Anthropological Review • Vol. 80(1), 37–55 (2017)

DE GRUYTER OPEN
ANTHROPOLOGICAL REVIEW
Available online at: www.deguyter.com/view/j/anre/
Journal homepage: www.ptantropologiczne.pl

Estimating age at death from an archaeological bone sample – a preliminary study based on comparison of histomorphometric methods

Barbara Mnich[1], *Janusz Skrzat*[2], *Krzysztof Szostek*[1]

[1]Department of Anthropology, Institute of Zoology, Jagiellonian Univeristy in Kraków, Poland
[2]Department of Anatomy, Collegium Medicum, Jagiellonian Univeristy in Kraków, Poland

ABSTRACT: The estimation of age at death is one of the most fundamental biological parameters, determined on skeletal remains in anthropological context. That is why, there is a constant need to improve applied methods. Histomorphometry, which uses microscopic analysis of bone tissue is suggested to be one alternative method. In general, this technique is based on measurements and the determination of the number and density of basic bone structural units, osteons. Osteon density is found to be related with age of the individual. The main goal of this research was to compare results of determined age at death, on the basis of ribs histology, comes from methods proposed by different authors. We analyzed ground cross sections of ribs from archeological origin. The presented methodology is simple in use and effective. Four different methods were tested (Stout and Paine 1992; Cho et al. 2002; Kim et al. 2007; Bednarek et al. 2009). The obtained age results were compared with each other as well as related to the age estimated by standard macroscopic method used in anthropology. Bednarek's method is recognized to be the most supportive for anthropological analyzes. Methodological issues connected with grinding methodology and results interpretation are also presented. Hypothesis about interpopulation as well as histological and dimorphic differences were confirmed.

KEY WORDS: histology, age assessment, ribs, archaeological bones

Introduction

Years of collecting information about the microscopic structure of bone tissue have enabled the development of a methodology for applying histological research in anthropological practice. The methodology involves histomorphometry, i.e., a quantitative research method based on analysing the microscopic structure of bone tissue using histological techniques, morphological analyses and metrical methods on a microscopic scale.

Original Article Received: December 21, 2016; Accepted for publication: February 6, 2017
DOI: 10.1515/anre-2017-0002

Assessment of age at death of the individual has proven to be the primary, as well as the most promising, application of histomorphometry. The method has the advantage of enabling the determination of the most probable chronological age. To date, age assessment methods used in physical anthropology are based on a macroscopic evaluation of changes within the skeleton that occur with age (Buikstra and Ubelaker 1994). Since these changes concern developmental and degenerative processes, they may occur at different rates in each individual, which corresponds to the biological development of the individual rather than chronological age (Bednarek et al. 2009). Another problem involves fragmented remains in which the elements of the skeleton that are crucial for age assessment are missing (e.g., the pelvis or the skull). In some cases, this could make age at death assessment completely impossible or limit it to the assignment of the individual into one of six age classes (*infans I, infans II, juvenis, adultus, maturus or senilis*) (Malinowski and Strzałko 1985). Moreover, using qualitative characteristics in macroscopic age at death assessment (e.g., the analysis of the pubic symphisis, auricular surface, sternal end of the rib or cranial suture obliteration), whereby the development degree of each characteristic is evaluated visually and attributed to a given age range, also seems problematic (Piontek 1985). However, this method of analysis may yield discrepant results due to the subjective classification of the development of each characteristic. On the other hand, macroscopic methods are relatively quick and easy-to-use with appropriate methodological preparation, which is why they remain the most popular in anthropological practice. Nonetheless, anthropologists have attempted to apply histomorphometry in age at death assessment, as quantitative methods such as histomorphometry are considered more objective by default, and clear and precise guidelines could ensure methodological reproducibility and repeatability. Also, some attempts have been made to analyse burned remains using histological methods (Absolonova et al. 2012). Incinerated remains are extremely difficult to analyse due to extensive tissue damage. Consequently, any method that could increase the amount of information obtainable from them would be extremely useful. In this case, histomorphometry can, to a certain extent, prove helpful. It is the methodology related to assessing an individual's age at the time of death and its practical application in anthropology which constituted the main subjects of research conducted by the authors of this article.

Histomorphometric research and its application in age at death assessment was pioneered by Kerley (1965), who was the first researcher to determine regression equations that allowed the age at death to be assessed based on the density of osteons visible in the cross-section of the femur, tibia and fibula. This was achieved by preparing a microscopy sample in the form of a ground section of the analysed bone. Kerley's discovery inspired numerous researchers who expanded their investigations with an analysis of other bones, determined new indicators and modified the assessment methodology itself (Singh and Gunberg 1970; Ericksen 1991; Stout and Paine 1992; Cho et al. 2002; Kim et al. 2007; Bednarek et al. 2009; Cannet et al. 2011). As a result, many different formulas have been found that enabled age at death assessment based on the histomorphometry of the cortical bone.

The aim of this study was to compare several methods for assessing age at death on the basis of ribs histology, proposed by different authors. It should be noted that no single, commonly used reference histomorphometric procedure exists to date encompassing the grinding technique, applied measurements and their analysis that could be considered to yield the most reliable results. Each of the methods presented in this article involves a slightly different methodological and statistical approach and is based on samples with different characteristics. Nonetheless, all methods applied in this study predominantly involve an indicator referred to as osteon density, hence the possibility to compare them. The comparison of the aforementioned methods will aim to bring us closer to answering the question of whether arriving at a universal model, even for a single type of bone (in this case, the rib) that would help determine an individual's age at death based on histological structure is possible at all. Therefore, the factors that may affect the differences in the obtained results will be discussed as well as whether their influence might be restricted. A histological assessment of age at death on the basis of the skeleton was compared to the biological age established by means of methods used in anthropology. In order to compare the results of histological analyses with calendar age, there some tests were carried out on bone samples of the individuals whose age was known.

Furthermore, the article will present a grinding and measurement procedure that constitutes a modification of currently used methods. The main goal was to simplify the methodology as much as possible to make the procedure effective, relatively quick, inexpensive and applicable without any special equipment, and ensure satisfactory results. This pragmatic approach stems from a desire to refute one of the most significant criticisms of histology concerning its applicability in anthropology, namely, that the method is difficult to use.

