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Assessment of Intra- and Intercostal Variation in Rib Histomorphometry: Its Impact on Evidentiary Examination*

ABSTRACT: Rib histological age estimation requires the evaluation of the middle third of the sixth rib. Human ribs have thin cortices and, when recovered, are often fragmented or absent, making it difficult to identify a specific midthoracic rib. This research explores the amount of microstructure variation in the middle third of the midthoracic ribs and determines whether the sixth rib age prediction equation can be applied to non-sixth ribs with similar accuracy. The amount of variability must be evaluated in order to meet the criterion for evidentiary examination. The sample consists of 120 cortical bone cross-sections from the middle third of ribs 3–8 removed from 20 cadavers. For each rib, osteon population densities (OPDs) and associated age estimates were calculated. The results demonstrate that non-sixth ribs can provide similar OPD values compared with those of the sixth ribs; however, individual variation proved to be significantly associated with bias, suggesting that individual factors influence the magnitude and direction of bias in non-sixth rib OPD values. This demonstrates the importance of evaluating multiple cross-sections (both intra- and inter-rib) to estimate age due to the normal remodeling variation within individuals.

KEYWORDS: forensic science, forensic anthropology, Daubert, Mohan, osteon population density, histology, histomorphometry

Histological and histodynamic parameters of bone remodeling for the ribs have been well documented in the medical community by H. M. Frost and colleagues (1–10) over a research period spanning several decades. Their clinical research evaluated remodeling rates from cortical bone biopsies of the fifth, sixth, seventh, and eleventh ribs across eight decades of life (2,4,5). These studies documented histomorphological differences in cortical bone remodeling rates from normal and pathological tissue. Using data and techniques from the previously mentioned studies, anthropologist Stout (11,12) developed a quantitative method that evaluates the entire cross-section from the middle third of the sixth rib to estimate adult age at death. At that time, the use of quantitative histology to estimate age at death was not a new tool in the field of anthropology. Methods had previously been developed using various long bones throughout the body (13–17). These methods require a specific number and size of microscopic fields within the cross-section or anterior wedge of the bone diaphysis to be evaluated. Stout (18) argued that these methods produce sampling errors attributable to the small proportion of the cross-section evaluated and caused difficulties in replicating microscopic field locations. Using skeletal elements with small cross-sections (i.e., the rib or clavicle) allows the observer to evaluate the entire cross-section with relative ease compared with elements with a larger cross-sectional area such as the femur.

The ribs are believed to be an ideal sampling location for histological studies due to the minimal biomechanical variation of the midthoracic region compared with the variation that occurs in the appendicular skeleton. For the most part, the ribs are not involved in complex biomechanical interactions or weight-bearing loads that may produce wide variability in bone remodeling (19–21). The main repetitive stress on rib tissue would be from respiration and because the biomechanical processes involved in breathing are similar among humans, the strain variability should be minimal (22). Tommerup et al. (21) observed levels of mechanical loading in the middle third of the seventh rib and femur midshaft from pigs that underwent a 20-week exercise program on a treadmill. The results indicated that weight-bearing exercise affected remodeling rates of the femur but had no statistically significant effect on the seventh rib. Therefore, the constant repetitive loading by the respiratory muscles and the increased mechanical forces involved in the work of breathing appear to be the primary strain-producing mechanism that affects activity-related bone remodeling within the rib cage.

Since the introduction of Stout's method of age estimation using sixth rib histomorphometry, Stout and colleagues (23–25) have continued to modify the method. One potential limitation using the rib methods that has not been addressed is the reliance on the middle third of the sixth rib as the standard for age estimation. Human ribs have thin cortices and are less resilient to taphonomic processes than other skeletal elements. Therefore, forensic and physical anthropologists are often confronted with incomplete or fragmentary remains in which the sixth rib is missing or difficult to identify from closely associated midthoracic ribs. Techniques developed for seriating ribs (26,27) are difficult to apply when ribs are missing (28). If the sixth rib is unavailable or cannot be positively identified, closely associated midthoracic ribs (namely the fifth and seventh) or a suspected sixth rib are often deemed adequate for use due to similarities reported in the clinical literature for bone remodeling kinetics of ribs 5–7 (2,4,5); however, the intercostal variation in rib histomorphometry and its effect on histologically derived age estimates have not been reported. Because of the guidelines established from the U.S. Supreme Court in Daubert v. Merill-Dow pharmaceuticals (29), and the Canadian

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courts in Regina v. Mohan (30), and in the wake of the United States v. Plaza (31), methods utilized or altered by forensic anthropologists may soon be challenged. The precision and accuracy of methods must be rigorously tested to meet requirements of evidentiary examination. The purpose of this study is to describe the variation of rib osteon population densities (OPDs) among individuals and across ribs. Given this study's small sample size, this is an exploratory analysis assessing whether systematic differences exist in rib histomorphometry to determine whether non-sixth ribs can be used for age estimation with an accuracy similar to the sixth rib established standard.

