DOCTOR OF PHILOSOPHY

A qualitative and quantitative analysis of the developing human lumbar vertebral column

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Appendix 6.1: μCT Scan Parameters Preliminary Study

Aims

The aim of this preliminary project was to identify optimal scanning conditions for the quantitative analysis of the lumbar vertebrae. In particular, the aim of this study was to identify whether the shortest scan condition produced an image stack that was significantly different to the scan condition considered most optimal, when assessing the trabecular architecture using histomorphometric parameters.

Methodology

Four conditions were considered, and two individuals were chosen, a perinate (L5 of SC-086) and a fetal individual (L1 of SC-096) to test each condition. Details of the conditions considered can be seen in Table 1.

Table 1: Details of the scan conditions tested to acquire the dataset.

Duration (hr:min:sec)	No. of Projections	No. of Frames per Projection
0:28:26	1682	2
0:56:53	1682	4
0:53:06	3141	2
01:46:11	3141	4

Each vertebra was scanned once at each condition, yielding 8 scans in total, and 4 scans per individual, following the protocol described in section 6.2.2.1. These were then post-processed as described in section 6.2.2.2. Images were analysed using ImageJ and the BoneJ plug-in (Doube *et al.*, 2010) on an Apple Mac Mini with 16GB RAM. Firstly, the image stacks were opened and cropped to the desired size, around ten image slices above and below the vertebra, to reduce the size of the image stack and hence the memory needed to process it. Once cropped, and rotated if required, the images were binarised globally using the threshold tool.

Three Volumes of Interest (VOIs) were assessed for each individual. Two VOIs were placed within the centrum while a further VOI was placed within one of the vertebral hemi-arches. Each VOI was repeatedly placed three times. Screenshots were taken during the first placing of the VOI and used to match individual trabecular features for each repetition to ensure that VOIs were as similar as possible. VOIs were placed using the BoneJ 'Fit Sphere' method. In the 'Fit Sphere' method, six points are used to create the sphere: one inferior point, one superior point and four central points placed on the same slice (Figure 6.10, red stars). Once set, the programme produces a sphere based on the location of the points placed. The outer cube was chosen for analysis as it most closely resembled the original cuboidal VOI being described. This VOI was inverted, resembling the original binarised scan. The properties of the outer cube were modified to the resolution of the scan. Six common histomorphometric parameters were assessed, a description of which can be found in Table 2.

Table 2: Histomorphometric parameters assessed when using ImageJ and BoneJ.

Parameter	Description
Bone Volume Fraction (BV/TV) %	The measure of trabecular bone volume relative to the total volume of the area of interest. Associated with mechanical competence as a higher percentage means more trabecular bone in a VOI.
Trabecular Thickness (Tb.Th) mm	The measure of thickness of trabecular struts within a VOI. A sphere is used to measure the thickness of each strut and an average is given. Higher values indicate more remodelling has occurred.
Trabecular Separation (Tb.Sp) mm	Similar to Tb.Th, a sphere is used, only this time to measure the space between trabecular struts. This also describes the extent of remodelling, with larger spacing indicating less remodelling occurring.
Structural Model Index (SMI)	Explains the dimensional form of trabecular struts, giving a value between 0 and 4. A value closest to 0= majority plate-like struts. 3= More rod-like struts. 4= Spherical shaped struts.
Connectivity Density (Conn.D)	Calculated the number of connected struts within a volume using the Eulor Principle.
Degree of Anisotropy (DA)	Isotropy is a measure of organisation. DA identifies the orientation of trabecular struts in relation to others using mean intercept length and Eigen values. Value of 0= total organisation, 1= total disorganisation.

Once all data had been collected, SigmaPlot was used to assess whether the VOIs of each scan condition differed significantly. Firstly, SC-086 L5 was analysed. Each parameter was analysed separately. As data sets were not normally distributed, a Kruskal Wallis One Way Analysis of Variance on Ranks was conducted. In cases presenting statistically significant data, the Tukey post-hoc test was utilised to identify where these significant differences occurred.

Results

For BV/TV, there was found to be a statistically significant difference between scan condition 1682/4 and 3141/2, as well as 1682/4 and 3141/4 at $p<0.05$. However, there was no significant difference found between 1682/2 and 3141/4. For Tb.Th, Tb.Sp, SMI, Conn.D and DA, no significant differences were found between scan conditions. The statistical analysis was computed a second time. On the realigned scans, BV/TV, Tb.Th, SMI and DA, showed no significant differences. However, for Tb.Sp, 1682/2 was found to differ significantly from 3141/4 at $p<0.05$, and for Conn.D, 1682/2 differed from 1682/4, and 1682/4 differed from 3141/2 at $p<0.05$.

Discussion

BV/TV was significantly different between SC-086 scans, however, was found not to be significant for SC-096. Similarly, Tb.Sp and connectivity were found to differ significantly between some scan conditions for SC-096, yet these results were not mirrored by SC-086 data. As the purpose of this preliminary study was to assess whether 1682/2 was an acceptable scan condition, the main focus was any statistically significant differences between the conditions 1682/2 and 3141/4, as it can be assumed that the latter parameter is the most effective due to its increase in projections and frames per projection. With this considered, only Tb.Sp for SC-096 L5 showed significant differences between 1682/2 and 3141/4. One explanation for this could be VOI placement. Two of the three VOIs selected included both trabecular bone and a section of the background scan. While it was possible to use trabeculae as cues for placing a majority of the points used to create each sphere, this became more difficult when placing points where no trabeculae was present. When computing the trabecular separation, values may have varied due to the potentially differing sizes of the free space surrounding the bone tissue within the volume of interest. This potential explanation is corroborated by the fact that no significant differences in trabecular thickness were found for this specimen. If the 3141/4 scan condition had adjusted the trabeculae in some way, this would have been reflected in both trabecular separation and trabecular thickness.

Conclusion

To conclude this preliminary study, it was decided that all individuals would be scanned at 1682 projections and 2 frames per projection, as the statistical analysis does not display any consistent differences between this scan condition and the optimal scan condition 3141/4. Furthermore, while the condition chosen indicates no difference in scan quality, it is also significantly more time efficient at only 28 minutes and 26 seconds, a 74% decrease in scan time compared to the longest condition tested.