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Forensic age estimation based on the trabecular bone changes of the pelvic bone using post-mortem CT

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Abstract

We analyzed the trabecular bone changes in the pubic bone (PB) and in the auricular surface (AS) of the ilium using 319 CT scans of cadavers to estimate the age. Although the sharpness of the trabecular structure decreases in CT images when soft tissues are present, we identified four phases for the changes in PB and five in AS; a juvenile trait in PB and a senile trait in AS helped narrow the age range. High correlation with age was identified for both sexes in PB (F 0.89; M 0.75) and in AS (F 0.85; M 0.71) used independently or combined (F 0.91; M 0.78). The old adults (> 60 years) could be evaluated with better accuracy and discriminated in several phases. We found low inter-observer error and low inaccuracy (about 6 years, mean for all age ranges). The method is robust with respect to slice thickness, display window and kernel within the tested ranges.

1. Introduction

Age estimation, combined with sex and ancestry assessment, is a key issue in forensic identification. The traditional methods evaluate macroscopically the morphological changes of the bones, such as cranial sutures [1], pubic symphyseal face [2], auricular surface [3] [4], and the sternal end of the ribs [5]. Their use in forensic cases requires time-consuming preparation of the bone, since all remains of soft tissues must be removed. Conversely, it has been suggested that 3D visualization of the bones could be generated from CT scans and the methods could then be applied to them, but their application is unreliable: some papers report good agreement between the modalities [6], others suggest using the methods developed on dry bones with caution and refer to reduce accuracy [7, 8] [8].

CT scans allow investigation of subsurface features of the bone, including features of trabecular bone. The structure of the trabecular bone changes with increasing age due to the imbalance of the processes of bone remodeling: consequently, the bone density decreases, the trabeculae lose their interconnection until they disappear, and therefore the medullar space increases [9], [10]. The first radiographic investigations of the trabecular bone were performed by Todd [11] who analyzed the pubic bone. Other bones have been investigated: the proximal epiphysis of the humerus [12], and the proximal epiphysis of the femur [13] used alone as the age indicator or combined with other bones [14]. X-rays of the clavicle and the calcaneus, together with the femur and the humerus, were investigated by Walker and Lovejoy [15]. The reliably of the method in forensic cases was tested by Lynnerup et al. [16], who obtained good results looking at the X-rayed trabecular changes in the proximal femur of living individuals. Computed Tomography (CT) was used to assess the trabecular structure changes on the pubic bone [17], the auricular surface [18] and the proximal epiphysis of the humerus and the femur [19]. Micro-CT scan, CT and X-ray were compared in a quantitative study of the trabecular changes in the pubic bone [20]. However, all these studies analyzed the trabecular bone of dry bones. The appearance of trabecular bone is different in post-mortem CT when the soft tissues are still in place: the sharpness of the images decreases and the fine trabecular structure can be seen with much less detail. The aim of this study was to develop a method to be applied directly on post-mortem CT, performed before the autopsy. We assessed the trabecular bone changes in the pubic bone (PB) and adjacent to the auricular surface (AS) using CT scans of cadavers without removing any tissue. We correlated the changes with increasing age and developed a scoring system to estimate the age-at-death using both areas independently and combined.

2. Materials and methods

2.1. CT scans sample

We used a sample of 319 CT scans of cadavers (145 females and 174 males) with an age range of 17 to 95 years (age distribution shown in Figure 1). The bodies were scanned within 3 days from the time of death and they showed no or very few signs of decomposition. CT scans were performed as part of the routine investigation of the Forensic Institute of the University of Copenhagen [21] during the period 2010-2013. A Siemens Somatom Sensation 4 Multislice spiral scanner was used with the following settings: 120 KV, 112 mAs (average value, Care Dose has been used). Others settings (slice thickness, slice increment and kernel) varied between some of the scans as follows: 1) 3mm slice thickness, 2 mm slice increment, smooth kernel B31f (N=151); 2) 1mm slice thickness, 1 mm slice increment, sharp kernel B60f (N=115); 3) 1 mm slice thickness, 1 mm slice increment, smooth kernel B30f (N=53). We intentionally included scans with these different settings to test the variation among them since there is no widely used standard protocol.

2.2. Description of the age determination system

A group of 50 males to represent decades from 20 to 70 years was randomly selected in order to define the new scoring system. We selected subjects with the same CT-scan settings (3mm slice thickness, 2 mm slice increment, smooth kernel B31f) and displayed them with a display window of [-150, +1000] Hounsfield Units (HU). This specific window display was chosen because it highlighted the trabecular bone in a visually adequate way. For all subjects, one or two slices were selected and classified in several distinct phases based on the density of trabecular bone. Presence of further features useful for age estimation was noted. The classifications were performed without knowing the age of the subjects. We could identify four phases for the changes of the trabecular bone in the pubic bone (PB) and five in the auricular surface (AS). Moreover, we identified a juvenile trait in the pubic bones and a senile trait for the auricular surface that helped in narrowing the age range in the young and in the old individuals respectively. Left and right sides could not be evaluated separately, since the observer could not exclude one during the evaluation of the other. The more advanced phase for either left or right side was selected in cases of disagreement. Having verified the relationship between the phases and the age-at-death, we applied the new scoring system to a larger sample, testing the applicability of the scoring when protocol settings varied (slice thickness, kernel).

A set of unisex descriptions was formulated for each phase for both bone (PB and AS) and a series of pictures (Figs 3,6) were selected as the best representative of each phase. Figures 3 and 6 show CT images of subjects scanned with 3mm slice thickness, smooth kernel and displayed with a window of [-150, +1000] HU.

2.2.1. Pubic Bone

Region of interest: all slices in axial view of the pubic bone where both left and right pubic symphyseal faces are present (Fig 2); the ischiopubic ramus was not considered. No suitable standard reference point could be identified for all subjects, so a variable number between 10 and 20 slices (depending on slice thickness, inclination and size of the pubic bone) have been evaluated. Each slice was attributed a phase and the more frequent one was taken to be the phase of the subject.

Final score

Two elements need to be scored to determine the final score: the phase and the presence of the juvenile trait. Thus, the final score is the sum of the phase of the trabecular density (from 1 to 4) and a “-1” point if the juvenile trait was present.

<u>Phase 1</u>: the trabecular bone is very dense and homogenous; the interior of the bone seems homogenously filled with trabecular bone. There is one dominant shade of grey, lighter than the surrounding soft tissues (Fig 3).

<u>Phase 2:</u> the trabecular bone is still predominantly dense and homogenous, but there are some areas (less than 20%) showing a less dense structure. There are two shades of grey, but the lighter one is dominant.

<u>Phase 3</u>: the trabecular bone further decreases in density; the trabecular bone is less dense than phase 2; furthermore, there are some areas with very large trabecular structure or areas where the trabecular bone is completely disappeared. Those areas cover less than 50% of the surface. There is no single dominant shade of grey.

<u>Phase 4:</u> The impression is that the majority (more than >50 %) of the area is “empty”. There are only few areas with remains of trabecular bone. The dominant shade is dark.

Juvenile trait (-1 point if present): the superior ossific nodule is visible, and is either unfused or partially fused (Fig 4). An unfused or partially fused superior ossific nodule was present in 6 females (4.14% of the sample; mean: 21 years; SD 3 years; range 17-26 years) and in 9 males (5.17%; mean 19.8 years, SD 2.2years, range 17-24 years).

2.2.2. Auricular surface of the ilium

Region of interest: axial view of the auricular surface of the ilium at the level of the 1st and 2nd sacral vertebrae (Fig. 5); a single slice was analyzed, since a suitable reference point could be identified in all individuals.

Final score

Two elements need to be scored to determine the final score: the phase and the presence of the senile trait. Thus, the final score is the sum of the phase of the trabecular density (from 1 to 5) and a "+1" point if the senile trait was present.

Phase 1: the trabecular bone is very dense and homogenous; the interior of the bone seems homogenously filled with trabecular bone. There is one dominant shade of grey, lighter than the surrounding soft tissues. No difference in density is present (Fig 6).

