BRIEF COMMUNICATION: SACRAL FUSION AS AN AID IN AGE ESTIMATION

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The degree of fusion at the anterior aspect of the sacral vertebrae has been scored in 242 male and female skeletons from the Lisbon documented collection, ranging in age from 16 to 59 years old. Statistical tests indicate a sex difference towards earlier fusion in young females compared with young males, as well as a clear association between degree of fusion and age. Similar results have been found in documented skeletal samples from Coimbra and Sassari, and the recommendations stated by these authors regarding age estimation have been positively tested in the Lisbon collection. Although more research from geographically diverse samples is required, a general picture of the pattern of sacral fusion and its associations with age and sex is emerging. We also provide a practical example of the usefulness of the sacrum in age estimation in a forensic setting, a mass grave from the Spanish Civil War. It is concluded that the scoring of the degree of fusion of the sacral vertebrae, specially of S1-2, can be a simple tool for assigning skeletons to broad age groups, and it should be implemented as another resource for age estimation in the study of human skeletal remains.
INTRODUCTION

The estimation of age at death of adult human skeletal remains is important for both Anthropology and Forensic Medicine and constitutes one of the main problems to be solved in these disciplines (Ritz-Timme et al., 2000; Rösing et al., 2007). In those skeletons with complete fusion of the epiphyses of limb long bones, if possible, the preferred method for age estimation is the observation and scoring of bone elements that still can show epiphyseal activity during the third decade of life. This preference is due to the narrower age ranges associated to maturation events in comparison with methods based on the multifactorial process of adult bone ageing (Schmitt et al., 2002). Among the elements which show epiphyseal activity during the third decade of life are the clavicle (Black y Scheuer, 1996; Schulze et al., 2006), vertebrae (Albert, 1998), ribs (Kunos et al., 1998), and iliac crest (Webb and Suchey, 1985). The sacrum can be included in this list, specifically the state of fusion of the anterior aspect of the sacral vertebrae, although few studies have focused in this bone since McKern and Stewart’s original paper (1957). Recently, Belcastro et al. (2007) reported the state of fusion of the sacral vertebrae in two documented collections (Sassari and Coimbra) widening the age range of the military sample from McKern and Stewart (1957), and including a female sample. Also in a recent study (Ríos et al., 2007), the present authors scored the state of fusion of the sacral vertebrae of female and male skeletons in the documented Lisbon collection with the objective of verify the utility of this bone as an aid in adult age estimation in a forensic context. Results from both research groups are similar and indicate the utility of the sacrum as a help for adult age estimation. We report here the results from the Lisbon sample, compare our results with that of Belcastro et al. (2007), and subsequently apply the recommendations to a case study, a mass grave from the Spanish Civil War.
MATERIAL AND METHODS
The osteological material comes from the documented skeletal Collection housed at the National Museum of Natural History in Lisbon (Portugal), and is mainly comprised by individuals buried between late 19th and early 20th centuries (Cardoso, 2006). Female (100) and male (142) individuals between 15 to 59 years of age were selected. The lumbo-sacral transition was examined in every case, and those with variants or anomalies were excluded for the study. Sex and age distribution are shown in tables 1 and 2.

In order to evaluate the application of sacral fusion variables in practical case of study 21 males from a mass grave of the Spanish Civil War (1936-1939) were used. This grave was exhumed with the objective of identification and return of the remains to the relatives who requested the exhumation. You can fin the age distribution in table…

Description of Variables.
We followed the methodology used by McKern and Stewart (1957) to score the state of fusion of $S_{1-2}$, $S_{2-3}$, $S_{3-4}$, and $S_{4-5}$. The description of the stages is as follows: 0, not fused; 1, fusion has started, less than 1/3 of the segment; 2, fusion in approximately ½ of the segment; 3, fusion more than 2/3 of the segment, but it is not completely fused; 4, completely fused. Belcastro et al. (2007) calculated for each sacrum in their sample a pattern of fusion defined by a sequence of four numbers corresponding to the cranio-caudal series of degrees of fusion between pairs of adjacent vertebrae (for example the pattern “1233” indicates $S_{1-2}=1$, $S_{2-3}=2$, $S_{3-4}=3$, $S_{4-5}=4$), as well as three fusion scores: total score $[\text{total} = (S_{1-2} + S_{2-3} + S_{3-4} + S_{4-5})/4]$, upper score $[\text{upper} = (S_{1-2} + S_{2-3})/2]$, and lower score $[\text{lower} = (S_{3-4} + S_{4-5})/2]$. We calculated the three fusion scores for each sacra from our sample. We calculated only the pattern x444, called type “a”, because according to Belcastro et al. (2007) it is the most informative for age estimation.

