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Evaluation of Morphological and Histological Adult Skeletal Age-at-Death Estimation Techniques Using Ribs

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ABSTRACT: Adult age-at-death estimation standards were applied to an independent sample ($N = 50, 55$) of documented ages 17.5 to 95 years. Estimates derived from the sternal rib end morphological and from the cortical rib histological techniques were compared with each other and to the documented ages. Comparisons to the documented ages reveal no statistically significant differences between the techniques. However, the comparison of each individual's estimates show a poor correlation ($r = 0.54$) despite the equal performance of the age estimations on the entire sample. Averaging of the two rib age estimates results in an estimate with a stronger Pearson's r (0.86) and a lower standard error of the estimate (7.5 years).

KEYWORDS: physical anthropology, age-at-death estimation, ribs

Skeletal age-at-death estimation, whether at the forensic scene or the archaeological site, is of the utmost importance if the remains are to be identified or classified [*1*]. In the forensic context, obtaining an accurate age at death estimate can eliminate a substantial proportion of missing individuals. Dissatisfied with the relative precision and applicability of existing adult skeletal age at death estimation techniques, researchers have turned to different skeletal locations that demonstrate predictable age related change to develop techniques. İşcan et al. [*2–5*] have demonstrated age related morphological change at the sternal end of the rib. Stout [*6*], and Stout and Paine [*7*] have demonstrated age related histological changes to rib cortical tissue.

İşcan and colleagues base their nine phase technique [*2–5*] on the age related morphological *modeling* of bone tissue that occurs at the sternal rib end. They observe changes, "in the form, shape, texture, and overall quality of the sternal rib" [*3*: p. 1096]. Separate reference standards for males and females have been created [*3,4*] as well as modifications to existing standards for use with black populations [*5*]. These reference standards are derived from the fourth right sternal rib ends collected from medical examiners' autopsies. The white reference sample age range is 16 to 90 years with a mean age of 44.4 years, and includes 108 males and 83 females. The black reference sample age range is 15 to 62 years with a mean of 34.9 years, and includes 49 males and 14 females.

Stout [*6*] has proposed a histological technique that samples from the middle third of the cortex of the sixth rib. This technique involves the quantification of age related bone

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remodeling units (osteons) over entire cross sections of rib cortex. It thus overcomes problems encountered by previous histological methods, such as sampling error with respect to cortical drift, field size standardization, and correction factors [*8,9*]. Histological variables are then used in regression equations to estimate age-at-death. The age distribution and sex ratio of the original reference sample has not been published. Recently Stout and Paine [*7*] have updated the original technique with a predicting equation derived from a new sample. The autopsy specimens used in the revised equation have an age range of 13 to 62 years with a mean age of 28.6 years, and include 7 females, 32 males, and one of undocumented sex. The sample racial distribution is 32 whites, 4 blacks, and 4 of undocumented race.

It has been argued that the rib represents an ideal location for study because it will show minimal biological variation [*4,9*]. Unlike techniques that use other skeletal locations such as the pubic and auricular surfaces, rib tissue is not influenced by childbirth, is not involved in complex biomechanical interactions [*10,11*], and is not directly responsible for weight bearing [*10*]. It is thus hypothesized that the rib morphological and histological techniques will generate age-at-death estimates similar to the individuals' documented ages. This would be a factor of the limited influence of biomechanical interactions shown to affect age related modeling and remodeling mechanisms [*12–14*]. It is further hypothesized that these estimates will also be similar to each other due to the common tissue origin used by both techniques.