Phase 2: the trabecular bone is still predominantly dense and homogenous as in phase 1, but there are some areas (less than 20%) showing a less dense structure. There are two shades of grey, but the lighter one is dominant.

Phase 3: the trabecular bone loses the appearance of density typical of the first two phases and the general impression is that the trabecular structure is less dense; areas with very large trabeculae are present, covering less than 50% of the surface. There is not a single dominant shade of grey.

Phase 4: the trabecular bone further decreases in density; the difference from phase 3 is the increasing number of areas with very large trabecular structure or areas where the trabecular bone has completely disappeared. These areas cover more than 50 %. There is no dominant shade of grey.

Phase 5: the trabecular bone is present in only a few areas and the general impression is that the majority of the region of interest is "empty". The dominant shade is dark grey.

Senile trait: at least one of the sacroiliac joints is fused (fused accessory facets alone are not considered as presence of senile trait) and / or the ossification of the anterior sacroiliac ligament (Fig. 7). The fusion of the sacroiliac joint and / or the ossification of the anterior sacroiliac ligament (senile trait) were present in 7 females (4.8%; mean 76 years; SD 12 years; range 57-90 years) and 21 males (12.1%; mean 70.3 years; SD 12.2 years; range 50-94 years).

2.3. Statistical analyses

Statistical analysis was performed using SPSS. Shapiro-Wilk test (W) was used to verify the normal distribution of the sample, considering the males and females both together and separately. Student t-test was used to calculate the differences of the mean ages of males and females in each phase, for both the PB and the AS. Paired t-test was applied to determine the effect of slice thickness on a sample of 30 individuals who had been scanned with both settings; we scored the two groups with at least 1 month interval. Paired t-test was again used to investigate differences between display windows on 61 auricular surfaces; we used a bone window [-1024, +1650] and custom windows [-150, +1000] HU. The evaluations again were performed with at least 1 month interval. Finally, independent sample t-test was used to determine difference between kernels (smooth kernel versus sharp kernel) on 50 individuals. Individuals scanned with a protocol different only in kernel were not available, thus we calculated the difference using the age error (estimated age – known age) both for the PS and the AS. All samples were randomly selected and the evaluations of AS and PB were carried out at different times to allow for independence of the results and to avoid bias.

Spearman correlation was calculated to measure the relationship between age-at-death and the final score. Scatter plots of the final scores against age-at-death were used to visualize the results. We calculated mean, standard deviation (SD) and range (min-max values) for each final score.

Intra- and inter-observer agreements were expressed with weighted kappa (wκ), specific for ordinal data, to give different weights to disagreements according to the magnitude of the discrepancy

[22]. The wκ values were interpreted following the indication of Landis and Koch [23]. The intra-observer test is based on the results of a person with 5 years of anthropological experience (CV); the second observer was a young student with experience in using CT scans for age estimation (MNH). The evaluations of AS and PB were again carried out at different times to allow for independence of the results and to avoid bias. Weighted kappa was calculated using MATLAB software.

New specimens, not included in the sample used for developing the method, were randomly selected for a validation test: 30 females (age range 23 to 89 years) and 30 males (age range 17 to 69 years). We calculated bias and inaccuracy using the following formula:

Inaccuracy = $\sum \left| \text{estimated} - \text{known} \right| / N$; Bias = $\sum (\text{estimated} - \text{known})/N$.

3. Results

Shapiro-Wilk Normality test showed that the sample used in this study had a normal distribution, both when considering males and females together (W= 0,975 p< 0.001) or separately (M: W=0.972, p< 0.01; F: W= 0.974, p< 0.01). Males and females were considered separately during the analyses because statistically significant differences were found between the mean ages of some phases both in the pubic bones (phase 2: t=2.964, p< 0.01; phase 4: t=3.703, p< 0.01), and in the auricular surface (phase 4: t=3.221, p< 0.01; phase 5: t=2.272, p< 0.05)

No significantly statistical differences were found for different slice thickness, both for the pubic bone (t=1,361 p>0.05) and the auricular surface (t=0.273 p>0.05). The display window also did not affect the evaluation (t=1.187 p>0.5). In addition, the statistical analysis shows no difference between a smooth and a sharp kernel both for the pubic bone (t=1.323 p>0.5) and the auricular surface (t=-0.082 p>0.5).

We found a substantial agreement in all tests if inter- and intra-observer error. The intra-observer agreements in the auricular surface and in the pubic bone were respectively wκ =0.77 (se= 0.079 C.I. [0.62-0.93]) and wκ =0.78 (se= 0.085 C.I. [0.61-0.95]). The values of the inter-observer agreement were slightly lower, but still reaching the threshold of the “substantial agreement”: wκ = 0.71 (se= 0.077 C.I. [0.56-0.86]) for the auricular surface and wκ = 0.61(se= 0.10 C.I. [0.41-0.80]) in the pubic bone.

3.1 Pubic bone

We found a correlation between score and age of 0.89 ($p<0.001$) in females and a slightly lower correlation in males 0.75 ($p<0.001$). The distribution of the results for males and females separately are shown in the scatter plots, where the final score (trabecular phase + juvenile trait) was plotted against known age (Fig 8). A total score of 0 was given for young individuals with trabecular bone phase 1, who also showed the juvenile trait. Tables 1-2 show the corresponding descriptive statistics: mean, standard deviation, minimum and maximum values and the 95% range. The descriptive statistics of the Suchey-Brooks (SB) method are reported for easier comparison.

3.2 Auricular surface of the ilium

A correlation of 0.85 ($p<0.001$) was found in females, and of 0.71 ($p<0.001$) in males. The distribution of the results for males and females separately are shown in the scatter plots, where the final score (trabecular phase + senile trait) was plotted against known age (Fig 9). A total score of 6 was attributed for individuals with a phase 5 for the trabecular bone and fusion of the sacroiliac joint or ossification of the sacroiliac ligament (senile trait). The mean, standard deviation and range for males and females separately are shown in Table 3. The descriptive statistics of the Buckberry-Chamberlain method are also given in Table 3, for a better comparison of the results.

3.3 Pubic bone and auricular surface: composite score

A composite score was obtained summarizing the score of the pubic bone and the auricular surface. We produced nine classes of age (total scores 9-10 were combined due to small sample size and completely overlapping of age ranges). We found a very high correlation in females (0.91; $p<0.001$), slightly lower in the males (0.78; $p<0.001$). Figure 10 shows the distribution of the total score versus the known age. In females, there is a very clear trend with age: the age intervals increased with known age, both for the mean and for age ranges (Table 4). In contrast, in males there was much more overlap of known age ranges between the total scores, especially between total scores of 4 and 5, where score 4 has a slightly higher mean age than score 5 (Table 4).

3.4 Validation test

Table 5 summarizes bias and inaccuracy for the complete sample (all ages) and for decades in males and females obtained in the validation test. The estimated age of the majority of the specimens fell within the interval of 1 SD: using the pubic bone 77% of females and 87% of males; using the auricular surface 73% of females and 83% of males. Using the composite score (AS+PB), known age fell in the 1 SD interval in 80% of females and in 77% of males.

3 Discussion

In this study to estimate age-at-death, we found that the trabecular bone changes of the pubic bone and the auricular surface could be evaluated in post-mortem CT, even though the presence of soft tissues decreased the sharpness of the CT images and thus blurred the trabecular microarchitectures. We distinguished some phases in the trabecular structure changes and developed a new method that be applied directly on post-mortem CT scans of fresh cadavers. In agreement with Lynnerup et al. [16], we demonstrated that the changes of the trabecular structure can be assessed without removing soft tissues, thus avoiding the time-consuming maceration process. We included CT scans with diverse slice thickness, display window and kernel to assess the impact of the machine settings and to verify how applicable our method was. Although statistical tests showed that the scoring system developed in this study is robust with respect to these settings, we suggest our method should be used on CT scans of fresh cadavers, scanned at 120 kV with slice thickness between 1 and 3 mm, a smooth kernel and a display window of [-150, +1000] HU. Slice thickness outside these ranges could have an influence on our method: larger slice thickness could cause loss of information, i.e. juvenile or senile traits may not be seen; lower slice thickness could increase the noise and make it difficult to distinguish among the phases. The same effect could result from too sharp a kernel. Another important aspect to consider is the state of decomposition of the body: we used only cadavers with no or very few signs of decomposition, since the presence of gas could alter the structure of the trabecular bone, creating bubbles on the bones [24].