Materials and methods

The study used part of the excavated material obtained from an archaeological site in Sanok (Podkarpackie Province, Poland) and dated to the Middle Ages. Samples were taken from 10 individuals (6 females and 4 males) aged between 19 and 50 years. Age at death assessment involved: fusion of skeletal elements, pubic symphysis and auricular surface age-related changes, teeth attrition, obliteration of cranial sutures. Sex determination involved: pelvis anatomy, ventral arc, the subpubic concavity, ischiopubic ramus ridge), greater sciatic notch shape, head of the femur and humerus diameter measurements and cranium morphology (including differences in nuchal crest, mastoid process, supraorbital margin, prominence of glabella and mental eminence) (Piontek, 1985; Buikstra and Ubelaker 1994). Rib assessment methods were used because of their several advantages over histological methods involving other bones. First of all, ribs are exposed only to constant biomechanical stress related to the chest's respiratory movements. Thus, we may exclude the effect of potential mechanical stresses which could influence the rate of bone remodelling e.g. in limb bones. (Crowder and Rosella 2007; Cannet et al. 2011). Furthermore, ribs are not subjected by default to detailed analyses during skeleton assessments, which is why interference into their integrity through

cutting does not significantly affect the information value of the entire skeleton. Bones were included in the analysis primarily if their osteological state also enabled, at least partially, a multi-factor sex and age at death determination using standard macroscopic methods. The analysis was performed on ribs no. 4–8, depending on their availability and state. Using different ribs is methodologically acceptable as ribs no. 3–8 show insignificant histological differences, and the obtained osteon density is comparable (Crowder and Rosella 2007). It was also assumed that left and right ribs show no histological differences, as most authors do not specify which side of the thorax the ribs used in their studies were taken from (Crowder and Rosella 2007). Juvenile individuals were excluded from analysis because the correlation between osteon numbers and age is poor up to approximately 20 years of age (Streeter 2010). Also excluded were ribs with visible pathological changes or signs of past fractures (Pfeiffer 1998). Several sections of 2–3 mm in thickness were taken from each individual with a hand rotary saw. Several sections should be taken because the bone may break or form an uneven surface during grinding or fall off from the microscope slide. The sections were taken at approx. 1/3 of the rib length, counting from the sternal end. The location lies on the boundary indicated by Stout and Paine (1992), Cho et al. (2002) and Bednarek et al. (2009), which is the middle 1/3 of the rib, and the spot indicated by Kim et al. (2007), i.e. the sternal end of the rib. At this stage, care should be taken to cut the rib perpendicularly to its longitudinal axis. Furthermore, there is a great risk of obtaining uneven sample thickness when grinding an unevenly cut section up to approx. 100 μm, making the sample practically useless. Two correctly ground samples were analysed to eliminate the effect of potential local intra-individual variation (Stout and Paine 1992; Crowder and Rosella 2007). Bone material in the form of dry ribs from 6 individuals of known age was also analyzed. Bone samples were made available by the Department of Anatomy and are in the deposit in Department of Anthropology Jagiellonian University.

Grinding methodology

Each collected bone section was ground by hand to below 100 μm of thickness according to a modified method by Maat et al. (2001). The effectiveness of hand grinding method was also confirmed by Boer et al. (2013). One side of the sample was ground with water using sandpaper with decreasing grit sizes (400, 1200 and 2000 grit sandpaper). A bone was considered sufficiently ground if, when viewed at a 45° angle, no cracks could be seen with the naked eye, and the surface reflected light. Next, the sample was rinsed in running water for a short time and left until completely dry. A drop of cyanoacrylate glue was applied in the centre of the microscope slide. After several seconds, the bone was attached to the slide with its smooth side and pressed evenly with moderate force for about a minute. The sample was then left to dry for 1–2 hours until the glue hardened but was not too brittle. The grinding process was repeated by hand for the other side of the bone using sandpaper with decreasing grit sizes (400, 1200 and 2000). 400 grit sandpaper was replaced with 1200 grit sandpaper when the bone started to become transparent when viewed against the light. Further grinding was performed with extreme care,

and its effects were examined frequently using a microscope until a satisfactory result was achieved, i.e., the sample was below approx. 100 μm thick, and bone microstructure was clearly visible. Once the desired thickness was obtained, the slide was rinsed in running water and left to dry. Samples obtained in this manner were ready for analysis. Optionally, the slide can be covered with a cover slip, for better quality of vision, but for this analyses it is not required. The method described above is extremely simplified and has the drawback of potentially contaminating the sample with sandpaper particles and bone dust. The contamination were minimised by frequently rinsing the slide and the grinding surface with water for short periods of time. The sample prepared according to the procedure described above is evenly thick, and the number of cracks is minimised, making basic structural elements (osteons and Haversian canals) clearly visible.

Microscope analysis

A Delta Optical IB-100 light microscope was used for assessments. The Motic Image Plus 2.0 software was used with a Moticam 1SP 1.3MP digital microscope camera for microscope analysis.

Histomorphometric age at death assessment was performed using methods developed by Stout and Paine (1992), Cho et al. (2002), Kim et al. (2007) and Bednarek et al. (2009). These methods were selected for this study because they represent research with groups of different geographical origins, which enables investigation into interpopulation variability. Furthermore, they were developed over the course of almost 20 years, which enables insight into histomorphological progress. The fact that all of them are based on using a relatively simple parameter of osteon density provides the opportunity to include them in anthropological practice, in particular, in age at death assessment.

The following procedure was used to analyse bone samples:

1. A series of six photographs was taken for each sample (three photographs on the inner-pleural and three on the outer-cutaneous side of the rib) using a video camera and a light microscope set to a magnification of 40× (10× eyepiece, 4x lens). A distance of at least 1 mm from the left and right edges of the photographs and the rib was maintained for the first and last photographs.
2. Any osteons located on the edges of the photograph were only counted if they were located on the right side of the photographs to avoid double counting. If a bone fracture or contamination were found, the affected areas were omitted, and the field of vision was moved slightly.
3. Intact and fragmentary osteons were counted in total by marking them on the photograph and counting the number of markings (Fig. 1).
4. The surface of the cortical bone was measured using the Motic Image Plus 2.0 ML software. The 'irregular' mode was used to enable the measurement of the surface area and perimeter of any outlined structure (Fig. 1). Fragments of cancellous bone on the side of the medullary cavity were excluded from the measurements, as were incomplete resorption bays located on the edges of the photograph (Cannet et al. 2011).
5. The surface area of osteons was measured using the 'circle 3 points' mode that enabled a circle to be created by

