

# A NEW QUANTITATIVE METHOD FOR THE MEASUREMENT OF SUBCHONDRAL BONE CHANGES IN ANIMAL MODELS OF OSTEOARTHRITIS.

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## Aim of the Study

Sclerosis of the subchondral bone is a hallmark in the pathology of osteoarthritis (OA). The aim of the present study was to quantify these changes in  $\mu$ -focal X-radiographs of knee joints of STR/1N mice - a mouse strain which develops knee OA spontaneously (Figure 1).

## Methods

The tibial heads of excised knee joints were imaged using a FeinFocus  $\mu$ -focal X-ray system (FXS-100.23) including a real-time Varian amorphous silicon detector (Figure 2) with the ability to view the bone specimens while positioning them anatomically correct in an antero-posterior view. Optimal X-Ray settings for image acquisition (photon energy and density) were found to be: 40 kV and 100  $\mu$ A. A special holding device was developed for juxtaposing a spherical calibration phantom (a 1.5mm diameter aluminum ball) to the tibial specimen during image acquisition (Figure 3), in order to allow for intensity calibration when rotating the specimens. The application such a phantom is new. Software modules were developed for the Visiopharm Integrator System, allowing for bone density measurements. Intensity calibration into arbitrary units (OAIx/D = mm aluminum) is automatically performed. Subsequently, regions of interest (ROI) were manually defined on the medial and lateral side of the tibial plateau of the knee joint (Figure 4). Average bone densities were in computed the marked ROI's.

Microfocal radiographs were obtained from 44 male STR/1N mice (40, 84 and 154 days of age) at the time of sacrifice. For each image, subchondral bone density was measured in the medial and lateral side of the knee joint, as described above. Specimens were randomized and ROI's marked with investigators "blinded" to the resp. age groups.

## \* Technical features of the FeinFocus X-Ray Microscope

- Resolution of the  $\mu$ -focal X-ray tube: 2 $\mu$ m; Maximal geometric magnification x1800; (for the mouse bones: x200 - 300)

- High-contrast detector and compact digital X-ray imager consisting of a large-area amorphous silicon sensor array with a gadolinium oxysulfide or cesium iodide scintillator, a command processor for fast image processing and acquisition (Frame rate/sec: 4 in high resolution mode; 30 in fluoroscopic mode & controlling software generating 12-bit grey-scale real-time images with film-like quality by means of automatic gain control.



Figure 2: FeinFocus X-Ray Microscope FXS-100.23 with Amorphous Silicon Real-Time Direct Digital Detector (DDD) - for technical details see footnote\*



Figure 3: Holding device for bone specimen and calibration sphere

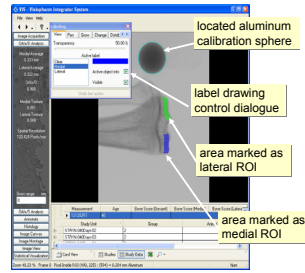


Figure 4: VIS user surface showing an X-ray image of a STR/1N mouse tibia with marked medial and lateral ROIs expanding 0.25 mm into the bone from the outer margin of the tibial plateau.

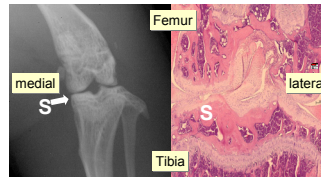


Figure 5: Sclerosis of the subchondral bone (S) in the medial tibial part of the knee joint of a 12-week-old male STR/1N mouse shown by  $\mu$ -focal radiography (A) and in a coronal histological section (B) in antero-posterior view; Histology: 7 $\mu$ m thick paraffin section, H&E-stained

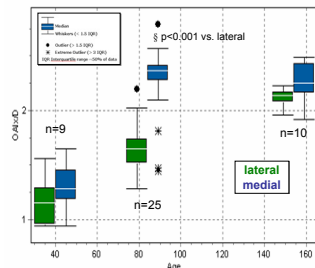


Figure 6: Age-dependent increase of the tibial subchondral bone density (OAIx/D = mm aluminum) in STR/1N mice. Note the asymmetry at the age of 84 days (significant difference between medial and lateral part,  $p < 0.001$  one-sided ANOVA). Three measurements per animal (see Figure 7) were averaged to further reduce the measurement noise.

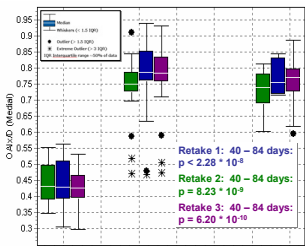


Figure 7: Age-dependent increase of the tibial subchondral bone density (OAIx/D = mm aluminum) in STR/1N mice in the medial and lateral side. One-way ANOVA was used to demonstrate age-dependency. For the lateral side a correlation of 0.84 was found between bone density and age. Three repeated measurements with repositioning were performed to assess the precision error of the method, which was found to be 4%-5%.

## Results and Conclusions

Male STR/1N mice show a profound sclerosis of the subchondral bone in the medial tibial region of the knee joints. A new quantitative method was developed for characterizing this subchondral bone sclerosis in small animal models of osteoarthritis (OA) using single-energy  $\mu$ -focal radiographic absorptiometry. An age-dependency study demonstrated a progressive subchondral bone sclerosis in STR/1N mice. For animals between 5 and 12 weeks of age, a significant age-dependent increase in subchondral bone density was found for both the medial and lateral side (see Figures 5). For the medial side, the increase in bone density seems to reach its maximum level after only 12 weeks, where it levels off. For the lateral side, the bone density continues to increase until the maximum level is reached after 21 weeks of age. Thus, asymmetric progression in density was evident, and appears to reach its maximum around 12 weeks of age. At this age moderate to severe cartilage destruction and subchondral bone sclerosis is evident histopathologically in the medial tibial plateau (see Figure 6). The precision error of this method is of major importance, as it is linked to the ability to measure longitudinal bone changes that one would expect in treatment studies. The only major source of variability is the operator interaction required for positioning of the specimens. The precision error was found to be about 5% for the medial side and 4% for the lateral side, based on three repeated measurements with repositioning of the bone specimen (Figure 7). The precision error did not seem to depend significantly on the age (disease progression).

Future studies are planned to prove that the method is also applicable for longitudinal *in-vivo* studies with minor modifications. The critical parameter here is the level of absorption due to soft tissue and ligaments around the knee joint. However, if the amount of soft tissue and ligament does not vary excessively over time and between animals, single-energy X-ray absorptiometry may prove to be a very useful method for quantifying the progression of bony changes and demonstrating the efficacy of structure-modifying drugs for OA.

## Acknowledgements:

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