A merit of our new method seems to be the possibility to estimate age-at-death in old adults (60+ years) more accurately than the macroscopic methods applied to the pubic bone and the auricular surface. Looking at the morphological changes of the pubic bone, it is possible to attribute a maximum age of +50 using the method of Todd [25], an age of 60±12.4 for females and 61.2±12.4 for males using the Suchey-Brooks method [2]. In contrast, we could estimate an age of 80.5 ±7.7 for females and 71.1 ±11 for males (Tables 1-2). One of the advantages of the Buckberry-Chamberlain method [3] over that of Lovejoy et al. [4] in evaluating the changes of the auricular surface is the greater accuracy in estimating age of older adults [26], indeed the age interval of the last stage (VII) is 72.25±12.7, versus the range 60 + years. We defined two older scores (5-6) in females with age ranges of 80±13 years and 83±6.1years; in males only the final score 6 (82.4±9.1 years) performed better than the Buckberry-Chamberlain method. Using the composite score (Table 4), three classes are over 60 years for the females, two for the males. However the classes of the composite score have to be tested on a larger sample, since some are based on few specimens and some have similar ranges of age, especially in the males.

Comparing our method with those based on X-ray investigation, we noticed that only a small number of phases have been identified in our method: four in the pubic bone, and five in the auricular surface. In contrast, Walker and Lovejoy [15] described eight phases of change in the clavicle and the femur, and Acsadi and Nemeskeri [14] described six classes for the proximal end of the humerus and the femur. A reason of the low number of phases could be that the structure of the trabecular bone cannot be distinctly viewed in post mortem CT scans, due to the presence of soft tissue. Similarly, Todd [11] and Lynnerup et al [16], who studied bones surrounded by soft tissues, identified only four phases. Nevertheless, our method performed better than these radiological methods in estimating the age of older individuals (60+ years): the last phase of the Walker and Lovejoy [15] method estimates an age of 60 + years, the last phase of the Acsadi and Nemeskeri [14] method provides an age estimate of 67.8±3.64 years, but with an actual range of 25 to 85 years. On the contrary, the lowest age in the last phase of our method is 48 years in males and 57 years in females.

The reliability of a method depends on different factors: variation between observers and among populations, accuracy and bias. In our test, the intra- and inter-observer agreements were substantial in the evaluation of the pubic bone and of the auricular surface and we concluded that our method could be applied in a consistent manner independently from the level of experience of the observers. Good results for bias and inaccuracy were obtained when we tested 60 new individuals: an inaccuracy of less than 10 years was found for the complete sample of males and females (all ages) and for each decade. As previously noted by Saunders et al. [27] and Martrille et al. [28], the combined method did not perform better than the single bone independently. In males, the best results were obtained using a single trait. In females, the PB results had the lowest inaccuracy in the decades 40-49, 60-69. In contrast to the results of inaccuracy obtained by Lovejoy et al. [29] and Martrille et al. [28], the inaccuracy of our method did not increase with increasing age. For our method, high inaccuracy was evident for the decade 40–49 in females and in the age range < 29 in males using auricular surface and in the decade 40–49 for both sexes using the pubic symphysis. Our results are more similar to those obtained by Walker and Lovejoy [15], who tested radiological method. Although the results of inaccuracy and bias for our method seemed better than those in the literature, especially for older adults, they need to be confirmed using a larger sample and tested on different populations. Finally, we need to incorporate our scoring method in the "transitional analysis" procedure [30] to estimate the probability of age distribution associated with each final score.

A possible limit of the method presented in this study is that again is based only on visual scoring of the criterion useful for age estimation, although we had access to digital data, and again the subjectivity and the experience play an important role. Future research will involve implementing our method and supporting the results with quantitative data. Previous studies have tested quantitative approaches in the quantification of the variation of the trabecular bone in femur [31] and humerus [32] and pubic bone [20]. Trabecular bone changes could be also investigated using the variation of the Hounsfield Units (HU), although particular attention has to be paid to factors such as the position and the dimension of the region of interest, the model and the setting of the CT scanner [33], that can influence the reliability and the reproducibility of any resultant method.

4 Conclusion

In conclusion, we developed a method for age estimation that can be applied directly on post-mortem CT scans of fresh cadavers. The method was robust with respect to slice thickness, display window and kernel within the tested ranges. From the validation test, age-at-death could be evaluated with a low inaccuracy (about 6 years) using the trabecular bone changes of both the auricular surface and the pubic bone and old adults could be discriminated with better accuracy than with the classic methods. However, the method should be validated on a larger sample to confirm inaccuracy and bias and should be tested for variation between populations.

Ethical issues

No formal ethical consent is needed from Danish Ethical committees to work with CT images of dead humans. Medico-legal autopsies are mandated by the police and CT scans are part of the routine investigation at the Department of Forensic Medicine (University of Copenhagen). The Department of Forensic Medicine adheres to Danish Standards Accreditation regarding data security. No personalized data can be exported from the systems. All personal data are removed from all images, as they have no use in the project; only age and sex data were retained.

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Females					Suchey-Brooks Females			
Final score	N	mean	SD	Range	Phase	mean	S.D.	95% range
0	6	21.0	3.0	17-26	1	19.4	2.6	15-24
1	17	26.7	6.1	17-38	2	25.0	4.9	19-40
2	36	42.6	9.0	27-59	3	30.7	8.1	21-53
3	55	60.0	10.7	31-81	4	38.2	10.9	26-70
4	31	80.5	7.7	66-98	5	48.1	14.6	25-83
					6	60.0	12.4	42-87

Table 1: Descriptive statistics of the female pubic bone related to this study (left) and as reported in the Suchey-Brooks method (from Brooks-Suchey 1990 Table1. Page 233)

Males					Suchey-Brooks Males			
Final score	N	mean	SD	Range	Phase	mean	S.D.	95% range
0	8	19.3	1.7	17-22	1	18.5	2.1	15-23
1	13	28.8	7.0	17-37	2	23.4	4.9	19-34
2	30	37.2	8.9	23-54	3	28.7	8.1	21-46
3	87	58.7	14.2	26-88	4	35.2	10.9	23-57
4	36	71.1	11.0	48-94	5	45.6	14.6	27-66
					6	61.2	12.4	34-86

Table 2: Descriptive statistics of the male pubic bone for this study (left) and as reported in the Suchey-Brooks method (from Brooks-Suchey 1990 Table1. Page 233)

Females					Males					Buckberry - Chamberlain				
Final score	N	mean	SD	Range	Final score	N	mean	SD	Range	Stage	N	mean	S.D.	Range
1	13	23.8	5.4	17-33	1	16	24.0	5.8	17-35	I	3	17.33	1.5	16-19
2	26	35.4	11.0	21-64	2	17	34.1	7.8	19-46	II	6	29.33	6.7	21-38
3	54	52.1	11.0	27-75	3	56	49.3	16.0	18-77	III	22	37.86	13.1	16-65
4	38	72.3	11.0	45-87	4	55	63.5	14.0	36-89	IV	32	51.41	14.5	29-81
5	11	80.0	13.0	57-98	5	25	67.4	10.0	48-87	V	64	59.94	13.0	29-88
6	3	83.0	6.1	79-90	6	5	82.4	9.1	71-94	VI	41	66.71	11.9	39-91
										VII	12	72.25	12.7	53-92

