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Ultrasonic characterization of post-mortem interval (PMI) of human bones

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Abstract. In order to improve the evaluation of the post-mortem interval (PMI) of human bone, experts in forensic anthropology from the french national gendarmerie are interested in new characterization methods and in particular non-destructive testing (NDT). If Nile Blue colorimetric methods currently allow dating up to a hundred years, these techniques alter the bone which is evidence in a criminal investigation. In order to avoid this degradation, the work presented hereby proposes a method for characterizing PMI using ultrasonic methods that have proven successful for the NDT of complex materials, including bones in medical applications. The objective is to identify relevant ultrasonic parameters representative of PMI. The propagation velocities of compression and shear waves are measured through parallelepiped samples of cortical bone taken from human femurs while respecting the anatomical orientation of the bone. These measurements carried out in the 3 directions of space make it possible to calculate the diagonal coefficients of the stiffness matrix C_{ij} . Measurements of the propagation velocity of compression waves are carried out in transmission, using Pinducer™ transmitter/receiver immersed in water. The measurement of shear wave velocities is made via a second non-immersed device using contact transducers. The results presented come from ultrasonic signals measured on bones, coming from individuals with similar pre-mortem parameters, whose PMI varies between 1 and 2500 years. The six diagonal coefficients of the stiffness matrix are represented and discussed in terms of the PMI. A first classification of bones is possible according to their PMI. In order to assist the analysis and interpretation of the results, a single parameter is proposed, the Trace of the stiffness matrix ($\text{Tr}(C_{ij})$), confirming the possible contribution of the ultrasonic techniques to be used for the dating of human bones.

1. Introduction

Bone is a particularly complex material to study because of its internal structure, its geometry and the differences between individuals. Many methods make it possible to study the degradation of bone over time after the death of an individual (i.e. phenomenon called diagenesis). These methods can be [1]:

biochemical estimation of the level of organic elements still present in bone; radioisotope measurement of levels of unstable chemical components still present in the bone; and histological study of the internal structural degradation of bone.

In the case of forensic anthropology, when a crime scene investigation is opened, a quantity of individualizing information is collected on the victim to define their biological profile and ultimately obtain information on the criminals. When only the bones remain of the victim (in particular cortical bone), one of the fundamental pieces of information is the estimation of the post-mortem interval (PMI): the time between the death of the individual and the discovery of the body. This interval determines the legal outcome or not of most of investigations: when PMI exceeds the limitation period (6-20 years), a court will not accept evidence according to the french Law. To determine PMI, thanatology and entomology methods can be used for intervals less than 1 year, but beyond, colorimetric methods and radioisotope methods should be favored. However, the methods accuracy and precision have limits due to the multiple parameters influencing the determination of PMI, such as pre-mortem parameters of the individual (age, sex, physical state, illness) and environmental parameters of place of discovery (humidity, temperature, burial, geological zone).

The French national gendarmerie forensic department (IRCGN) was interested in new characterization methods and in particular non-destructive testing (NDT). Since Nile Blue colorimetry methods currently allow dating up to a hundred years, these techniques alter the bone which is evidence in a criminal investigation [2]. In order to avoid this degradation, the work presented hereby proposes a method for characterizing PMI using ultrasonic techniques that have proven successful for the NDT of complex materials [3], including bones in medical applications [4].

The motivation of this study is that mechanical behavior depends on composition and structure of the material. Bone mechanical behavior requires the use of hypotheses to reduce the number of parameters in order to approach the bone using simple models. Many works have proposed to circumvent the problems linked to the geometry of the bone by studying small samples [5-7]: these studies attempt to approach the mechanical behavior of the bone by considering the medium as infinite. The mechanical behavior of the bone is thus associated with an anisotropy model whose parameters are the stiffness coefficients.

In this paper, we propose to study the stiffness coefficients of bone and their behavior according to PMI. These coefficients will make it possible to approach the behavior of the bone by an anisotropy model and to judge the relevance of this model in the post-mortem time. Independently of the model, the evolution of the measured coefficients will allow us to know if the degradation of the bone matrix linked to diagenesis allows a distinction between different groups of individuals with similar PMI.

