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Sacral fusion as an aid in age estimation

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ABSTRACT

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 from 16 to 59 years old in age. 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 by other authors 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 for 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 S_{1-2}, 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.

1. Introduction

The estimation of age-at-death of adult skeletons from both archaeological and forensic contexts constitutes a fundamental problem to be solved by skeletal biologists [1,2]. The age of subadult skeletons may be determined with a low degree of error due to the narrow age ranges associated to dental and skeletal maturation events. In adult skeletons with complete fusion of the epiphyses of the long bones, the preferred method for age estimation is the observation and scoring of bone elements that continue to show epiphyseal activity during the third decade of life (clavicle [3,4], vertebrae [5], ribs [6], iliac crest [7], sacrum [8]). In completely mature skeletons, the degenerative processes that characterize adult age estimation display broader age ranges and greater variation between individuals [9]. The sacrum, specifically the state of fusion of the anterior aspect of the sacral vertebrae, has been used for age estimation in young adults, although few studies have focused on this bone since McKern and Stewart's original paper [8]. Recently, Belcastro et al. [10], reported the state of fusion of the sacral vertebrae in two documented collections (Sassari and Coimbra). This study widened the age range of the military sample from McKern and Stewart [9], and included a female sample. Also in a recent study [11], 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 verifying 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 for adult age estimation. The results from the Lisbon sample are presented here along with an application of the current results to a case study; a mass grave from the Spanish Civil War.

2. 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 of individuals that died between late 19th and early 20th centuries [12]. Female (100) and male (142) individuals between 15 and 59 years of age were selected. The lumbo-sacral transition was examined in every case, and those individuals with variants or anomalies were excluded from the study. Sex and age distribution are shown in Tables 1 and 2. In order to test the results from the Lisbon sample, and from Belcastro et al. [10], the degree of sacral fusion of 21 skeletons exhumed from a mass grave is discussed. Details of the mass grave are given below and in Ríos [13].

2.1. Description of variables

The scoring method used by McKern and Stewart [9] was used 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 1/2 of the segment; 3, fusion more than 2/3 of the segment, but it is not completely fused; 4, completely fused. Following Belcastro et al. [10], the frequencies of the pattern of fusion was calculated. For example, an individual with a pattern x444

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Table 1
Percentages of degree of fusion at every sacral location by sex and AGE1

