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Estimation of Age at Death Using Cortical Histomorphometry of the Sternal End of the Fourth Rib

ABSTRACT: Given the often fragmentary nature of unidentified human remains, and the importance of using multiple criteria to estimate age at death, it is essential to have a variety of methods that use different anatomical sampling sites. In this study, osteon population densities (OPDs) were determined from transverse sections removed from an area immediately adjacent to the sternal ends of 60 autopsy rib samples. Regression analysis was performed using age at death as the dependent variable and OPD as the independent variable. The results of a "training set/test set" strategy to evaluate the performance of the histological age predicting model indicates that it provides reasonably reliable and accurate age estimates. A multiple regression model using both OPD and the mean age for a rib's morphological age according to the phase method of Işcan et al. [7,8] is also presented. This later age predicting model is recommended when both methods are applicable.

KEYWORDS: physical anthropology, bone, histomorphometry, age at death, sternal rib, osteon population densities

Methods to estimate age at death are important for the study of skeletal remains, whether the context is bioarchaeological, paleontological, or forensic in nature. Since estimates should be based upon more than one indicator, and skeletal remains are often incomplete, the availability of a variety of methods that are applicable to different skeletal elements is essential. Histological age estimation methods not only add to our arsenal of available methods, but have the added benefit of being applicable to extremely fragmentary remains. The application of histological age estimation methods, however, is limited by the fact that they require sampling from relatively specific anatomical locations on specific bones.

Ahlqvist and Damsen [1] provide a method that can be applied to the femur, and Kerley and Ubelaker [2] one that is applicable to the femur, tibia, and fibula. Since both methods require the removal of complete transverse cross-sections from the mid shafts of major long bones that are also important for standard osteometric analysis, there is often reluctance to employ them unless the bones are fragmentary. Several methods have

been introduced that attempt to minimize invasiveness. Thompson [3] has developed a method that requires the removal of only a small core of bone, while Singh and Gunberg [4] and Ericksen [5] provide methods that use wedges that do not completely transect the bone.[3] The use of small wedges and cores of bone, however, introduces sampling error that can outweigh the benefits of the reduced invasiveness. Stout and Paine [6] developed an histological age estimation method that involves the use of complete transverse sections from two bones that are not routinely used in osteometric analysis, the middle third of the sixth rib and mid shaft of the clavicle.

This paper reports the results of a study designed to develop an histological age prediction method that samples the fourth rib. Because this technique involves taking bone samples from the area adjacent to the sternal end used in the morphological aging technique developed by Işcan et al [7,8], both methods can be applied to the same bone sample, thus minimizing the amount of tissue that is required for analysis.

Materials and Methods

The sample consisted of 60 sternal rib ends taken at autopsy. The age range for the sample is 11 to 88 years with a mean and standard deviation of 39.2 ± 19.09 years. All were classified as being morphologically White on the basis of gross examination at autopsy. Since the ribs used in this study were drawn from the original sample used to develop the Işcan et al. [7,8] sternal rib method, histologically determined ages for each specimen can be compared with their morphological (phase) as well as reported age.

A 3 to 5 mm thick transverse cross-section was removed from within an area approximately 20 mm from the sternal end of each rib. Two thin (50 to 100 m) sections were prepared for histological analysis following routine petrographic procedures [9]. The microscopic analysis used in this study follows the method described by Stout and Paine [6] for the middle third of the sixth rib which employs oculars fitted with a Merz counting reticule for area measurement and field delineation.[4] The following histomorphometrics were determined.[5]

> INTACT OSTEON DENSITY (P_i), the number of osteons per unit area that have 90% of their Haversian canal perimeters intact, i.e. unremodeled
> FRAGMENTARY OSTEON DENSITY (P_f), the number of osteons per unit area for which 10% or more of the perimeters of their Haversian canals have been remodeled by subsequent generations of osteons
> OSTEON POPULATION DENSITY (OPD), the sum of P_i and P_f.

