#### SHORT COMMUNICATION



# Technical note: age estimation by using pubic bone densitometry according to a twofold mode of CT measurement

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Received: 19 March 2020 / Accepted: 15 June 2020 / Published online: 22 June 2020  $\odot$  Springer-Verlag GmbH Germany, part of Springer Nature 2020

#### Abstract

In forensic anthropology, age estimation is a major element in the determination of a biological profile and the identification of individuals. Thus, many anatomical structures have been studied, such as the pubic symphysis, which is a source of major interest due to its late maturation. One of the most well-known methods of assessment is the Suchey-Brooks (SB) system based on the morphological characteristics of the pubic symphysis. The aim of this study was to propose linear regression formulae in order to deduce chronological age from bone density, using both Hounsfield unit (HU), and mean bone density (mBD) values of the pubic symphysis. Moreover, we intended to test the reliability and then to explore the feasibility of using HU instead of mBD values for age estimation. We built retrospectively a reference sample of 400 pubic symphyses using computed tomography at a French hospital and a test sample of 120 pubic symphyses. Equations were created to establish linear regression models for age estimation. Inaccuracy and bias were calculated for individuals aged more or less than 40 years. We highlighted homogeneous mean absolute errors for both HU and mBD values, most of them being less than 10 years. Moreover, we reported a moderate overestimation for younger individuals and a very small underestimation for older individuals. This study proposes a correlation between the bone density and age of individuals with a valuable level of reliability. Finally, HU measurements seem to be suitable for linking bone density with the age of individuals in forensic practice.

Keywords Forensic anthropology · Age estimation · Mean bone density · Hounsfield unit · Pubic bone · Computed tomography

# Introduction

In forensic anthropology, establishing an age at death is a major element for the determination of a biological profile

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and the identification of individuals [1-4]. In this respect, many anatomical structures have been studied, such as the pubic symphysis, which is a source of major interest due to its late maturation [5]. One of the most commonly used and tested methods of assessment is the Suchey-Brooks (SB) system based on the morphological characteristics of the pubic symphysis [6-8]. This method entails some drawbacks, such as a lack of precision, especially for older individuals [9–12]. Besides, bone density represents a relevant biological indicator for age estimation [13] and seems to highlight an interesting correlation with age of individuals [14–16]. By protecting the integrity of the material, the contribution of computed tomography (CT) in measuring this continuous parameter appears significant [13, 17, 18] and could enable a higher precision in age estimation [5, 16].

CT is particularly used in radiological practice for measuring bone density. In this respect, mean bone density (mBD) seems to be the reference value [19, 20], even though Hounsfield units (HU) values are increasingly the target of many specialized studies [21, 22]. The aim of this study was to propose linear regression formulae from a reference sample in order to deduce chronological age from bone density, using both HU and mBD values, at the pubic symphysis. Moreover, we intended to test the reliability of the models by calculating the accuracy and precision from a test sample and then to explore the interest of using HU instead of mBD values for age estimation.

## **Materials**

#### **Reference sample**

We undertook a retrospective study of pubic bones from male and female adults undergoing clinical multi-slice computed tomography (MSCT) in a hospital in Toulouse, France, between 2015 and 2017. The MSCTs were mainly requested in the clinical context of general condition alteration or suspicion of abdominal disease. Patients with a known history of bone disease or pelvic trauma, as well as those with materials causing artifacts, such as hip replacements, were excluded. A total of 200 males (mean age 58.5 years; median age 60 years; minimum age 17 years; maximum age 94 years; SD 17.1) and 200 females (mean age 62.3 years; median age 65 years; minimum age 15 years; maximum age 99 years; SD 19.2) were included (Fig. 1).

#### Test sample

The test sample was built retrospectively and randomly by including male and female adults undergoing clinical MSCT in a hospital in Toulouse, France, in 2018. A total of 60 males (mean age 62.4 years; median age 61 years; minimum age 21 years; maximum age 97 years) and 60 females (mean age 51.6 years; median age 52.5 years; minimum age 20 years; maximum age 94 years) were recorded.

According to French law, the results of medical imaging examinations may be used retrospectively without the patient's consent when those examinations have been conducted for clinical purposes and have been recorded anonymously (Article 40–1, Law 94–548 of 1st July 1994).