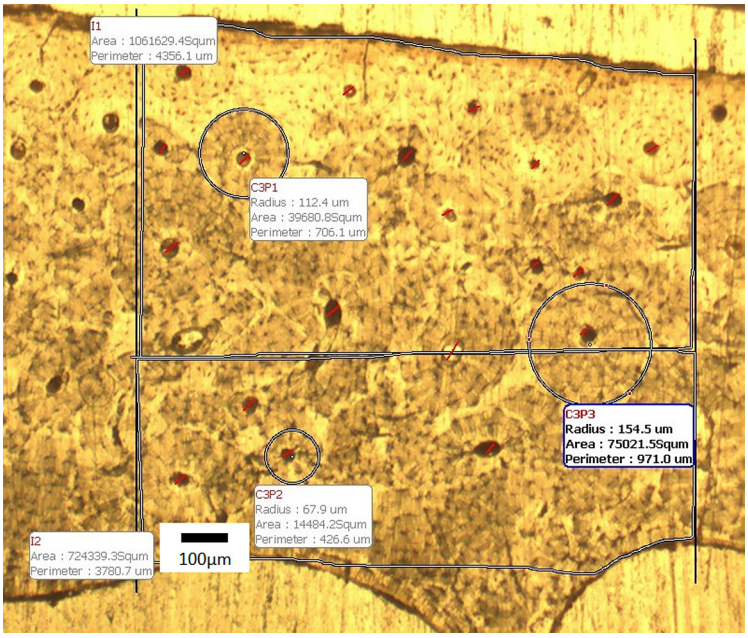

Fig. 1. Example of a photograph of a ground bone sample. Selected measurements are shown, i.e., the surface area of the cortical bone, I1 and I2, the surface area of selected osteons C3P1, C3P2, and C3P3, and markers specifying the location of the osteons (red lines)

marking any three points. The software measured the circumference and surface area of the circle based on these points (Fig. 1).

6. The estimated age at death of each individual was obtained by introducing formulas developed by each author. Further result analysis was performed using the Statistica 10 statistical analysis software.

Age at death assessment methods

Stout and Paine (1992) were the first to use ribs analysis to assess the age-at-death. They defined the analysed structures in detail, which allowed other researchers to apply the methodology in similar studies. The following parameters were measured:

1. Cortical area, i.e., the total surface area of the cortical bone in all analysed areas of a given sample.
2. Intact osteon density, i.e., the number of secondary osteons in which at least 90% of the Haversian canal diameter did not show signs of remodelling divided by the cortical area.
3. Fragmentary osteon density, i.e., the number of remodelled osteons in which at least 10% of the Haversian canal diameter showed signs of remodelling (e.g., due to the presence of a resorption bay) divided by the cortical area.
4. Total visible osteon density, i.e., the sum of intact and fragmentary osteon densities.

Table 1. Comparison of equations of applied methods

Method	Equation	r^2	SEE
Stout and Paine (1992)	Ln(Age)=2.343 + 0.050877(OPD)	0.721	3.9
Cho et al. (2002)	(Age) = 37.982 + 1.400 (OPD) – 670.138 (OA)	0.569	12.68
Kim et al. (2007)	For unknown sex		
	(Age) = 1.014 (OPD) – 790.651 (OA) + 37.022	0.826	4.971
	For known sex		
	(Age) = 1.056 (OPD) – 851.295 (OA) + 2.926 (Sex) + 36.132	0.839	4.821
	Sex: 0 – Male. 1 – Female		
Bednarek et al. (2009)	Males		
	(Age) = 2.684 × (OPD) – 3.358	0.578	5.5
	Females		
	(Age) = 2.443 × (OPD) + 5.687	0.622	5.5

OPD – osteon population density, OA – osteon area, r^2 – coefficient of determination, SEE – standard error of estimate.

The authors of this article decided to use the total visible osteon density in order to eliminate a potential error caused by a subjective classification of osteons into intact or fragmentary.

Ten years after Cho's team (2002) conducted research during which they determined population-specific age at death assessment equations. Two additional measurements were performed:

1. Mean osteonal cross-sectional area, i.e., the mean surface area of at least 25 intact osteons (with an intact cement line) within a single sample. Intact osteons with a shape considerably different from a circle were excluded from measurements.
2. Relative cortical area, i.e., the ratio of the cortical area to the total area in the rib cross-section.

The method developed by Kim et al. (2007) was based on bone samples from a Korean population and showed histomorphometric differences between sexes. A number of measurements developed by Stout and Paine (1992) and Cho et al. (2002) were performed. Because the material analysed in this study came from Sanok in Poland, equations developed based on samples from Poland were used. Bednarek et al. (2009) conducted research according to a methodology suggested by Stout and Paine (1992) with only a single modification, i.e., the use of the Lucia 4.80 image analysis software to measure surface area. The correlation between age and total osteon density was determined separately for males and females.

Table 1 shows equations used for each method presented in this article. All methods express density as the number of osteons per mm^2, and surface area in mm^2. In order to assess age at death using all four methods described above, the following series of measurements and calculations had to be conducted: cortical area, total number of osteons within the analysed area of a given sample, total visible osteon density and mean osteonal cross-sectional area.

Results

Individuals with known age at death were analyzed. For all samples, we demonstrate differences between known and predicted age for all tested methods (Table 2).

In the case of archaeological remains, each of the four methods yielded the predicted age at death of a given individual. Age at death for males and females was determined with different equations depending on the method or with the equation for unknown sex. The obtained results were compared to age at death assessed with standard macroscopic methods used in anthropology (Piontek 1985; Buikstra and Ubelaker 1994) (Table 3).

Taking advantage of the fact that histomorphometric methods enable the determination of approximate chronological age, results were first compared between each histological method. Subsequently, the obtained results were compared to those yielded by macroscopic methods, as these methods can usually estimate a given individual's age range at the time of death.

Separate charts were created for four selected individuals to provide a detailed perspective on age at death estimated according to a given method (Fig. 2 A–D).