OPD served as the independent variable for regression analysis to generate an age predicting equation. A "training set/test set" strategy was used to evaluate the performance of the histological age predicting model. Ten test cases were drawn sequentially from the complete sample. The test cases were created as follows: The complete sample was sorted by I.D., which has no relation to age at death. The first 10 samples were withheld, and a predicting equation was generated from the remaining sample of 50 and tested against the 10 test cases. The test cases were returned to the sample, and the procedure was repeated using the next 10 cases. Sub sampling continued until all 60 samples had

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[3]Thompson's [3] method is applicable to the femur, tibia, humerus, and ulna; the Singh and Gunberg [4] method is applicable to the posterior border of the ramus of the mandible, and midshaft of the femur and tibia; and Ericksen's [5] method can be applied to the anterior midshaft of the femur only.

[4]The combination of eyepieces and objective produced a grid area of 0.37 mm[2].

[5]The histomorphometric variables were averaged over at least two rib cross-sections per individual.

served as test cases. After testing the model, all but one of the test cases were returned to the sample, and a final predicting equation was generated using the total sample of 59 individuals. During the testing procedure the rib sample of an 80 year old female was determined to be an outlier and, therefore, was excluded from the sample used to generate the final predicting equation. She will be discussed separately. In addition, multiple regression was performed using OPD and the mean age of a specimen's assigned phase as independent variables.

Results

An analysis of covariance (ANCOVA) found no statistically significant difference in age adjusted OPD between the sexes (Table 1), therefore, the data for males and females were combined for analysis. Applying the locally weighted least squares (LOWESS) smoothing algorithm [11,12] to a scatter plot of age against OPD reveals that their relationship is non-linear and approximates a quadratic shaped function (Fig. 1). Table 2 presents the resulting histological age predicting equation. The results of a "training set/test set" evaluation of the histological model are presented in Table 3. An analysis of variance for repeated measures found no significant differences for the means for known ages at death, histologically estimated ages, and those based upon rib phase analysis ($P > 0.6$) among the 6 test sets. Mean absolute differences between known age and histologically estimated age range from 4.8 years to 11.2 years, with a mean of 8.8 $\pm$ 0.98 years for the combined test sets. This compares favorably with the range of 3.2 years to 8.1 years and combined test set mean of 5.7 $\pm$ 0.86 years for the difference between known age and the predicted age based upon sternal rib phase, especially considering the fact that these same ribs were included among those used by Işcan et al. [7,8] to define their phases.

Discussion

There are a number of reasons why an histological age estimating technique that uses the sternal rib should prove useful. By using the rib rather than a major long bone, such

TABLE 1—*Results of analysis of covariance (ANCOVA) to test for a difference in OPD by sex.*

Test for interaction between sex and age (homogeneity of slopes):
Dep var: OPD N: 60 multiple r: 0.801 Squared multiple r: 0.642

Analysis of Variance

Source	Sum-of-squares	DF	Mean-square	F-ratio	P
Sex	2.005	1	2.005	0.160	0.690
Age	1191.203	1	1191.203	95.342	0.000
Sex* age	10.457	1	10.457	0.837	0.364
Error	699.665	56	12.494		

Covariance analysis of sex differences adjusted for age:
Dep var: OPD N: 60 multiple r: 0.798 Squared multiple r: 0.637