AGE	FEM						MAL					
	N	0	1	2	3	4	*N*	0	1	2	3	4
S_{1-2}												
<20	8	100	0.0	0.0	0.0	0.0	11	90.9	9.1	0.0	0.0	0.0
20–24	13	23.1	23.1	15.4	15.4	23.1	15	73.3	6.7	6.7	6.7	6.7
25–29	13	15.4	15.4	7.7	30.8	30.8	7	28.6	0.0	28.6	28.6	14.3
30–34	9	0.0	11.1	11.1	22.2	55.6	12	25	0.0	16.7	16.7	41.7
35–39	5	0.0	0.0	40	60	0.0	10	20	0.0	0.0	10	70
40–44	6	0.0	0.0	16.7	16.7	66.7	16	0.0	0.0	6.3	18.8	75
45–49	9	0.0	0.0	0.0	11.1	88.9	30	0.0	3.3	0.0	26.7	70
50–54	27	0.0	0.0	0.0	7.4	92.6	32	0.0	0.0	0.0	9.4	90.6
55–59	22	0.0	0.0	0.0	9.1	90	25	0.0	0.0	0.0	8	92
S_{2-3}												
<20	8	37.5	12.5	12.5	0.0	37.5	11	72.7	9.1	0.0	0.0	18.2
20–24	13	0.0	7.7	0.0	0.0	92.3	15	13.3	0.0	6.7	0.0	80
25–29	13	0.0	0.0	0.0	0.0	100	7	0.0	0.0	0.0	0.0	100
30–34	9	0.0	0.0	0.0	0.0	100	12	0.0	0.0	0.0	0.0	100
35–39	5	0.0	0.0	0.0	0.0	100	10	0.0	0.0	0.0	0.0	100
40–44	6	0.0	0.0	0.0	0.0	100	16	0.0	0.0	0.0	0.0	100
45–49	9	0.0	0.0	0.0	0.0	100	30	0.0	0.0	0.0	0.0	100
50–54	27	0.0	0.0	0.0	0.0	100	32	0.0	0.0	0.0	0.0	100
55–59	22	0.0	0.0	0.0	0.0	100	25	0.0	0.0	0.0	0.0	100
S_{3-4}												
<20	8	25	12.5	0.0	0.0	62.5	11	54.5	9.1	18.2	0.0	18.2
20–24	13	0.0	0.0	7.7	0.0	92.3	15	13.3	0.0	0.0	6.7	80
25–29	13	0.0	0.0	0.0	0.0	100	7	0.0	0.0	0.0	0.0	100
30–34	9	0.0	0.0	0.0	0.0	100	12	0.0	0.0	0.0	0.0	100
35–39	5	0.0	20	0.0	0.0	80	10	0.0	0.0	0.0	10	90
40–44	6	0.0	0.0	0.0	0.0	100	16	0.0	0.0	0.0	0.0	100
45–49	9	0.0	0.0	0.0	0.0	100	32	0.0	0.0	0.0	0.0	100
50–54	27	0.0	0.0	0.0	0.0	100	33	0.0	0.0	0.0	0.0	100
55–59	22	0.0	0.0	0.0	0.0	100	25	0.0	0.0	0.0	0.0	100
S_{4-5}												
<20	7	12.5	12.5	0.0	12.5	50	11	72.7	0.0	0.0	0.0	27.3
20–24	13	0.0	0.0	0.0	0.0	100	15	13.3	13.3	0.0	0.0	73.3
25–29	13	0.0	0.0	0.0	0.0	100	7	0.0	0.0	0.0	0.0	100
30–34	9	0.0	0.0	0.0	0.0	100	12	0.0	0.0	0.0	0.0	100
35–39	5	0.0	0.0	0.0	20	80	10	0.0	0.0	0.0	0.0	100
40–44	6	0.0	0.0	0.0	0.0	100	16	0.0	0.0	0.0	0.0	100
45–49	9	0.0	0.0	0.0	0.0	100	32	0.0	0.0	0.0	0.0	100
50–54	27	0.0	0.0	0.0	0.0	100	33	0.0	0.0	0.0	0.0	100
55–59	22	0.0	0.0	0.0	0.0	100	25	0.0	0.0	0.0	0.0	100

would correspond to the cranio-caudal series of degrees of fusion between pairs of adjacent vertebrae (S_{1-2} = 0–4, S_{2-3} = 4, S_{3-4} = 4, S_{4-5} = 4). Two age variables were used, AGE1, a 5-year interval grouping, and AGE2, the broad age groups defined by Belcastro et al. [10] with an additional subadult age group: SB (subadults, <20 years old), YA (young adults, 20–34 years old), MA (middle adults, 35–49 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 bioarchaeological studies [14].

2.2. Statistics

Chi-square and Mann–Whitney tests were used to analyze sex differences for the degree of sacral fusion within every age group defined by the variables AGE1 and AGE2. The Chi-square is a statistical test used to examine whether two categorical variables are independent or not, in this case sex and degree of fusion. The Mann–Whitney is used to test whether two independent samples that are defined by a grouping variable (sex) come from the same population, and it is used when the assumption of normality or equality of variance is not met or when the data are not quantitative but they follow an ordinal scale. To test for an association of the degree of fusion of the sacral vertebrae with age, the Chi-square test and the non-parametric Spearman's correlation coefficient were applied. All the statistics were calculated with SPSS 14.0.