Figure 2, insert here

The obtained results show that the higher limit of the variability range achieved using the method by Stout and Paine (1992) overlapped with the age range achieved using macroscopic methods in only one of 10 cases (Fig. 2A). In individuals belonging to the *adultus* age

Table 2. Results of comparison between real and estimated age

Sample	Mean OPD	Mean OA	Sex	Age yrs	Stout and Paine (1992) b	ΔCAE-Ac	Cho et al. (2002) d	ΔCAE-Ac	Bednarek et al. (2009) e	ΔCAE-Ac	Kim et al. (2007) f	ΔCAE-Ac	Notes
s1	14.15	0.0193	M	60	21.39	38.61	44.83	15.17	34.62	25.38	34.61	25.39	Numerous resorptive bay's
s2	28.75	0.0100	M	68	44.95	23.05	71.51	–3.51	73.80	–5.80	57.96	10.04	
s3	29.23	0.0094	M	73	46.07	26.93	72.59	0.41	75.10	–2.10	58.98	14.02	
s4	19.62	0.0139	M	79	28.25	50.75	56.16	22.84	49.30	29.70	45.05	33.95	Very thin cortical layer
s5	30.12	0.0123	M	81	48.21	32.79	71.91	9.09	77.49	3.51	57.47	23.53	
s6	14.91	0.0169	M	78	22.24	55.76	47.51	30.49	36.67	41.33	37.46	40.54	Very thin cortical layer
					Mean difference	37.98		12.42		15.34		24.58	

OPD – osteon population density (number of osteons/mm^2), OA – osteon area (mm^2), [a]M – male, [b]Age for males and females, [c] ΔCAEA – difference between calendar and estimated ages, [d]Age for males and females, [e]Age for males, [f]Age for males

Table 3. Results of age estimation by macroscopic and histomorphometric methods.

Sample	Sex	I	Mean OA	Mean OPD	II	III	IV	V	VI	VII	VIII
Sanok 112	F	19–22	0.0195	8.39	15.95	36.67	30.13		31.33		26.17
Sanok 219	M	25–30	0.0274	12.52	19.69	37.17	28.07	26.05		30.24	
Sanok 102	M	25–30	0.0224	12.94	20.11	41.07	32.42	30.71		31.37	
Sanok 135	F	19–20	X	1.46	11.22						9.26
Sanok 242	F	35–44	0.0223	9.29	16.70	36.06	28.83		29.91		28.38
Sanok 35	F	35–40	0.0168	15.53	22.95	48.48	39.50		41.17		43.64
Sanok 40	F	30–35	0.0268	11.61	18.79	36.28	27.61		28.51		34.04
Sanok 94	M	28–34	0.0171	7.57	15.31	37.12	31.18	29.57		16.96	
Sanok 101	M	25–29	0.0253	4.93	13.38	27.93	22.02	19.81		9.87	
Sanok 162	F	47–50	0.0228	16.92	24.63	46.38	36.13		37.49		47.03

Roman numerals indicates the age of individuals estimated by different methods: I – age estimated by macroscopic methods, II – histomorphometric age for female and male Stout and Paine (1992), III – histomorphometric age for female and male Cho et al. (2002), IV – histomorphometric age for female and male Kim et al. (2007), V – histomorphometric age for male Kim et al. (2007), VI – histomorphometric age for female Kim et al. (2007), VII – histomorphometric age for male Bednarek et al. (2009), VIII – histomorphometric age for female Bednarek et al. (2009). Sex – F – female, M – male. OA – osteon area (mm2), OPD – osteon population density (number of osteons/mm2), X measure not possible.

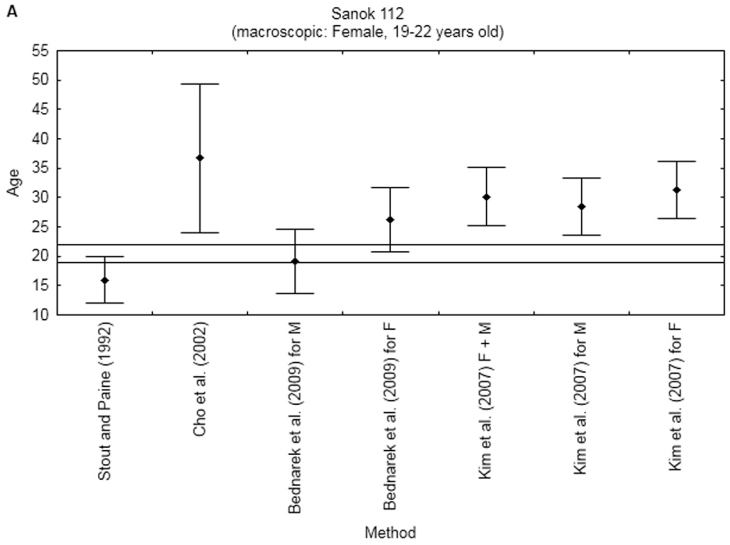

Fig. 2. A–D. Charts with histomorphometric age, estimated by different methods (error bars corresponding to the standard error range). Horizontal lines indicate a given individual's age range assessed using macroscopic methods (bolded line – more probable age based on verification by histological method)
Fig. 2. A

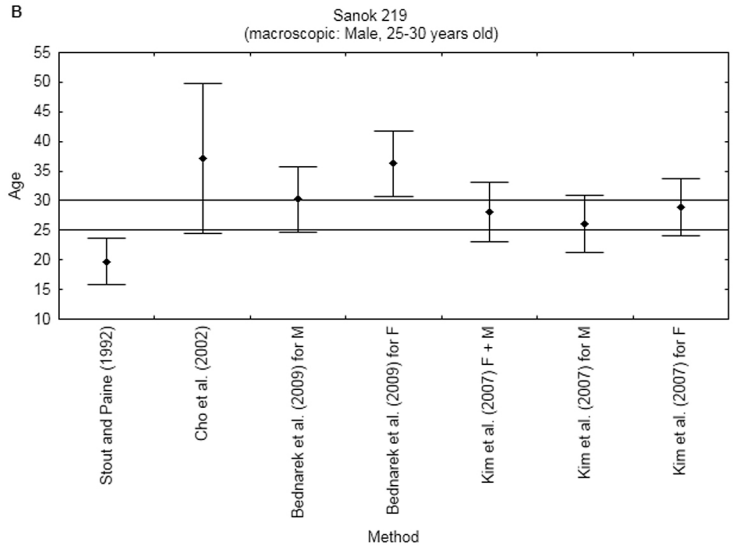

Fig. 2B

class (Sanok 219, Fig. 2B and Sanok 102, Fig. 2C), the higher range limit for the method approached the values obtained using standard morphological methods. However, the older the individual, the more the result obtained using the method by Stout and Paine (1992) differed from those obtained using other methods. On the contrary, variability ranges obtained using the method by Cho et al.