This study is hereby presented as follows. Firstly the theoretical background regarding the ultrasonic wave propagation in cortical bone will be presented. Only compression and shear waves were assumed to be transmitted in the sample (direct transmission with no reflection or diffraction). The wave velocities were assumed to be independent of the frequency over the frequency range studied and the waves attenuations were neglected. Secondly, the experimental technique used to study samples with different PMI is presented. Finally, results will be presented and the correspondences and discrepancies are discussed. In order to assist the analysis and interpretation of the results, the Trace of the stiffness matrix ($\text{Tr}(C_{ij})$) is used as unique parameter confirming the possible contribution of the ultrasonic techniques for the dating of human bones.

2. Theoretical models of ultrasonic wave propagation in cortical bone

In this section, the theory behind the proposed non-destructive evaluation method is presented, starting with the different models of bone anisotropy and the study of the ultrasonic wave propagation in homogeneous solids.

In this study, we focused on on the diaphyseal/cortical zone of the human long bone considered as an elastic medium (inhomogeneous and anisotropic). Pre-mortem human bones are mainly Haversian, and which are usually considered to be transversely isotropic [5-7]. However, we will investigate post-mortem bone in order to check if this behavior is conserved during the aging after death and its homogeneity.

The notion of anisotropy describes a material whose mechanical behavior varies depending on the axis of study and the nature of the stress applied. These theoretical notions being widely presented in the literature [8-10], only a brief reminder is proposed allowing us to introduce the elements necessary for understanding anisotropy.

The objective of mechanical monitoring of a material is to predict its evolution when it is subjected to stress. Hooke's law relates the stress T_{ij} applied to a solid material to the strain S_{kl} through the elastic stiffness tensor C_{ijkl} by the following formula:

$$T_{ij} = C_{ijkl} S_{kl} \quad (1)$$

The index $ijkl$ are integer numbers from 1 to 3 according to the axes (x, y, z) of geometric system in Cartesian coordinates. Thus, the maximum possible number of stiffness constants in Hooke's law is $3^4 = 81$.

The displacement of matter following ultrasonic stimulation remains within the elasticity limit: the material can return to its initial state after the loading cycle. This remark allows us to define the system as being in static equilibrium, ensuring the condition of motion equilibrium. This condition forces the symmetry of the tensors T and S , allowing us to use Einstein's notation and thus simplify the writing of Hooke's law:

$$\begin{array}{cccc} 11 & 12 & 13 & 1 & 6 & 5 \\ 21 & 22 & 23 & = & 6 & 2 & 4 \\ 31 & 32 & 33 & & 5 & 4 & 3 \end{array} \quad (2)$$

This transformation implies that Hooke's law can then be written:

$$T_i = C_{ij} S_j \quad (3)$$

The index i and j being between 1 and 6, the tensor C_{ij} is represented in the form of a matrix of 6 columns and 6 rows and is composed of 36 coefficients. This number of coefficients can be reduced to 21 thanks to the symmetric character of the tensor corresponding to the triclinic system. On the other hand, the organization of anatomical elements explains anisotropic behavior of cortical bone. Thus, the degree of anisotropy of the cortical bone makes it possible to approach it by a material with orthotropic symmetry [11], where z is denoted as the axis of symmetry.

Taking into account orthotropic symmetry, the tensor can then be reduced to 9 coefficients:

$$\text{Orthotropic } C_{ij} = \begin{bmatrix} C_{11} & C_{12} & C_{13} & 0 & 0 & 0 \\ C_{12} & C_{22} & C_{23} & 0 & 0 & 0 \\ C_{13} & C_{23} & C_{33} & 0 & 0 & 0 \\ 0 & 0 & 0 & C_{44} & 0 & 0 \\ 0 & 0 & 0 & 0 & C_{55} & 0 \\ 0 & 0 & 0 & 0 & 0 & C_{66} \end{bmatrix} \quad (4)$$

Modeling as a transversely isotropic elastic solid material [5-7,12,14] and isotropic were also considered [13] allowing reduction to 6 or 3 coefficients.

$$\text{Transversely isotropic } C_{ij} = \begin{bmatrix} C_{11} & C_{12} & C_{13} & 0 & 0 & 0 \\ C_{12} & C_{11} & C_{13} & 0 & 0 & 0 \\ C_{13} & C_{13} & C_{33} & 0 & 0 & 0 \\ 0 & 0 & 0 & C_{44} & 0 & 0 \\ 0 & 0 & 0 & 0 & C_{44} & 0 \\ 0 & 0 & 0 & 0 & 0 & C_{66} \end{bmatrix} \quad (5)$$

The study of these matrices can be done by monitoring certain parameters. In particular, the choice of model can be defined using anisotropy ratios [15]. In the case of the transversely isotropic model, the expected C_{11}/C_{22} and C_{44}/C_{55} ratios are close to 1.