An unrestricted cumulative probit analysis, commonly referred to as transition analysis, was also performed in order to determine the mean age at transition for each phase of the S_{1-2} fusion for males and females. For a complete discussion of transition analysis, see Boldsen et al. [15] and Steadman et al. [16]. Probability density function (pdf) plots were created in "R". Transition analysis was performed only for the S_{1-2} segment because frequencies obtained in the other segments in both sexes did not show enough variation in the age at transition. A sample with younger age ranges would be necessary in order to perform such an analysis.

2.3. Test of accuraccy of the sacrum in age prediction

Two tests were developed to evaluate the utility of the sacrum for age estimation. First, we applied the recommendations of Belcastro et al. [10] to our Lisbon sample. It is important to state that the scoring system used by these authors differs slightly from that used in the present study. Their system is as follows: 0, non-fused; 1, less than 50% fusion; 2, more than 50% fusion; 3, complete fusion (also if a body fusion "scar" is still visible). So, in order to compare their results, we use the equivalences described in Table 3. In this regard, it is necessary to state that only 17 sacra at the Lisbon collection presented a McKern and Stewart's score 2 at any site, and therefore the possible error assumed with the equivalence to score 1 of Belcastro et al. [10] is limited. Their first recommendation states that "with Degree 0 or 1 at any site the specimen can reliably be attributed to the YA age class". The second recommendation states that "patterns 'b' (xx33, xxx3), 'c' (xxxx), and 0333 are most frequent in the YA age class".

Second, we apply the recommendations that emerge from the present work and Belcastro et al. [10] results to the skeletons exhumed from a mass grave from the Spanish Civil War. This grave was exhumed with the objective of identification and return of the remains to the relatives who requested the exhumation. In the same grave 46 skeletons were found, spatially separated into 2 groups of 21 and 25 skeletons. The identification process initially focused on the group of 21 skeletons due to compatibility of testimonies regarding grave location and number of men disappeared. Archaeological findings and documentary evidence from the prison archives, which include an official document of 21 men released from jail and dissapeared the same date were used to narrow the focus of the investigation. The

Table 2
Percentages of degree of fusion at every sacral location by sex and AGE2

AGE	FEM						MAL					
	N	0	1	2	3	4	*N*	0	1	2	3	4
S_{1-2}												
SB	8	100	0.0	0.0	0.0	0.0	11	90.9	9.1	0.0	0.0	0.0
YA	35	14.3	17.1	11.4	22.9	34.3	34	47.1	2.9	14.7	14.7	20.6
MA	20	0.0	0.0	15	25	60	56	3.6	1.8	1.8	21.4	71.4
OA	49	0.0	0.0	0.0	8.2	91.8	55	0.0	0.0	0.0	9.1	90.9
S_{2-3}												
SB	8	37.5	12.5	12.5	0.0	37.5	11	72.7	9.1	0.0	0.0	18.2
YA	35	0.0	2.9	0.0	0.0	97.1	34	5.9	0.0	2.9	0.0	91.2
MA	20	0.0	0.0	0.0	0.0	100	56	0.0	0.0	0.0	0.0	100
OA	49	0.0	0.0	0.0	0.0	100	55	0.0	0.0	0.0	0.0	100
S_{3-4}												
SB	8	25	12.5	0.0	0.0	62.5	11	54.5	9.1	18.2	0.0	18.2
YA	35	0.0	0.0	2.9	0.0	97.1	34	5.9	0.0	0.0	2.9	91.2
MA	20	0.0	0.0	0.0	5	95	56	0.0	0.0	0.0	1.8	98.2
OA	49	0.0	0.0	0.0	0.0	100	55	0.0	0.0	0.0	0.0	100
S_{4-5}												
SB	8	12.5	12.5	0.0	12.5	50	11	72.7	0.0	0.0	0.0	27.3
YA	35	0.0	0.0	0.0	0.0	100	34	5.9	5.9	0.0	0.0	88.2
MA	20	0.0	0.0	0.0	5	95	56	0.0	0.0	0.0	0.0	100
OA	49	0.0	0.0	0.0	0.0	100	55	0.0	0.0	0.0	0.0	100

