A statistical method for reassociating human tali and calcanei from a commingled context

ABSTRACT: In a commingled context, assessing that a talus and a calcaneus correspond to the same individual could become a primary step for accurately sorting human remains. For this purpose, the lengths and widths of the trochlea, posterior calcaneal articular surface, and posterior talar articular surface were measured in 197 individuals (105 males, 92 females) from the Athens Collection. A total of 12 highly accurate equations for reassociating tali and calcanei were developed, using simple and multiple linear regression analysis and they were found to be suitable for sorting commingled human remains. Bilateral asymmetry and sex did not have an effect on the accuracy of the method.

KEYWORDS: forensic science, forensic anthropology, commingled remains, osteometric sorting, talus, calcaneus

One of the primary objectives of physical anthropology involves the reconstruction of the biological profile of decomposing or skeletonized individuals. In forensic anthropology, this information is fundamental for the identification of unknown human remains. In bioarchaeological contexts, it can provide crucial demographic knowledge on the ancient population under study (1). The applicability of the osteological methods applied for these purposes relies on the fundamental condition that all skeletal elements to be analyzed for each individual correspond to the same skeleton.

The term “commingling” is used to denote the mixing of skeletal remains of different origins within a single osteological context (2). Apart from mixed human remains, it is common to collect animal bones as well. Nevertheless, the term usually refers to skeletal elements which are associated with two or more individuals. Commingled human remains can be recovered from both bioarchaeological and contemporary osteological contexts. In archaeology, they are usually located within mass graves or cemeteries, whereas, in forensic contexts, they are often scattered among other remains of mass disasters.

Nowadays, DNA analysis can provide the most accurate results for sorting commingled human remains. However, there are a number of reasons for which this analysis may not be possible. For example, some degraded bone samples may not be suitable for DNA analysis. This is frequent in moist environments, but other burial conditions may lead to the fragmentation of DNA making its PCR amplification difficult, if not impossible. In addition, there may be issues of DNA contamination. Moreover, certain burial contexts such as mass graves may require a significant input of anthropological techniques, especially in cases of commingling of skeletal remains. Here, anthropological techniques can be used for an initial sorting of the remains in order to speed up the identification process. Finally, the cost associated with DNA analysis may be prohibitive in many countries or projects related to mass graves, where a large number of identifications is required. Therefore, there is still a need for reliable anthropological methods for sorting commingled skeletal remains from a forensic or an archaeological context.

Until recently, the traditionally-used methods for sorting commingled human remains were entirely based on macroscopic observation. Their main criteria involve the morphological compatibility between two associated articular surfaces as well as the presence of similar surface characteristics between two or more skeletal elements (i.e., texture, coloration, density). This similarity is due to various taphonomic or pathological factors which had a similar effect on multiple bones of the same skeleton (2-4). Another criterion used involves the frequent presence of osteoarthritic changes in both articular surfaces of the two bones that articulate (5).

However, the accuracy of these visual techniques may be influenced by subjectivity in estimating the degree of similarity and compatibility between two skeletal elements. Furthermore, their concept relies on the assumption that both bones of the same individual are preserved in the same osteological context. These practices often also require the comparison of compatibility across all possible pairs of bones found in a commingled context, which can be an extremely time-consuming task when multiple specimens are involved. These fundamental limitations could be greatly reduced through the systematic development of more objective methods for sorting commingled human remains. If these techniques present high precision and accuracy, they could substantially increase the level of certainty in associating a series of mixed bone elements with the same individual.

Small and compact human bones are highly resistant to taphonomic change and usually found intact in the field (6). The talus and calcaneus are often recovered undamaged in forensic cases. These bones are compact and highly resistant to taphonomic factors, such as scavenging, while they are additionally protected by footwear (7,8). Furthermore, these two bones are more easily recognizable because of their relatively large size as well as distinctive form. Previous research has shown that, in absence of other widely-utilized skeletal elements (9), these two bones can provide accurate sex and stature estimation (10-14).

Consequently, in cases where an osteological context under study is commingled, assessing that a talus and a calcaneus safely correspond to the same individual could become a useful step for sorting and identifying commingled human remains. On this basis, this study aims at developing a new and more accurate method for reassociating commingled human tali and calcanei.

