H79 Reducing Observer Error Through Choice of Histological Evaluation Technique

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After attending this presentation, attendees will understand the importance of scrutinizing techniques used in methods of skeletal analysis in order to limit the amount of observer error.

This presentation will impact the forensic community and/or humanity by underlining the importance of identifying and differentiating method error from biological variability. Addressing the issue of method repeatability is essential for selecting current, as well as developing new, macroscopic, and microscopic methods of skeletal analysis in the field of forensic anthropology.

The traditional approach to the estimation of adult age at death has been a macroscopic, morphological evaluation. Another approach utilizes a microscopic, histomorphological evaluation, which is often presented as less subjective and more accurate than gross morphological methods. Compared to morphological methods histological methods of age estimation have been tested haphazardly, leading to a literature that is confusing, inconsistent, sometimes conflicting in terms of recommended methodology, and reported rates of precision and accuracy. The application of histological methods has been criticized from a number of different perspectives ranging from problems with intraand inter-population variation caused by environmental and hormonal influences on skeletal physiology to the influence that skeletal preservation holds over effectively using such methods. Furthermore, research demonstrates that there will be a spatial and temporal variance in remodeling dynamics in response to mechanical loading. These concerns may all be deemed secondary to the concerns inherent in the methods themselves. This paper focuses on an aspect of observer error related to the histological technique in order to determine the best technique needed to develop more precise histological methods of age estimation. Differentiating method error from biological variability will provide a baseline interpretation of histological methods as useful age predictors for skeletal analysis. Furthermore, a more solid methodological foundation for future histological methods will be established.

The Singh and Gunberg (1970), Thompson (1979), and Ericksen (1991) techniques for collecting histomorphometric data were compared in this research allowing for the comparison of precision amongst three different data collection techniques. The Singh and Gunberg method uses osteon counts per open circular microscopic field. The Thompson method requires a 100-square grid to perform the point count method. The Ericksen method uses a combination of microstructure counts per rectangular photographic field and predominant microstructure type per square in a 100-squared grid. To evaluate intraand inter-observer error, 30 femur thin-sections were randomly selected from a larger sample of 187 femoral sections. A *true* test of inter-observer error would require individuals with a certain level of familiarity with the methods or histological analysis in general. Instead, three individuals (1 per method) with little to no histological experience were used, thus testing the ability for novices to learn the methods. The author refers to this error as the novice application error. Each observer was given an extensive tutorial at the microscope, the article associated with the method that they were selected to perform, and Chapter 7 from *Biological Anthropology of the Human Skeleton* (Robling and Stout, 2000). This chapter provides a comprehensive discussion on the application of cortical bone histomorphometry for the estimation of age at death. Two statistical methods of observer error analysis were employed. The first analysis followed the procedure outlined by Bland and Altman (1986, 1995) for testing the repeatability of methods. The second followed the procedure outlined by Nichol and Turner (1986), which is a commonly used method for error analysis in anthropology. Observer error was evaluated for the microstructure counts and age estimates between the two trials.

The intra-observer results indicate that only the Thompson method passed repeatability standards. This method uses a predictive variable that consists of two variables. Evaluating the constituent variables individually produced some repeatability failures, indicating that the combined variable will reduce the amount of potential error in determining the microstructure type. Therefore, it is not surprising that the other methods, which use single and not combined microstructure counts, failed repeatability standards. The largest variable intra-observer error occurred using the Ericksen method. Higher error levels can be expected in the Ericksen method using the subjective determination of the predominant microstructure per square in a 100-squared grid.

The novice application error demonstrates that, for the most part, the novice observers were unable to repeat the results of the primary investigator, thus indicating that more experience is necessary to perform histological analyses. The Singh and Gunberg method produced the highest percentage of novice application error (43% for the variable and 25% for the age estimates) suggesting that open microscopic field methods have potential to produce higher levels of observer error.

Histological methods of age estimation have been presented in the literature as being more objective than the traditional gross morphological methods. This analysis shows that the histological evaluation technique chosen is an important factor in the amount of objectivity, accuracy, and observer error that is expected or

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introduced. Open microscopic field evaluations, such as that of the Singh and Gunberg method, provide no reference points for counting structures. In older individuals, with large numbers of intact and fragmentary osteons, one can easily loose their place in the microscopic field. Using a grid in the microscope provides points of reference and reduces observer error. The Thompson method demonstrated the lowest intra-observer error, which may be due to a combination of the point count grid method and simple variable definitions. Furthermore, methods that employ the principles of stereology, such as the point-count method, allow for changes in grid size, and are less subjective compared to methods that are based on assessing the percentage of a microstructure in a gridded area.

Precision, Histology, Histomorphometry

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