Table 3
Proposal for equivalence of degrees of fusion between Belcastro et al. (2008) and the present work

Belcastro et al. (2008)	Present work
0: Absence of fusion.	0: Absence of fusion
1: Less than 50% fusion.	1: Fusion has started, less than 1/3 2: Fusion has started, approximately 1/2
2: More than 50% fusion. 3: Complete fusion.	3: More than 2/3 fused 4: Complete fusion

names on the list from the prison archives corresponded to the people the relatives were looking for. The findings from the osteological study supported the identification of the group, DNA tests were requested and so far, the identities of nine skeletons have been confirmed [13].

3. 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 of sacral fusion; (3) test of the utility of the sacrum for age estimation.

3.1. 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 S_{1-2} for the YA group (Table 4), and the Mann–Whitney test also indicates a significant sex difference in the degree of sacral fusion at S_{4-5} for the YA group. The oldest individual with degree 0 at S_{1-2} for the female sample is 25 years old, and the oldest male cases presented the following ages: 30, 31, 34 and 35 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 S_{1-2}, S_{2-3}, S_{3-4}, S_{4-5} were 0.0/0.0, 37.5/18.2, 62.5/18.2, 50/27.3 (Table 1).

With regard to the association with age, the Chi-square test (Table 5) indicates that the degree of fusion at the four sacral locations is not independent of AGE2, and this conclusion is also supported by the Spearman correlation (Table 6), which indicates a clear association between the degree of fusion at the four sacral locations and the two age variables AGE1 and AGE2.

The results from the transition analysis indicate that the mean age of complete fusion of S_{1-2} in males is 37 years and 35 years in females as seen in Tables 7 and 8. Females also show a different distribution of transitional phases in the stages of fusion compared to males, with greater separation in intermediate transitional phases; this can be seen in Figs. 1 and 2. In females transition from

Table 4
Chi-square and Mann–Whitney tests for sex differences at every sacral location by AGE2 ($p = 0.05$)

	S_{1-2}	S_{2-3}	S_{3-4}	S_{4-5}
Chi square				
YA				
x^2	11.440	4.125	4.125	4.371
p	0.022	0.248	0.248	0.112
MA				
x^2	6.326	CTE	0.594	2.837
p	0.176		0.441	0.092
OA				
x^2	0.028	CTE	CTE	CTE
p	0.867			
Mann–Whitney				
YA				
u_{MW}	411	559	559	525
p	0.023	0.286	0.286	0.038
MA				
u_{MW}	492.5	CTE	542	532
p	0.330		0.444	0.094
OA				
u_{MW}	1335	CTE	CTE	CTE
p	0.867			

CTE indicates that in all cases the state of fusion was the same (4, complete fusion).

Table 5
Chi-square for association between degree of fusion and AGE2 by sex

	S_{1-2}	S_{2-3}	S_{3-4}	S_{4-5}
MAL				
x^2	111.805	106.633	106.54	95.588
p	0.000	0.000	0.000	0.000
FEM				
x^2	104.671	61.592	46.826	39.654
p	0.000	0.000	0.000	0.000

Table 6
Spearman correlation for the association between degree of fusion and AGE1 and AGE2 by sex

	S_{1-2}	S_{2-3}	S_{3-4}	S_{4-5}
MAL				
AGE1				
r_S	0.671	0.449	0.449	0.442
p	0.000	0.000	0.000	0.000
AGE2				
r_S	0.676	0.452	0.441	0.441
p	0.000	0.000	0.000	0.000
FEM				
AGE1				
r_S	0.685	0.375	0.284	0.256
p	0.000	0.000	0.002	0.007
AGE2				
r_S	0.674	0.380	0.273	0.258
p	0.000	0.000	0.004	0.006

degree 0 to degree 1 occurs at 21.1 years, whereas in males the transition occurs at 25.6 years.