Materials and Methods

The sample consists of skeletal tissue from 11 male and nine female cadavers from two university teaching facilities in Ontario. The individuals died during the late 1980s or early 1990s, most likely between 1989 and 1990. Ages range from 59 to 89 years, with a mean age of 74.6 years. Before this research, cross-sections from the middle third of ribs 3–8 were embedded in a sectioning medium by Dudar (22) in conjunction with his analysis of the sixth ribs from these individuals.

Thick-sections were removed from the rib histoblocks by one of the authors (C. C.) using a water-cooled Struers Accutom™ (Struers, Cleveland, OH) geological diamond blade saw. Each thick-section was washed in an ultrasonic water bath to remove debris and then allowed to air dry. Once dry, one side of each thick-section was polished using a cloth and polycrystalline diamond paste on a Buehler™ (Buehler, Markham, Ontario, Canada) variable-speed polishing unit. The thick-sections were washed again, dried, and fixed to a microscopic slide using an epoxy. Thick-sections were prepared for histological analysis by grinding them to an approximate thickness of 50–80 μm on a Buehler™ variable-speed grinding unit using 200 and 400 grit paper. A final polishing using the cloth and diamond paste was performed to remove grit striations from the thin-sections. Another individual coded the thin-section slides, and the age and sex of the samples were withheld until all histomorphometrics were collected.

Histomorphometric data were collected using a binocular transmitted light Olympus (Olympus, Markham, Ontario, Canada) BX41 microscope at $\times 100$ magnification ($\times 10$ eyepiece and $\times 10$ objective). Four histomorphometric variables as defined by Cho et al. (25) were evaluated:

1. *Cortical area to total area ratio (CA:TA):* the area of bone between the periosteum and endosteum divided by the area of bone within the periosteal envelope.
2. *Intact osteon density:* The number of osteons per unit area that have 90% of their Haversian canal perimeters intact or unremodeled, divided by the cortical area.
3. *Fragmentary osteon density:* The number of osteons per unit area, in which 10% or more of the perimeters of their Haversian canals, if present, has been remodeled by subsequent generations of osteons. This includes interstitial lamellae that are remnants of pre-existing osteons and no longer contain a Haversian canal. The total fragmentary osteon counts are divided by the cortical area.
4. *OPD:* sum of the intact and fragmentary osteon densities.

Stout's (12) method of collecting histomorphometric variables was followed with the exception of measuring cortical areas. Instead of using the Merz counting reticule for calculating cortical cross-sectional areas, thin-sections were scanned and then traced using the freeform polygon function in the Image Pro Plus 4.5 software.

Statistical Analysis

Bias and inaccuracy for rib OPDs and corresponding age estimates were calculated to examine the relationship (direction and magnitude) of the difference in values if another rib other than the established standard (sixth rib) were used to estimate age at death (32). In this study, bias is defined as the difference between OPDs and age estimates of the non-sixth ribs from the sixth ribs. The average bias is calculated by $\Sigma(x_i - x_6)/n$ where i = rib 3, 4, 5, 7, and 8 respectively. Inaccuracy is the average absolute value of the bias or $\Sigma|(x_i - x_6)|/n$.

A linear model was fit to assess the effect of individuals and rib number on the bias. Variance among OPD and corresponding age estimates may be correlated within individuals. To account for this interperson correlation, $J - 1$ dummy variables were added to the general linear model where J is a fixed effect that equals the number of individuals in the sample. A second approach was used incorporating a multilevel model, which treats the effects of the individuals to be random. Although both approaches account for interperson correlation, the second approach reduces the number of parameters that fit in the model and generalize the differences due to any individual variability found in samples. This approach, given the small number of individuals selected from the population, may limit the generalizations made. All statistical analyses were performed on SAS statistical software (version 8.2).

