Diagnostic and Interventional Imaging (2016) **97**, 651—655

ORIGINAL ARTICLE /*Muskuloskeletal imaging*

Micro-CT evaluation of rheumatoid arthritis mouse model disease progression: Manual tracings versus semi-automated routines

A.C. Antill[a], **D.H. Ballard**[a,*], **A.M. Hollister**[b,c], **E.J. Rogers**[d], **S. Yang**[e], **S.J. Lokitz**[b,f]

[a] *Department of Surgery, Louisiana State University Health Shreveport, 1501 Kings Highway, Shreveport, 71130 Louisiana, USA*
[b] *Department of Radiology, Louisiana State University Health Shreveport, 1501 Kings Highway, Shreveport, 71130 Louisiana, USA*
[c] *Department of Orthopaedic Surgery, Louisiana State University Health Shreveport, 1501 Kings Highway, Shreveport, 71130 Louisiana, USA*
[d] *San Antonio Military Medical Center, 3551 Roger Brooke Dr, 78234 Fort Sam Houston, USA*
[e] *Department of Cellular Biology, Louisiana State University Health Shreveport, 1501 Kings Highway, Shreveport, 71130 Louisiana, USA*
[f] *Center for Molecular Imaging and Therapy, Biomedical Research Foundation of Northwest Louisiana, 1505 Kings Hwy, 71103 Shreveport, USA*

KEYWORDS
Collagen induced arthritis;
Computed tomography;
Disease index;
Micro-CT;
Rheumatoid arthritis

Abstract
Purpose: The primary goal of this study was to demonstrate the value of micro-CT imaging in a rheumatoid arthritis (RA) mouse model. The secondary goal was to assess whether manual correction of the articular surface regions of interest (ROI) identification of the semi-automated methods may result in more effective assessment of bone volume and density loss.
Materials and methods: Collagen-induced arthritis (CIA) was induced in six DBA/1J mice at 12 weeks of age and three other DBA/1J identical mice served as controls. Micro-CT images were acquired at baseline and at four, seven, and nine weeks post-induction. Disease was monitored via ROI analysis, and ROIs were first generated using semi-automated techniques. These ROIs were manually manipulated so that a variety of edge irregularities were corrected. Effort was focused on the proximal and distal humerus and the distal femur. ROI volume and density were calculated, and data were compared. A histologic analysis of the study mice was also performed after the last time frame.
Results: There was a significant difference between the volume data comparison between the manually manipulated data and the semi-automated routine data across all time frames and

* Corresponding author.
E-mail addresses: aantil@lsuhsc.edu (A.C. Antill), davidballardmd@gmail.com (D.H. Ballard), annahans@mac.com (A.M. Hollister), emily.j.rogers120@gmail.com (E.J. Rogers), syang1@lsuhsc.edu (S. Yang), slokitz@biomed.org (S.J. Lokitz).

http://dx.doi.org/10.1016/j.diii.2015.12.007

across both humeri and femurs. There was no significant difference in densities calculated in Hounsfield units across any of the time frames, humeri or femurs, except for one time frame.
Conclusion: Our findings suggest that the manual correction technique of semi-automated data can be used to quantify and evaluate bone volume, density, and joint surface architecture changes in a RA mouse model.

Structural damage from rheumatoid arthritis (RA) disease progression includes articular cartilage degradation and bone erosion [1]. Micro-computed tomography (CT) has been shown to be an effective imaging modality to document the progression of RA [2—4]. Yang et al. demonstrated that a semi-automated method using CT imaging analysis software can detect bone volume and density loss in disease progression of a mouse model of RA [4]. However, observations from this study showed that the semi-automated method responded to noise properties of the CT images tracing errors, which may have affected study results. The primary goal of this study was to further demonstrate the value of micro-CT imaging in the RA mouse model. The secondary goal was to assess whether manual correction of the articular surface regions of interest (ROI) identification of the semi-automated methods may result in more effective assessment of bone volume and density loss.

Materials and methods

Following Animal Care and Use Committee approval and in accordance with Institute for Laboratory Animal Research's Guide for the Care and Use of Laboratory Animals, nine DBA/1J male mice 12 weeks of age were used in this study: three of these mice made up the control group and six were in the experimental group. The collagen-induced arthritis (CIA) model was used [5,6]. Induction in the six experimental mice was achieved at the start of the study by intradermal injection of 100 μg chicken collagen type II and 100 μg of *Mycobacterium tuberculosis* in emulsified in complete Freund's adjuvant solution into the base of the tail. At three weeks post-injection, a booster of 100 μg chicken collagen type II emulsified in incomplete Freund's adjuvant was injected intraperitoneally into the six experimental mice. Micro-CT images were acquired using the Gamma Medica Triumph (Gamma Medica Inc, Northridge, California, USA) at baseline (pre-induction) and at four, seven, and nine weeks post-induction to assess disease progression. Anesthesia for image acquisition was achieved with isoflurane gas. Micro-CT images were obtained using the following parameters: 15 μm resolution, 12 mm slices, 1024 projections at 80 kvp and 130 μA. Disease was monitored via ROI analysis of the articular surfaces of bilateral proximal and distal humeri as well as the distal femurs. These ROIs were first generated by the semi-automated ROI tools available in Analyze 10.0 (AnalyzeDirect, Inc., Overland Park, Kansas, USA) and these data were reported in the previous Yang et al. study [4]. The ROIs were manually manipulated so that the defined areas correctly included the bone and joint surface and excluded soft tissue; additionally, a variety of edge irregularities introduced from the semi-automated routines were corrected (Fig. 1). Effort was focused on the proximal and distal humerus and the distal femur, including the metaphysis and epiphysis. ROI volume and density were calculated and expressed in Hounsfield units using Analyze 10.0. We compared the semi-automated data — the original ROI data presented in the Yang et al. study [4] — and the manually manipulated data of the present study. This was performed by comparing all of the data from the manually manipulated ROIs of humeri or femurs at a specific time frame across all of the disease and control mice to their respective time frame across all of the control mice data from the semi-automated ROIs.

Control and experimental mice (two of the three control mice and five of the six experimental mice) were euthanized after final imaging for joint dissection, preparation, and sectioning. These sections were stained with hematoxylin and eosin and prepared for histological analysis by light microscopy. Joints investigated were examined by a blinded procedure and classified for the severity of joint disease. Four histologic parameters were assessed: inflammation, formation of pannus, hyperplasia of synovia, and erosion of cartilage or bone and graded on a numbered scale that included ''normal'', ''slight inflammation'', ''moderate inflammation'', and ''severe inflammation''.

Differences in quantitative variables were searched for using Student *t* test with Microsoft Excel (Microsoft Corporation, Redmond, Washington). The ROIs were then rendered and viewed using Meshlab (sourceforge.net) to visually inspect the surface renderings.

Results

The volume and density analysis of the manually manipulated method is displayed in Table 1. This represents a comparison of all of the disease model mice humeri or femurs across a time frame compared using a t test to the original induction time frame data. The volume analysis showed a significant difference in volumes at later time frames with inspection of humeri but not at the first time frame post disease induction. When comparing the femur volumes, there was no significant difference at the 4 week or 9 week time frame. Analysis of density showed a similar pattern as in which significant differences calculated were only significantly different at later time frames (Table 1).