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Original Communication

Accuracy and sampling error of two age estimation techniques using rib histomorphometry on a modern sample

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ABSTRACT

Most age estimation methods are proven problematic when applied in highly fragmented skeletal remains. Rib histomorphometry is advantageous in such cases; yet it is vital to test and revise existing techniques particularly when used in legal settings (Crowder and Rosella, 2007). This study tested Stout & Paine (1992) and Stout et al. (1994) histological age estimation methods on a Modern Greek sample using different sampling sites.

Six left 4th ribs of known age and sex were selected from a modern skeletal collection. Each rib was cut into three equal segments. Two thin sections were acquired from each segment. A total of 36 thin sections were prepared and analysed. Four variables (cortical area, intact and fragmented osteon density and osteon population density) were calculated for each section and age was estimated according to Stout & Paine (1992) and Stout et al. (1994).

The results showed that both methods produced a systemic underestimation of the individuals (to a maximum of 43 years) although a general improvement in accuracy levels was observed when applying the Stout et al. (1994) formula. There is an increase of error rates with increasing age with the oldest individual showing extreme differences between real age and estimated age.

Comparison of the different sampling sites showed small differences between the estimated ages suggesting that any fragment of the rib could be used without introducing significant error. Yet, a larger sample should be used to confirm these results.

1. Introduction

Age estimation of skeletal remains associated with forensic cases requires an appropriate choice of methodology use to meet specific legal expectations related to expert witness testimony and peer reviewed criteria.[1] Therefore, scientific methods used should be standardised in peer reviewed publications before they can be applied to court cases.[2] The vast majority of available age estimation methods specific to adult skeletal remains rely on degenerative changes to bone joint surfaces and are employed using macroscopic observation techniques [e.g.[3–6]]. Most of these methods are not suitable for highly fragmented remains with incomplete bone inventory; remains that are often encountered by forensic anthropologists.[7] As a result age assessment using bone fragments examined under the microscope may provide age estimation answers. As with any age assessment, the accuracy of the result depends in part to training and experience.

Microscopic techniques focus on the variation of microanatomical patterns in the bone cortex such as cortical area and a count of bone features (for example, secondary osteons).[8,9] Although the reliability and feasibility of histological methods use to estimate age have been demonstrated[10]; the disadvantages –e.g. the destructive and time-consuming nature of the technique, the necessity for specialised equipment and training and the required observer's experience are considered significant drawbacks for its applications in forensic investigations.[11] The alternative use of manual thin sectioning techniques[12,13] instead of expensive equipment can however reduce the cost significantly. Thus,

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histological analysis still has potential as the skeleton is subjected to inter-population variation in metabolism and bone microstructure is a record of past metabolic events.

The development of methodologies based on bone histomorphometry has increased in number and bone regions during the last 45 years. Some of these studies have focused on the differentiation between animal versus human remains[14,15]; the analysis of pathological conditions and their impact on bone microstructure[16,17]; and histological determination of age at death.[7,18–21] The age assessment rests on the observation of changes in cortical bone microstructure features during the life of the individual. The methodology is based on the remodelling process in which older bone is replaced through the activity of bone cells: osteoblasts or bone forming cells, and osteoclasts or bone resorbing cells.[22] They both work in coordination resulting in the basic structural units known as secondary osteons or Haversian systems.[23] As remodelling occurs throughout life, the changes experienced by these units will constitute the basis of aging methods and the principle applied in age estimation techniques.

The first aging technique using histological features was developed by Kerley in 1965. He used bone cross sections from the femur, tibia and fibula. He created multiple age regression formulae per bone using quantitative variables correlated to age.[9] History has shown that only the Kerley equation using intact osteon numbers of femoral bone remains in common practice by anthropologists.[24] Since 1965, the femur has been subjected to attempts to improve the predictive power of histological assessment for age estimation.[25,26] As part of this process several attempts also developed equations that were population specific to improve the equations accuracy.[27–29]