Analysis of Variance

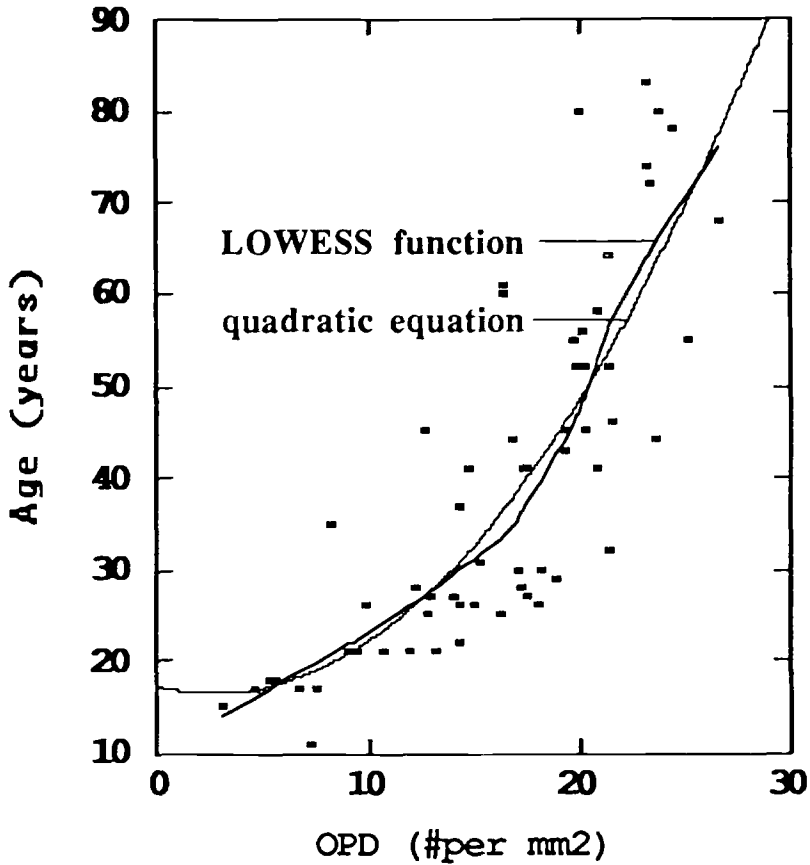| Source	Sum-of-squares	DF	Mean-square	F-ratio	P
Sex	11.376	1	11.376	0.913	0.343
Age	1191.580	1	1191.580	95.646	0.000
Error	710.122	57	12.458		

FIG. 1—*Scatterplot of age against osteon population density. Both the locally weighted least squares (LOWESS) [11,12] and quadratic smoothing methods are illustrated. Table 2 presents the equation fitting the quadratic regression curve.*

TABLE 2—*Age predicting equations.*

Histological Predicting Equation
Age = 18.389-0.731 (OPD) + 0.110 (OPD)^2
N = 59
Mean OPD ± SEM = 16.01 ± 0.753/mm^2
Mean Age ± SEM = 38.51 ± 2.405 yrs.
Regression Standard Error of Estimate = 10.43 years
Multiple R^2 = 0.693

Multiple Regression Predicting Equation
Age = 8.599 − 0.697 (OPD) + 0.623 (phase age) + 0.058 (OPD)^2
N = 54
Mean OPD ± SEM = 16.01 ± 0.753
Mean Age ± SEM = 38.51 ± 2.405 years
Mean Phase Age ± SEM = 39.48 ± 2.674
Regression Standard Error of Estimate = 7.182 years
Multiple R^2 = 0.865

TABLE 3—*Test of age predicting model using repeated samplings*[a]

| | Known Age (A) | Mean Estimated Ages by Method | | | Accuracy** | | |
		Phase (B)	Histology (C)	Multiple Regression (D)	B—A	C—A	D—A
			[Test Set 1]				
N	10	9	10	9	9	10	9
Mean	33.4	37.7	39.2	38.8	8.1	8.7	5.8
Standard error	4.36	6.80	3.42	5.69	3.17	1.61	2.53
			[Test Set 2]				
N	10	8	10	8	8	10	8
Mean	35.1	32.8	35.4	32.9	4.47	4.81	3.32
Standard error	6.31	6.89	5.20	6.71	1.81	1.66	1.76
			[Test Set 3]				
N	10	9	10	9	9	10	9
Mean	44.9	45.1	38.3	43.4	5.0	9.4	5.7
Standard error	6.84	7.38	5.15	6.71	1.78	3.00	2.10
			[Test Set 4]				
N	10	9	10	9	9	10	9
Mean	37.8	35.8	34.4	35.0	41.5	11.2	6.8
Standard error	7.30	6.75	4.33	5.53	2.15	2.69	2.12
			[Test Set 5]				
N	10	10	10	10	10	10	10
Mean	51.2	52.4	48.9	52.7	6.5	9.7	6.2
Standard error	6.25	6.87	6.38	7.00	1.78	2.60	2.14
			[Test Set 6]				
N	10	10	10	10	10	10	10
Mean	32.8	34.2	40.5	36.6	3.2	8.7	4.5
Standard error	3.27	3.26	5.09	4.15	1.76	2.65	2.05
			[Combined Test Sets]				
N	60	55	60	55	55	60	55
Mean	39.2	39.9	39.5	40.2	5.7	8.8	5.4
Standard error	2.43	2.67	2.05	2.51	0.86	0.98	0.85

