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ORIGINAL ARTICLE

Test of a new components method for age-at-death estimation from the medial end of the fourth rib using a modern Spanish sample

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Abstract Recently, Verzeletti et al. (Am J Forensic Med Pathol 31:27–33, 2010) developed a new components method for the estimation of age-at-death from the sternal end of the fourth rib. This approach consists of the assignment of numerical scores for several age-related morphological characteristics, which are then inserted into a regression equation for predicting chronological age. The present study tested the accuracy of the new components method on a sample from Spain, consisting of 58 males and 36 females. The results demonstrated that the regression equation devised by the aforementioned authors for an Italian male sample was less accurate when applied to the Spanish population sample. This was true for separate male and female samples, as well as a combined-sex sample. A pair of population-specific regression formulae was then generated from the Spanish sample data in an attempt to improve the accuracy of the age estimate. The new equation which incorporated sex as an independent variable did not yield more accurate age estimates than a non-sex-specific equation. Furthermore, both of these formulae provided only marginally more accurate results for the Spanish sample compared to the original Italian equation. Thus, the standard errors of the estimate associated with these population-specific models (8.1–8.9 years) were still noticeably larger than the 5.2 years observed by Verzeletti and colleagues in their Italian study. Given the high standard errors associated with the age estimates for the Spanish population sample, the new rib components method should be used in conjunction with other adult aging techniques.

Keywords Forensic anthropology · Age estimation · Ribs · Linear regression · Spain

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Introduction

The accurate estimation of chronological age-at-death is an integral part of establishing the biological profile (osteobiography) of unidentified human remains. A multitude of methods have been developed for assessing this demographic characteristic in mature individuals, in which growth and development are complete. The more commonly utilized techniques are based on the evaluation of degenerative and regressive changes observable in various skeletal structures, including the face of the pubic symphysis [1, 2], auricular surface of the ilium [3–5], cranial sutures [6, 7], and dentition [8, 9].

Adult age assessment from morphological changes of the medial end of the rib began with the pioneering work of İşcan and colleagues, whose initial components method [10] was quickly replaced by the phase method [11, 12]. Since then, numerous studies have evaluated, revised, and attempted to improve these methods, employing direct macroscopic examination [13–17] as well as computer-based imaging techniques [18]. In one recent investigation, Verzeletti et al. [19] developed a new components method, which examined the age-related metamorphosis of the articular surface (*A*), anterior/posterior walls (*B*), and superior/inferior edges (*C*) of the sternal extremity of the fourth rib. For each specimen, a numerical score was assigned for each of these parameters and several regression equations for predicting adult age were then developed for the sample. The best correlation between morphological characteristics and age-at-death was obtained through the formula: $\mathrm{Age} = 10.43(AB)^{0.53}$. This equation provided reasonably accurate age estimates for the white Italian male sample utilized in that study, with a standard error of 5.2 years for the entire sample.

Several earlier studies [20, 21], however, have reported differences in the rate and morphological pattern of age-related changes in the sternal extremity of the ribs between various human groups, and thus, the applicability of this new method must be evaluated for each population, particularly if the technique is to be employed in medicolegal proceedings. Therefore, the objective of the present study was to test the accuracy of the new components method, including the abovementioned regression equation, developed by Verzeletti et al. [19] on a modern sample from Southern Spain. An additional aim was to generate new regression formulae utilizing the Spanish dataset to determine if the accuracy of the age estimate is improved through the use of population-specific models.

Materials and methods

The present study examined the sternal end of the right fourth rib belonging to individuals of Spanish descent. The skeletal material was collected from decedents undergoing postmortem examination at the Forensic Pathology Service, Institute of Legal Medicine, in Seville, Spain, between March 2011 and February 2012. The selected sample consisted of 94 adults, including 58 males and 36 females, chosen from different age groups. Although not part of the original study by Verzeletti et al. [19], the latter group was included to assess the accuracy of the components method for females, as well as males, given that the sex of an individual may not always be reliably determined for remains recovered from forensic contexts. The ages-at-death for the sample ranged from 18 to 86 years (mean age, 51.3±18.5 years) in males and 23 to 89 years (mean age, 56.5±18.3 years) in females. The age distribution of the study sample is depicted in more detail in Fig. 1. Only non-pathological specimens, as assessed by macroscopic observation, were included in the investigation.