Materials and Methods

The skeletal material analyzed originates from the human skeletal collection of the National and Kapodistrian University of Athens known as the “Athens Collection” (15). This osteological sample was gathered from cemeteries in the greater area of the city of Athens. It is housed at the Department of Animal and Human Physiology, Faculty of Biology. A total of 197 adult individuals (105 males, 92 females) was included in the study. Their biological age ranged between 22 and 99 years and they lived during approximately the second half of the 20th century. All individuals are documented for their sex, age-at-death, cause of death, occupation, and place of birth. The sample utilized did not include specimens with pathological lesions, taphonomic alterations, as well as antemortem or perimortem skeletal trauma.

The lengths and widths of the trochlea, posterior calcaneal articular surface, and posterior talar articular surface were measured. These measurements follow Martin's definitions as mentioned below and they are illustrated in Fig. 1 (16):

- Talus' trochlear length (TTL): the maximum length of the trochlea at the midline that bisects the articular surface longwise.
- Talus' trochlear width (TTW): the width of the upper trochlear articular surface that bisects the trochlea transversely, vertical to the trochlear length.
- Talus' length of the posterior articular surface for the calcaneus (TAL): the direct distance from the anterior-lateral intersection point of the midline of the posterior articular surface for the calcaneus and the margin of this articular surface to the posterior-medial intersection point of the midline.
- Talus' width of the posterior articular surface for the calcaneus (TAW): the maximum width of the posterior articular surface for the calcaneus vertical to the length of the posterior articular surface for the calcaneus.
- Calcaneus' length of the posterior articular surface for the talus (CAL): the direct distance from the anterior-lateral intersection point of the midline of the posterior articular surface for the talus and the anterior margin of this articular surface to the posterior-medial intersection point of the midline.
- Calcaneus' width of the posterior articular surface for the talus (CAW): the maximum width of the posterior articular surface for the talus vertical to the length of the posterior articular surface for the talus.

Measurements were obtained using a digital sliding caliper (Absolute Digimatic Caliper®, Mitutoyo) of 0.01 mm accuracy and were recorded in millimeters with a calibration of 0.01 mm. After a period of three months, 60 individuals were randomly selected and all their dimensions were measured again by the first author (IA) for the repeatability analysis.

The data obtained were computed and analyzed in the SPSS software package (IBM Inc., version 22 for Windows). The intraobserver error was estimated by performing consecutive paired t-tests between the first and the second measurements. The degree of significant correlation among measurements was calculated using the Pearson's correlation coefficient (r). Simple and multiple regression analyses were performed for the development of mathematical equations for reassociating an individual's talus with its corresponding calcaneus. In all regression analyses, the talar dimensions were used as independent variables. The standard error of the estimate (SEE) was utilized as a measurement of the functions' overall accuracy.

In the simple regression analyses, one of the talus' length measurements (TAL or TTL) was used to predict CAL, while one of the talus' width measurements (TAW or TTW) was used to predict CAW. The two multiple regression equations utilized all talar measurements as predictors for one calcaneal measurement (CAL or CAW).

All analyses were conducted separately for each anatomical side (left and right). Furthermore, it was evaluated whether the functions based on the tali of one anatomical side (left or right) were equally accurate for predicting calcaneal dimensions for the opposite anatomical side. For each function developed, the predicted scores for each anatomical side were compared to the measurements of the opposite one, using consecutive dependent t-tests (17).

The normality of all variables was evaluated using a Kolmogorov-Smirnov test, while homoscedasticity was verified through the use of scatter plots (17,18). The presence of significantly influential points was tested using Cook's distances (17). For detecting multicollinearity, the variance inflation factors were utilized (18).

Finally, an artificially commingled assemblage of tali and calcanei from both anatomical sides belonging to 20 individuals were compiled, in order to evaluate the introduced methods' accuracy in a real-case simulation. The individuals utilized for the blind test were of known sex and age-at-death and did not belong to Athens Collection. Nevertheless, they originate from a cemetery population comprised of individuals who lived in Athens during the second half of the 20th century. All available tali were measured and the two multiple regression formulae were used to predict the calcaneal measurements for each anatomical side. The possible matches for each talus according to the predicted value calculated and the maximum range of error were further examined by the first author (IA) for the purpose of segregating the final match. This process involved an evaluation of morphological compatibility between the associated articular surfaces, as well as an evaluation of color, texture and pathological lesions of the bones. The correct classification rate was calculated as the number of all correct predictions divided by the number of individuals used in the blind test.

Results

Table 1 presents the descriptive statistics (mean, standard deviation, and range) for each measurement utilized, for left and right tali and calcanei separately. Intraobserver repeatability analysis found the differences between the two sets of measurements to be small and not statistically significant (p–value>0.05).