Results

Intraperson OPD Variation

The intraperson analysis assesses the variation in OPDs that occurs among ribs 3–8 in relation to the sixth rib. Table 1 shows the average bias and inaccuracy values for each individual in the sample. The magnitude and direction of the bias associated with non-sixth rib vary across individuals (OPD range: -14.90 to 1.30). Measures of bias indicate a trend for the non-sixth ribs to produce OPD values lower than those of the sixth rib. Overall, 14 of the 20 individuals had OPD values lower than the sixth rib. Evaluating variable plots for each individual consistently reveals one or more rib within the series that deviates from the others. Figs. 1 and 2 demonstrate the variability between CA:TA values and total number of secondary osteons counted (N.On.) by taking two of the 20 individuals who represent the range of variation within the data set. The trend line in CA:TA values throughout the ribs of an individual may be mirrored by N.On. values (see Fig. 1) or N.On. values may strongly deviate with little changes to corresponding CA:TA values in an individual (see Fig. 2). Large differences in N.On. values do not necessarily equate to large differences in CA:TA values. In other words, a rib cross-section with a larger cortical area does not always have a higher number of total osteon counts. Additionally, Figs. 3 and 4 demonstrate the range of variability in OPD within the rib cage by examining two other individuals within the data set. OPD values may be similar (see Fig. 3) or may vary considerably from the sixth rib values (see Fig. 4); however, the eighth rib values consistently deviate the furthest.

Interperson OPD Variation

The interperson analysis assesses the variation in OPDs among ribs of the same number in the pooled rib sample in relation to the sixth rib. In order to determine the interperson relationship between sixth rib and non-sixth rib OPD values, the samples were pooled by rib number. Overall, non-sixth rib values were posi-

TABLE 1—*Average intraperson inaccuracy and bias values comparing pooled non-sixth rib values with the sixth ribs.*

	Individual	OPD	Age Estimate (years)		Individual	OPD	Age Estimate (years)
1	Bias	− 2.90	− 8.50	11	Bias	− 9.50	− 27.3
	Inaccuracy	5.26	15.10		Inaccuracy	4.86	14.02
2	Bias	− 8.50	− 30.30	12	Bias	− 4.50	− 12.70
	Inaccuracy	1.18	3.36		Inaccuracy	2.82	8.02
3	Bias	− 10.40	− 30.0	13	Bias	1.30	4.00
	Inaccuracy	6.82	19.66		Inaccuracy	5.28	15.38
4	Bias	− 4.60	− 13.30	14	Bias	− 3.30	9.50.
	Inaccuracy	2.92	8.50		Inaccuracy	5.22	14.96
5	Bias	− 1.70	− 5.0	15	Bias	− 6.80	− 19.50
	Inaccuracy	1.76	5.08		Inaccuracy	4.08	11.70
6	Bias	− 5.20	− 14.80	16	Bias	− 14.90	− 42.90
	Inaccuracy	2.48	7.14		Inaccuracy	5.52	15.86
7	Bias	− 8.50	− 24.40	17	Bias	− 13.00	− 37.40
	Inaccuracy	2.75	7.78		Inaccuracy	5.26	15.10
8	Bias	− 3.20	− 9.20	18	Bias	− 5.50	− 15.80
	Inaccuracy	3.34	9.58		Inaccuracy	3.52	10.14
9	Bias	− 8.90	− 25.40	19	Bias	− 10.10	− 29.20
	Inaccuracy	2.92	8.34		Inaccuracy	4.84	13.98
10	Bias	− 3.40	− 9.90	20	Bias	− 10.50	− 30.10
	Inaccuracy	3.10	8.90		Inaccuracy	2.78	7.84

OPD, osteon population density.

tively correlated with sixth rib values, indicating that there is significant agreement among measurements (Fig. 5). As expected, the eighth rib demonstrates the largest variance. Measures of bias indicate that all of the non-sixth ribs, except the fifth rib, produce OPD values lower than those from the sixth ribs. Overall, four of the five ribs compared produce average OPD values that are lower than those of the sixth rib. Ribs 5 and 7 produce bias values that are closest to zero (zero being the value for the sixth rib). It is evident from subject to subject that there is variation in which rib approaches the sixth rib values; however, the eighth rib OPD values frequently deviate the most. Inaccuracy for the non-sixth ribs is similar, with the exception of the eighth, which demonstrates a slightly higher value. Table 2 lists the average bias and inaccuracy values between the pooled sixth and non-sixth ribs.