Table 3: Descriptive statistics of the auricular surface for this study divided by sex (left) and as reported in the Buckberry-Chamberlain method (Buckberry and Chamberlain, 2002, Table 12 pag 237)

Composite score	Females				Males			
	N	mean	SD	Range	N	mean	S.D.	Range
1	4	19.8	2.2	17-22	5	19.2	1.9	17-22
2	10	24.4	4.6	17-32	8	24.3	6.4	17-35
3	10	29	6.4	21-38	12	29.3	6.3	18-37
4	12	37.8	6.2	26-48	9	37.1	8.1	26-46
5	25	46.6	9.6	27-64	14	35.3	7.4	23-45
6	32	56	10.4	31-75	41	54.5	13.5	26-77
7	20	65.9	8.3	52-81	40	60.3	13.3	36-88
8	19	79.4	6.9	57-89	28	70	9.4	53-89
9	13	82.5	10.1	67-98	17	72.8	12.3	48-94

Table 4 Descriptive statistics of the composite score

Known age (years)	Females				Males			
	N	AS	PB	composite score	N	AS	PB	composite score
<29	5				2			
Inacc.		5	2.54	2.48		9.05	8.25	8.15
Bias		2.84	1.1	0.64		9.05	8.25	8.15
30-39	8				3			
Inacc.		3.8	5.29	3.55		2.9	1.4	1.37
Bias		-2	1.84	-3.35		-2.9	0.2	0.1
40-49	5				9			
Inacc.		9.6	7.52	8.48		6.06	8.98	7.92
Bias		4.16	4.96	4		3.86	1.42	1.48
50-59	6				8			
Inacc.		3.1	4.8	1.2		7.64	3.43	5.16
Bias		-3.1	4.8	0.8		-2.16	3.2	1.66
60-69	3				6			
Inacc.		8.9	1	5		2.98	6.76	5.63
Bias		-8.9	-1	-5		-1.68	-5.07	-2.3
>70	3				0			
Inacc.		8.9	6.67	5.43		-	-	-
Bias		-8.03	-6.67	-5.43		-	-	-
All ages	30				30			
Inacc.		6.33	5	4.52		5.73	6.05	5.96
Bias		-2.16	0.21	-1.54		0.6	0.9	1.05

Table 5 Inaccuracy and bias.

Fig 1: Sample age distribution by decades.

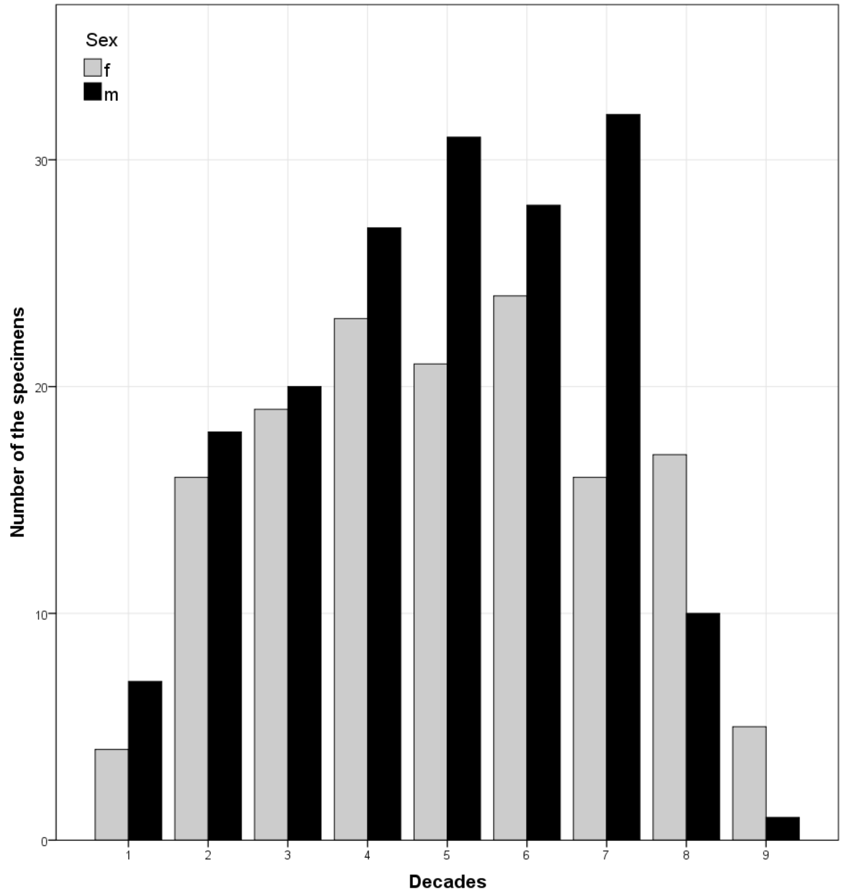


Fig 2: Region of interest in the pubic bone: (a) axial view; (b) 3D visualization of the bone. The rectangle indicates the area to evaluate.

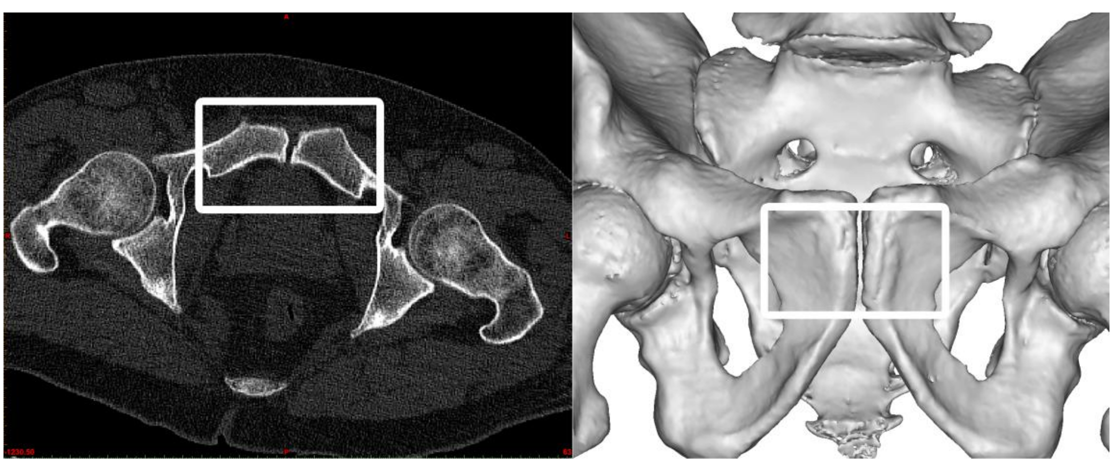


Fig. 3: Pubic bone phases. All slices are displayed with a window of [-150, +1000] HU. No alteration of brightness or contrast was applied. The right image is an exact copy of the left one with annotations. Phase 1: 17-year-old male. The trabecular bone is very dense and filled all the interior of the bone; there is one dominant shade and it is lighter than the surrounding tissues. The ossicle (marked with a circle) is not completely fused. A final score of 0 was assigned: 1(for the phase) -1 (for presence of the ossicle). Phase 2: 34-year-old male. The trabecular bone entirely fills the interior of the bone, but there are some areas with lower density (outlined with thin white lines). Phase 3: 46-year-old female. The trabecular bone is less dense, and it is possible to see large trabecular structures (highlighted with white lines); there are also areas where the trabecular bone has completely disappeared (outlined with thin white lines). Phase 4: 71-year-old female. The "empty" areas cover more than 50% (outlined with thin