3.2. Sex and age associations with pattern of sacral fusion

We will focus our attention on the pattern x444 that includes the following degrees of fusion: $S_{1-2} = 0–4$, $S_{2-3} = 4$, $S_{3-4} = 4$, $S_{4-5} = 4$ (0, non-fused; 4, complete fusion). In Table 9 the distribution of this pattern according to AGE2 is seen. The Chi-square and Mann–Whitney tests fail to find a significant sex difference for the distribution of the pattern in any age group (Table 10), although for the YA group the *p*-values for both tests are nearly significant (0.058 and 0.087, respectively, for Chi-square and Mann–Whitney). On the other hand, in Table 11, the Spearman correlations indicate a clear association between this pattern and AGE1 and AGE2 for both sexes.

Table 7
Mean age-at-transition and standard deviation of the sacral fusion of S_{1-2} for males from the Lisbon skeletal collection

S_{1-2}	Mean age at transition	Standard deviation
0–1	25.6	8.7
1–2	27.1	8.7
2–3	29.9	8.4
3–4	37.0	12.7

Table 8
Mean age-at-transition and standard deviation of the sacral fusion of S_{1-2} for females from the Lisbon skeletal collection

S_{1-2}	Mean age at transition	Standard deviation
0–1	21.1	3.7
1–2	23.6	6.2
2–3	26.7	9.4
3–4	35.0	14.1

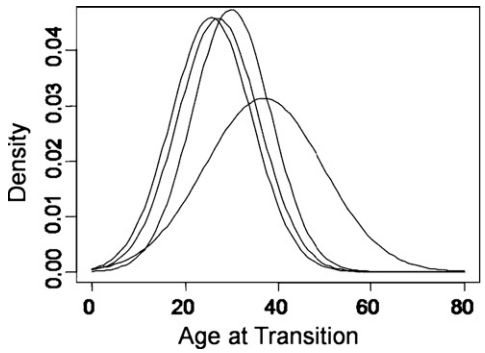

Fig. 1. Age-at-transition distribution for the sacral fusion of S_{1-2} for males from the Lisbon skeletal collection.

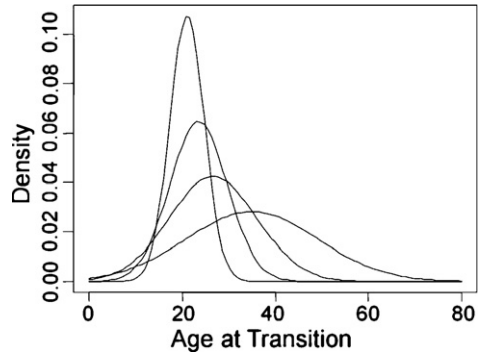

Fig. 2. Age-at-transition distribution for the sacral fusion of S_{1-2} for females from the Lisbon skeletal collection.

3.3. Test of the utility of the sacrum for age estimation

3.3.1. Test in the Lisbon sample of the Sassari and Coimbra findings

With regard to the first recommendation of Belcastro et al. [10], at the Lisbon collection all the cases with degree 0 or 1 (including degrees 1 and 2 of McKern and Stewart [9]) at S_{2-3}, S_{3-4}, and S_{4-5} belonged to the YA group (Table 12). But six cases presented degree 0 or 1 at S_{1-2} and belonged to the MA group: three males of 35 (degree 0), 45 (degree 1) and 40 years old (degree 1), and three females of 35, 35 and 42 years old, all with degree 1. This means that 10.7% of those cases with degree 0 or 1 at any site are misclassified. If we restrict our observation to those cases with degree 0, only one case out of 35 is misclassified (2.8%), and with degree 1 the error rate is 5 out of 21 (23.8%).

