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The Use of Histomorphology to Estimate Age

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ABSTRACT: The purpose of this paper is to discuss some of the major factors that can affect age-at-death predictions when using histomorphological methods. Although evidence suggests that some of the currently available methods are more reliable and accurate, and there are a number of factors other than chronological age that can affect bone remodeling, histomorphological methods, when properly applied, are valuable tools for anthropology and forensic medicine. It is suggested that both accuracy and reliability are maximized when the histomorphometrics of as many anatomical sampling sites as possible are sampled and the resultant ages are averaged.

KEYWORDS: physical anthropology, musculoskeletal system, histomorphology, human identification, bones, age at death

The accurate determination of age at death for skeletal remains is particularly important in anthropology and forensic medicine. Histological methods are potentially very useful because of their reported superior accuracy for the adult age ranges, and the fact that they can be applied to fragmentary remains. Initial applications of these methods have often been somewhat disappointing, however, particularly when attempted by individuals with minimal experience and training in bone histology. The purpose of this paper is to discuss some of the major factors that can influence the accuracy and reliability of histological aging methods.

It is essential that anyone attempting to employ histological methods be familiar with the biological basis for them. Most methods are based upon the fact that in larger vertebrates, such as humans, bone remodeling is continuous throughout their lives. It is characterized by the sequential resorption and formation of relatively consistent packets of lamellar bone, sometimes referred to as basic multicellular units of remodeling (BMUs). In cortical bone, each BMU results in the production of histomorphological structures known as Haversian systems or osteons, which represent a durable and lasting record of past remodeling activity in a bone. Most of the currently available histological aging methods are based upon the resulting association between number of osteons per unit area and age reported by a number of researchers [*1-3*]. The application of this phenomenon to the problem of age estimation was demonstrated by Kerley in 1965 [*4*]. Since this pioneering work, a number of authors have introduced histological aging methods that are primarily modifications of Kerley's method [*4*], and provide predicting formulas which use different bones or microscopic field locations or both [*5-8*].

Comparisons among histological methods suggest that precision and accuracy differ among them. Bouvier and Ubelaker [*9*] found Kerley's [*4*] predicting equations for the femur to be more accurate than Ahlqvist and Damsten's [*5*] modified method. This compari-

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son, however, utilized a subsample of the original sample from which Kerley's formulas were derived. One might, therefore, expect his formulas to demonstrate better predictability than formulas derived from different population samples. Also note that this comparison employed a field size and prediction equation which differed from the more recently reported revisions to Kerley's method [*10*].

A comparison of the reliability and accuracy among all of Kerley's revised osteonal age predicting equations [*10*] and that of Ahlqvist and Damsten [5] was undertaken using an independent sample by the author [*11*]. It was concluded that averaging the ages resulting from each of Kerley's regression equations is the method of choice when both accuracy and reliability are taken into consideration (Table 1).

Three additional histological aging methods that should be discussed are those of Singh and Gunberg [*7*], Thompson [*6*], and Stout [*8*]. Singh and Gunberg's method uses multiple linear regression using three histomorphological variables: number of complete osteons, average number of lamellae per osteon, and average Haversian canal diameter. They provide predicting equations based upon various combinations of the three variables for the anterior midshaft of the femur and tibia, and the posterior border of the mandibular ramus. Accuracy is claimed to be within six years of the true value in 95% of human males. An attempt by the author to evaluate independently this method for the femur and tibia obtained poor results [11]. Errors in age prediction ranged from 12 to 49 years of actual age. This extremely high inaccuracy may have resulted in part from methodological factors. I used approximately 100-μm-thick ground, undecalcified sections, while Singh and Gunberg [*7*] used 10-μm-thick decalcified sections. It is reported that section thickness can affect histomorphometrics [*12*]. Sampling error may also be significant for this method, since they employ only two randomly chosen microscopic fields per bone.

Thompson [*6*] has developed a method that uses only a 0.4-cm-diameter core of bone from the anterior midshaft of 4 long bones: femur, tibia, humerus, and ulna. It involves multiple regression formulas based upon various combinations of 19 variables, and has the advantage of being less invasive than other methods which require the removal of complete transverse sections of cortical bone. Thompson reports standard errors of estimation ranging from 10.57 years for the ulna to 6.21 years for the humerus. The use of only a single small core, however, would seem to introduce considerable sampling error. Don Ortner cautions that deviations from the horizontal plane when removing cores can significantly affect the results (personal communication). The method has yet to be subjected to an independent evaluation.

TABLE 1—*Ranking of histological aging methods on the basis of both accuracy and reliability* [11].[a]

1. Mean regression (age estimated by averaging the results of each of Kerley's [*10*] regression equations).
2. Femur osteon fragment regression formula.
3. Femur intact osteon regression formula.
4. Kerley's profile method (1965) [*4*].
5. Tibia intact osteon regression formula.
6. Ahlqvist and Damsten's (1969) modified method [5].
7. Fibula osteon fragment regression formula.
8. Fibula intact osteon regression formula.
9. Tibia osteon fragment regression formula.

[a]Accuracy and reliability based upon mean differences between predicted and known age and predicted ages by two observers, respectively. Based upon an independent sample of 13 cadavers with an age range of 13 to 102 years.

Stout [8] has introduced a method which employs the number of osteons and osteon fragments per square millimetre for the middle third of the sixth rib. It remains to be established whether other ribs can also be used. The method has the advantage of being applicable when intact long bones are not available or, as a result of their importance for other purposes, cannot be sectioned. To minimize the effects of sampling error, several complete cross sections of rib are read per individual. Moreover, since biomechanical factors have been shown to influence bone histomorphometry [13-16], nonweightbearing bones, such as the rib, may more reliably reflect age associated changes in histomorphometry.