white lines). **[IMPORTANT: DOWNLOAD AND USE THE HIGH RESOLUTION FIGURE]**

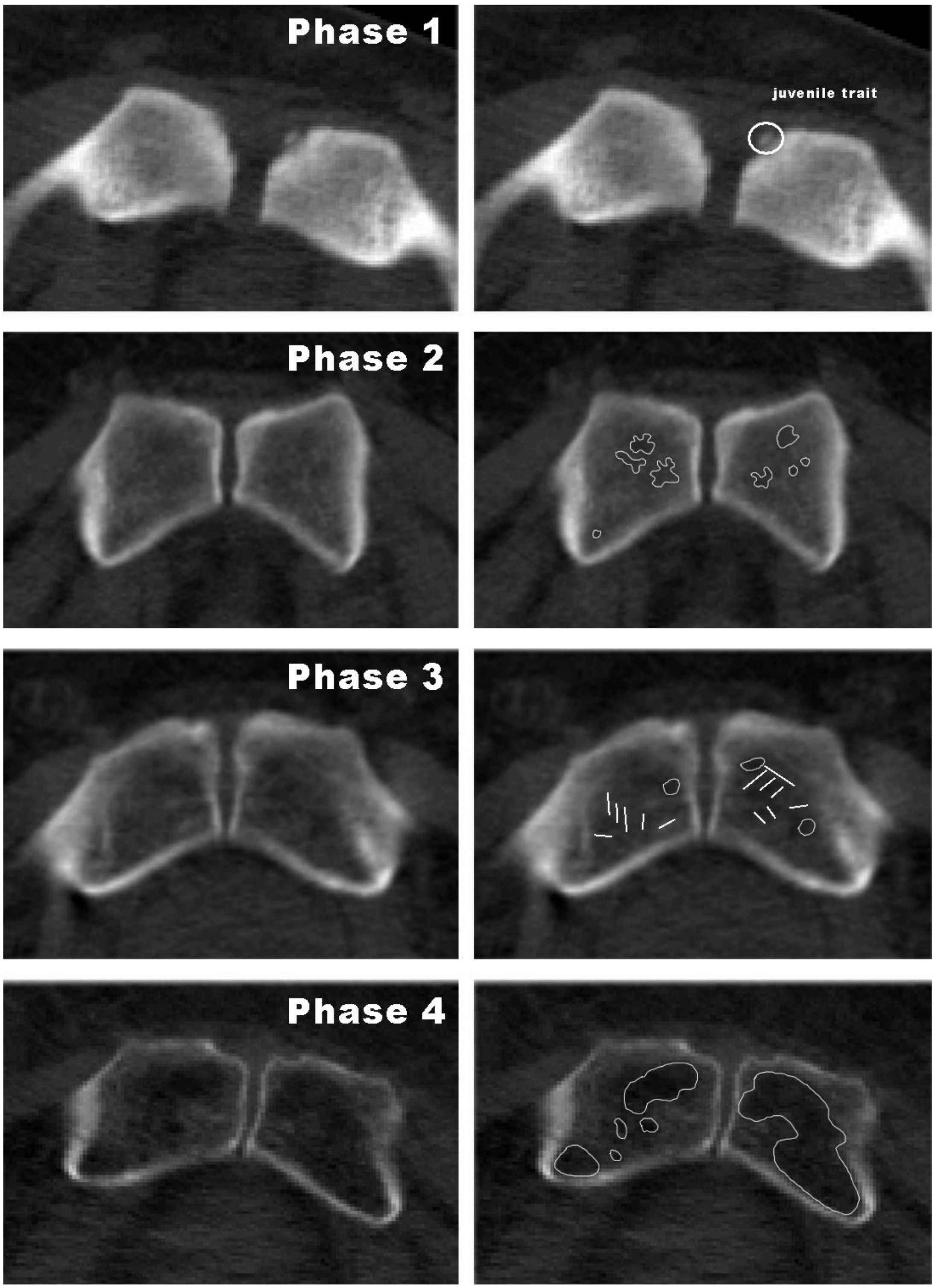


Fig 4: Juvenile trait in the pubic bone: (left) the superior ossific nodule is visible in an18-year-old male; (right) the superior ossific nodule partially fused and in a 21-year-old male.

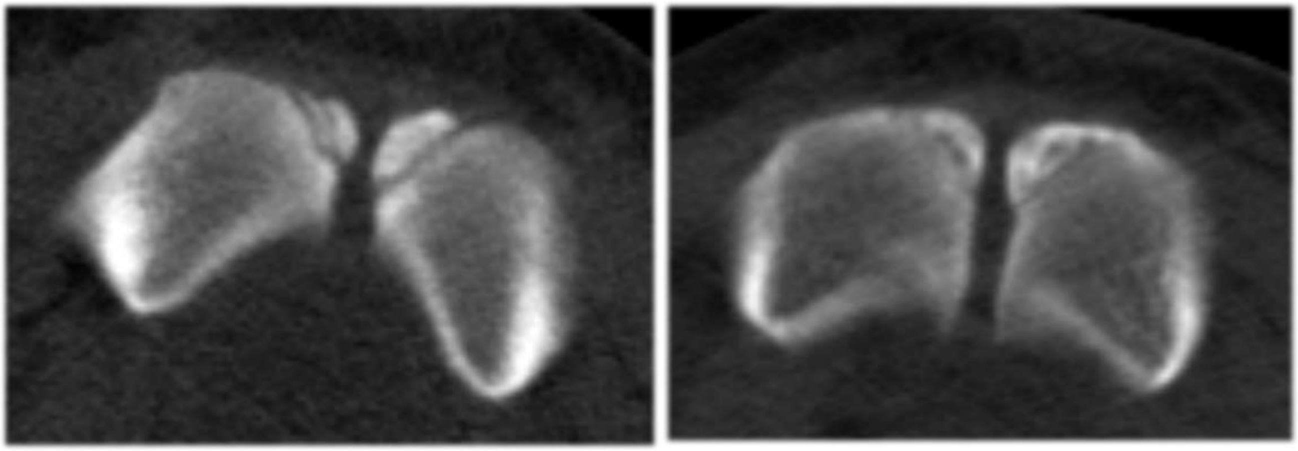


Fig 5: Region of interest in the auricular surface: (a) axial view; (b) sagittal view.

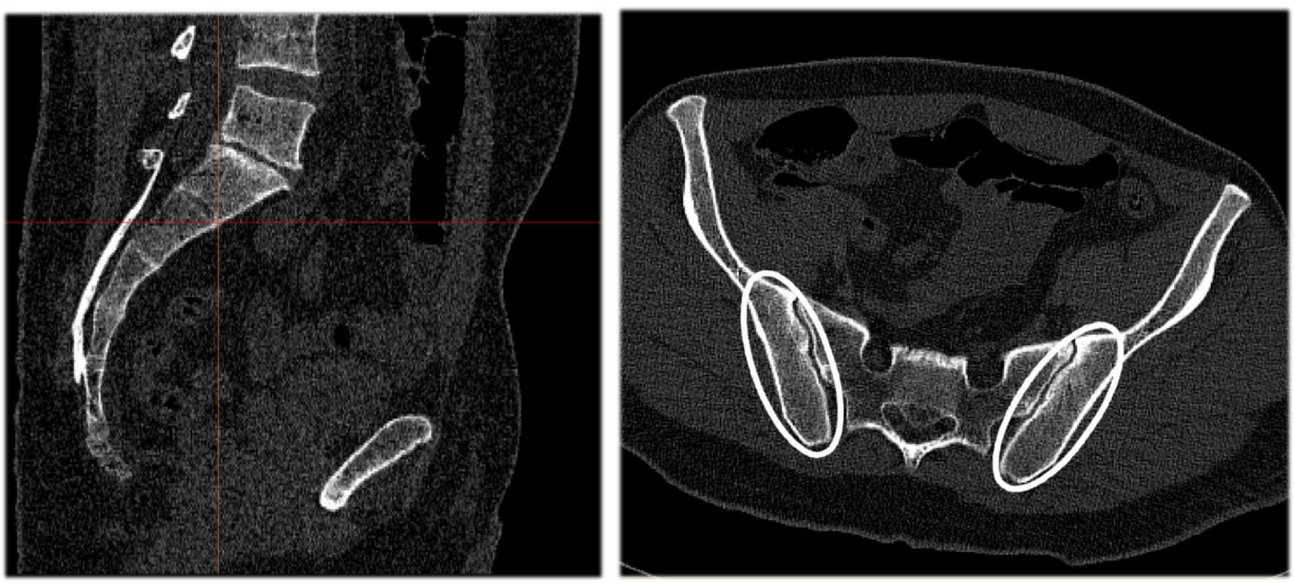


Fig 6: Auricular surface phases. All slices are displayed with a window of [-150, +1000] HU. No alteration of brightness or contrast was applied. The right image is an exact copy of the left one with annotations. Phase 1: 17-year-old male. The trabecular bone is very homogenous; no difference in density is visible; the dominant shade is lighter than the surrounding tissues. Phase 2: 22-year-old female. The density is very similar to phase 1, but there are some areas of lower density (outlined with thin white lines). Phase 3: 68-year-old female. The trabecular bone is generally less dense than phase 1 and 2; there are areas with further decrease in density (outlined with thin white lines), but they are not "empty", trabecular bone can be see inside these areas. Phase 4: 74-year-old male. the trabecular bone