With regard to the second recommendation, at the Lisbon collection all the cases with pattern 0333 belong to the YA group except a 35 years old male skeleton. This is also true for the pattern 1333: all but one case belong to the YA group. With regard to the patterns "b" (xxx3, xx33), and "c" (xxxx), only 13 skeletons displayed these patterns, all of them belonging to the YA group, with an age range of 15–23 years.

3.3.2. Test in a forensic context

In order to illustrate the usefulness of the sacrum for adult age estimation the skeletal analysis from a recently excavated mass grave is discussed. As explained previously, the identities of nine skeletons have been confirmed, and there is ample archival, archeological and osteological evidence that the 21 skeletons exhumed correspond to the 21 men believed to be at the location

Table 9
Distribution of the percentages and absolute frequencies of type "a" patterns (x444) according to AGE2 in Lisbon

	FEM				MAL			
	N	YA	MA	OA	*N*	YA	MA	OA
0444	3.6 (4)	100.0 (4)	0.0 (0)	0.0 (0)	8.3 (13)	84.6 (11)	15.4 (2)	0.0 (0)
1444	5.4 (6)	100.0 (6)	0.0 (0)	0.0 (0)	1.28 (2)	50.0 (1)	50.0 (1)	0.0 (0)
2444	4.5 (5)	60.0 (3)	40.0 (2)	0.0 (0)	3.8 (6)	83.3 (5)	16.7 (1)	0.0 (0)
3444	14.5 (16)	50.0 (8)	25.0 (4)	25.0 (4)	14.1 (22)	22.7 (5)	54.5 (12)	22.7 (5)
4444	71.8 (79)	15.2 (12)	15.2 (12)	69.6 (55)	72.4 (113)	6.2 (7)	36.3 (41)	57.5 (65)
Total	110				156			

Table 10
Chi-square and Mann–Whitney tests for sex differences in type "a" patterns by AGE2

	Chi square		Mann–Whitney	
	x^2	*p*	u_{MW}	*p*
YA	9.126	0.058	360.5	0.087
MA	4.004	0.405	485	0.664
OA	0.07	0.936	2057	0.936

Table 11
Spearman correlations for the association between type "a" patterns and AGE1 and AGE2 by sex

	FEM	MAL
AGE1		
r_S	0.577	0.553
p	0.000	0.000
AGE2		
r_S	0.575	0.545
p	0.000	0.000

Table 12
Percentages of misclassified cases at the Lisbon collection following the recommendations of Belcastro et al. [10]

	Degree of fusion	*N*	MA (misclassified) (%)
S_{1-2}	0	35	2.8 (1/35)
	1	21	23.8 (5/21)
			Total 10.7 (6/56)
S_{2-3}	0	11	0.0
	1	4	0.0
S_{3-4}	0	9	0.0
	1	5	0.0
S_{4-5}	0	10	0.0
	1	3	0.0
Pattern 0333		16	6.25 (1/16)
Pattern 1333		19	5.26 (1/19)
Pattern xx33		3	0.0
Pattern xxx3		1	0.0
Pattern xxxx		9	0.0

where the grave was found. The exact ages at death of the 21 men were obtained by cross-checking testimonies, archival information from birth certificates, and individual military and prison records [13]. The age distribution of the 21 men released from prison, and subsequently dissapeared, was as follows: 18, 20, 21, 22, 23, 23, 23, 24, 24, 26, 27, 36, 42, 42, 47, 48, 50, 50, 54, 55, and 61. During the osteological study, the first step was to establish the compatibilty of the sex and age profile estimated from the skeletons with the sex and age profile from testimonies and archival information. The degree of fusion of the anterior aspect of the sacral vertebrae was recorded when available. Table 13 shows the state of fusion of the available sacral vertebrae for those skeletons whose age was estimated to be less than 30 years (all with active fusion at the clavicle except individuals 4 and 8, both with a scar at the clavicle interpreted as recent fusion). Table 14 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 (except the sacrum) and by observation of pubic symphisis, auricular surface and, when possible, sternal end of the fourth rib. In both tables we follow the equivalence proposed in Table 3. If we apply the recommendations of Belcastro et al. [10], supported by the results from the Lisbon sample, the skeletons 2, 7, 8, 10, 14, 15 and 20 from Table 13 would be assigned to the YA age class, where other maturity indicators (long bones, pelvis, sternum, vertebrae, clavicle) had already placed them. Individuals 5 and 6 would be the only ones from Table 14 that