Materials and Methods

The independent test sample originates from university anatomy teaching facilities of southern Ontario, and documented archaeological cemeteries: the Harvie site (southwestern Ontario), and the historic St. Thomas Anglican Church site in Belleville, Ontario. Whenever possible, fourth right sternal ends were sampled for morphological estimation of age-at-death. When the fourth right rib end was not preserved, then the next closest rib end has been found to furnish a satisfactory estimate [*15,16*]. The middle third of the right sixth rib was sampled for histological age-at-death estimation. Documented ages at death and sex were released upon request for the cadavers, and supplied by G. Boyce for the St. Thomas remains, and by Lazenby et al. [*17*] for the Harvie remains. The total sample consists of 55 individuals, with an age range of 17.5 to 95 years and a mean of 61.8 years (see Fig. 1). Morphological assessment of age-at-death could only be completed on 50 individuals, 31 males and 19 females, due to preservation difficulties. Histological assessment involved five more females for a total of 55 estimates.

Cadaveral material required the removal of adhering soft tissue and cartilage before morphological estimation could proceed. The preparation protocol of İşcan et al. [*3*] was followed. This consists of soaking the samples in a warm water bath for several weeks, then boiling them gently for 10 to 15 minutes to loosen the costal cartilage. A mild commercial enzyme detergent, Bioad™, was added to the bath to speed the digestion. This was not part of İşcan's original protocol. The archaeological component required no additional preparation. All rib ends were coded and pooled by an independent investigator. A blind assessment of age-at-death for each rib end was completed twice, separated by at least two months, before the code was broken. Age-at-death estimates for each individual are the reported mean values for the second assessed phase.

Histological preparation of the archaeological specimens consisted of sonicating 2 cm samples of cortical bone tissue in a water and mild detergent solution to remove as much dirt as possible. Cadaveral samples were degreased in trichloroethylene for 24 to 48 h. The samples were then air dried for several days before embedding in Struers Epofix™ under vacuum. Two thin sections were cut from each sample block approximately 1 cm

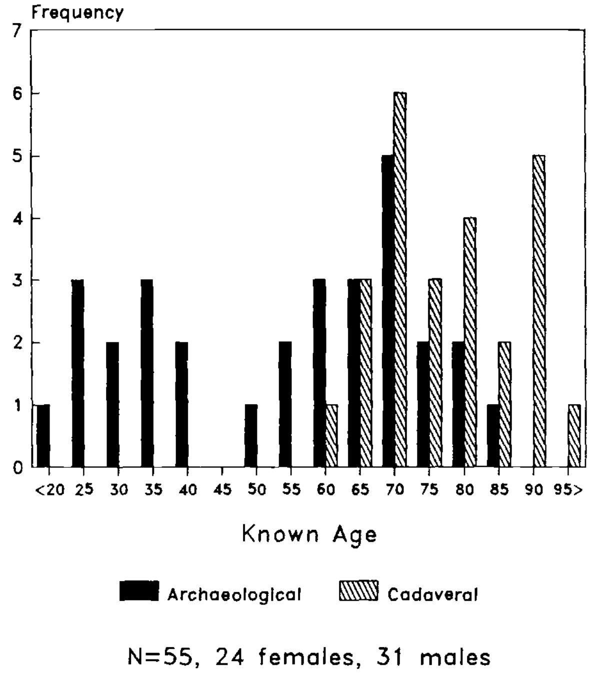

FIG. 1—*Age distribution of sample.*

apart with a low-speed Accutom wafering saw. The samples were mounted on glass slides and hand ground and polished to approximately 100 μ thickness.

Stout's [*6*,*7*] method of collecting histomorphometric variables was modified slightly. Their use of a Merz counting reticule for quantifying cortical cross sectional area (CA) was replaced by enlarging and tracing the thin sections of the rib cortex and then digitizing the tracings with the SLCOMM™ software program for collecting biomechanical data [*18*]. This change to the protocol was implemented to increase the efficiency in collecting CA data. Total remodeled bone unit counts (TRC) were made from entire cross sections of two thin sections per individual at 100x with a polarizing light microscope. Visible osteon creations (VOC) were calculated by dividing TRC by CA. The VOC values for each of the two thin sections were averaged and age-at-death estimates were calculated utilizing Stout's [*6*] and Stout and Paine's [*7*] published regression equations. Documented ages-at-death were not released until all histomorphometric assessments were complete. Intraobserver error in collecting VOC values was evaluated in two trials on ten randomly selected slides separated by at least two months.