further decrease in density; some "empty" area can be identified (outlined with thin white lines). Phase 5: 94-year-old male. The "empty" areas, with very dark color, cover more than 50% of the interior of the bone (outlined with thin white lines). There are fusion of the joints and ossification of the sacroiliac ligament. A final score of 6 was assigned: 5 (for the phase) + 1 (for the senile trait). **[IMPORTANT: DOWNLOAD AND USE THE HIGH RESOLUTION FIGURE]**

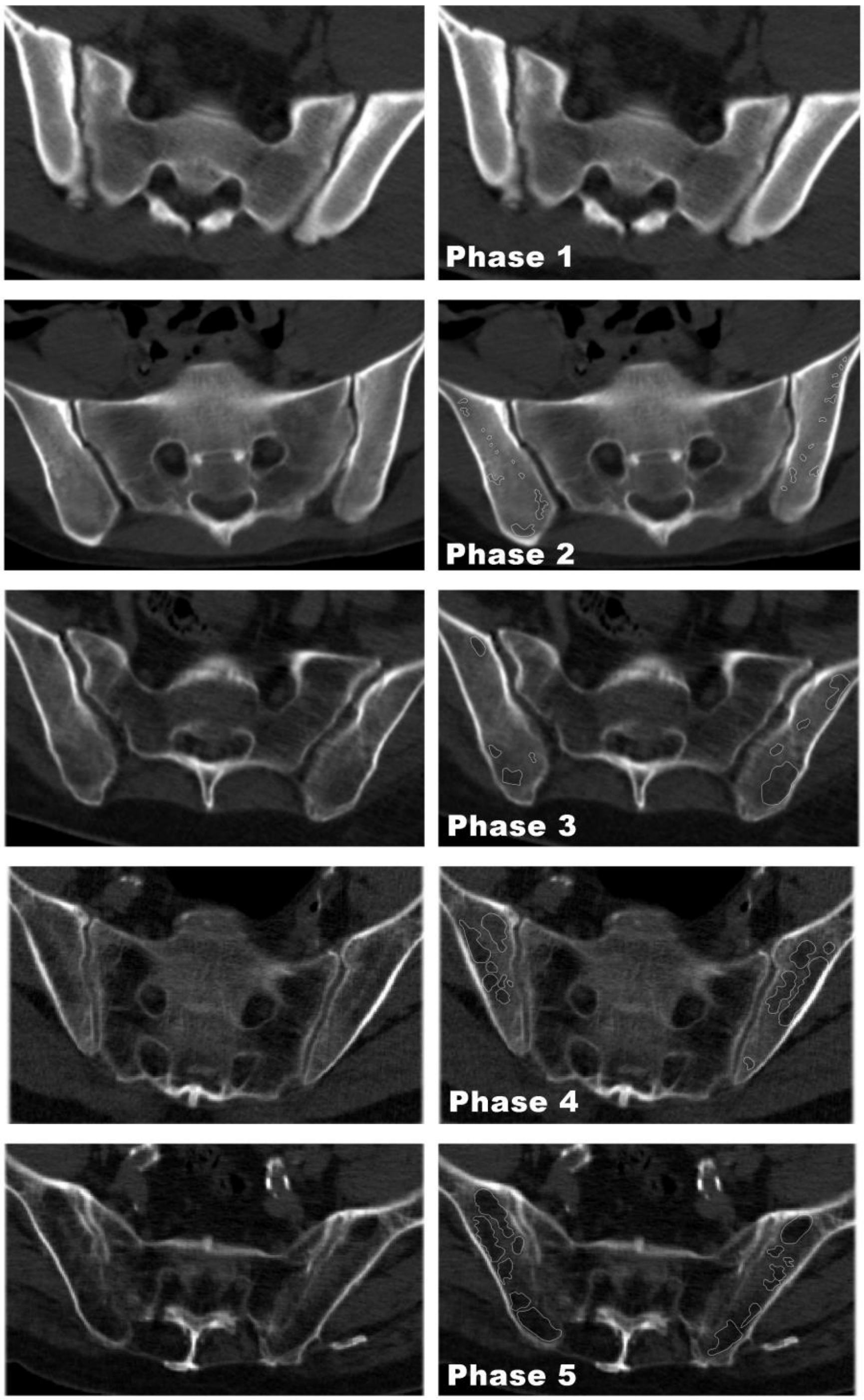


Fig 7: Senile traits of the auricular surface: the sacroiliac joints are fused and the left sacroiliac ligament is ossified in a 83-year-old male.

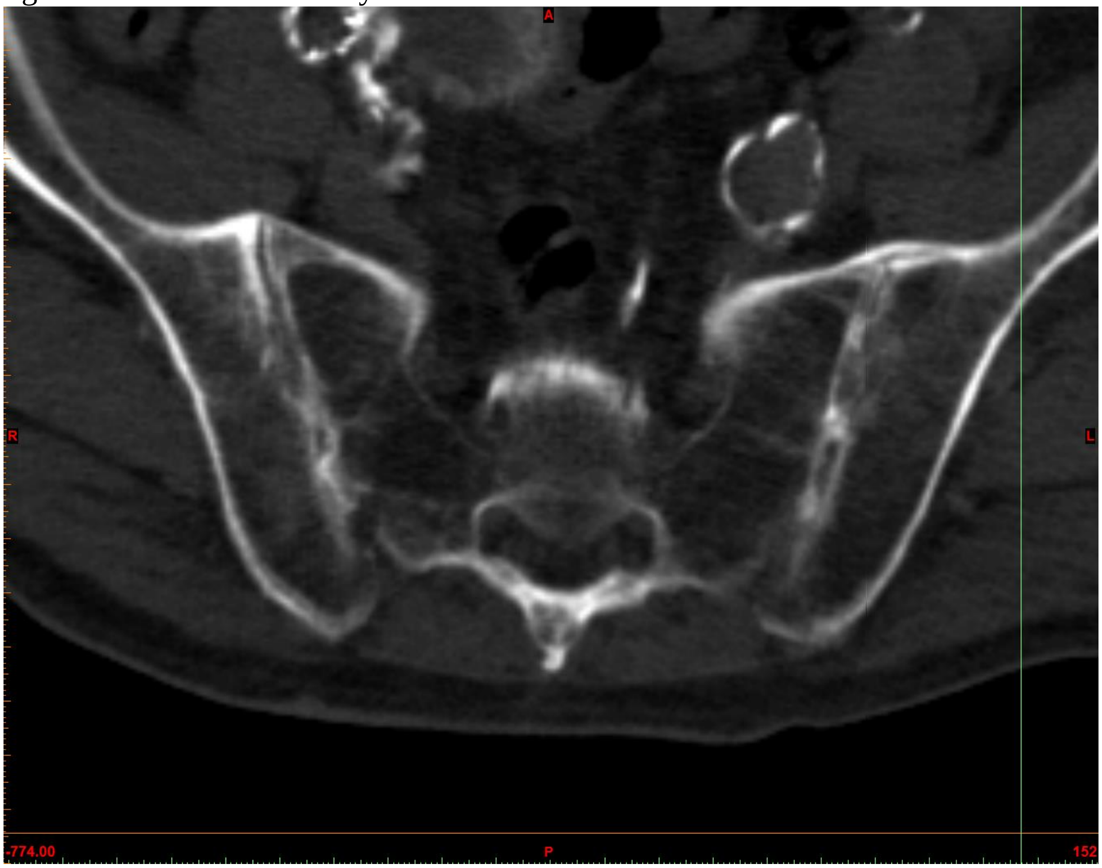


Fig 8: Scatterplot of the final score of the pubic bone (PB) against known age. Females on the left, males on the right.