To assess whether significant over- or underestimation occurs across ribs (measured by bias), a general linear model was fit using bias as the dependent variable. The results from this analysis show that there is no significant difference in bias for OPD values

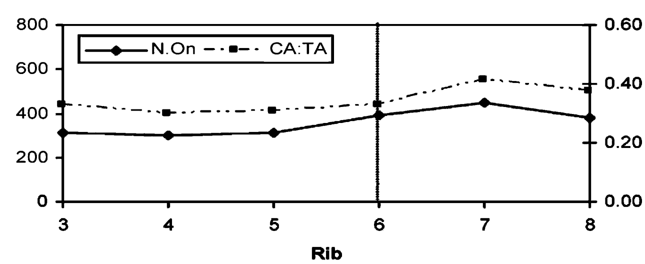

FIG. 1—*N.On. and cortical area to total area ratio (CA:TA) values of an individual demonstrating similar trend lines, indicating that changes in one value are reflected in the other. The Y1 axis (left) represents N.On. and the Y2 (right) axis represents CA:TA.*

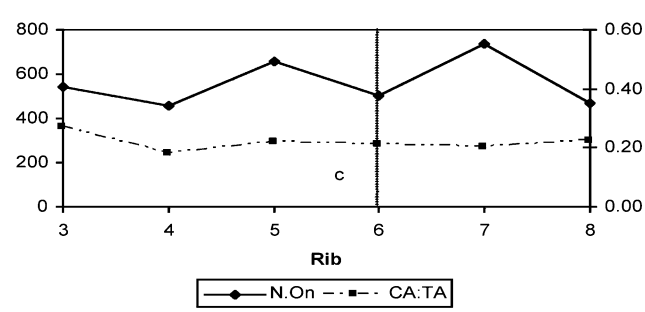

FIG. 2—*N.On. values vary notably between ribs of an individual, while cortical area to total area ratio (CA:TA) values remain fairly constant from rib five to eight. The Y1 axis (left) represents N.On. and the Y2 (right) axis represents CA:TA.*

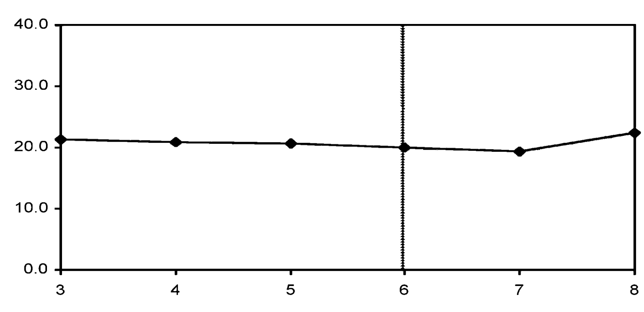

FIG. 3—*Osteon population density values may be similar among ribs within the thorax of an individual with one rib, typically the eighth rib, deviating from the others.*

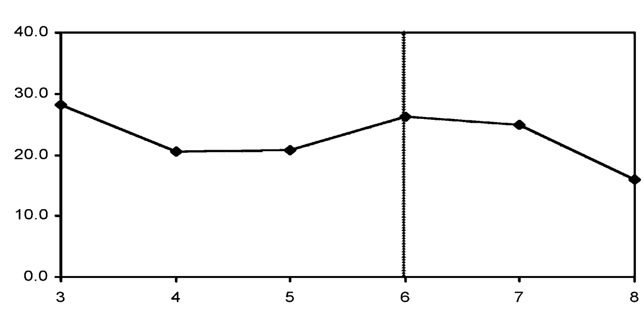

FIG. 4—*Osteon population density values may vary significantly among ribs within the thorax of an individual.*