Results

Before detailed analyses could be conducted, it was necessary to determine which of the published histological regression equations would be used [*6*,*7*]. Figure 2 illustrates the implementation of both equations. The Stout and Paine 1992 revised equation appears to consistently underage the independent sample when compared to an ideal relationship (a line with a slope of one) between known and estimated age. Stout's earlier 1986 equation has generated estimates both above and below the line with a slope of one. Based on a one tailed t-test, the means of the two histologically estimated age distributions

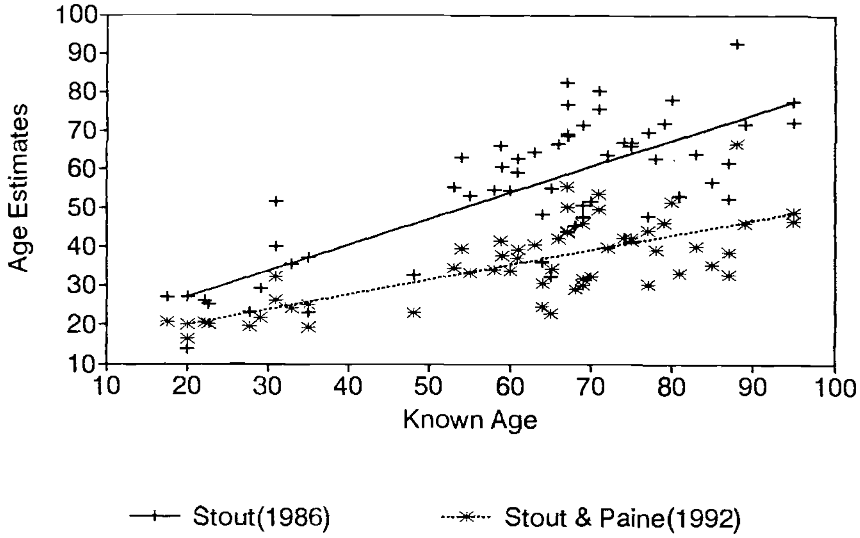

FIG. 2—*Histological age estimation: the rib regression equations.*

are significantly different from each other ($P < 0.001$, 54 df). Thus Stout and Paine's [7] revised equation was not used for the subsequent comparisons.

The mean of the histological estimates is 55.2 years with a standard deviation of 17.4 years. Pearson's correlation coefficient r between estimated and known age-at-death equals 0.76 with 53 df (see Fig. 3). The proportion of the variance of the age estimates that can be explained by the regression on documented ages, or the coefficient of determination r^2, equals 58%. The standard error of the estimate is 11.4 years. No significant over or underaging was found using a Chi-square goodness of fit test. Intraobserver error in determining the total remodeled bone density, VOC, for each individual is 8.8% from trial one to trial two.

