheumatol* 2000;19(2):92-8.

28. Zink A, Braun J, Listing J, Wollenhaupt J. Disability and handicap in rheumatoid arthritis and ankylosing spondylitis--results from the German rheumatological database. German Collaborative Arthritis Centers. *J Rheumatol* 2000;27(3):613-22.

29. Maksymowych WP, Jhangri GS, Fitzgerald AA, LeClercq S, Chiu P, Yan A, et al. A six-month randomized, controlled, double-blind, dose-response comparison of intravenous pamidronate (60 mg versus 10 mg) in the treatment of nonsteroidal antiinflammatory drug-refractory ankylosing spondylitis. *Arthritis Rheum* 2002;46(3):766-73.

30. Karberg K, Zochling J, Sieper J, Felsenberg D, Braun J. Bone loss is detected more frequently in patients with ankylosing spondylitis with syndesmophytes. *J Rheumatol* 2005;32(7):1290-8.

31. Braun J, Landewe R, Hermann KG, Han J, Yan S, Williamson P, et al. Major reduction in spinal inflammation in patients with ankylosing spondylitis after treatment with infliximab: results of a multicenter, randomized, double-blind, placebo-controlled magnetic resonance imaging study. *Arthritis Rheum* 2006;54(5):1646-52.

32. Baraliakos X, Davis J, Tsuji W, Braun J. Magnetic resonance imaging examinations of the spine in patients with ankylosing spondylitis before and after therapy with the tumor necrosis factor alpha receptor fusion protein etanercept. *Arthritis Rheum* 2005;52(4):1216-23.

33. Wanders A, Heijde DV, Landewe R, Behier JM, Calin A, Olivieri I, et al. Nonsteroidal antiinflammatory drugs reduce radiographic progression in patients with

ankylosing spondylitis: A randomized clinical trial. *Arthritis Rheum* 2005;52(6):1756-1765.

### Figure legends

Figure 1: Magnetic resonance image of the transversal section of the spine used for clarification of the localisation of the histological specimens taken in this study.

Figures 2: a-c, Immunohistochemical examination for cathepsin K in patients with ankylosing spondylitis (AS); d, Intervertebral disk of a patient with degenerative disk disease (DDD). AP=articular process, SP=spinous process, ID=intervertebral disk.

Figures 3: a/b, Percentage of AS and DDD patients with positive cathepsin K cells at different sites of the vertebra. AP=articular process, SP=spinous process, ID=intervertebral disk. The occurrence of cathepsin K positive cells is higher in AS compared with DDD at all sites examined in this study.

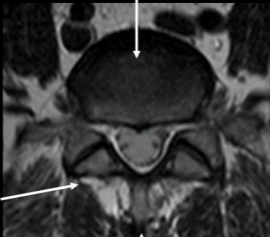
Figures 4: a-c, Immunohistochemical examination for MMP-1 in patients with ankylosing spondylitis (AS); d, Intervertebral disk of a patient with degenerative disk disease (DDD). AP=articular process, SP=spinous process, ID=intervertebral disk.

Figures 5 a/b, Percentage of AS and DDD patients with positive MMP-1 cells at different sites of the vertebra. AP=articular process, SP=spinous process, ID=intervertebral disk. The occurrence of MMP-1 positive cells is higher in AS compared with DDD particularly in the AP.

Figures 6a/b. Expression of RANK ligand in the spinous process in a patient with ankylosing spondylitis (AS) and a patient with degenerative disk disease (DDD).

# Figure 1

Intervertebral disc (ID)



Transverse process (TP)

Spinous process (SP)

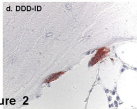
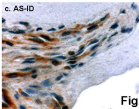
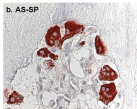
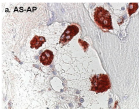


Figure 2

**Figure 3 a/b**

**Mononuclear cells**

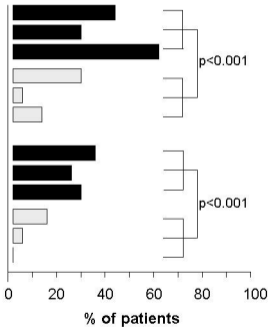
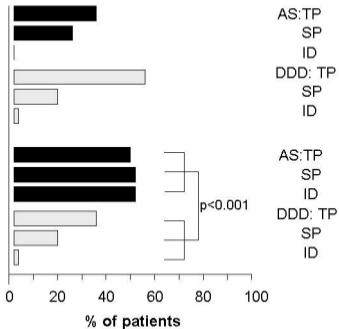
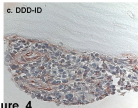
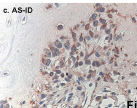
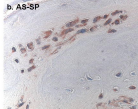
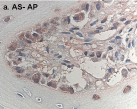


Figure 3 c/d

Large multinucleated cells

Cells attached to bone





**Figure 4**



Figure 5 a/b

Mononuclear cells



$p < 0.01$

Fibroblast-like cells

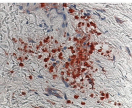


0 20 40 60 80 100

% of patients

MMP-1 positive cells

a. AS-SP



b. DDD-SP

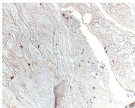


Figure 6



## Expression of cathepsin K and MMP-1 indicate persistent osteodestructive activity in longstanding ankylosing spondylitis

Michel Neidhart, Xenofon Baraliakos, Christian Seemayer, Caren Zelder, Renate E Gay, Beat A Michel, Heinrich Boehm, Steffen Gay and Jürgen Braun

*Ann Rheum Dis* published online August 4, 2008

---

Updated information and services can be found at:  
<http://ard.bmj.com/content/early/2008/08/04/ard.2008.092494>

---

### Email alerting service

*These include:*

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

---

### Topic Collections

Articles on similar topics can be found in the following collections

- [Immunology \(including allergy\)](#) (5112)
- [Inflammation](#) (1243)
- [Ankylosing spondylitis](#) (414)
- [Calcium and bone](#) (722)
- [Connective tissue disease](#) (4228)
- [Degenerative joint disease](#) (4616)
- [Musculoskeletal syndromes](#) (4924)
- [Rheumatoid arthritis](#) (3241)

---

### Notes

---

To request permissions go to:  
<http://group.bmj.com/group/rights-licensing/permissions>

To order reprints go to:  
<http://journals.bmj.com/cgi/reprintform>

To subscribe to BMJ go to:  
<http://group.bmj.com/subscribe/>