Two age variables were used: AGE1, a 5 year interval grouping, and AGE2, the broad age groups defined by Belcastro et al. (2007) plus an additional subadult age group: SB (subadults, <20 years old), YA (young adults, 20 to 34 years old), MA (middle adults, 35 to 49 years old), OA (older adults, over 50 years old). The first variable provides detailed information on the variability of sacral fusion in relation to age while the second one is commonly used in bioarchaeology studies (Buikstra and Ubelaker, 1994).
Statistics
Sexual differences for the degrees of sacral fusion were analysed inside every age group defined by the variables AGE1 and AGE2 by the Chi square, Mann-Whitney and T-test for independent samples. To look for an association of the degree of fusion of the sacral vertebrae with age, the Chi square test and Spearman correlation were applied. All the statistics were calculated with SPSS 14.0.

**Application of sacral fusion variables in practical case**
The variables of the sacrum: state of fusion, pattern of fusion “a” and the three scores of fusion were evaluated in each individual of the skeletons exhumed from a mass grave from the Spanish Civil War in order to see their utility in the age estimation. This grave was exhumed with the objective of identification and return of the remains to the relatives who requested the exhumation. 46 skeletons were found in the same ditch but spatially separated in two groups of 21 and 25 skeletons. The identification process focused on the first group of 21 skeletons due to compatibility of testimonies, archaeological findings and documentary evidence from the penitentiary archives. The findings from the osteological study supported the identification of the group, DNA tests were requested and confirmed the identities of eight skeletons (Ríos, in preparation).

RESULTS
For sake of clarity results will be described in three parts refering to: (1) sex and age associations with degree of sacral fusion; (2) sex and age associations with pattern and scores of sacral fusion; (3) test of the utility of the sacrum for age estimation.

**Sex and age associations with degree of fusion of pairs of adjacent vertebral bodies.**
The degree of fusion of the sacral vertebrae for each sex is shown in table 1 for AGE1 and in table 2 for AGE2. Although we illustrate the degree of fusion for both age variables, for a clearer presentation of the results we will focus our comments on significant differences for AGE2 except when indicated. Chi square and Mann-Whitney tests indicate the presence of significant sex differences in the degree of sacral fusion at S1-2 for the YA group (table 3), and the Mann-Whitney test also indicates a significant
sex difference in the degree of sacral fusion at S4-5 for the YA group. The oldest individual with degree 0 at S1-2 for the female sample is 25 years old, and the oldest male cases presented the following ages: 30, 31, 34, 34 and 39 years old. This sex difference can be also seen at the SB age group (<20 years old) at every sacral location: the female/male percentages of degree 4 at S1-2, S2-3, S3-4, S4-5 were 0.0/0.0, 37.5/18.2, 62.5/18.2, 50/27.3.

With regard to the association with age, the Chi square test (Table 4) indicates that the degree of fusion at the four sacral locations is not independent from AGE2, and this conclusion is also supported by the Spearman correlation (Table 5), which indicates a clear association between the degree of fusion at the four sacral locations and the two age variables AGE1 and AGE2.

Sex and age associations with pattern and scores of sacral fusion.

We will focus our attention in the type “a” pattern or x444, that includes the following degrees of fusion: S1-2= 0 to 4, S2-3=4, S3-4=4, S4-5=4. In table 6 we can see the distribution of the type “a” pattern according to AGE2. The Chi square and Mann-Whitney tests fail to find a significant sex difference for the distribution of type “a” pattern in any age group (Tables 7 and 8), although for the YA group the p values for both tests reach almost significant values (0.058 and 0.087 respectively for Chi square and Mann-Whitney). On the other hand, in table 8 we can observe that the Spearman correlations indicate a clear association between type “a” patterns and AGE1 and AGE2 for both sexes.

With regard to the total, upper and lower scores defined by Belcastro et al. (2007), table 9 shows the mean values for these scores. It can be observed that for the SB and YA groups the mean scores for the female sample are higher than for the male samples, while for the MA and OA groups the mean scores are very similar. For the YA group the T-test for independent samples indicates that this difference is statistically significant for the total and upper scores. The Spearman correlations for the scores and the two age variables AGE1 and AGE2 (table 10), indicate that there is a significant association between age and the sacral fusion scores except for the lower score for the female sample. For the male sample, although significant, the lowest values of the Spearman coefficients also belong to the lower score.
Application of sacral fusion variables in practical case

As explained previously, there is ample evidence that the 21 skeletons exhumed correspond to the 21 men looked for at the location where the grave was found. The exact ages at death of the 21 men were obtained crossing testimonies and archival information (birth certificates and individual military and penitentiary files). The age distribution was as follows: 18, 20, 21, 22, 23, 24, 25, 26, 27, 36, 42, 42, 47, 48, 50, 50, 54, 55, 61. During the osteological study, the first step was to establish the compatibility of the sex and age profile estimated from the skeletons with the sex and age profile from testimonies and archival information. During the skeletal study the degree of fusion of the anterior aspect of the sacral vertebrae was recorded when available. Table 7 shows the state of fusion of the available sacral vertebrae for those skeletons whose age was estimated under 30 years old (all with active fusion at the clavicle except Nº4 and Nº8, both with a line of recent fusion at the clavicle). Table 8 shows the state of fusion of the available sacral vertebrae for the skeletons whose age was estimated over 30 years old due to complete epiphyseal fusion of all the preserved bones and by observation of pubic symphisis, auricular surface and sternal end of the fourth rib. If we apply patter of fusion “a” and the three scores of fusion, the skeletons Nº 2, 4, 7 (doubtful due to state of preservation), 8, 10, 14, 15 and 20 from table 11 would be assigned to the YA age class, where other maturity indicators (long bones, pelvis, sternum, vertebrae, clavicle) had already placed them. Skeletons Nº5 and 6 would be the only ones from table 12 that could be included in the YA category. Skeleton Nº5, with a Klippel-Feil syndrome at the level of the first and second cervical vertebrae, presents a degree of fusion 0 and skeleton Nº6 presents a degree of fusion 1 at S1-2.