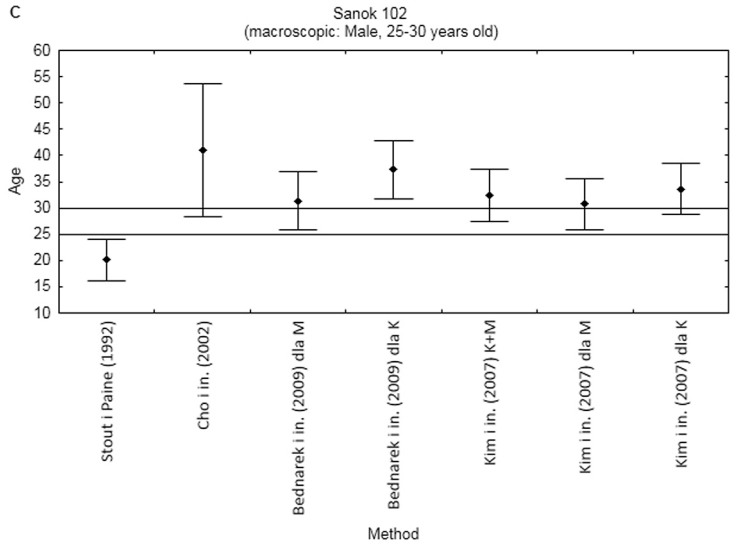

Fig. 2C

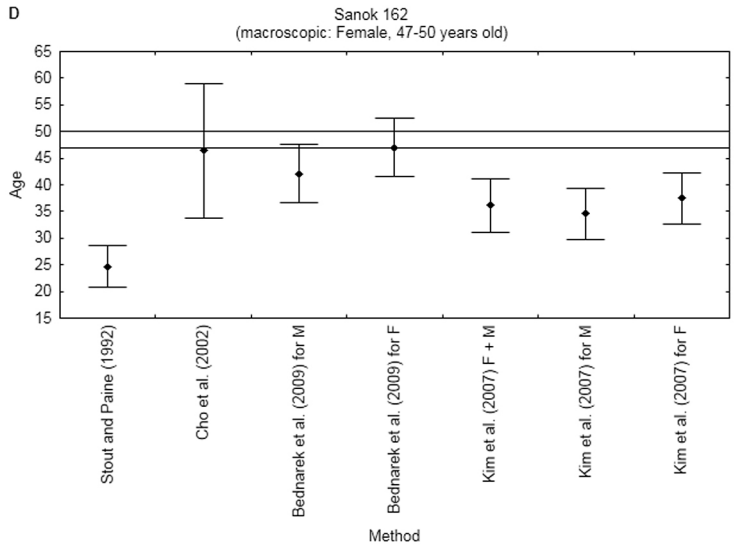

Fig. 2D

(2002) overlapped with those obtained using macroscopic methods in nine samples out of 10 (except Sanok 112, Fig. 2A). Two age at death values estimated using the method by Cho et al. (2002) fell within the range determined using macroscopic methods (Sanok 101 and Sanok 242), and three age at death values deviated from the range by no more than approx. 3 years (Sanok 162, Sanok 40 and Sanok 94). It is worth noting that the method by Cho et al. (2002) even estimated four of the remaining individuals to be approx. 15 years older than what macroscopic methods indicated (Sanok 112, Fig. 2A). However, the method by Cho et al. (2002) involves the largest standard error among all methods applied in this study, which may explain why the method yielded such varied results. The methods by Kim et al. (2007) and Bednarek et al. (2009) yielded the results that were the most similar to each other as well as to the results obtained using macroscopic methods. The most prominent difference between the two methods concerned age at death assessed for females. The method by Bednarek et al. (2009) yielded higher age values for females than the method by Kim et al. (2007). However, comparison of these two methods has certain limitations resulting from the measurement methodology. Bednarek et al. (2009) relied solely on osteon density, while Kim et al. (2007) introduced an additional parameter, the mean osteonal cross-sectional area, which may have affected the final result. Both methods assumed that males and females show histomorphometric differences. The results obtained in this study seem to justify this assumption. Age at death assessed using both methods for each sex was closer to values obtained using macroscopic methods than what would have been obtained with the expressions for the opposite sex than the analysed individual's sex. For example, sample Sanok 102 was described macroscopically as a male in age range of 25–30

years. Histological age at death obtained by Bednarek et al. (2009) method for males is 31.37 years. Opposite sex estimation (female instead of male) would give a highly distinct result, i.e. 37.29 years, which is less consistent with macroscopically estimated age at death. Similar situation applies to sample Sanok 40. Macroscopically: female 30–35 years old. Equation for females estimates age as 34.04 years, but equation for males, 27.79, which emphasises the importance of right sex assessment, if we want to use histological method differentiating separate equations for both sexes.

In the Sanok 135 sample, because of only few osteons in bone slice, reliable measurements of mean osteon area were not possible. What is more, age at death obtained by two possible methods (Stout and Paine 1992 and Bednarek et al. 2009) gave lowered results. The most probable cause is weak correspondence between formation of the osteons and age in individuals under 20 years old (Streeter 2010). In this case, using standard anthropological methods, the most probable age range was estimated at 19–20 years. It only confirms that the discussed histomorphometric methods are not preferable in young individuals, which complies with the assumptions taken by the authors of those methods.

The method by Stout and Paine (1992) shows a clear tendency towards considerably lowered results compared to other methods. In turn, the method by Cho et al. (2002) frequently yields higher age values than other methods. This causes a considerable spread of results for a given sample, even up to about 25 years (Sanok 35). The Kruskal–Wallis non-parametric test and its multiple comparisons found statistically significant differences in results between the methods by Stout and Paine (1992) and Cho et al. (2002) ($Z=4.594421$, $p=0.0003$) and between the methods by Stout and Paine (1992) and Kim et al. (2007) for females ($Z=3.129164$, $p=0.0003$). In the case of differences in the latter pair of methods, the test result is not completely justified, as Stout and Paine (1992) introduced an equation for both sexes in total, whereas the observed difference concerns only the equation for females.

The results show clearly a similar difference between the predicted age at death for male and female individuals in both methods, the one suggested by Kim et al. (2007) and the one by Bednarek et al. (2009). Both methods, when based on the same osteon density, indicate lover age at death estimation for male than for female. The equation for an unknown sex by Kim et al. (2007) yielded values that were located predominantly between those obtained separately for male and for female but showed a much greater variation. Thus, determining an individual's age at death prior to histomorphometric analysis using the method by Kim et al. (2007) reduces the error range when assessing age at death. Incorrect sex determination could also significantly affect results when using the method by Bednarek et al. (2009), albeit in this case, the variability ranges for each sex overlap to a greater degree. However, determining age at death assessment methods that do not take into account sexual dimorphism is also extremely important, even if such methods involve a greater error. The reason for that is the fact that diagnostic characteristics that enable sex determination are sometimes lost in the bone material.