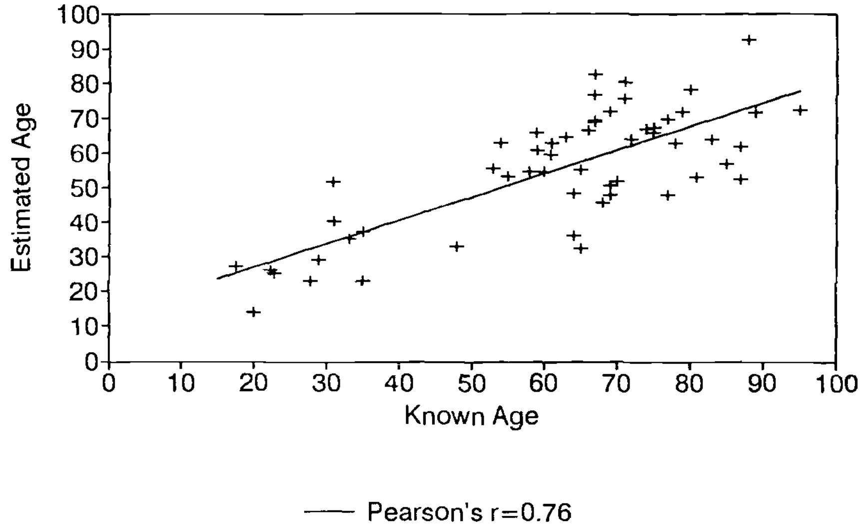

FIG. 3—*Histological age estimations.*

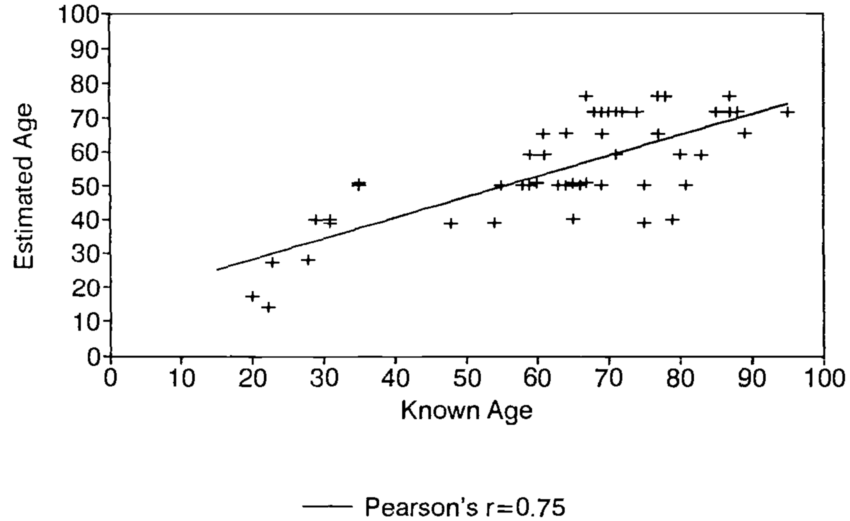

FIG. 4—*Morphological age estimations.*

The mean of the morphological estimates is 54.9 years with a standard deviation of 15.5 years. Pearson's r equals 0.75 with 48 df (see Fig. 4). The coefficient of determination equals 56% and the standard error of the estimate is 10.4 years. A significant ($0.01 < P < 0.001$) proportion of estimates fell below the documented ages as determined by a Chi-square goodness of fit test, suggesting that the sample is being underaged. Intraobserver error shows 34% deviation from initial phase assessment; however all deviations between trials are by one phase only.

There is no significant difference between the regression slopes of the morphological estimates versus known age, or the histological estimates versus known age. This was determined using Edwards [*19*] modified t-test (101 df).

Tests of inaccuracy and bias as defined by Lovejoy et al. [*20*] were applied to the overall sample, to the sample divided into age classes, and to the normalized sample (see Table 1). Bias is defined as the mean over- or under-prediction. Inaccuracy is defined as the average absolute error of age estimation, without reference to over- or under-prediction. There is a trend toward increasing inaccuracy and bias with increasing chron-

TABLE 1—*Bias*[a] *and inaccuracy*[b] *of morphological and histological estimates.*

Age range	N	Bias morphological	Bias histological	Inaccuracy morphological	Inaccuracy histological
17.5–29	6	1.1	1.0	5.5	4.6
31–35	5	11.9	4.5	11.9	9.3
48–59	7	−7.7	0.03	7.8	5.8
60+	37	−11.9	−10.5	13.2	13.4
Overall	55	−6	−6	10.9	10.8
Normalized[c]		−1.6	−1.2	9.5	8.3

[a] Bias is defined as the mean over- or under-prediction.