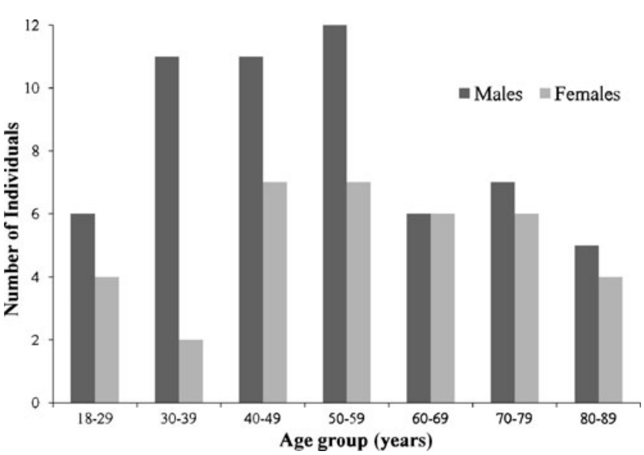

Fig. 1 Age distribution of the utilized sample (n=94)

During autopsy, a short segment of the sternal end of the right fourth rib along with its costal cartilage was excised from the cadavers with a large clipper tool. The specimens were preserved in a small glass container filled with water for approximately 3 weeks. The bones were later boiled gently to remove the remaining soft tissue including costal cartilage. The specimens were allowed to dry at room temperature.

The rib segments were examined and chronological age-at-death was estimated according to the method defined by Verzeletti et al. [19] for their Italian sample. This system consists of the assignment of a numerical score for several parameters of the sternal extremity of the rib based on the form, texture, and bone quality of the specimen. The morphological changes in the articular surface (A) and anterior/posterior walls (B) are described in ten and nine different classes, respectively, each one associated with a specific numerical score. A third parameter of the medial end of the fourth rib, namely superior/inferior edges (C), was also examined by Verzeletti and colleagues [19]. The morphological changes for this variable are described in eight different classes again each one associated with a specific numerical score. In addition, for all parameters, intermediate scores can be assigned to a rib segment in which the sternal end displays the morphological pattern of two sequential classes. Descriptions, illustrations, and reference photographs of the different morphological classes and corresponding numerical scores for the abovementioned parameters are provided in the original article [19]. The assignment of scores for all rib specimens in the current study was completed by the first author (PJM).

The relationship between age-at-death and the three morphological characteristics within each sex was evaluated for the Spanish population sample using Spearman's rank correlation coefficient (a nonparametric alterative to Pearson's coefficient). The assigned values for each specimen in the study sample were then inserted into the previously enumerated regression equation $\left[\mathrm{Age} = 10.43(AB)^{0.53}\right]$ devised by Verzeletti et al. [19] for predicting adult age-at-death. The age estimates derived from this regression formula were then compared to the actual ages of the specimens to assess the accuracy of the components method among individuals of Spanish descent. Following recent studies [22–24], bias [Σ(estimated age−actual age)/n] and inaccuracy or absolute error [Σ|(estimated age−actual age)|/n] of the method were computed for the entire sample, as well as for age classes (e.g., 18–29, 30–39, 40–49, etc.). The reliability of the regression equation was also assessed by calculating the standard error of the estimate (SEE). This analysis was performed separately for each sex given that the onset, rate, and pattern of age-related metamorphosis of the sternal extremity of the rib differ between males and females [11, 12]. However, as previously stated, the sex of an individual may not be known with any great degree of certainty in many forensic situations, and thus, the bias, inaccuracy, and standard error of the method were also evaluated for a combined sample consisting of both male and female specimens.