Table 4 shows a comparison between the age at death assessed by Bednarek et al. (2009) method and the age at death

Table 4. Application of histomorphometry to precise age estimated by macroscopic methods

Sample	Macroscopic methods (age and sex)	Age based on all (the most accurate) histomorphometric methods	Comments	Age by Bednarek et al. (2009)
Sanok 112	19–22 F	Result not clear	Conformity to Bednarek et al. (2009) method for age 19 male sex only	26.17
Sanok 219	25–30 M	30	–	30.24
Sanok 102	25–30 M	30	–	31.37
Sanok 135	19–20 F	Result not clear	All methods lowered the estimated age in comparison to macroscopic age	9.26
Sanok 242	35–44 F	35	35 yrs by: Cho i in. (2002). Results from other methods: under 35 yrs	28.38
Sanok 35	35–40 F	40	–	43.64
Sanok 40	30–35 F	35	By: Bednarek et al. (2009) Cho et al. (2002)	34.04
Sanok 94	28–34 M	Result not clear	Great dispersion of results	16.96
Sanok 101	25–29 M	25	Most methods under 25 yrs	9.87
Sanok 162	47–50 F	47	–	47.03

F – Female, M – Male

assessed by macroscopic methods. It presents how histomorphometry may be applied in the age at death estimation procedure. Of course, "effectiveness" can only be understood here as the convergence between age values obtained using histological methods and standard methods, as we lack the actual, documented age of the analysed individuals at the time of death. Consequently, this is the only way in which histomorphometric methods can be compared and how their usefulness may be evaluated.

Disscussion

This study was based on a relatively small number of samples since it was intended to be a methodological research considering the analysis and comparison of histological methods applied in age at death estimation. Analyses regarding the usefulness of histomorphometric measurements have not been conducted so far, hence the results of this study may prove interesting. The potential reasons for the observed differences between the applied methods may be especially worth discussing.

Modern material analyzes (influence of involution and disease processes on the results interpretation)

Analyzing the results from six individuals of known calendar age, the average method deviations should be noticed. It gives following results, for Stout and Paine (1992) 37,98 years, range from 23,05 to 55,76; Cho et al. (2002) 12,42 years, range from –3,51 to 30,49; Bednarek et al. (2009) 15,34 years, range from -5,80 to 41,33 and Kim et al. (2007) 24,58 years, range from 10,04 to 40,54 (Table 2). Observed high discrepancies between real and histological age might be connected with advanced involutionary and/or disease processes, which are

visible in 3 samples (s1, s4, s6). Firstly, the compact substance of the individuals' ribs was much thinner from the compact substance of the ones whose age was determined correctly, or even in comparison to the bones from Sanok. Secondly, an abnormally high number of resorption sinuses in an already thin bone cortex was observed. Both of these factors caused a very low number of observable osteons within the ground section, which led to the age at death being strongly underestimated in the calculation. After excluding this three outliers, differences between real and estimated age significantly decreased and present as follows: for Stout and Paine (1992) 27,59 years, range from 23,05 to 32,79; Cho et al. (2002) 2,00 years, range from –3,51 to 9,09; Bednarek et al. (2009) –1,46 years, range from –5,80 to 3,51 and Kim et al. (2007) 15,87 years, range from 10,04 to 23,53. The lowest variation presents Cho et al. (2002) and Bednarek et al. (2009). method. Results obtained by these two methods hover around known calendar age. Negative mean deviation (mean underestimation of age at death) in Bednarek method and positive mean deviation (mean overestimation of age at death) in Cho method could be the consequence of an insufficient number of samples. To conclude, both methods, have similar variability and the most precisely estimate age at death of individual. Despite small number of samples, it can be clearly seen that every degenerative or disease-related change in bone histology influences the reliability of the methods used. Accordingly, each sample must be considered and research individually.

Historical material analyses (methodological issues)

Differences in accuracy between the applied methods may stem from the varying age distribution of the analysed individuals that was used to determine regression equations. No age group should dominate in a sample. Otherwise, the obtained regression equations may be unreliable, leading do under- or overestimation of the age at death of individuals in a given age range (Stout 1998 in Reichs 1998). Perhaps this was why the method by Stout and Paine (1992) provided lower age values compared to other methods used in this study. Stout et al. (1996) themselves introduced an appropriate correction with respect to the clavicle. Stout's team noticed that age at death assessment was less accurate in individuals' aged over 40 years, a fact they explained by a relatively low mean age of individuals they used to determine the correlation (28.6 +/– 12.9 years). The hypothesis that a similar conclusion can be drawn with respect to the ribs stems from the fact that the regression for the ribs was determined based on the same material, i.e. bones of the same individuals, as the regression for the clavicle.

A good example of development in histomorphometric research is the age range that enables an accurate assessment of an individual's age at death. Initial studies estimated the range to begin practically at birth and end at 95 years (Kerley 1995). Today, some researchers estimated the lower limit of the range at 17 years (Cho et al. 2002) and 20 years (Kim et al. 2007), and the upper limit at about 70 years (Kim et al. 2007). The results of this study also confirm the existence of a lower limit for standard histomorphometric methods, below which age at

death assessment is virtually meaningless. All applied methods inaccurately assessed both samples taken from the youngest individuals, i.e. those whose age at death was estimated macroscopically at 19–20 years (Sanok 135) and 19–22 years (Sanok 112), usually overestimating the age of the Sanok 112 sample and underestimating the age of the Sanok 135 sample. On the other hand, macroscopic methods are relatively reliable with respect to individuals up to the juvenis age class. Reasons why histomorphometric age at death assessment is inaccurate with respect to juvenile individuals comprise the still on-going development of primary osteons followed by secondary osteons, the large area of the non-remodelled osseous tissue, and the presence of drifting osteons. However, a descriptive method has been developed for ages up to 21 years that allows an individual to be qualified into one of four age ranges (<5, 5–9, 10–17 and 17–21 years) (Streeter 2010). Membership in a given stage of bone development is based on the assessment of the following factors: the presence and amounts of woven (thick-fibre) bone and lamellar bone, the presence and advancement stage of bone remodelling, and the general histology of the cortical bone on the inner and outer sides of the ribs (Streeter in Crowder and Stout 2012).