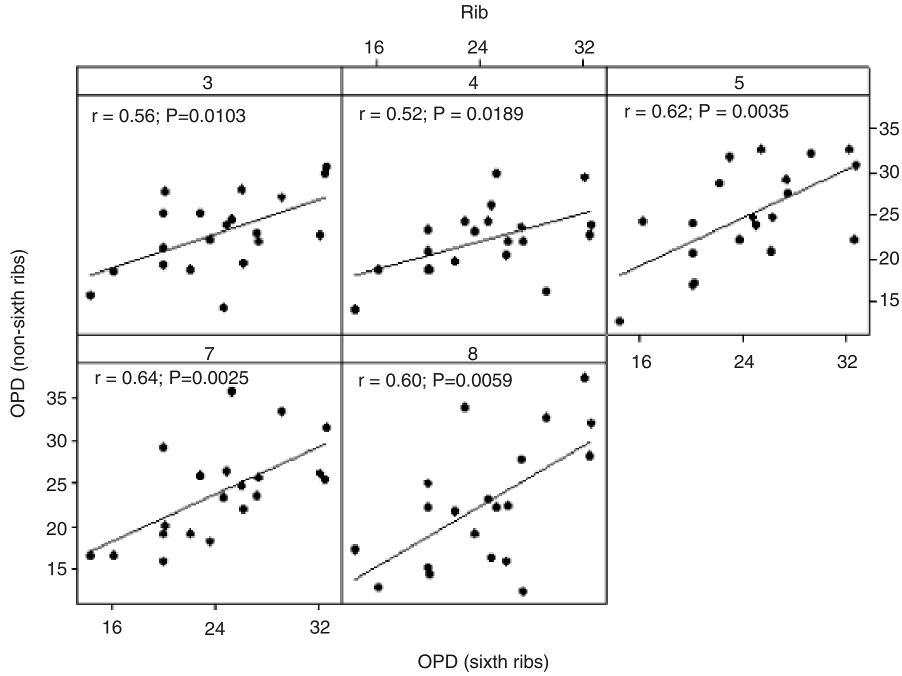

FIG. 5—*Correlation of non-sixth rib osteon population density (OPD) values with sixth rib OPD values.*

or corresponding age estimates between ribs when the values were pooled; however, there are significant differences between individuals (Table 3). The hierarchical model also demonstrates that OPD values from non-sixth ribs do not differ significantly from the sixth rib (Table 4). Twenty-six percent of the variability (also known as the interclass correlation coefficient) seen in the bias values can be attributed to the individual. This indicates that bias is significantly clustered within individuals. The effect of rib was not significantly associated with bias once adjusting for individual variation, which is consistent with the generalized linear model. Both analyses demonstrate that once accounting for the individual, rib number does not significantly affect the bias.

Discussion and Conclusions

Forensic casework often involves disarticulated, fragmentary, or missing ribs. This may cause uncertainty in rib seriation and the sixth rib may not be positively identified. Although it is often difficult to determine rib number, the ribs of the middle thorax (4–7) can usually be identified apart from the others. The results of this study indicate that non-sixth ribs can provide similar OPD values compared with those of the sixth ribs, which is the established standard rib for histological age estimation. The differences in OPD bias values of ribs 3, 4, 5, 7, and 8 were not statistically significantly different from zero. This potentially implies that when a non-sixth rib is evaluated, deviations in age estimates from the sixth ribs will also be nonsignificant. Interestingly, individual variation did prove to be significantly associated with bias. This indicates that there are individual factors that are influencing the magnitude and direction of bias in OPD measurements. Understanding the factors within an individual that influence bias would be an important step in improving histological age estimations. This study was an exploratory analysis intended to examine whether significant remodeling differences occur and how these differences affect age estimates. While it should be noted that the individual sample size for this research is small and nonsignificant values could be a result of lack of power, the conclusions from this study are further strengthened by similarity to the results produced in the clinical literature regarding intraperson bone turnover rates for pathological and nonpathological ribs 5–7 (1–8).

While the results from this study indicate that non-six ribs 3–8 may be used interchangeably for estimating age at death, it is important to note that the eighth rib is the most variable of the

TABLE 2—*Average interperson inaccuracy and bias values by rib number.*

Rib	OPD	Age Estimate (years)
3		
Bias	– 1.45	– 4.17
Inaccuracy	3.66	10.54
4		
Bias	– 2.29	– 6.58
Inaccuracy	3.68	10.56
5		
Bias	0.57	1.61
Inaccuracy	3.53	10.15
7		
Bias	– 0.34	– 0.96
Inaccuracy	3.54	10.13
8		
Bias	– 1.65	– 4.75
Inaccuracy	4.78	13.74
Overall		
Bias	– 1.03	– 2.96
Inaccuracy	3.83	11.02

TABLE 3—*Results of the General Linear Model with the individual as the fixed factor.*

Source	df	SS	MS	*F*	*p* Value
Rib	4	103.13	25.784	1.46	0.2241
Individual	19	943.48	49.66	2.80	0.0008*
Error	76	1346.09	17.71		
Total	99	2392.71			

*Significant at $\alpha = 0.05$.