Considering ultrasonic wave perturbation of the material, combining the motion conservation equation, Hooke's law, and the kinematic relations between the strains produced by the particle displacement due to wave propagation, the wave equation can be deduced [10]. The progressive plane wave solution leads to the Christoffel's equation for which the phase velocities are solutions to an eigenvalue problem. The diagonal coefficients of the stiffness matrix can then be associated with the propagation phase velocities with Einstein's notation:

$$C_{ij} = \rho V_i^2 \quad (6)$$

with ρ the mass density. With the knowledge of this last quantity and measuring the velocities of pure compressional and shear waves propagating along the three principal axes, the diagonal elements of the stiffness matrix, can be therefore computed, as well as the Trace of the stiffness matrix and the anisotropy ratios.

3. Experimental technique

In this section, the experimental technique is presented, starting with the experimental protocol, following samples conditioning, the description of the ultrasonic measurement and finishing with the hypotheses to be checked. The measurement technique is based on classical approach for the analysis of ultrasonic echoes in locally parallel samples.

Experimental protocol

The experiments were performed on 11 individual femur samples belonging to 4 different post-mortem interval (PMI) groups. Each individual sample was cut into cubes (3 cubes per individual) in order to carry out the ultrasonic measurements. The dimensions of samples are sized with digital caliper and mass density is controlled using a weighing machine and a density kit.

Ultrasonic waves measurements are carried out in transmission mode following the three axes of the cubic samples. Compression waves are measured using transducers immersed in water, while shear waves are measured via a second non-immersed device using contact transducers. All received ultrasonic signals were recorded using a digital oscilloscope and the propagation velocities were determined using propagation path length and time-of-flight detection. Finally the diagonal elements of the stiffness matrix are computed from the mass density and the velocities.

Samples conditioning

The human bones delivered by the IRCGN have already been cleaned and washed before their arrival. This work focused on samples of cortical bone from adult human femurs. Pre-mortem parameters (age, sex, physical state, illness) allowing determination of the identity of individual and exact circumstances of their discovery are unknown. However, the samples provided come from similar discovery environments according to the IRCGN.

The set of human bones studied is composed of 11 individuals of different PMI: three from 1 to 3 years (Group 1), three from 10 to 13 years (Group 2), three from around 50 years (Group 3) and two of archaeological ages (Group 4). The group 1 is considered as control group (similar to medical studies), group 2 has PMI close to the limitation period, group 3 has PMI beyond limitation period and group 4 represents extreme situation. The sample summary is shown in Table 1.

Table 1: Summary of PMI groups and porosity measurements.

Group 1 (G1)	1 year ($\phi = 5.8 \%$)	3 years ($\phi = 10.7 \%$)	3 years ($\phi = 5.2 \%$)
Group 2 (G2)	10 years ($\phi = 5.3 \%$)	11 years ($\phi = 11.6 \%$)	13 years ($\phi = 7.9 \%$)
Group 3 (G3)	~50 years ($\phi = 6.4 \%$)	~50 years ($\phi = 4.1 \%$)	~50 years ($\phi = 5.6 \%$)
Group 4 (G4)	500 years ($\phi = 6.1 \%$)	2500 years ($\phi = 5.0 \%$)	

X-ray micro-tomography scans on the bones entrusted by the IRCGN were carried out in the Laboratory of Mechanics and Acoustics of Marseille before their cutting in cube. Each individual bone was cut into three parallelepipeds (hereinafter named cube) of dimension (3-6 x 5 x 5 mm): the X-ray images made it possible to verify the porosity of the samples (Table 1). Even if the dimensions between individuals vary, the cutouts sought to maximize the dimensions of the sides, particularly in the radial axis, in the cortical thickness: these dimensions are between 3 and 6 mm. Some samples, which had thin cortical thickness and/or had high porosity, made difficult data analysis (in red in Table 1): for these samples in particular, other cubes were cut at different locations on the bone.

The cuts were made using a diamond saw (IsoMet™ Low Speed Saw, Buehler, Lake Bluff, IL, USA) with a slow rotation, following directions shown in Figure 1A. This saw, particularly suited to cutting fragile samples, makes it possible to hold the sample and apply weight to it when cutting to control the pressure on the saw. The rotating blade is bathed in an oil bath to limit friction with the sample when cutting. Finally, the arm holding the sample can be shifted above of the blade without having to touch the sample, thus ensuring the parallelism of the opposite faces of the cube.