The upper limit to which the obtained results can be reliable is a more controversial issue. Kim et al. (2007) and Wu et al. (1970) directly stated that the number of osteons reaches its maximum at the age of 60 years, and individuals in the senilis age range can display degenerative changes that affect the results. However, it is difficult to determine whether the maximal number of osteons has been achieved, as the highest age of individuals analysed in this study was almost 50 years. Bone remodeling rate also depends on many physiological and pathological factors, so when osteon density reaches maximum value is open to discussion (Frost 1987). In addition, the samples were collected from individuals whose cause of death is unknown; therefore it is difficult to conclude whether such histological image is a result of pathological processes or age, which alters the bone structure anyway. Obviously, this constitutes a limitation to the application of histomorphometry and makes it necessary to consider each result on a case-by-case basis more reasonably.

It might be subjected to scrutiny whether histology might help to establish how the most probable chronological age (obtained by histomorphometry) modifies the age which is assessed by the macroscopic analysis of bone material. Each method aims to yield the most probable age of a given individual at the time of death, taking into account the variability range of the method. It might be examined how each method would modify the estimated age at death obtained by macroscopic methods. A large discrepancy between results following such an analysis would allow researchers to consider the effect of potential population variability. A comparison between histological age and macroscopic age, showed that histomorphometry did not constitute a decisive tool in every case. Nonetheless, it was of considerable help in most of the individuals (Sanok 112, 219, 102, 162, 35, 40, 242), as it has indicated whether a given age at death of the individual is closer to the lower or upper limit of the range obtained with macroscopic methods. And that could be found as a useful tool for anthropologists. The performed analyses showed

that the method suggested by Bednarek et al. (2009) might be regarded as the most effective one.

As with any branch of science, anthropology is not satisfied by only using well-established methodologies. Rather, it constantly seeks new solutions to expand its capacity for interpreting bone material. Histomorphometry is one of the tools that aid this pursuit. This study provided an example of the application of histomorphometry in age at death assessment, as age constitutes one of the major, and often problematic, parameters determined during analysis of bone material.

The process of grinding a sample and performing appropriate measurements and calculations is not complicated. While it obviously relies on experience, once a researcher learns the technique and obtains the necessary equipment, they could easily implement histomorphometry into their anthropological research. Validation processes which include sample grinding, photographing, measurements and calculations will be required and would unambiguously confirm the usefulness of the method in anthropological practice.

Interpopulation variation

Interpopulation variation is one of the most recently and widely discussed factors that may affect differences in bone remodelling (Stout 1998 in Reichs 1998; Cho et al. 2002). Kerley (1965), the pioneer of histomorphometric research, indicates a lack of a visible effect of the geographical origin on changes in the histological properties of bones. However, later publications disagree, providing evidence for considerable differences in estimated age at death using the same method among different populations (Reichs 1998). Other studies indicate that Inuit populations display higher osteon density than European and North American populations (Thompson and Guiness-Hey 1981 cited in Reichs 1998). As of today, it seems that determining regression equations for particular geographical areas is necessary (Cho et al. 2002; Kim et al. 2007; Bednarek et al. 2009). Analysis of histomorphometric parameters in American populations of African and European origin indicated significant differences between these groups (Cho et al. 2002), which motivated authors to determine population-specific regression equations based on osteon density, mean osteon cross-sectional area and the ratio between cortical bone to the total surface area of the cross-section. Kim et al. (2007) analysed bones of Korean origin and arrived at an equation for assessing age at death. Kim's team then compared the results obtained using their method and methods by other authors (Stout and Paine 1992; Cho et al. 2002), and noted considerable differences. The method by Stout and Paine (1992) yielded only 29.7% results to an accuracy of +/– 5 years, 20.8% to an accuracy of +/– 5–10 years and as much as 49.5% to an accuracy of over +/– 10 years. The method by Cho et al. (2002) yielded values with a similar distribution: 17.2%, 35.2% and 47.6%, respectively. These results suggest histological differences between the American and Korean populations.

This study did not arrive at direct or unambiguous conclusions regarding the effect of the geographic factor. Nonetheless, the application of population-specific equations was justified by the results obtained using the method by Bednarek et al. (2009), developed for the Polish population, as it was the most effective

method applied in this study. This method proved most useful both in the case of a model being a sample of known age and in reference to anthropologically estimated age at death of bone remains. Results obtained using the method by Kim et al. (2007) for the remains from Sanok are more difficult to interpret. In most cases, these results deviated only slightly from those obtained using the method by Bednarek et al. (2009). On the other hand, those cases where the differences were greater indicate that research should be done on the effect of the additional variable used in the method by Kim et al. (2007), i.e., the osteon cross-sectional area. However, researchers should keep in mind that unambiguous and dependable conclusions can only be drawn based on bones of individuals of which the actual age is known, which is why population-specific age at death assessment equations require model studies based on contemporary samples. As far as developing age at death assessment equations for historical populations is concerned, the only alternative would be to use written sources such as cemetery records that would confirm a given individual's age at the time of death. Another factor that could be taken into consideration is the historical period in which given remains originated, i.e., the question should be considered whether the remodelling ratio of the cortical bone changed over the years. When Kerley (1965) attempted to test his method using remains dated from 500 to 5000 years ago, he did not mention the possibility of his sample differing from the contemporary population. Most publications do not attempt to explain the cause of observed differences in bone remodelling depending on sample origin. Only very general reasons, if any, are given, e.g., genetic or dietary factors (Stout 1998 in Reichs 1998). Pfeifer (1998), however, suggests that environmental factors dominate over genetic ones.

