The use of micro-CT analysis for evaluation of bone and joint damage in experimental arthritis.

Malin C Erlandsson¹, Ing-Marie Jonsson¹, Mattias Svensson¹, Karin Andersson¹ and Maria I Bokarewa¹

¹Göteborg University, Sahlgrenska Academy, Department of Rheumatology and Inflammation research, Göteborg, Sweden

Rheumatoid Arthritis (RA) is a chronic joint inflammation, which affects about 1% of the population worldwide. Damage of skeletal bone is a frequent consequence of RA and the major course of disability in RA patients. Here we present micro-CT evaluation of changes in joint surfaces and periarticular bone density in two experimental models of arthritis, collagen II-induced arthritis (CIA) and mBSA-induced arthritis. At present arthritis scoring is done clinically (in CIA) and histologically (in CIA and mBSA-arthritis) and depends on the quality of histological sections and the experience of the evaluator.

Aims
In this study we assessed the place of radiographic examination of the skeletal bone and joints in CIA and mBSA-induced arthritis models and compared its sensitivity with clinical and histological evaluation.

Methods
Induction of arthritis: The mice are immunized with collagen or mBSA. Arthritis in the collagen-immunized mice starts to develop spontaneously after the booster sc injection of collagen. CIA is clinically visible and affects paw joints. Severity of clinical arthritis was scored in 3 joint groups in the hind paws, ankle, foot and toes every other day until termination 3 weeks after second immunisation. Each joint group can have a clinical score between 0 and 3. Arthritis in the mBSA-immunized mice is induced by the injection of mBSA in the knee joint. Arthritis develops only in the injected knee joint and is not visible clinically.

Micro-CT: The left hind paw of CIA mice and mBSA-injected knee joints were fixed in 4% buffered formalin and scanned ex vivo with our µCT Skyscan1176 at 9μ voxel size, 0.2 mm Al filter, frame averaging 4. The dataset were reconstructed using NRecon 1.6.8.0, toes aligned in data viewer and saved as a TRA dataset each for the 3 middle toes. In CIA mice, the region of interest was chosen with the reference line in the middle of the joint space of metatarsophalangeal joints and a selection of 150+250 lines, which will correspond to 2.25 mm. In mBSA-arthritis mice, the region of interest included 250 proximal lines of the fibula (2.25 mm). The analysis was made with custom processing, low threshold 100, radius 3, region of interest shrink-wrap, despeckle and 3D analysis.

Histological analysis: After scanning the specimens were decalcified, embedded in paraffin, and 4 microm sections were prepared for histological examination. The sections were stained with eosin/hæmatoxylin, coded, and evaluated for the signs of inflammatory cell accumulation in synovial tissues (synovitis) and of bone/cartilage destruction. Synovitis was defined by membrane thickness of more than two cell layers, and scored as follows: 1, mild; 2, moderate; and 3, severe synovitis. The presence of destructions was scored by severity as 1, mild; 2, moderate and 3, severe destruction.
Results

**Collagen induced arthritis**

(A) Clinical arthritis evaluation.

(B) 3D reconstruction of paws with different clinical score of arthritis. The region of interest (red box) was analysed for tissue volume (TV), bone volume (BV), and bone surface (BS). The progressive loss of BV is clearly seen in figures B2 and B3. (C) The reduction of BV/TV correlated to clinical arthritis in MTP joints at termination. (D, E) The plot visualises that only MTP joints with highest clinical score 3 display significant changes of the bone parameters measured by microCT.

**Figure 1.** Micro-CT evaluation of the left hind paw in collagen induced arthritis. (A) Clinical arthritis evaluation. (B) 3D reconstruction of paws with different clinical score of arthritis. The region of interest (red box) was analysed for tissue volume (TV), bone volume (BV), and bone surface (BS). The progressive loss of BV is clearly seen in figures B2 and B3. (C) The reduction of BV/TV correlated to clinical arthritis in MTP joints at termination. (D, E) The plot visualises that only MTP joints with highest clinical score 3 display significant changes of the bone parameters measured by microCT.
Figure 2. 3D reconstruction of fibula with different histological score of arthritis. (A) The region of interest (red box) was analysed for tissue volume (TV), bone volume (BV), and bone surface (BS). The progressive loss of bone volume (BV) is clearly seen in figures A2 and A3. (B) Histological arthritis scores (synovitis + erosions) of the knee joint correlated with the analysed bone variables. (C) The data were dichotomised into no/mild damage (score 0-1) or severe bone damage (score 2-3) and compared by bone parameters (Figure 2C). Statistically significant differences were detected between the knees with no/mild and with severe arthritis.

Conclusion
Micro-CT scan is a valuable tool for registration of bone and joint surface changes in the experimental arthritis models. The currently available methods of CT-scan analysis permit distinction of severe bone and joint damage. New methods with enhanced sensitivity for cartilage damage and moderate bone loss would broaden the use of microCT-scan and improve reliability of the results.