The regression formulae developed, along with r, r^2, and SEE values are summarized in Tables 2 and 3. The resulting equations show very strong correlations, as Pearson's correlation coefficients (r) were found to be between 0.69 and 0.93 (p-value <0.05). All variables were found to be normally distributed. Scatter plots confirmed the presence of homoscedasticity in all regression analyses performed (17). As far as the two multiple regression equations are concerned, no multicollinearity issues were observed among variables (18). The Cook's distances for all specimens were below 1.00, showing that there were no significantly influential points (17).

Regarding the simple regression analyses, the correlation between the dimensions of the talar posterior articular surface (TAL and TAW) and their corresponding calcaneal ones (CAL and CAW) was stronger than the association between trochlear (TTL and TTW) and calcaneal measurements (CAL and CAW). As a consequence, both formulae based on the talar posterior articular surface showed higher accuracy (SEE=1.06–1.25) for reassociating the pairs. The multiple regression equations also presented high multiple correlation coefficients (r=0.85–0.93) and low SEE (SEE=1.03–1.20). For further demonstrating the accuracy of the present method, Tables 2 and 3 show the percent ratio of individuals with residuals of 1.00 mm or less and 2.00 mm or less. Overall, the higher accuracy rates were presented by the multiple regression formulae (Table 3), whereas the least accurate functions were the ones who used trochlear measurements as predictors.

For future application of each function for reassociating tali and calcanei in a set of commingled human remains, the measurements of each talus must be multiplied by their corresponding coefficients. Then, the resulting products should be summed up and added to the constant. The outcome of this calculation is the predicted calcaneal dimension for that talus. If one of the commingled calcanei presents approximately the same value, then it probably belongs to the same individual.

All twelve equations showed highly similar SEE between males and females, with the difference ranging between 0.04 and 0.34 mm. Concerning the applicability of the functions on bones of different anatomical sides, the results of the paired t-tests showed that the predicted scores of each side were significantly similar to the observed scores of the opposite side (p-value > 0.05). This suggests that the measurements of right tali can potentially be used for predicting the dimensions of left calcanei, and vice versa.

Based on the results of the blind test, left tali and calcanei were correctly reassociated in 17 out of the 19 cases (89.5%). Similarly, 16 of the 18 (88.9%) right tali were accurately reassociated with their corresponding calcanei.

Discussion

The statistical method presented in this study comprises a practical, accurate, and less expensive way of sorting commingled human remains. Its accuracy rates demonstrate that osteometric reassociation of the subtalar joint is possible, especially when the multiple predictor variables are used. Sex did not have a significant effect on the method's accuracy, as the two sexes presented very similar error rates. Furthermore, the results demonstrated that bone dimensions of one anatomical side can be used to predict the bone dimensions of the opposite side. Therefore, the formulae proposed in this study are applicable on mixed-sex samples comprising specimens of both anatomical sides. On this basis, the introduced method could potentially be used for sorting tali and calcanei of different anatomical sides.

There is a number of studies concerning commingling, which are summarized in Ubelaker (19) and Byrd (20). Nevertheless, the use of statistical methods for sorting human skeletal remains has drawn relatively limited scientific attention, in spite of the promising results. Buikstra et al. (21) proposed a metric method for estimating the likelihood that two isolated vertebrae belong to the same individual, which was successfully applied in two forensic cases with possible commingling. London et al. (22,23) found a significant correlation between the femoral head and the acetabulum, proposing that osteometric sorting of the hip joint is possible when supported by visual reassociation.

By contrast, Rösing and Pischtschan (24) concluded that osteometric sorting has limited contribution to commingling. They utilized long bone and cranial measurements to develop regression models. As discussed in Byrd and Adams (25), their poor results may be mainly attributed to small sample size as well as various methodological issues.

Byrd and Adams (25) developed several regression models and introduced a new method of sorting based on several long bone measurements. The obtained metric data were summed up and converted into a single natural logarithm, which was used to predict the dependent variable. The same methodology was presented by Byrd (20) and Byrd and LeGarde (26). They used several statistical models to compare different bones, paired elements and articulating bones. Their results showed that, when bone lengths were used, the correlation coefficients of the regression models were high. Nevertheless, in commingled contexts, long bones are often not found intact and their lengths cannot be accurately measured. Additionally, these authors recommended that, for each forensic case, experts should develop new statistical models using the available specimens of a reference skeletal collection. However, this proposition does not take into account that the prediction accuracy of regression methods for reassociating human remains is not yet verified for most bones of the human skeleton.