Subsequently, a pair of new linear regression formulae was developed from the Spanish sample data to determine if the accuracy of the age estimate is improved through the use of a population-specific model. For the first equation, actual chronological age was entered as the dependent variable while the product of the two morphological parameters (AB), as well as the sex of the individual, was entered as independent variables. For the second equation, the same variables were entered into the regression model with the exception of sex, which was excluded from the analysis. The former equation may be useful in forensic situations in which the sex of an individual is known, while the latter may be applicable in cases where sex cannot be reliably determined. The bias, inaccuracy, and standard error of the estimate were then obtained for these sample-specific regression formulae and compared to those previously observed employing the equation devised by Verzeletti and colleagues [19]. The accuracy measures for the two population-specific models were calculated from cross-validated (leave-one-out) age estimates. The leave-one-out technique holds out one specimen at a time, generates a regression model based on the remaining specimens, and then uses this model to provide an age estimate for the held out specimen. This process is repeated until the ages of all specimens in the sample are estimated. The cross-validation procedure, therefore, yields an unbiased estimate of the prediction error for the study sample given that the same data are not used for building the model and evaluating its accuracy.

Another important consideration for this new components method of estimating age-at-death is the ability to reliably assign numerical scores to each individual rib segment. To assess possible observer error (repeatability), numerical scores for articular surface morphology (A), anterior/posterior wall morphology (B), and superior/inferior edge morphology (C) were assigned a second time by the same author (PJM) for 30 randomly selected specimens from the original sample, with a minimum of 1 week between scoring sessions. The difference between the two sets of values (scores), for the morphological variables (A, B, and C), was then analyzed using the Wilcoxon matched-pairs signed-ranks test. Intra-rater reliability was further evaluated using Cohen's weighted kappa statistic, which assesses the proportion of agreement between scoring sessions corrected for chance.

Results

The results of the Wilcoxon matched-pairs signed-ranks test demonstrate that there was no statistically significant intra-observer difference between the paired set of scores for articular surface morphology ($Z=-0.183$, $p=0.855$), anterior/posterior wall morphology ($Z=-1.203$, $p=0.229$), or superior/inferior edge morphology ($Z=-1.069$, $p=0.285$). Likewise, weighted kappa values for the three morphological variables were $k=0.70$ (articular surface), 0.63 (anterior/posterior walls), and 0.72 (superior/inferior edges), indicating substantial agreement between scoring sessions [25]. These combined results suggest that numerical scores can be reliably assigned to the three morphological variables used in this study.

Spearman's rank correlations between age-at-death and both articular surface morphology and anterior/posterior wall morphology were highly significant ($p<0.0001$) in males and females. Correlation coefficients for the male sample were $r_s=0.897$ and $r_s=0.837$ for articular surface morphology (A) and anterior/posterior wall morphology (B), respectively. Similar correlation coefficients were observed for the female sample (A: $r_s=0.783$; B: $r_s=0.880$). The correlation between age-at-death and superior/inferior edge morphology was also significant in males ($p<0.0001$, $r_s=0.593$) and females ($p<0.0001$, $r_s=0.489$); however, these correlation coefficients represent only weak to moderate associations between the variables. These results suggest that the morphological changes associated with this variable will not contribute to the age estimate, as was the case in the original Italian study [19], and thus, this parameter is not considered further in the present investigation.