Table 13
Degree of fusion of the sacral vertebrae and other skeletal elements for the 11 skeletons whose age was estimated under 30 years old

N	S_{1-2}	S_{2-3}	S_{3-4}	S_{4-5}	Other active maturity indicators	Compatible identities
2	0	0	–	–	Long bones, pelvis, scapula, vertebrae, clavicle	18 (identified)
20	0	0	–	–	Vertebrae, ribs, sternum, clavicle	21 (identified)
7	0	–	–	–	Clavicle, vertebrae	24 (identified)
8	0	3	3	3	Epyphiseal scar visible at clavicle	26 (identified)
15	0	–	–	–	Long bones, pelvis, vertebrae, clavicle	20 (tentative identification)
14	0	0	0	0	Pelvis, clavicle	22, 23, 23, 23, 24, 27
26	3	3	–	–	Vertebrae, clavicle	22, 23, 23, 23, 24, 27
10	0	0[a]	–	–	Clavicle	22, 23, 23, 23, 24, 27
19[b]	3	3	–	–	Clavicle, vertebrae	22, 23, 23, 23, 24, 27
4	2	2	–	–	Epyphiseal scar visible at clavicle	22, 23, 23, 23, 24, 27
12[c]	–	–	–	–	Sternum, vertebrae, clavicle	22, 23, 23, 23, 24, 27

[a] Doubtful due to poor preservation.
[b] Unequivocal anatomical variation at the lumbosacral transition with a supernumerary lumbar vertebra. This observation should preclude any further inquire in the state of fusion of the sacrum but we include the state of fusion at what we considered to be S_{1-2} for illustrative purposes.
[c] Non-observable due to poor state of preservation.

Table 14
Degree of fusion of the sacral vertebrae and other skeletal elements for the 10 skeletons whose age was estimated over 30 years old

N	S_{1-2}	S_{2-3}	S_{3-4}	S_{4-5}	Compatible identities
5*	0	3	3	3	48 (identified)
6	1	3	3	–	36, 42, 42, 47, 50, 50, 55
1	2	3	3	–	36, 42, 42, 47, 50, 50, 55
3	3	–	–	–	48 (identified)
9	3	3	–		54 (Identified)
11	3	3	3	3	36, 42, 42, 47, 50, 50, 55
13	3	–	–	–	36, 42, 42, 47, 50, 50, 55
23	3	–	–		36, 42, 42, 47, 50, 50, 55
33	3	3	–	–	36, 42, 42, 47, 50, 50, 55
16	3	3	3	–	61 (identified)

* Klippel-Feil at C2C3.

could be included in the YA category. Individual 5, with a Klippel–Feil syndrome at the level of the first and second cervical vertebrae, presents degree of fusion 0 at S_{1-2}, and individual 6 presents a degree of fusion 1 at S_{1-2}.

4. Discussion

As confirmed for most skeletal elements and different stages of the ontogeny, a significant sex difference towards an earlier maturation of the female sample is confirmed in the YA group (20–34 years) for degree of fusion of the sacral vertebrae (Tables 4, 7 and 8), and almost significant in the frequencies of x444 patterns (Table 10). This sex difference is also found in the SB group, which includes skeletons from 15 to 19 years old, although narrower age groups and larger samples should be used to study sacrum maturation in these years of accelerated development. The association of sacral fusion with age is confirmed for degree of fusion and frequencies of x444 patterns (Tables 5, 6 and 11). In this regard, the results from the Lisbon sample are similar to those found by Belcastro et al. [10], for Coimbra and Sassari samples. The compatibility of the observations in three different collections indicates that a clear picture of sex differences and age associations for sacral fusion is emerging.

