A18 Histological Variables at Multiple Locations and the Effect on Age Estimation

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After attending this presentation, attendees will: (1) appreciate the importance of sampling location in the human rib for accurate results utilizing histological age methods; and, (2) understand how differences in bone remodeling along the length of the rib may help or hinder successful application of rib aging methods.

This presentation will impact the forensic science community by demonstrating the need for in-depth analysis of the variance of histological structures. Increased understanding of the rib's bone biology will serve to improve sampling procedures and accuracy of histological aging methods.

Current histological methods for estimating adult age at death were developed exclusively from cross sections of the mid-shaft sixth rib; however, in forensic practice, it is not uncommon for histological samples to come from fragmented or previously segmented ribs, leading to uncertainty of sampling location.[1,2] The potential for error increases when the sampling location on the rib is uncertain and utilizing a section beyond the mid-shaft (either anterior or posterior) may result in erroneous age estimates.

Additionally, Frost recommended a minimum of 50mm^2 of cortical bone be read when assessing skeletal remodeling, which has resulted in the common practice of reading two to three serial sections in the ribs and averaging the results to account for local variation.[3] Problems have arisen when there is not enough bone to create or insufficient time to properly analyze multiple serial sections. This study sought to determine the importance of the mid-shaft distinction for age assessment by analyzing histological variables at multiple sampling locations along the length of the rib.

Histological sections were obtained from a single left or right sixth rib from five male postmortem human subjects (82-90 years, *Standard Deviation* (*SD)*=6.24 years). Three seriated sections were taken from each of three locations, posterior (25%), middle (50%), and anterior (75%), resulting in a total of nine sections from each individual (45 sections in total). All slides were imaged at 100x magnification and observed on the microscope using bright field and polarized light at 200x magnification. The following histological variables were quantified and analyzed: (1) Osteon Population Density (OPD): $[OPD_{(Intact)} + OPD_{(Fragmentary)}]$/Ct.Ar; and (2) Cortical Area (Ct.Ar.) per mm^2.

Ct.Ar was manually traced and measured from still images using a digitizing tablet in ImageJ software, while OPD was collected live from the microscope. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) 24.

Serial sections were compared using a repeated measures Analysis Of Variance (ANOVA), which revealed no significant differences between Ct.Ar or OPD in any of the serial sections at the three locations. For the location comparisons, paired *t*-tests were used to examine OPD and Ct.Ar. at the anterior, middle, and posterior sites. Preliminary results indicate that OPD does not differ significantly between the anterior and middle locations (p=0.762) or between the anterior and posterior locations (p=0.131), although the middle and posterior locations are significantly different (p=0.05). Results for Ct.Ar revealed that there are significant differences between all three locations (anterior-middle: $p < 0.001$, anterior-posterior: $p < 0.001$, and middle-posterior: p=0.002).

Overall, results indicate that there is no significant difference in OPD and Ct.Ar between serial sections; however, significant differences exist between location sections for all variables analyzed. Due to histology's two-dimensional approach to bone, a three-dimensional structure, the absence of significant differences in the observed variables between serial sections is not surprising. Significant differences in Ct.Ar. at all locations reflect variation in the loading environment along the length of the rib. Though Ct.Ar. differences between the anterior and middle sites are significant, they are small by comparison to the differences between either of those locations and the posterior section. This may explain the observed trend of a lower OPD in the posterior sections than in the other locations.

These results suggest that it may be possible to obtain accurate age estimates using only one slide, rather than averaging multiple serial sections. Furthermore, these results indicate the importance of a mid-shaft location in histological age estimation; however, if the sampling location is uncertain, a more anterior section should be taken, as the histomorphology of the anterior rib is more consistent with that of mid-shaft. Future studies should explore Ct.Ar. and OPD in a more diverse sex and age sample to determine if the pattern observed in this study is consistent throughout the population.

Reference(s):

1. Stout S.D., Pain R.R. Brief communication: Histological age estimation using rib and clavicle. *American Journal of Physical Anthropology.* 87 (1992): 111–115.
2. Cho H., Stout S.D., Madsen R.W., Streeter M.A. Population-specific histological age-estimating method: A model for known African-American and European-American skeletal remains. *Journal Forensic Science.* 47 (2002): 12–18.
3. Frost H.M. Tetracyline-based histological analysis of bone remodeling. *Calcified Tissue Research.* 3 (1969): 211–237.

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