Rodríguez et al. (27) successfully applied Byrd's method to a small artificially commingled assemblage based on multiple measurements of nine skeletal elements. However, the small sample size, ranging between two and four individuals for each test performed, suggests that the results should be interpreted with caution.

Despite the many advantages, statistical reassociation of human remains should be used with caution when the commingled individuals are of similar size. If the metric difference between two calcanei is less than the error ranges of the developed regression functions, the metric method is not applicable as a stand-alone technique. In these cases, as demonstrated by the blind test, the combined application of both metric and macroscopic techniques can provide a rather accurate assessment. Therefore, in cases where individuals of very similar size are examined, the final match should be confirmed by visually evaluating the morphological compatibility between the associated articular surfaces.

A recent study has reported that linear measurements provide a more accurate sorting of human skeletal remains compared to the application of three-dimensional geometric morphometric techniques (28). Nevertheless, future research could further evaluate the applicability of these methods by developing a precise landmark-based technique for reassociating two bones with adjoining articular surfaces. Such an approach could retrieve accurate shape information for each articulation by incorporating the use of semilandmarks on the three-dimensional areas of two associated articular surfaces (29).

The development of sorting metric techniques is considered as an on-going project. Future research should continue to examine metric techniques for sorting bones of the human skeleton. The present study produced promising results regarding the subtalar (or "talocalcaneal") articulation. In the future, if similar metric methods are developed for other large diarthroses such as the ankle, the knee, the elbow and the shoulder, statistical association may become a standard tool for accurately sorting human remains.

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TABLE 1–*Descriptive statistics.*

Side	Measurement	N	Range	Mean		Std. Deviation
				Statistic	Std. Error	
Right	TTL	181	15.68	33.7520	0.23058	3.10220
	TTW	181	11.52	31.0310	0.18942	2.54839
	TAL	181	13.08	33.4587	0.21765	2.92817
	TAW	182	9.30	21.8397	0.14713	1.98484
	CAW	187	9.43	22.1544	0.14390	1.96783
	CAL	187	14.91	29.9327	0.20501	2.80344
Left	TTL	179	14.75	33.5605	0.22297	2.98310
	TTW	179	12.12	30.8981	0.19237	2.57369
	TAL	180	13.75	33.3385	0.22692	3.04446
	TAW	181	9.87	21.7235	0.15215	2.04703
	CAW	187	8.95	21.9525	0.14749	2.01687
	CAL	187	13.19	29.8181	0.20797	2.84388

TABLE 2–*Simple regression models.*

				Correct sorting	
	SEE	r	r^2	error 0-1mm	error 0-2mm
Right					
CAL=0.883*TAL+0.375	1.06	0.93	0.86	68.57%	94.86%
CAL=0.625*TTL+8.848	2.02	0.69	0.48	42.53%	71.26%
CAW=0.832*TAW+3.936	1.06	0.85	0.7	69.71%	94.86%
CAW=0.565*TTW+4.579	1.34	0.76	0.54	60.92%	85.63%
Left					
CAL=0.850*TAL+1.482	1.25	0.9	0.81	65.29%	90.59%
CAL=0.673*TTL+7.186	1.99	0.71	0.5	43.02%	71.51%
CAW=0.832*TAW+3.824	1.11	0.83	0.71	67.82%	91.38%
CAW=0.573*TTW+4.185	1.35	0.75	0.55	58.72%	89.53%

TABLE 3–*Multiple regression models*.

	SEE	r	r^2	Correct sorting error 0-1mm	Correct sorting error 0-2mm
Right					
CAL=0.021*TTL+0.077*TTW+0.730*TAL+0.141*TAW-0.684	1.03	0.93	0.87	71.84%	95.4%
CAW=0.038*TTL+0.135*TTW-0.050*TAL+0.711*TAW+2.780	1.05	0.85	0.73	69.54%	95.98%
Left					
CAL=0.079*TTL-0.010*TTW+0.703*TAL+0.175*TAW+0.204	1.20	0.91	0.82	67.44%	91.86%
CAW=0.102*TTL+0.058*TTW+0.066*TAL+0.575*TAW+1.977	1.08	0.85	0.72	66.86%	93.6%

Figure Legend

FIG. 1–*Measurements taken (TTL: Talus' trochlear length, TTW: Talus' trochlear width, TAL: Talus' length of the posterior articular surface for the calcaneus, TAW: Talus' width of the posterior articular surface for the calcaneus, CAL: Calcaneus' length of the posterior articular surface for the talus, CAW: Calcaneus' width of the posterior articular surface for the talus).*