However, bearing in mind that our scoring system is slightly different from theirs, there are some differences betweent our results and those of Belcastro et al. [10]. At S_{1-2}, absence of fusion (degree 0) is present until 29 years for females and until 35 years for males in Lisbon, whereas it is present until 54 years for females and 34 years for males in Sassari and 29 years for females and males in Coimbra. We have to conclude that more research is needed in geographically diverse samples in order to estimate the extent of population differences in sacral fusion and its possible impact on age estimation.

With regard to the utility for age estimation in adult skeletal remains, the three main recommedations of Belcastro et al. [10], for age estimation have been positively tested in the Lisbon sample. No error was observed when applying degree 0 or 1 at S_{2-3}, S_{3-4}, and S_{4-5}, and when applying patterns xx33, xxx3, and xxxx. However, the age of the skeletons with these scores and patterns at the Lisbon collection ranges from 15 to 23 years old, and if we are studying complete remains, this age range corresponds to a time during which several aspects of the skeleton can be used to accurate estimate age. On the other hand, the error rate ranges from 2.8% of sacra misclassified using degree 0 at S_{1-2}, to a 23.8% misclassified sacra when using degree 1 at the same site. With only one case misclassified out of 35 for degree 0 at S_{1-2}, 35 years old seems a reasonable upper limit for age estimation when a degree 0 is observed at S_{1-2}. When applying the patterns 0333 and 1333, and the total score, the error rate is approximately 6% (Table 12). These error rates are low and these results, especially those obtained from S_{1-2}, are interesting because they include individuals from 18 to 35 years (including three cases of >40 years). Aging methods easy to score that can be applied to estimate the age of individuals after about age 25 are especially useful, because the number of active ephyphiseal centers quickly diminishes in these individuals. These observations are relevant for age estimation in those cases where it is expected to find skeletons with ages between 20 and 30 years old, specially males: at the three documented collections studied, for the age range 20–24 years old, the percentage of male cases with degree 0 at S_{1-2} ranges from a minimum of 57.9% for Sassari to a maximum of 73.3% for Lisbon, and for the age range 25–29 years old, from a minimum of 9.1% to a maximum of 29.2% for Sassari. In ce 10% of missclassified sacra in the blind test carried out by Belcastro et al. [10], on 31 sacra from the Frassetto collection.

On the other hand, the forensic case study verifies that the degree of fusion of the sacral vertebrae can be helpful in assigning skeletons to broad age groups where the comparison of the skeletal age profile with the documented age distribution is a key step in the identification or exclusion of groups of skeletons exhumed from mass grave contexts. In this case, the age profile estimated from the degree of fusion at S_{1-2} indicates the presence of at least 7 skeletons with an estimated age less than 34 years old, an age distribution compatible with the actual age distribution of the 21 disappeared men. This would include the problematic case 5, already genetically identified as an adult man of 48 years old. This case could be an extreme of variation in fusion at S_{1-2}, similar to the 54-year-old female observed at Sassari, or as indicated before, the presence of a Klippel–Feil condition at the second and third cervical vertebrae could be related to a delay in fusion at S_{1-2}, although we mention this just as a suggestion without further support. The important point to address is that, for instance, if we knew from antemortem information that the group we are looking for is totally composed of over 35 years old men, the age profile estimated from the degree of fusion at S_{1-2} would indicate us the possibility that we have not found the 21 men we were looking for. As mentioned before, the utility of the sacrum in a mass grave context is relevant when the antemortem information states the presence of young adults, especially between 20 and 30 years old.

The results from the present work support that the scoring of the degree of fusion of the sacral vertebrae, especially degree 0 at S_{1-2}, is useful for age estimation and therefore we conclude that it should be implemented as another tool, simple and informative, in the study of human skeletal remains.

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