DISCUSSION

As confirmed for most skeletal elements and different stages of the ontogeny (CITA), a significant sex difference towards an earlier maturation of the female sample is confirmed in the YA group (20 to 34 years) for degree of fusion of the sacral vertebrae, fusion scores (total, upper and lower), and almost significant in the frequencies of x444 patterns (TABLES Nº, Nº, Nº respectively). The association of sacral fusion with age is statistically confirmed for degree of fusion, frequencies of x444 patterns and the total
and upper fusion scores (TABLES Nº, Nº, Nº respectively). These results from the Lisbon sample are similar to those found by Belcastro et al. (2007) for the Sassari and Coimbra samples regarding sex differences and age associations. Bearing in mind that our scoring system is slightly different from theirs, and this precludes a more detailed comparison, we conclude that most of our results are similar to theirs with some exceptions, which are related with the intra and inter-populational variability. For degree of fusion of the sacral vertebrae these authors also reported for their female samples a statistically significant higher frequency of degree 3 in the YA class, and for their male samples a higher frequency of degree 0 in the YA group, as well as a statistically significant association of the degree of fusion with age. But it is interesting to note that at S1-2, absence of fusion (degree 0) is present in females until 29 years in Lisboa and Coimbra, whereas is present until 54 years in Sassari. In males until 39 years in Lisboa, 34 years in Sassari and 29 years in Coimbra, showing a big variability inter and intra-population.

For the fusion scores (total, upper and lower) Belcastro et al. (2007) found higher scores for the female sacra than for the male sacra in the YA group, and stated that “the correlation between mean score and relative age class is equal or very similar in the entire sacrum and the upper part while it is low in the lower part (albeit always significant, except for the lower score for Coimbra males and females)” (CITA). Finally, for the pattern of fusion our results are in agreement with the findings of Belcastro et al. (2007), who found a significant increase in the frequency of pattern 3333 (equal to pattern 4444 in our Lisbon sample) with age in both sexes and both collections (Sassari and Coimbra), and only found a significant sex difference for the Sassari sample, where patterns 2333 and 3333 were significantly more frequent in the YA females.

Similar results from three different documented collections indicate us that a clear picture of sex differences and age associations for sacral fusion is emerging, although it would be interesting to sample other geographically diverse documented collections.

Similar results are observed by Belcastro et al. (2007), who reported. Again, and always bearing in mind that our scoring system is slightly different from theirs, similar results have been observed by Belcastro et al. (2007), who found higher scores for the
female sacra than for the male sacra, and stated that “the correlation between mean score and relative age class is equal or very similar in the entire sacrum and the upper part while it is low in the lower part (albeit always significant, except for the lower score for Coimbra males and females)”.

It is also important to note that some differences between the Lisbon and Sassari and Coimbra samples have been observed, although the different scoring systems preclude more detailed comparisons. For instance at S1-2, absence of fusion (degree 0) is present until 29 years for females and until 39 years for males in Lisboa, whereas is present until 54 years and 29 years in Sassari and Coimbra females respectively, and until 34 years and 29 years in Sassari and Coimbra males respectively, and we would recommend other researchers to follow the Belcastro et al. (2007) methodology for scoring the degree of fusion of the sacral vertebrae for his simplicity and because the interobserver error has already been tested (in ongoing research projects at the Lisbon collection we plan to rescore our sample).

With regard to the utility for age estimation in adult skeletal remains, the forensic case study verifies that the degree of fusion of the sacral vertebrae can be helpful in assigning skeletons to broad age groups (young, middle and older adults) in an specific context where the comparison of the skeletal age profile with the documented age distribution is a key step in the identification of groups of skeletons exhumed from mass grave contexts.

DISCUITIR which is also present at the MA age category in the three studied male samples: Sassari 6.9%, Coimbra 23.4%, Lisbon 1.8%.

For instance, if we knew from ante mortem information that the group we are looking for is totally composed of over 40 years old men (in fact any age distribution with a minimum of 14 men over 40 years old would be incompatible), the age profile estimated from the degree of fusion of the sacral vertebrae would indicate us that probably we have not found the grave we were looking for. This usefulness obviously extends to paleodemographic studies in archeological series. We have to conclude that more research in diverse geographic samples is desirable, and that the scoring of the degree of fusion of the sacral vertebrae should be implemented as a simple and informative tool in the study of age estimation in human skeletal remains.