[b] Inaccuracy is defined as the average absolute error of age estimation, without reference to over- or under-prediction.

[c] Normalized error measures are the unweighted averages over all decades.

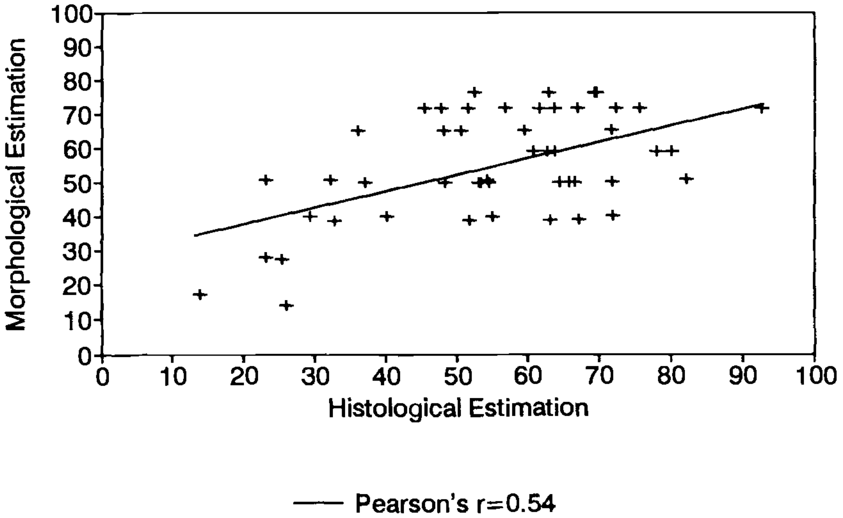

FIG. 5—*Histological vs. morphological age estimations.*

ological age. In the overall and normalized sample, there are no significant differences between the estimates derived from the two techniques.

In a final comparison, Fig. 5 illustrates the result of plotting the individual estimations against each other. Individuals histologically estimated at 50 years span a morphological estimate range of 39 to 76 years. Individuals morphologically estimated at 50 years span a histological estimate range of 23 to 82 years. A weak Pearson's r of 0.54 was determined ($P < 0.01$). This may be in part the contribution of the grouping of morphological estimates into mean ages as determined by phase assignments. The distribution however, is so broad about the line of best fit, despite the fact that the same sample is being

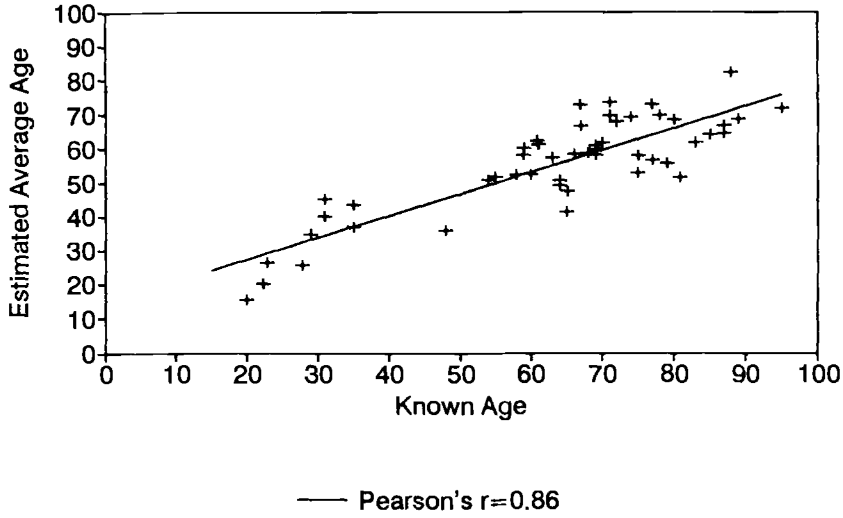

FIG. 6—*Average of morphological and histological age estimates.*

correlated against itself, that this comparison suggests fundamental differences between these techniques.

