A New Method for Histological Age Estimation of the Femur

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After attending this presentation, attendees will understand the current issues with histological age estimation and be introduced to a new method that uses the anterior midshaft of the femur. In addition, newly defined and tested histological variables will be presented and distributed.

This presentation will impact the forensic community by providing a new method for histological age estimation. This will result in a higher quality of forensic practice and reduce errors in histological analysis.

Estimating adult age is problematic owing to biological variability in skeletal age indictors and their differential response to environmental factors over an individual's life. Furthermore, when standard age indicators (e.g., pubic symphyses and sternal rib ends) are absent or altered by post-depositional taphonomic factors, anthropologists often resort to less accurate methods such as cranial suture closure. In order to improve age estimates, the use of multiple age indicators and various modalities of assessment should be considered. Histological methods are based on the continuous turnover of primary cortical bone with secondary cortical bone, which has been argued to occur at a more predictable rate than other degenerative changes. Despite this, a histological approach is typically not employed owing in part to inherent methodological issues ranging from subjective definitions to difficulty reproducing microscopic field sizes. This research evaluates histological age estimation using the anterior femur and explores the biological limitations of bone turnover as an age indicator. The study builds upon previous histological methods, recognizing their importance and impact to our current understanding of bone turnover as an age indicator.

The sample includes femur cross-sections of known age individuals from two histological collections. The sample consists of 101 individuals (51 males, 49 females) from the Ericksen collection (1) and 15 individuals (8 males, 7 females) from the Kerley collection (2). Prior to this study, research was performed to redefine and validate histological variables. The variable definitions are available to attendees as a supplemental document provided by the authors. The following variables were collected:

1. Surface Area (Sa.Ar.) per mm^2
2. Intact Secondary Osteons (N.On.):
3. Fragmentary Secondary Osteons (N.Fg.On.)
4. Intact Secondary Osteon Density (P_I) per mm^2

5. Fragmentary Osteon Density (P_F) per mm^2
6. Osteon Population Density (OPD): sum of P_I and P_F
7. Mean Osteonal Cross-Sectional Area (On.Ar) per mm^2
8. Mean Anterior Cortical Width (Ant.Ct.Wi.) per mm^2

Histomorphometric data was collected using a transmitted light microscope and a firewire digital camera. The topographic sampling method was modeled after Iwaniec and colleagues (3) and Stout and Paine (4). The method evaluates ten columns from the periosteal to the endosteal cortex located at the anterior femur midshaft. Using a Merz counting reticule at 200x magnification (field area = 0.2304 mm^2), 50% of the microscopic fields were evaluated in each column by alternating fields. This sampling strategy accounts for 95% of the remodeling variability within the anterior cross-section. Principles of stereology were followed, thus different magnifications or counting reticules may be employed to collect the histological variables. Osteon areas and cortical widths were calculated using imaging software.

Statistical analyses were performed in SPSS 19 to examine the relationship between age and cortical bone histomorphometrics. Stepwise linear regression was used to develop the prediction equation and bootstrap methods were performed to assign measures of accuracy to sample estimates. Two variables (P_F and On.Ar.) required log transformation to meet normality requirements. Analysis of observer error was performed using Bland and Altman's (5) procedure for testing the repeatability of methods.

To examine the relationship between histomorphometrics and age, a general linear model was employed. Pearson correlations show moderate and strong relationships with age for all collected variables except PI. Due to this finding it was determined that the constituent variables for OPD should remain separate in the regression model. A slight level of collinearity between predictor variables was recognized and influenced the selection of variables. A one-way ANOVA indicated that all variables, with the exclusion of PI (p=0.256), demonstrate significant sex differences at the 0.05 level. Stepwise regression analysis of the male dataset produced a model using PF-log and On.Ar.-log as predictors, while the female model selected PF-log and Ant.Ct.Wi. as predictors. The standard error of the estimate is 13.24 years and 10.02 years, respectively. In the event that sex cannot be determined, a general equation was developed using PF-log and On.Ar.-log, providing a standard error of 12.07. Observer error results indicate the method passed repeatability standards set by the authors.

Current histological methods demonstrate significant issues that affect their reliability and accuracy. The method developed from this research demonstrates several advantages over previous methods. The method is based on validated variables, accounts for 95% of the spatial variation in osteons within the anterior cortex, and is not restricted to a specific field size or magnification. The results of the study indicate that histological analysis of the anterior femur provides reliable age estimates. Considering the biological variation in both macroscopic and microscopic adult age indicators, the standard error in this study is similar to that of previous studies with large sample sizes. One of the most prevalent issues regarding adult age estimation is the inability to accurately age older adults. The described regression model is most accurate for individuals over 50 years of age. Bearing in mind that the elderly are a rapidly growing percentage of North American populations and that unidentified adults are a common occurrence in the forensic setting, this research will significantly increase the accuracy of estimating age for older adults.

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4. Stout SD, Paine RR. 1992. Brief communication: Histological age estimation using rib and clavicle. Am J Phys Anthropol 87:111-115.
5. Bland JM, Altman DG. 1986. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 8476: 307–310.