Table 1 provides a summary of the accuracy of the regression equation of Verzeletti et al. [19] when applied to the Spanish population data. This model provided a slight underestimation of the actual age (−0.61 years) for the combined-sex sample. For males, ages were slightly overestimated using this formula, with a sample bias less than 1 year (0.78 years). However, neither of these bias values are significantly different from zero (males, $p=0.459$; combined, $p=0.473$). Ages for females, on the other hand, were significantly underestimated by nearly 3 years (−2.85 years, $p=0.043$). Although bias was relatively low, particularly for males, inaccuracy or absolute error was roughly six and a half years for all three groups (male, 6.64; female, 6.35; combined, 6.53). As can be seen in Table 1, the regression equation of Verzeletti et al. [19] was most accurate (lowest absolute error) in the youngest age group (18–29) for the various samples. Inaccuracy generally increased until the six (50–59) or seventh (60–69) decade of life and then improved slightly for those below the age of 80 years. The regression formula was least accurate in the oldest cohort (80–89), in which the ages of all specimens were underestimated.

Table 1 Accuracy of the age estimate using the regression equation devised by Verzeletti et al. [19]

Age group (years)	Male sample			Female sample			Combined sample		
	n	Bias	Inaccuracy	n	Bias	Inaccuracy	n	Bias	Inaccuracy
18–29	6	1.93	3.10	4	−0.53	1.27	10	0.94	2.37
30–39	11	4.84	6.75	2	−0.84	0.84	13	3.97	5.84
40–49	11	5.77	6.93	7	−1.64	5.97	18	2.89	6.56
50–59	12	3.86	6.21	7	−4.41	10.76	19	0.80	7.89
60–69	6	−7.43	7.94	6	2.71	4.24	12	−2.36	6.09
70–79	7	−3.70	5.01	6	−4.52	5.59	13	−4.08	5.28
80–89	5	−11.74	11.74	4	−11.39	11.39	9	−11.58	11.58
All ages	58	0.78	6.64	36	−2.85	6.35	94	−0.61	6.53

The linear regression analysis for the Spanish population data, which incorporated sex as an independent variable, yielded the following model:

$$\text{Age} = 1.386(AB) - 2.865(\text{sex}) + 23.681$$

where AB is the product of the scores for the two morphological variables (A and B) and *sex* is a variable equal to 1 for males and 0 for females. The second analysis, which excluded sex as an independent variable, yielded the following linear regression model:

$$\text{Age} = 1.376(AB) + 21.726.$$

The cross-validated accuracy results obtained for these models (Tables 2 and 3) followed a similar pattern to those observed for the regression equation of Verzeletti et al. [19]. The formulae were generally the most accurate (lowest absolute error) when applied to younger individuals (18–29). However, the ages for the majority of these specimens were overestimated, and thus, bias and inaccuracy values for this age group were higher than for the equation of Verzeletti et al. [19]. Again, inaccuracy then increased for the middle decades (40–69), followed by a slight increase in accuracy for both males and females between the ages of 70 and 79 years. All individuals in their ninth decade (80–89) were underestimated, and thus, bias and inaccuracy values were the largest for this cohort. However, the sample-specific equations were less biased for this age group, as well as for the previous category (70–79), than for the equation of Verzeletti et al. [19].

Overall, the two sample-specific regression formulae yielded bias and inaccuracy results comparable to those for the original Italian model (Tables 1, 2, and 3). Surprisingly, the population-specific equation which incorporated sex as an independent variable did not provide more accurate age estimates than the non-sex-specific equation. Inaccuracy or absolute error values were very similar between the two models, particularly for the combined-sex samples (sex-specific, 6.57; non-sex-specific, 6.51). Furthermore, none of the bias values for the two formulae derived from the Spanish population data are significantly different than zero, including those for male (1.11, p=0.289) and female (−1.79, p=0.218) samples for the non-sex-specific equation.