Since 1992, a number of histomorphometric studies have been carried out on ribs using micro-anatomical variables in the attempt to determine higher accuracy rate in age prediction.[7,30] In doing so, new variables based on osteon features were introduced in creating age estimation equations.[7,20,31–33]

As a result of the early work done on ribs,[7] they are now often used as they can be easily obtained during standard autopsy procedures without further dissection as it would be required for sampling long bones. In addition, the available data on normal and pathological rib physiology[7,17,34] and the fact that the rib allows for a full examination of the entire section under the microscope, make them an ideal bone to work with on future research endeavours. The fragmented nature of archaeological ribs, however, can lead to poor identification to their location and/or rib number which potentially can cause problems of sampling rib micro-features [e.g. 1]. This matter is one of the research questions we will address in this study. A second goal is to test the applicability of two existing methods using rib criteria to assess age using a modern autopsy sample. The last objective of this study concerns the estimation of inter-observer error rates in quantifying micro-anatomical features (fragmentary and intact osteons) on rib thin sections.

One of the first studies using rib histomorphometry for age estimation of unknown human remains was carried in the early 1990's.[7] The original sample consisted of 40 individuals; the middle third of the 6th left rib was processed following the standards of histological preparation outline by Stout and Paine.[7] To test their rib equation the authors applied it to 12 rib test samples from autopsies. Their results showed that the standard errors from actual age to estimated age were −2.7 to +9 years for the rib, and −8.1 to +20.6 for the clavicle. The combined formula gave an error of −2.5 to +14.5 years. Most of the individuals fell within 95% of confidence interval for the estimated ages.[7] The lower mean absolute differences between actual and predicted age was for the combined formula suggesting that histological age estimation yields more accurate results when both skeletal elements are examined. It is worth mentioning the new variable created by the authors: osteon population density (OPD) as a combination of intact and fragmentary osteons; both are the product of cortical remodelling. OPD has become a standard variable used by bone histologists working on age assessment in an anthropological context.[24] This new parameter has been extensively used in recent histological research due to its high correlation with age.[20]

There have been several attempts to use both macro and micro-anatomical rib features to estimate age-at-death. Stout et al.[35] used sections extracted from the sternal end of the 4th rib to estimate age at death incorporating the combination of two age related changes: morphological changes and microscopic bone structures. The first method was based on *İşcan* et al.'s[3,36] technique for aging individuals through the sternal rib end and the microscopic approach was carried out by applying Stout and Paine's method.[7] Two estimation formulae were generated: one used histological features only and the second equation applied both micro and macro-assessment techniques. Multiple variables included in the equation provided more accurate results (SEE = 7.18) than each method applied separately. This suggests that the multifactorial approach is preferable when possible. Dudar et al.[30] also tested both techniques finding no statistically significant differences between them although more accurate results were produced by using the combined micro and macro variables.

The purpose of this paper is to report the results of the histological age estimates using both Stout and Paine[7] and Stout et al.[35] methods on a contemporary Greek sample. The objectives are the following:

a) To test the accuracy of applying the two equations developed from US populations on a contemporary Greek sample and to verify if population specific formulae are required.
b) To explore the effect of sampling error on age estimation on six different sampling sites along the rib in order to compare the accuracy levels between different sampling areas.

2. Material and methods

2.1. Sample

The histological sample consists of six 4th left ribs (with known sex and age) from a Modern Cretan Collection.[37] Due to difficulties in finding complete ribs, the selection of the sample was limited in number of specimens and individual's profile; all the specimens selected were females and only the fourth rib was available for analysis. Although Stout and Paine's[7] technique was developed from the 6th rib, studies have shown that the methodology can be reliably applied on other ribs.[1] According to this publication, the histological findings of the fourth rib can be interchangeable with the sixth rib. With regards to known age-at-death, the sample represents an age range from 19 to 58 years old with a mean age of 36 years (Table 1).

Table 1
Rib sample and real age.

Rib Nº	Age
Rib_53	19 y.o.
Rib_88	27 y.o.
Rib_28	29 y.o.
Rib_180	35 y.o.
Rib_194	46 y.o.
Rib_6	58 y.o.
Mean	36 y.o.