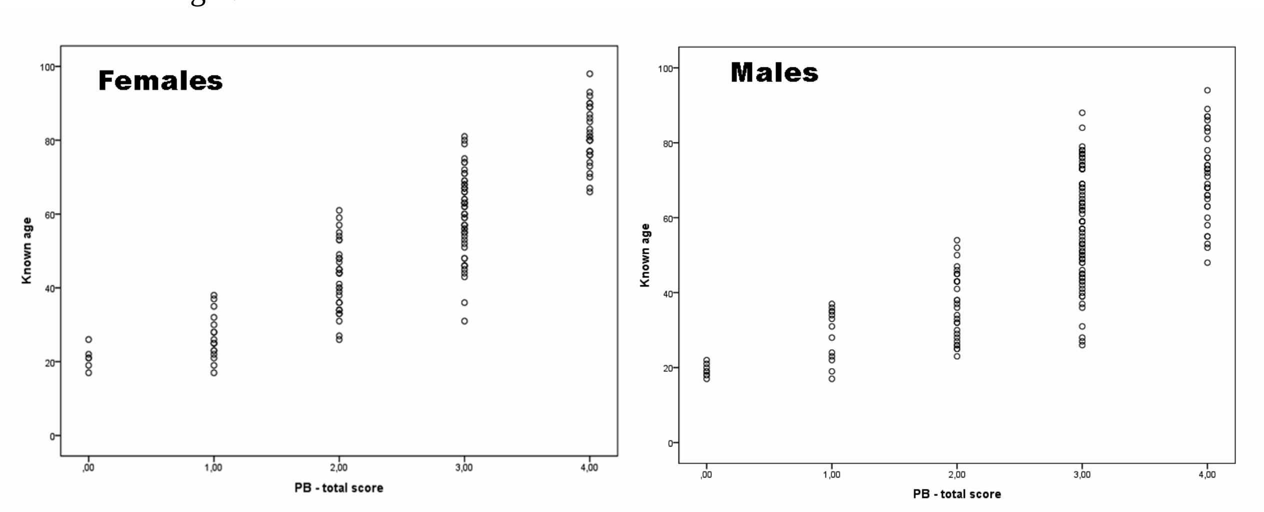


Fig 9: Scatterplot of the final score of the auricular surface against age at death. Females on the left, males on the right

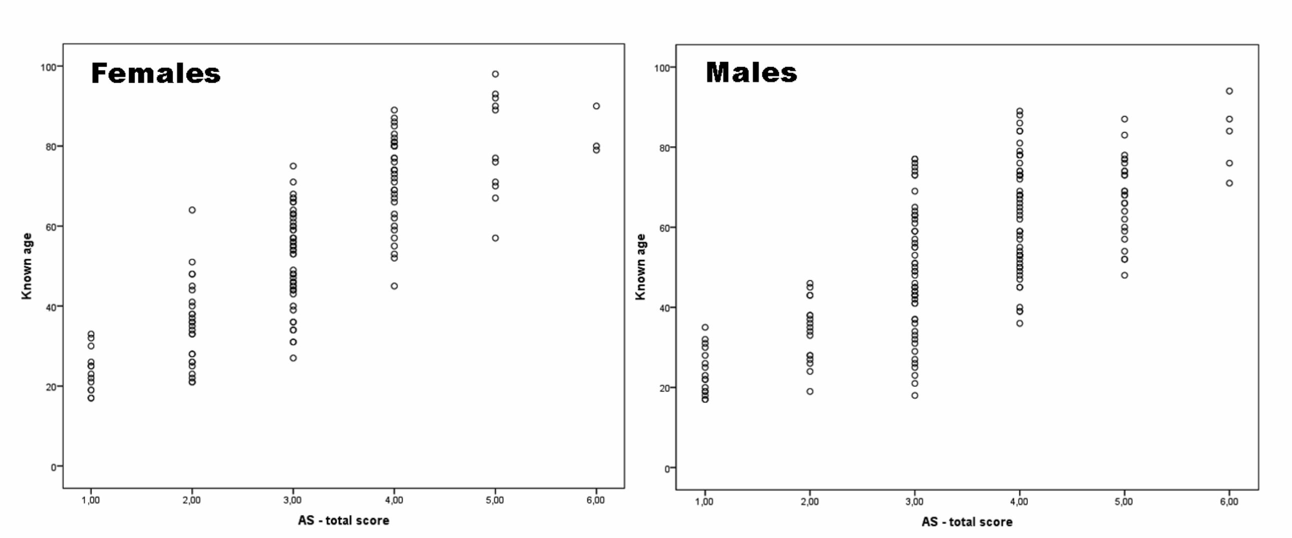
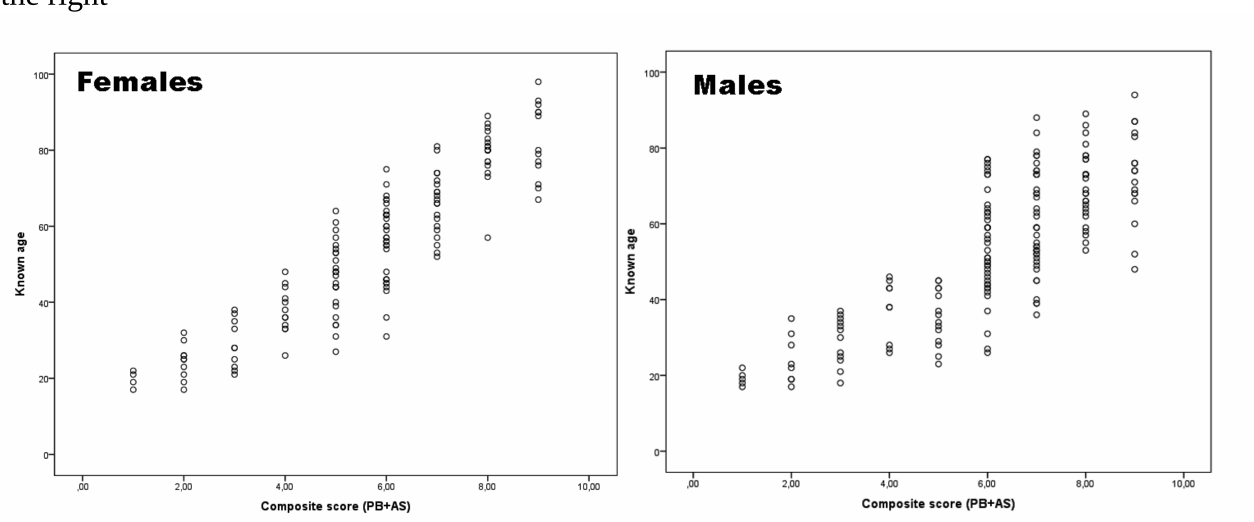
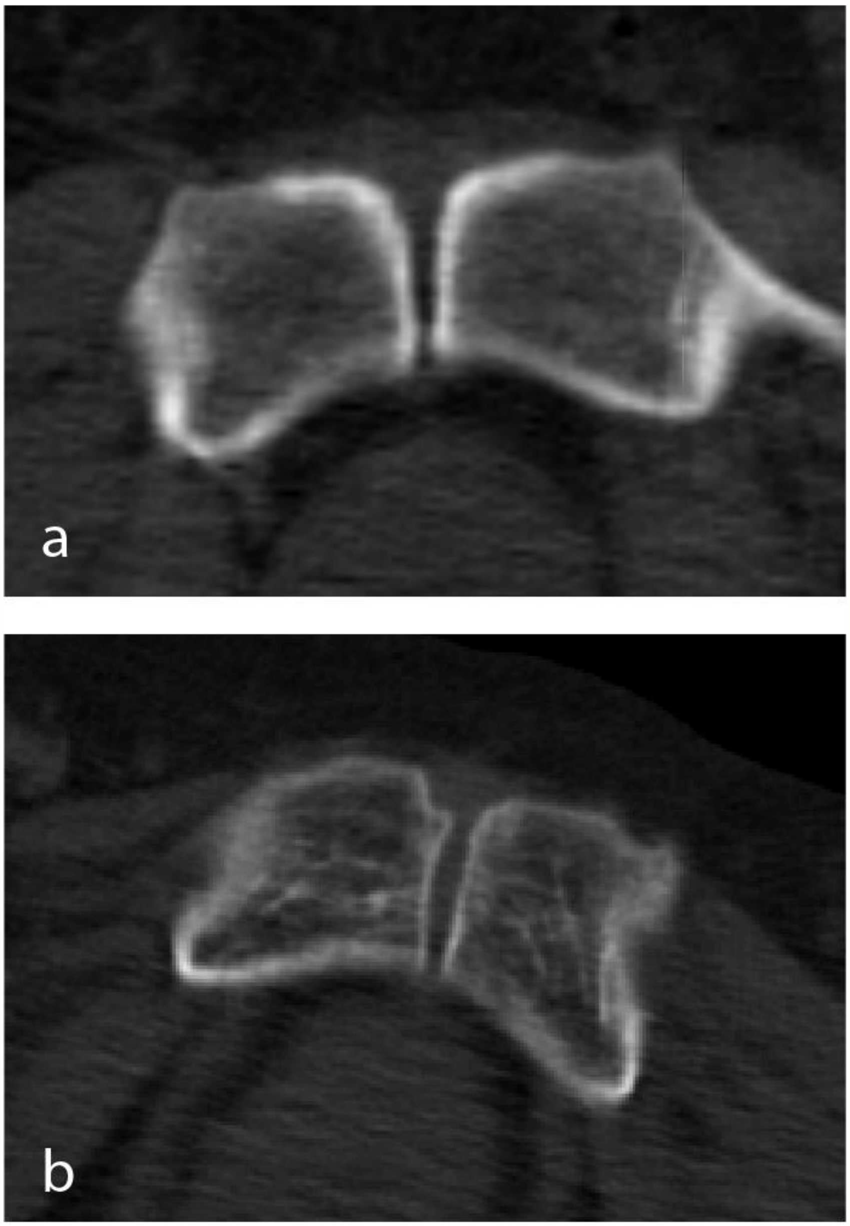