The standard error of the estimate for the regression equation of Verzeletti et al. [19] when applied to the Spanish population data is presented in Table 4. The number of specimens falling within 1 and 2 standard errors of the estimated age, expressed as a percentage of the total number of specimens in the sample, is also provided in the table. The model of Verzeletti et al. [19], which was generated from a sample consisting only of male specimens, is

Table 2 Accuracy of the age estimate using the sex-specific linear regression equation derived from the Spanish data

Age group (years)	Male sample			Female sample			Combined sample		
	n	Bias	Inaccuracy	n	Bias	Inaccuracy	n	Bias	Inaccuracy
18–29	6	4.81	4.93	4	5.24	5.24	10	4.98	5.05
30–39	11	3.49	5.14	2	1.42	1.42	13	3.17	4.57
40–49	11	3.67	5.59	7	−0.39	5.22	18	2.09	5.45
50–59	12	1.97	5.90	7	−3.18	11.15	19	0.08	7.84
60–69	6	−9.80	10.13	6	6.22	7.49	12	−1.79	8.81
70–79	7	−2.52	4.69	6	−0.92	6.09	13	−1.78	5.34
80–89	5	−10.97	10.97	4	−7.62	7.62	9	−9.48	9.48
All ages	58	0.00	6.33	36	0.00	6.95	94	0.00	6.57

Table 3 Accuracy of the age estimate using the non-sex-specific linear regression equation derived from the Spanish data

Age group (years)	Male sample			Female sample			Combined sample		
	n	Bias	Inaccuracy	*n*	Bias	Inaccuracy	*n*	Bias	Inaccuracy
18–29	6	5.77	5.77	4	3.15	3.15	10	4.72	4.72
30–39	11	4.55	5.83	2	−0.53	0.53	13	3.77	5.02
40–49	11	4.72	6.06	7	−2.26	5.72	18	2.01	5.93
50–59	12	3.08	6.26	7	−4.88	11.24	19	0.15	8.09
60–69	6	−8.62	9.35	6	4.42	6.25	12	−2.10	7.80
70–79	7	−1.23	3.88	6	−2.60	5.59	13	−1.87	4.67
80–89	5	−9.62	9.62	4	−9.19	9.19	9	−9.43	9.43
All ages	58	1.11	6.40	36	−1.79	6.68	94	0.00	6.51

unsurprisingly the least accurate for the female sample, with the highest standard error and the fewest individuals within 2 SEE. The standard errors of the estimate, as well as the number of individuals falling within 2 SEE, for the two population-specific formulae were similar, although error values for all three samples (male, female, and combined) were slightly lower for the non-sex-specific model (Table 4). The standard errors for both these formulae were also very similar to those generated from the equation of Verzeletti et al. [19].

Discussion and conclusions

The estimation of chronological age-at-death is an important component in the analysis of unidentified human remains from medicolegal contexts. Several methods for assessing adult age from the dentition [26, 27] and acetabulum [28] have been developed or tested for use in the Spanish population. However, in many forensic situations, these skeletal elements may not be available for analysis. Furthermore, a recent study [23] has demonstrated that the traditional techniques for assessing age-at-death from the pubic symphysis [1] and auricular surface of the ilium [3, 5], which are commonly employed by forensic practitioners in Spain, may not provide highly accurate estimates for this population group. Therefore, there remains a need for the development of adult aging methods with proven accuracy or known accuracy rates for use in the modern Spanish population.

This study tested the accuracy of the new components method for age-at-death estimation from the medial end of the fourth rib, developed by Verzeletti et al. [19], on a sample from Southern Spain. The results demonstrated that numerical scores for articular surface morphology and anterior/posterior wall morphology can be reliably assigned to individual rib specimens. However, the regression equation devised by the abovementioned authors for their Italian male sample was less accurate when applied to the Spanish population sample. This was true for the separate male and female samples, as well as the combined sample which included all specimens.