## **MSCT**

Examinations were performed using an Optima 64-detector row CT scanner (General Electric Medical Systems). A Picture Archiving and Communication System (PACS, McKesson Medical Imaging Group, Richmond, BC, Canada) used by the hospital enabled us to acquire MSCT images. The image matrix was  $512 \times 512$  pixels and a softtissue filter was used. Axial reconstructions were performed every 1.25 mm with a slice thickness of 1.25 mm.

Owing to the differences in CT acquisition settings for every case, a phantom was used as an imaging standard to



Fig. 1 Histograms showing the distribution by age of the reference sample (A male, B female)

calibrate and normalize bone density values with every patient. The software used for the phantom was QCT PRO<sup>TM</sup> (Bone Mineral Densitometry Software CT Calibration Phantom, Mindways Software, Austin, TX, USA).

Scans were saved as digital imaging and communications in medicine (DICOM) files.

## Methods

## Measures of density and data recovery

Images were analyzed, and measurements were taken using Osirix software (OsiriX MD 10.0, https://www.osirix-viewer. com). The raw density values were in HU. Using the



Fig. 2 Positioning of ROIs on the rods of the phantom (A) and on right and left publs (B)

methodology of the preliminary study [14], a measurement corresponding to a 1.6-cm<sup>2</sup> circle was performed on each of the five rods of the phantom, from the least radio-opaque (the highest HU value) to the most radio-opaque (the lowest HU value). These regions of interest (ROIs) were named "ROI1," "ROI2," "ROI3," "ROI4," and "ROI5." The bone densities of the various rods of the phantom were known and permitted the calibration (Fig. 2a).

The pubis pubic bone measurements were made on an axial plane on the left and right pubis. The ROIs were named RMR and LMR (respectively for right medium region and left medium region) and were placed on the slice that depicted the largest section of the pubic auricular surface. The ROIs were placed in the center of the cancellous bone, without reaching the cortical bone (Fig. 2b).

The calibration of measurements was performed according to the phantom values for transforming the HU into mBD  $(mg/cm^3 aqueous equivalent of K_2HPO_4^1)$  [23].

Thus, this process enabled the use of two types of measurement for the purpose of meeting the initial aims: on the one hand, a phantomless value before calibration (HU value), and on the other hand, a phantom-based value after calibration (mBD value).

#### Statistical analysis

All statistical analyses and graphical representations were conducted with R 3.0.2 software (R Development Core Team, http://www.R-project.org). For the measurements of the density, intra- and interobserver variabilities were tested using the intraclass correlation coefficient (ICC) on the density values (mg/cm<sup>3</sup>) of 25 patients of each sex. The patients were chosen randomly, and the measurements were performed on the right and left pubic bones.

Pearson's correlations were undertaken between bone density in HU and mBD values and age for each sex. Linear regression equations were run for males and females.

The reliability of the reference sample was tested by the calculation of inaccuracy and bias, both before and after calibration. Mean errors between the calculated and real ages were determined for individuals aged more or less than 40 years old, according to the type of measurement (HU or mBD values). Inaccuracy was calculated as:  $\sum |estimated age - real age|/n$ , showing the average magnitude of the absolute error. Bias was calculated as:  $\sum (estimated age - real age)/n$ , expressing the tendency for either over- or underestimation of age.

## Results

## Intra- and inter-observer variability of the bone density measurements

The intra- and inter-observer variabilities of the bone density measurements were excellent, with ICC values of 0.96 and 0.86 (p < 0.0001) for males and 0.99 and 0.95 (p < 0.0001) for females, respectively.

## Statistics of the left and right pubis bone density

The normality of the left and right pubis mBD values was confirmed by the Shapiro test. A *t* test was performed to confirm no difference between the left and right sides (t = 0.1 and p = 0.91 for males and t = 0.3 and p = 0.75 for females).

