

Comparison between Static Histomorphometric Measures Conducted by Traditionally 2D Histomorphometry and 3D μ -CT in Human Tibial Biopsies

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The golden standard for obtaining information on the trabecular bone structure is through histomorphometry performed on histological sections. However, the preparation and handling of the bone samples is tedious and time consuming and the procedure is destructive. Recently developed high resolution μ -CT scanners have made it possible to acquire histological sections non-destructively. The aim of this study was therefore to compare the histomorphometric measures obtained by 3D μ -CT with traditional 2D histology performed on human tibial biopsies obtained from human cadavers. We wanted to know whether the technique of obtaining 3D data sets by μ -CT and quantifying the 3D bone structure with histomorphometric measures would lead to superior, inferior, or the same results compared to the outcome of traditional histomorphometry. Positive results would open the way to develop new 3D measures based on μ -CT data sets that are not possible to conduct with traditional histological techniques.

Trabecular bone biopsies were obtained from the medial side of the proximal tibial metaphysis 17 mm below the knee joint with a 7 mm compressed-air driven diamond drill bit. The direction of the drilling was strictly parallel to the frontal plane. The tibial site was chosen due to its easy access, and its rich trabecular network. The biopsies were embedded in methyl methacrylate before μ -CT scanning in a μ CT 40 scanner (Scanco Medical, Switzerland) at a resolution of $20\ \mu\text{m} \times 20\ \mu\text{m} \times 20\ \mu\text{m}$. 3D volumes were created from the entire length of the trabecular bone biopsies. From a central 2 mm thick portion of each biopsy orientated along the main axis of the trabeculae, sixteen $10\text{-}\mu\text{m}$ -thick sections grouped in 8 disector pairs were cut with a microtome and stained with aniline blue (modified Masson trichrome). Digital images of the sections were obtained with a flatbed image scanner at a resolution of $10\ \mu\text{m} \times 10\ \mu\text{m}$. Due to drilling residue an approximately 0.5 mm wide region along the edge of the biopsy was excluded in the numerical analyses for both techniques. Trabecular bone volume (BV/TV), connectivity density (CD), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp) was measured in both modalities.

Very strong correlations were found between the static histomorphometric measures obtained by 3D μ -CT and traditional 2D histomorphometry. In particular BV/TV and CD showed highly significant correlations between the two techniques ($r > 0.9$ in both cases). We relate this strong correlation to the careful preparation of the bone biopsies.

This study has shown that it is possible to perform non-destructive static histomorphometry using high resolution μ -CT techniques of an equal quality as traditional histomorphometry. The result is seen as an opportunity to develop new quantitative measures for the 3D data set acquired by μ -CT, to apply new 2D quantitative measures to sections of these 3D data sets, and it opens the possibility to perform quantitative analysis of the trabecular bone structure *in vivo*. This can potentially affect future non-invasive diagnostic tools for osteoporosis and osteopenia that can take both the bone density and the bone structure into account. Furthermore, it will then also be possible to conduct longitudinal studies of the changes in trabecular bone structure during drug interventions, immobilization, or microgravity conditions.

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