[a]See methods section for sampling and test procedures. All mean ages and standard errors are in years.
**Mean absolute differences.

as the femur, it does not interfere with the application of osteometric methods. In terms of practicality, the sternal end of the rib provides an easily accessible anatomical sampling site. It also minimizes the amount of bone sample required for age estimation, since gross morphological changes in the sternal end of the rib [7,8] can be observed on the same bone sample.

In the hands of an experienced histomorphometrist, the histological method developed for the sternal rib can produce reasonably reliable and accurate estimates of age at death. As with any method that is based upon biological phenomena that are inherently variable, its use in conjunction with other methods greatly enhances reliability. A number of sources of error that affect histological age estimation have been discussed elsewhere Stout [13]. Error can be minimized, however, if one relies on multiple indicators of age. When estimating age using histomorphology, Stout and Gehlert [14] suggest that averaging age estimates from several different kinds of bone samples, for example, femur, tibia, fibula, rib, clavicle, provides greater accuracy and reliability. A recent study by Dudar et al. [18], compared the use of sternal rib morphology method [7,8] and histomorphometry of the middle third of the sixth rib [6]. It reports that averaging age estimates by both methods increased the correlation between estimated age and known age, and reduced the standard error of estimate significantly. This same study found morphological and histological age estimates to have a relatively low correlation ($r = 0.54$) [18]. In the current study, the Pearson product moment correlation between morphological phase age, and histological age is somewhat higher ($r = 0.776$), but still relatively low. The correlation between OPD and morphological phase age is ($r = 0.766$). This suggests that the histological and morphological methods reflect different age-associated factors and the use of multiple regression is justified. In order to take advantage of the benefits of using multiple criteria, a multiple regression model using both OPD and the mean age for a rib's assigned phase is provided. Results of the application of this model to the 6 test sets (Table 3) reveal that, while the mean absolute differences from known age of 5.7 years and 5.4 years produced by the phase method and multiple regression model respectively are similar, the range of absolute differences of 3.3 years to 6.8 years for multiple regression is narrower than that for the phase method alone, which ranges from 3.2 years to 8.1 years.

The test sample from an 80 year old female that was excluded from the final analysis serves to illustrate how anomalous histological age estimates can be identified. Frost [15] describes how the mean tissue age for adult compacta can be affected by factors such as growth, modeling, and cortical drift. This problem is clearly illustrated by Wu et al.'s [16] description of the histomorphometry for a 70 year old female with osteogenesis imperfecta, in whom severe scoliosis produced significant cortical drift, resulting in a mean tissue age of only a few years. Although cortical drift is relatively inactive after skeletal maturity, it can account for over 30% of a bone's compacta in people over 50 years of age [15]. This source of error can be particularly significant for individuals exhibiting senile kyphosis, which is a common spinal deformity found among individuals after the fifth decade [17]. An experienced histomorphometrist should be able to identify the existence of such drifts on the basis of abnormal amounts and locations of primary bone, thin cortices, and sub-normal osteon size. The 80 year old female in the current study is considered an outlier on the basis of these criteria.

These results should not be viewed as a comparison of the relative accuracy and reliability of the histological and phase methods, since the test sample was part of the original study to develop the phase method's age criteria. Such testing would require applying both methods to an independent sample.

It is recommended that when estimating age at death using the sternal rib, the multiple regression model provided here is the method of choice. When circumstances do not

permit phase analysis, the histological age estimating method can provide reasonable age estimates.