## Pearson's correlation between bone density and age

The results revealed a significant negative correlation between mBD and age for male (correlation coefficient (R) = -0.54; coefficient of determination ( $R^2$ ) = 0.3; and  $p < 2.2 \times 10^{-16}$ ) and female individuals (R = -0.61,  $R^2 = 0.37$ , and  $p < 2.2 \times 10^{-16}$ ). These results were similar to the correlation between the HU value and age for male (R = -0.59,  $R^2 = 0.35$ , and  $p < 2.2 \times 10^{-16}$ ) and female individuals (R = -0.69,  $R^2 = 0.48$ , and  $p < 2.2 \times 10^{-16}$ ). The regression lines, confidence, and prediction intervals are presented in Figs. 3 (for males) and 4 (for females).

<sup>&</sup>lt;sup>1</sup> This method defines a stable bone equivalent material used as a bone reference standard to calibrate all scanning systems.





## **Regression equations**

Tables 1 (for males) and 2 (for females) present the linear regression equations, including the correlation coefficient (*R*), the coefficient of determination ( $R^2$ ), and the standard error of the estimate (SEE), according to the method of bone density measurement (before calibration: HU value; after calibration: mBD value).

### Reliability of the reference sample

Table 3 (for males) and Table 4 (for females) show the inaccuracy and bias for individuals aged more or less than 40 years old according to the method of bone density measurement.

Inaccuracy was slightly higher for males aged over 40 years than for males aged 40 years or less, regardless of the calibration or not. For females, inaccuracy was roughly the same in all groups. Generally, bias was very low.

## Discussion

While methods in forensic anthropology are inevitably subject to further evolution due to the search for reliability and precision, a few studies have tended to use bone mineral density for age estimation [13, 15, 17, 18, 24, 25]. Our study reported a correlation coefficient value between bone density and age of -0.54 for males and -0.61 for females, similar to the correlation between the HU value and age, and substantially the same as in the preliminary study (-0.55 for females and -0.62 for males) [14]. These results are in accordance with the values in the literature, namely, those reported by Lopez-Alcaraz et al. [15]. More broadly, the best correlation found in the literature is -0.76 and regards the area of Ward in the proximal extremity of the femur [13]. Moreover, in light of our results, the intra- and inter-observer variabilities were excellent and reinforce the observation that bone mineral density is a reliable criterion for age estimation.

In addition to showing a significant correlation with age, bone density has the advantage of being easy to measure by CT and to constitute a continuous parameter. The latter seems to run counter to the morphological bony criteria used in the SB system, which are likely to show reproducibility [2, 4, 26–28]. Moreover, we may consider it more interesting to use a quantitative criterion such as bone density in order to develop linear regression models for age estimation.

The methodological originality of our study can be found in the use of phantoms to calibrate and transform raw



**Fig. 4** Scatter plot of the HU value (**C**) and mean bone density (mBD;  $mg/cm^3$  aqueous equivalent of  $K_2HPO_4$ ; **D**) vs. age in females

Table 1         Regression equations f	for	males
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	R	$R^2$	Regression equation	SEE
Before calibration	- 0.59	0.35	HU = -1.51 (age) + 168.67	8.97
After calibration	-0.54	0.3	mBD = -1.26 (age) + 165.28	8.24

scannographical measurements (HU) into density values. Thus, this approach enables the creation of a general and uniform standard for all measurements, whatever the CT acquisition parameters.

Regarding the reliability of our reference sample, we firstly chose to separate the individuals into those aged more or less than 40 years. Indeed, this value is often considered as the threshold beyond which age estimation by the different methods is inaccurate and underestimated [9–12, 29–34]. As regards our results concerning bias with mBD values, we observed a moderate overestimation for younger individuals and a very small underestimation for older individuals. This point is well supported in the literature [20, 35].

Concerning inaccuracy, we highlighted homogeneous mean absolute errors for both sexes and age groups, most of them being less than 10 years. These results are promising and could enable consideration of the use of this linear regression model for particular forensic casework. Curate et al. reported a mean absolute error of always over 10 years by applying the Fernando Castillo and Lopez Ruiz method to skeletal samples, which is barely acceptable for forensic cases [17, 24].