Therefore, two new regression equations were generated from the Spanish sample data in an attempt to improve the accuracy of the age estimate using the new rib components method. The first model incorporated sex as an independent variable, while for the second model, this variable was excluded. Neither of these population-specific formulae provided a notable increase in accuracy for the Spanish sample

Table 4 Comparison of the standard error of the estimate for regression equations using the new components method

Equation	Reference	SEE	Sample	Accuracy (%)[a]	
				1 SEE	2 SEE
$\text{Age} = 10.43(AB)^{0.53}$	Verzeletti [19]	8.10	Male	69.0	98.2
		8.77	Female	72.2	91.7
		8.27	Combined	70.2	95.7
$\text{Age} = 1.368(AB) - 2.865(\text{sex}) + 23.681$ $(\text{sex} : \text{male} = 1,\ \text{female} = 0)$	Current study	8.13	Male	77.6	96.6
		8.98	Female	75.0	94.4
		8.32	Combined	73.4	95.7
$\text{Age} = 1.376(AB) + 21.726$	Current study	8.06	Male	65.5	98.3
		8.91	Female	83.3	94.4
		8.30	Combined	73.4	96.8

[a] Percentage of individuals in the sample whose actual chronological age fell within ±1 and ±2 standard errors of the estimate

compared to the original Italian equation. Furthermore, the standard errors of the estimate associated with both of these models (8.06–8.91 years) were still appreciably larger than the 5.2 years observed by Verzeletti et al. [19] in their original study. The overall lower accuracy for the Spanish sample, compared to the Italian sample, may be due to populational variation in the rate and degree of age-related metamorphosis of the medial end of the ribs. Conversely, the greater variability observed in the current study, which utilized a larger sample of males as well as females, may more accurately reflect the relationship between morphological changes of the sternal rib end and chronological age present in human populations or may simply be due to chance.

For both population-specific regression formulae, as well as the original Italian equation, chronological age in the Spanish sample was generally overestimated in younger individuals (<60 years) and underestimated in older individuals (>60 years), particularly those in the oldest cohort (80–89). This is in agreement with the results of most other skeletal aging techniques, including methods that rely on morphological changes in the sternal extremity of the ribs [29, 30]. The underestimation recorded for the oldest age group in the current study may be due, in part, to the fact that several individuals in the Spanish sample were above 84 years of age, the maximum age in the original Italian sample, and thus beyond the upper limit of the prediction interval established by Verzeletti et al. [19] for the new components method.

Although the original Italian regression equation generally has lower standard errors of the estimate than the two population-specific formulae, the maximum difference in error values between the three models is only two tenths of a year (2.5 months). In addition, the sample-specific models provide age estimates with a greater number of individuals falling within both 1 and 2 SEE, particularly for the female sample. Furthermore, the two population-specific formulae provide relatively unbiased age estimates for females, while the equation of Verzeletti et al. [19] underestimates the age of this group by roughly 3 years. Therefore, it is suggested that the population-specific regression formulae should be preferred when estimating the chronological age of individuals of Spanish descent. These equations can be used to obtain an age estimate for an individual specimen as well as an *approximate* 95 % confidence interval for the predicted value (±2 SEE).

It is interesting to note, given that female ribs begin to show age-related morphological changes before males [12], that the population-specific regression equation which did not include sex as an independent variable generally provides more accurate age estimates for the Spanish sample than the sex-specific model. The non-sex-specific equation provides slightly lower standard errors of the estimate for male, female, and combined-sex samples, as well as a greater number of individuals falling within 2 SEE. Furthermore, this model may provide a more useful method in most forensic contexts as the sex of the decedent does not have to be assessed prior to analysis, which may add a level of uncertainty to the age estimate.

However, given the high standard errors associated with the age estimates (~8–9 years), the new rib components method should be used in conjunction with other adult aging techniques whenever possible, as suggested previously by numerous researchers [31–34]. Nonetheless, one of the advantages of the method devised by Verzeletti et al. [19] for estimating chronological age-at-death is that the technique is reliable and relatively easy to apply, as no specialized technical equipment or training is needed. In addition, rib specimens are easily acquired during autopsy from well-preserved or partially decomposed human remains. Therefore, the new components method devised by Verzeletti et al. [19], together with the population-specific linear regression models derived in the present study, can be used by forensic anthropologists and pathologists working in Spain to provide a statistical indication of the chronological age-at-death for unidentified adult human remains.

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Conflict of interest The authors declare that they have no conflict of interest.

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