References

[1] Ahlqvist, J. and Damsten, O., "A Modification of Kerley's Method for the Microscopic Determination of Age in Bone," *Journal of Forensic Sciences*, Vol. 14, No. 2, April 1969, pp. 205–212.

[2] Kerley, E. R. and Ubelaker, D. H., "Revisions in the Microscopic Method of Estimating Age at Death in Human Cortical Bone," *American Journal of Physical Anthropology*, Vol. 49, 1978, pp. 545–546.

[3] Thompson, D. D., "The Core Technique in the Determination of Age at Death in Skeletons," *Journal of Forensic Sciences*, Vol. 24, No. 4, 1979, pp. 902–915.

[4] Singh, I. J. and Gunberg, D. L., "Estimation of Age at Death in Human Males from Quantitative Histology of Bone Fragments," *American Journal of Physical Anthropology*, Vol. 33, 1970, pp. 373–382.

[5] Ericksen, M. F., "Histological Estimation of Age at Death Using the Anterior Cortex of the Femur," *American Journal of Physical Anthropology*, Vol. 84, 1991, pp. 171–179.

[6] Stout, S. D. and Paine, R. R., "Histological Age Estimation Using Rib and Clavicle," *American Journal of Physical Anthropology*, Vol. 87, 1992, pp. 111–115.

[7] Işcan, M. Y., Loth, S. R., and Wright, R. K., "Age Estimation from the Rib by Phase Analysis: White Males," *Journal of Forensic Sciences*, Vol. 29, No. 4, 1984, pp. 1094–1104.

[8] Işcan, M. Y., Loth, S. R., and Wright, R. K., "Age Estimation from the Rib by Phase Analysis: White Females," *Journal of Forensic Sciences*, Vol. 30, No. 3, 1985, pp. 853–863.

[9] Stout, S. D., "Histomorphometric Analysis of Human Skeletal Remains," *Reconstruction of Life from the Skeleton*, M. Y. Işcan and A. R. Kennedy, Eds., Alan R. Liss, Inc., New York, 1989, pp. 41–52.

[10] Wilkinson, L., "*SYSTAT: The System for Statistics*, SYSTAT, Inc., Evanston, 1989.

[11] Wilkinson, L., "*SYGRAPH: The System for Graphics*, SYSTAT, Inc., Evanston, 1989.

[12] Cleveland, W. S., "LOWESS: A Program for Smoothing Scatterplots by Robust Locally Weighted Regression," *The American Statistician*, Vol. 35, p. 54.

[13] Stout, S. D., "Methods of Determining Age at Death Using Bone Microstructure," *Skeletal Biology of Past Peoples*, S. R. Saunders and M. A. Katzenberg, Eds., Wiley-Liss, Inc., New York, 1992, pp. 21–35.

[14] Stout, S. D. and Gehlert, S. J., "The Relative Accuracy and Reliability of Histological Aging Methods," *Forensic Science International*, Vol. 15, 1980, pp. 181–190.

[15] Frost, H. M., "Secondary Osteon Populations: An Algorithm for Determining Mean Bone Tissue Age," *Yearbook of Physical Anthropology*, Vol. 30, 1987, pp. 221–238.

[16] Wu, K., Schubeck, K. E., Frost, H. M., and Villaneuva, A., "Haversian Bone Formation Rates Determined by a New Method in a Mastodon, and in Human Diabetes Mellitus and Osteoporosis," *Calcified Tissue Research*, Vol. 6, 1970, pp. 204–219.

[17] Ortner, D. J. and Putschar, W. G., *Identification of Pathological Conditions in Human Skeletal Remains*, Smithsonian Contributions to Anthropology, No. 28, Smithsonian Institution, Washington, 1981.

[18] Dudar, J. C., Pfeiffer, S., and Saunders, S. R., "Evaluation of Morphological and Histological Adult Skeletal Age-at Death Estimation Techniques Using Ribs," *Journal of Forensic Sciences*, Vol. 38, No. 3, 1993, pp. 677–685.

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