Moreover, we were interested in studying the potential differences between the use of HU and bone density values, specifically regarding inaccuracy and bias. Mean bone density as described seems to be the gold standard in the radiological literature [19, 20], and dual X-ray absorptiometry measurements are particularly used to quantify osteoporosis. However, HU values seem to be increasingly used in CT in regard to large-scale studies [21, 22]. Incidentally, they may be of interest in medical practice through a variety of clinical applications [36]. Whereas it seems important to consider the CT model and the kilovolt settings, which could have an effect on HU values [21, 37-39], some radiological studies have demonstrated a very substantial correlation between phantomless and phantom-based bone density values [19, 20]. Actually, we noticed almost no differences between the two groups (before and after calibration) for the reliability of

 Table 2
 Regression equations for females

	R	$R^2$	Regression equation	SEE
Before calibration	- 0.69	0.48	HU = -2.15(age) + 211.67	10.36
After calibration	-0.61	0.37	mBD = -1.86(age) + 209.91	11.06

 Table 3
 Mean absolute error (inaccuracy) and mean error (bias) for males

Before calibration			After calibration		
Age	п	Inaccuracy	Bias	Inaccuracy	Bias
≤40	8	7.8	- 0.9	7.4	2
>40	52	9.8	-0.3	11.4	-0.7
Total	60	9.5	-0.4	10.8	-0.3

our sample, no matter what the age and sex of the individuals. Thus, it would not appear to be particularly necessary to use the phantoms to calibrate and normalize bone density values for age estimation. We can hypothesize that HU measurements are sufficient to link bone density with the age of individuals in forensic practice.

In this study, we used a hospital sample with living individuals to deduce bone density values from CT. For living subjects, the evolution of bone density is well known and described in the literature, depending on, among others, the age and the gender of individuals. However, with bones of cadavers, where marrow and soft tissues tend to disappear, these changes seem to be much more unpredictable. Indeed, they are prone to taphonomic alterations, such as through the action of air or putrefaction gas on the bones [40]. Similarly, it is known that radiological images, such as by CT, are often subject to artifacts that could distort bone density measurements [25].

From a significant French reference sample, this study proposes methodological and forensic benefits. On the one hand, the use of bone density, a continuous parameter, seems to be useful for age estimation because of its reproducibility. On the other hand, we report regression equations correlating bone density with the age of individuals, with a valuable level of reliability. Thus, we can hypothesize that bone density measurements could sharpen age estimation by the SB method, especially for older individuals. In addition to this possible application in forensic practice, we could also consider using this method for age estimation in living individuals. However, this point meets with some problems. Firstly, that implies the

 Table 4
 Mean absolute error (inaccuracy) and mean error (bias) for females

Before calibration				After calibration	
Age	n	Inaccuracy	Bias	Inaccuracy	Bias
≤40	18	6.9	- 1.6	8.2	2.8
>40	42	7.3	0.7	7.2	- 0.06
Total	60	7.1	0.02	7.5	0.75

application of CT radiation in a non-therapeutic context which can involve some ethical issues. Secondly, in terms of accuracy, we highlighted that the SEE values appear to be very high, exceeding 8 years for males and 11 years for females, which is absolutely not acceptable in forensic practice, especially for typical legal age thresholds (between 12 and 21 years in most of the countries). Thus, in order to apply the minimum age concept [41] and avoid age overestimation in this relevant age range [16], it seems to be interesting to extend this study to a wider population and to consider many factors, such as ethnicity, physical activity, and socioeconomic status, which affect bone density changes.

To conclude, the authors suggest that age estimation could be achieved by multiple linear regression models, which may contribute to more exhaustive and accurate. Furthermore, it would be relevant to apply the regression equations obtained from our sample of virtual pubic bones to dry bones and to study other anatomical regions to link bone density with age.

Acknowledgments The authors wish to particularly thank Mrs. Agathe BASCOU and Mrs. Eulalie PEFFERKORN, students in physical anthropology at the University of Toulouse (Paul Sabatier), for participating in this work.

Authors' contribution Olivier Dubourg: conceptualization, writingoriginal draft, formal analysis, investigation. Marie Faruch-Bilfeld: resources, software. Norbert Telmon: supervision, validation, project administration. Pauline Saint-Martin: methodology, investigation, validation, writing-reviewing and editing. Frédéric Savall: methodology, conceptualization, validation, formal analysis, writing-reviewing and editing.

## **Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflicts of interest.

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