Fig 10: Scatterplot of the composite score (PB + AS) against know age. Females on the left, males on the right



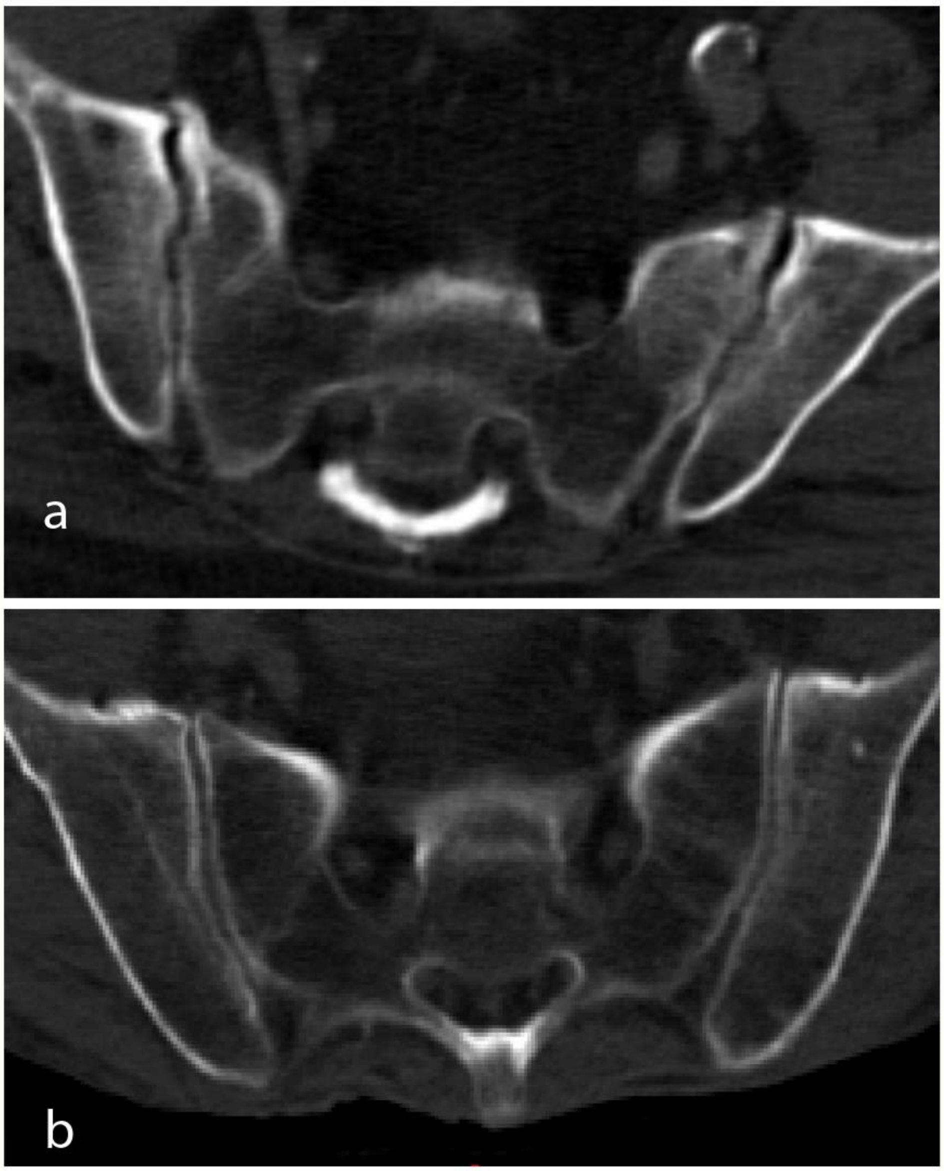
Supplementary data

Fig.1_supplementary_data: Pubic bone: a- the interior of the bone is completely filled with trabecular bone. The trabecular bone is quite dense, even though in the central part of the bone there are some small areas with decreased density. Phase 2 was assigned (age-at-death: 19-year-old male). b- Trabecular bone is not as dense as in image a, and it is possible to see very large trabecular structures. Phase 3 was assigned (age-at-death: 69-year-old- female). **[IMPORTANT: DOWNLOAD AND USE THE HIGH RESOLUTION FIGURE]**



Villa C., Hansen M.N., Buckberry J., Cattaneo C., Lynnerup N. 2013. *Forensic age estimation based on the trabecular bone changes of the pelvic bone using post-mortem CT*. Forensic Science International 233(1-3):393-402. 1

Fig.2_supplementary_data: Auricular surface phases: a- the trabecular bone appears dense, but not homogenous inside the bone. There are areas with less density covering about 30-40% of the entire bone. These areas are not "empty"; only one small rounded area in the upper part of the auricular surface on the left seems "empty". Phase 3 was assigned (age-at-death: 63-year-old male). b- All the interior bone is filled with large trabecular bone, but in this case, only few trabeculae are clearly visible. There are some "empty" areas in the lower part of both auricular surfaces. Phase 4 was assigned (age-at-death: 73-year-old male). **[IMPORTANT: DOWNLOAD AND USE THE HIGH RESOLUTION FIGURE**



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