Sexual dimorphism

Another most problematic subject in histological analyses, after interpopulation differences, is the presence of sexual dimorphism or lack thereof. Kerley's (1965) original study indicates a lack of sexual dimorphism. The method developed by Singh and Gunberg (1970 in Reichs 1998) was based on bone samples collected only from male individuals. They suggested, however, that their method might be applied to female individuals as well. Methods by Stout and Paine (1992) and Cho et al. (2002), used in this study, also involve calculations for both sexes together, as does the method by Cannet et al. (2011). Data presented in Reich (1998), suggesting that separate regression equations should be determined for men and females. Samson and Branigan (1987 in Reich 1998) noted differences between sexes in the standard error for several histomorphometric characteristics. They explained these differences through an increased rate of bone remodelling in postmenopausal females due to a decreased level of oestrogens in the body. Kim et al. (2007) reached similar conclusions. They stated that sex has a significant effect on bone remodelling processes and osteon density due to increased activity of basic multicellular units (BMUs), caused by menopause. Their claim is confirmed by the fact that differences in values of all histological parameters they analysed were statistically different between sexes in the 40–49 years range. Note that the mean age at which Korean enter men-

opause is 46.9 years (Kim et al. 2007). Bednarek et al. (2009) also pointed out the importance of differences due to sexual dimorphism, which is why they developed separate regressions for women and men. Based on the obtained results, the authors of this study find the claim about histological differences between sexes compelling. The equations designed for the sex of the individual usually assessed the age at death more accurately. As Stout (in Reichs 1998) mentions, "It is possible that the amount of variation between sexes may differ among bones with different remodeling rates, cortical areas, and biomechanical environments". Therefore, histological methods of age at death assessment as well as the macroscopic methods require great caution. There is a certain risk of wrong sex determination, which, as has been mentioned, may have a significant effect on the obtained results.

To sum up, a comparison of several age at death assessment methods based on rib samples, followed by result analysis allowed for methodologically important observations. Bednarek's et al. (2009) methods seems to be the most useful for age at death estimation on Polish bones samples. It might seem that the large number of factors directly and indirectly affecting the obtained results and their interpretation could make it difficult to develop a universal equation for age at death assessment of any individual regardless of sex, origin or health. Further research has been adjusted to the characteristics of a given group and aimed at eliminating some of the variables affecting the results. However, it appears that histomorphometry might be considered as a useful complementary method for age at death estimation.

Acknowledgements

Publication was co-financed by the Department of Anthropology of the Jagiellonian University and Department of Anatomy of the Collegium Medicum, Jagiellonian University.

Author contribution

BM made laboratory and microscope analysis, collected the literature and wrote the manuscript; JS collected contemporary human ribs. KS provided the main concept of the paper. All authors critically read and approved the final manuscript.

Conflict of interest

The Authors declare that there is no conflict of interests regarding publication of this paper.

Corresponding author

Barbara Mnich, Department of Anthropology, Institute of Zoology, Jagiellonian University in Kraków, Gronostajowa 9, 30-387 Kraków
e-mail address: barbara.mnich@uj.edu.pl

References

Absolonova K, Veleminsky P, Dobisikova M, Beran M, Zocova J. 2012. Histological Estimation of age at death from the compact bone of burned and unburned human ribs. J Forensic Sci 58(S1):35–4.

Bednarek J, Bloch-Bogusławska E, Engelgardt P, Wolska E, Śliwka K. 2009. Validity of histomorphometric rib assessment for age at death prediction. Problems of Forensic Sciences LXXX:403–10.

Buikstra J, Ubelaker DH. 1994. Standard for data collection for human skeletal re-

mains, Fayetteville**:** Arcansas Archaeological Survey Research. 44.

Cannet C, Baraybar JP, Kolopp M, Meyer P, Ludes B. 2011. Histomorphometric estimation of age in paraffin-embedded ribs: a feasibility study. Int J Legal Med 125:493–502.

Cho H, Stout S, Madsen RW, Streeter M. 2002. Population specific histological age estimating method: a model for known African-American and European-American skeletal remains. J Forensic Sci 47(1):12–18.

Crowder Ch, Rosella L. 2007. Assessment of Intra- and Intercostal Variation in

Rib Histomorphometry: Its Impact on Evidentiary Examination. J Forensic Sci 52(2):271–6.

Ericksen MF. 1991. Histologic estimation of age at death using the anterior cortex of the femur. Am J Phys Anthropol 84:171–9.

Frost HM. 1987. Secondary osteon populations: An algorithm for determining mean bone tissue age. Yearb Phys Anthropol 30:221–38.

Kereley ER. 1965. The microscopic determination of age in human bone. Am J Phys Anthrop 23:149–64.

Kim Y-S, Kim D-I, Park D-K, Lee J-H, Chung N-E, Lee W-T, Han S-H. 2007. Assessment of histomorphological features of the sternal end of the fourth rib for age estimation in Koreans. J Forensic Sci 52(6):1237–42.

Malinowski A, Strzałko J. 1985. Antropologia. Warszawa–Poznań: Państwowe Wydawnictwo Naukowe.

Maat GJR, Van Den Bos RPM, Aarents MJ. 2001. Manual preparation of ground sections for the microscopy of natural bone tissue: Update and modification of Frost's "Rapid manual method". Int J Osteoarchaeol 11:366–74.

Pfeiffer S. 1998. Variability in osteon size in recent human population. Am J Phys Anthropol 106:219–27.

Piontek J. 1985. Biologia populacji pradziejowych. Zarys metodyczny. Poznań: Uniwersytet im. Adama Mickiewicza.

Reichs KJ. 1998. Forensic Osteology: Advances in the Identification of Human, Second Edition. Springfield, Illinois: Charles C Thomas Publisher LTD.

Samson C, Branigan C. 1987. A new method of estimating age at death from fragmentary and weathered bone. In: A Boddington, AN Garland and Janaway RC (editors). Death, Decay and Reconstruction: Approaches to Archaeology and Forensic Science. Manchester, UK: Manchester University Press. 101–8.

Singh U, Gunberg DL. 1970. Estimation of age at death in human males from quantitative histology of bone fragments. Am J Phys Anthrop 33:373–82.

Stout SD, Paine RR. 1992. Brief communication: Histolological age estimation using rib and clavicle. Am J Phys Anthropol 87:111–5.

Stout S.D., Porro M.A., Perotti B., 1996. Brief Communication: A test and correction of the clavicle method of Stout and Paine for histological age estimation of skeletal remains. Am J Phys Anthropol 100:139–42.

Stout SD. 1998. The application of histological techniques for age at death determination. In: Reichs KJ (editor). Forensic Osteology: Advances in the Identification of Human. 2nd edition. Springfield, Illinois: Charles C Thomas Publisher LTD.

Streeter M. 2010. A four-stage method of age at death estimation for use in the subadult rib cortex. J Forensic Sci 55(4):1019–24.

Thompson DD, Guiness-Hey M. 1981. Bone mineral-osteon analysis of Yupik-Inupiaq skeletons. Am J Phys Anthropol 55:1–7.

Wu K, Schubeck KE, Frost HM, Villanueva A. 1970. Haversian bone formation rates determined by a new method in a mastodon, and in human diabetes mellitus and osteoporosis. Calcif Tissue Res 6:204–19.