The three cubes from each individual are cut one after the other along the axis of the bone fibers: the samples allow to perform three measurements (on the three cubes) on a length of ~1.5 cm (3 x 0.5 cm) along the axis of the fibers (Figure 1B). This cutting protocol allows to question the local homogeneity of the bone along the fibers.

Before carrying out compression waves measurements, the cubic samples were degassed (also allowing measurement of mass density using the Archimedes' principle), while for the shear wave measurements the samples were dried to facilitate the wave propagation. The mass density was therefore obtained using a weighing machine (Ohaus PX323M) and a density kit (Voyager 610, Ohaus Corporation, FlorhamPark, NJ, USA).

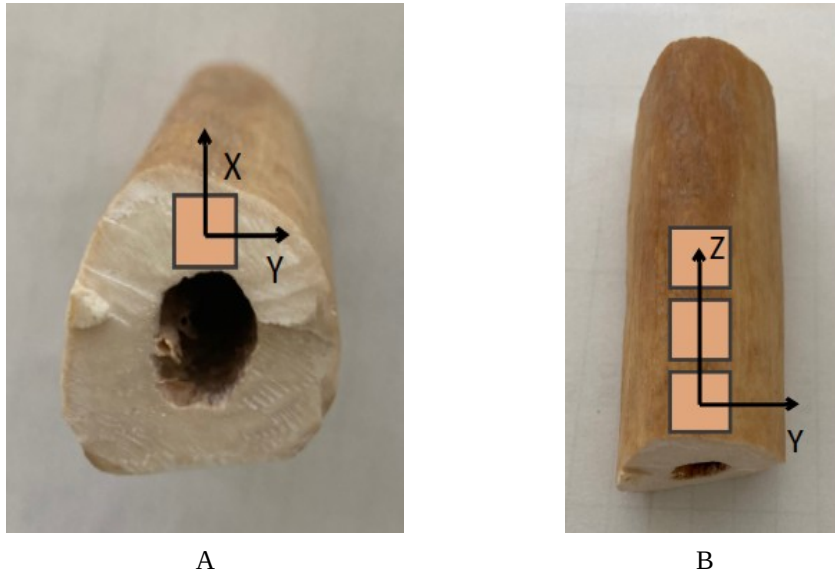


Figure 1: Bone sample conditioning. A) XY plane view. B) YZ plane view.

Ultrasonic measurements

Figure 2 shows the principle of measurement of ultrasonic velocities V_{ii} of locally parallel sample. The experiments were carried out in the transmission mode between two coaxial transmitter/receiver transducers (5 MHz Pinducer™, CTS Valpey Corporation, Hopkinton, MA, USA) as described in [15]. Each transducer was driven using a pulse-receiver generator (Sofranel 5077 PR, Olympus, USA) including the voltage amplifier. The radio-frequency signals (RF-signals) were conveyed from the oscilloscope (Agilent DSO1514A, Santa Clara, CA 95051, USA) to a personal computer using a USB interface file transfer, and stored. All processing algorithms were implemented using Matlab (The MathWorks, Inc., Natick, Massachusetts, USA).

In order to operate with well-defined coupling conditions an immersion technique was used [16-18]. Measurements were performed, one without the sample (reference measurement) and the other with the sample. This procedure was repeated five times per cube axis. The energy delivered by the transmitting transducer and the distance to the receiving transducer remain invariant for both measurements.

In order to calculate the last three diagonal coefficients of the stiffness matrix, the shear wave velocities were measured. These waves propagate poorly in the water, a contact measurement is necessary. To measure the propagation velocities shear waves in the three planes, a test bench was created: as for the measurements in immersion, this bench has already been used to characterize children's bones [15]. As in immersion technique, the experiments were carried out in the transmission mode between two coaxial transmitter/receiver transducers (2.25 MHz Panametrics V154, Olympus, Waltham, Massachusetts, USA, Figure 3) [19]. Measurements were performed, one with a reference sample (i.e. a homogeneous and isotropic parallelepiped aluminum sample, 3 mm thick) and the other with the bone sample. The coupling medium used between the transducers and the samples was a viscous Shear Waves Coupling suitable for transverse stimulation unlike aqueous coupling. The shear

measurements were made according to a plane: an axis of propagation i and an axis of polarization j for the velocity V_{ij} . In total, six velocity measurements can be made according to the three axes and reversing the direction of propagation and polarization.