There are a number of additional factors that can significantly affect the accuracy and reliability of most histological methods. The application of histological predicting equations to samples other than those from which they were derived has often produced results that are considerably less accurate than one would expect given their published correlation coefficients. This apparent reduction in predictability is, in part, due to the overfitting of data when second and third order regression is employed. The addition of terms to regression equations increases the fit to a particular set of data, but it also tends to reduce the accuracy of prediction when applied to different samples [17]. Future studies to develop predicting equations should attempt to balance the number of parameters and degree of fit.

Most histological methods require that particular field locations within bone sections be sampled. For these methods, the use of microscopic field sizes different from those prescribed has been shown to cause errors in age estimates. The introduction of correction factors has been recommended to adjust for field size differences that result from the use of different combinations of objectives and oculars [10]. Because of the spacial variation that can exist within the cortex of a bone [18,19], however, merely adjusting measures in proportions to differences in field sizes may be of limited use. Research has shown that as the difference between observed and recommended field size increases, the variance of the differences between known and predicted age increases, leading to a significant reduction in reliability. Stout and Gehlert [20] investigated the effects of field size differences on Kerley's method [10]. It was found that although mean differences in counts for different field sizes did not differ significantly after the introduction of a correction factor for field size, the variance was significantly lower for the field size of 2.06 mm^2 which is most similar to that reported by Kerley and Ubelaker [10] (Table 2). Thus, reliability is reduced when field sizes

TABLE 2—*Mean differences between actual ages in years and those estimated from counts of three different histological parameters at three different field sizes* [20]. *(N = 20).*[a]

Histological Parameter		Field Size		
		2.83 mm^2	2.27 mm^2	1.06 mm^2
Intact osteons	MD	−2.15	−2.35	−2.56
	s	15.13	6.43+	14.38
	t	−0.64	−1.63	−0.79
Osteon fragments	MD	−4.29	−2.07	0.20
	s	10.61	6.46+	12.19
	t	−1.81	−1.43	0.07
Nonhaversian canals	MD	−7.55	−8.62	−9.08
	s	14.37	12.54	12.74
	t	−2.35[b]	−3.07[c]	−3.19[c]

[a]MD = mean difference, s = standard deviation, *t* = *t* value for test of significance of mean difference from zero, and + = variance (s^2) is, by the *F* statistic, smaller than those for all other measures at the $p = 0.05$ level of confidence.

[b]$p < 0.05$.

[c]$p < 0.01$.

significantly different than Kerley's [*10*] are used, even when a correction factor is introduced.

As noted above, most histological methods use various sampling techniques, rather than determining histomorphometrics for entire sections of bone. This can introduce a possible source of variation known as incoherence, the random variation of one unit of tissue to an adjacent comparable unit [*19*]. In addition, noxious stimuli, such as fracture or bone lesion as a result of metastases or phlebitis can lead to a local phenomenon known as the regional acceleratory phenomenon [*21*]. A single section from one bone, therefore, may not be representative of the skeleton as a whole. For this reason, it is recommended that several sections from more than one bone should be examined and their values averaged. This may explain why, as mentioned above, averaging predictions from all of Kerley's regression formulas was found most reliable. Age predictions based upon a single bone can be highly unreliable. Histomorphometric age predicting methods are based upon observing the cumulative evidence of bone remodeling, usually osteonal. Note, however, that age is not the only factor influencing bone remodeling [*22,23*]. Table 3 provides a list of some of the factors known to influence osteonal remodeling, and therefore, affect the accuracy of age prediction. A bone sample from one individual, therefore, may have more osteons than a comparable bone from another individual because it is older or because remodeling is abnormally increased or decreased in one or both of the individuals. A further discussion of how many of these factors affect bone remodeling can be seen in Frost's recent study [*24*].

The following is a list of the major recommendations for those undertaking age estimation using histomorphological methods.

1. Use a field size as close to 2.06 mm^2 as possible when employing Kerley's method [*10*].
2. Follow the original method as closely as possible, for example, magnification, sample preparation, field location, and so forth.
3. It is best to base your age estimate upon several sections from each of several skeletal elements.
4. When the appropriate portions of major long bones are available, it is recommended that an age estimate be based upon the average of the age estimates from Kerley's [*10*] regression formulas for each variable and bone.
5. Be aware of possible pathognomonic factors.
6. Consult an experienced histomorphometrist, such as some of those cited in this paper.

Conclusion

In conclusion, there are a number of methodological and physiological factors that can affect the accuracy and reliability of histological methods now available for age estimation.

TABLE 3—*Factors known to influence osteonal remodeling and accumulated osteon populations* [24].

Age, chronologic	Regional trauma
Life span	Paralysis
Sex	Mechanical usage
Maturation, skeletal	Acute mechanical disuse
Species	Nutrition
Hormones	Metabolic alkalosis
Electrolyte disorders	Metabolic acidosis
Metabolic	Vitamins
Genetic disorders	Genetic structural disorders
Toxic agents	Microdamage
Radiation damage	Drugs
Bone growth	Mean tissue age
Bone remodeling patterns	Mechanical strain

The author's purpose in discussing these factors is not to discourage the use of these methods, but rather to inculcate a realistic degree of caution and to suggest possible steps that can be taken to minimize their effects.

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