It is a recommended practice when estimating age-at-death of unknown skeletal remains to use as many indicators as possible to derive a final age estimation [*20*]. The average of morphological and histological age estimates for each individual was plotted against known age (see Fig. 6). The average age estimation plot reveals a tighter distribution of estimates. A full 74% of the variability is explained by merging these techniques. The mean of the average age estimates is 55.2 years with a standard deviation of 14.3 years. Pearson's r is now 0.86, and the standard error of the estimate drops to 7.5 years.

Discussion

As determined by statistical analyses of the morphological and histological estimates and known age, there appear to be only slight differences in the values of the techniques for estimating adult age-at-death at the sample level. Pearson's r, the coefficient of variation, the standard error of the estimate, and inaccuracy and bias overall, are nearly identical. The slopes of the regression lines for the estimates versus known age are not significantly different. The morphological technique has been shown, however, to have a higher intraobserver error, and to significantly underage the sample. This under estimation of age-at-death may be due to the use of some nonfourth ribs with rib four standards when preservation was a problem. Eight morphological estimations were carried out with nonfourth ribs. Of these eight, one estimate was below by one phase of the expected phase (as set by known age), two were below the expected phase by two phases, one was above by one phase, and four coincided with the expected phase. However, there is no change in the results of the Chi-square goodness of fit test if one excludes the nonfourth rib estimations. Despite the differences noted, the morphological and histological techniques estimate the documented ages-at-death on the entire sample equally well.

It would however be premature to conclude that estimates derived from morphological and histological methodologies are similar to each other. Figure 5 plots each morphological estimate against its histological counterpart for that individual. The poorer correlation coefficient of this comparison illuminates differences within the techniques that were not apparent when the entire sample was analyzed. On a case-by-case basis the morphological and histological estimates do not correlate strongly with each other due to the different age related mechanisms of modeling and remodeling used by the respective techniques.

Frost [*14*] hypothesizes that modeling and remodeling mechanisms are activated at different levels of mechanical usage to avoid them working at cross purposes when adaptations to the skeleton are required. The minimum effective strain (MES) range for bone tissue modeling is proposed to be 1500 to 3000 microstrain [*14*]. Strains at or above that magnitude activate the modeling mechanism that increases additions of new cortical bone. The remodeling MES is lower, approximately 100 to 300 microstrain. However, mechanical usage above this threshold causes a depression of the remodeling mechanism, thus conserving bone. Activation of remodeling occurs below this MES range. Modeling thus appears to be activated by overloading the skeleton and remodeling is a response to underloading.

The different strain activation thresholds seem to affect the age related morphological and histological indicators in such as way that if one estimation technique underages (due to inactivity, or not contributing to age related change) then the other other will overage (due to an active mechanism, or contributing to age related change). Averaging the two estimates for each individual shows the best performance; this is the result of averaging an over- and an under-estimate.

The revised histological age-at-death predicting equation of Stout and Paine [7] significantly underestimated age-at-death in this documented age independent test sample. Revisions involved using a reference sample that under represented older individuals (less than one third of the sample is over 30 years of age). The resulting regression equation may underage independent samples in a manner similar to that observed by Bocquet-Appel and Masset [21] and Jackes [22] with the McKern and Stewart pubic symphysis technique [23]. The youthful reference sample used to derive the predicting equation could not avoid accurately estimating the youthful test sample of Stout and Paine [7].

Conclusions

On their own, the sternal rib end and rib histological age-at-death estimation techniques fulfill their objectives. Careful and consistent applications of the investigated methodologies should provide reliable and accurate estimates. However, combining the age related modeling and remodeling mechanisms used by the techniques has the distinct advantage of controlling for more of the variability due to biomechanical influences. The average age estimate demonstrates improved performance over either indicator alone, and is a highly recommended practice. Age-at-death estimation may thus be made with greater confidence and provide greater assistance in the positive identification of "found" human remains.

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