TABLE 4—*Residual variance from the multi-level model without (I) and with (II) the rib as a covariate in the model.*

	Variance Parameter	Subject	Estimate	Standard Error	*p* Value
I	Intercept-only model				
	Intercept	Individual	6.31	3.23	0.0270
	Residual		18.12	6.32	<0.0001
	Unexplained variance = 74.2%				
II	Random intercept and rib in the model*				
	Intercept	Individual	6.39	3.23	0.0260
	Residual		17.72	2.87	<0.0001
	Unexplained variance = 73.5%				
Variance in bias explained by the addition of rib to the model = 2.21%.					

*The fixed effects of the ribs were not statistically significant.

non-sixth ribs. This suggests that the larger variability in histomorphology may be caused by differences in the biomechanical environment of the vertebro-costal (false) ribs, ribs that do not attach directly to the sternum, compared with the vertebro-sternal (true) ribs. Unlike ribs 3–7, which have direct contact with pectoralis minor and the abdominis rectus muscle, there are no additional anterior muscle groups that attach to the eighth ribs. Furthermore, the distance and angle from the sternal attachment, via the costal cartilage, may generate more variability in the mechanical loading. Ribs 2–11 have similar muscle attachments for the three intercostal muscles (external, internal, and innermost intercostal muscles) and the two serratus posterior muscles (inferior and superior) that are involved in respiration. During inspiration and expiration, the muscles contract and relax, pulling the ribs superiorly toward the first rib or inferiorly toward the twelfth rib. The serratus anterior muscle originates from the first eight ribs and attaches to the inferior angle of the scapula. Its main biomechanical stress occurs during pushing activities. The action of the serratus anterior is to draw the scapula forward around the thoracic wall. It has also been noted to assist in raising and everting the ribs when the shoulders are in a fixed position (19,20,33,34). It is possible that the mechanical loading of serratus anterior on the ribs may differ depending on the distance of muscle origin to the insertion on the inferior angle of the scapula.

The large variance demonstrated in some of the intraperson OPD values indicates that remodeling may be occurring at different rates among ribs of the same person. Significant sampling error can occur within and between cross-sections, with the latter's sampling error increasing dramatically as the amount of cortical area evaluated decreases (7). A rib cross-section with 10 mm^2 of cortical area may demonstrate several 100% differences in bone formation rates between serial bone cross-sections (6). Frost (7) recommends that a minimum of 50 mm^2 of cross-sectional bone in nonpathological individuals should be analyzed, to minimize sampling error. Stout (11) asserts that the time necessary to increase sample area must be weighted against the gains. He recommends the evaluation of two rib cross-sections per individual. Crowder (35) reports that incoherence variation in the ribs is more pronounced in older individuals, who have higher fragmentary OPDs. Evaluating multiple thin-sections may not increase the accuracy of the age estimate, but it does provide for an assessment of the amount of intersection remodeling variability (35).

While large amounts of spatial variation or incoherence within the rib cage may be normal, it is important to realize the potential bias that a sample consisting of cadavers may produce. Mortality profiles for medical cadavers are heavily skewed due to the advanced age of the individuals. It is theorized that an asymptote occurs sometime after 50 years of age when new osteon creations remove all evidence of older ones. If this has occurred, histological methods will underestimate age. Chronic disease, prolonged periods of bed rest, or the long-term use of medication may alter bone metabolism and change the rate of bone remodeling (36,37). It is assumed that if remodeling in the ribs is altered due to these circumstances, it will not be localized to a particular rib within the rib cage.

Because of the Daubert and Mohan, guidelines established for determining the admissibility of evidence methods used or altered by forensic anthropologists may soon be challenged. The forensic specialist must perform more rigorous scientific trials on new and existing methods, especially if the method is being used: (1) well outside of the reference sample, (2) on an element other than the one for which the method was developed, or (3) if the method has been altered on the premise of specialized knowledge that has not been adequately tested. Before this research, any of the midthoracic ribs were assumed to be acceptable for use with histological age estimation methods developed from the sixth rib. This research validates the *a priori* assumption that midthoracic ribs are acceptable for age estimation if the sixth rib is unidentifiable or unavailable. This research also demonstrates the importance of evaluating multiple cross-sections (both intra- and inter-rib) to estimate adult age at death due to the normal remodeling variation within individuals. To improve histological age estimation, further studies are needed exploring the individual factors that affect cortical bone turnover within the rib cage.

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