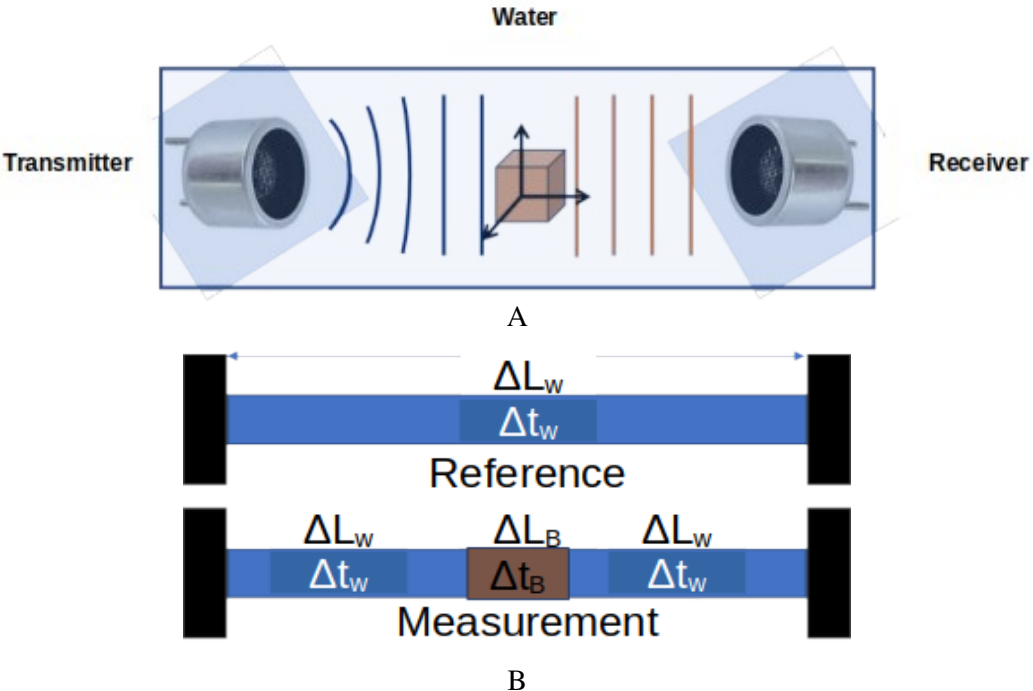


Figure 2: Immersion method. A) Principle. B) Definition of distances and propagation times.

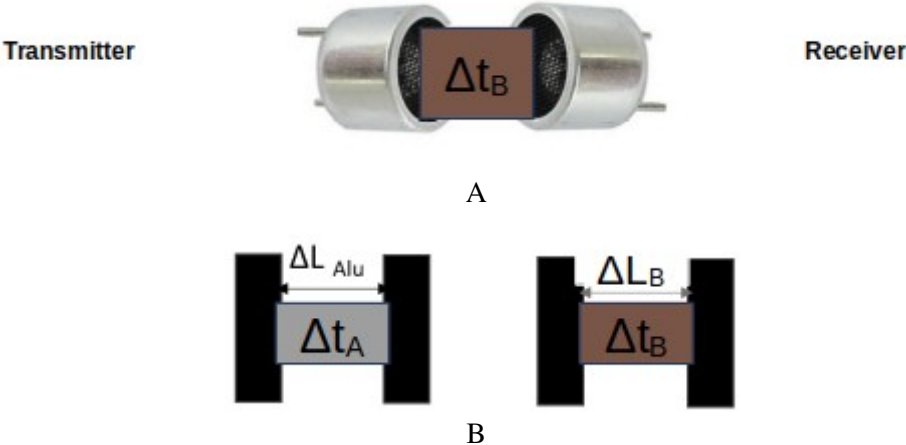


Figure 3: Contact method. A) Principle. B) Differential measurement using reference aluminum and bone samples.

For the frequency range studied, the average wavelength for longitudinal and shear waves in human cortical bone is approximately of ~0.8 mm, which let us to study bone structure at mesoscopic scale. This wavelength is sufficient to avoid echo overlapping according to our samples sizes, and therefore to be able to compute wave velocities using temporal signal processing. Thus, time-of-flight difference between measurement with bone sample and reference sample (denoted Δt), is determined by cross-correlation of the recorded RF-signal. Finally, compression and shear waves velocities are determined using equations 7 and 8 respectively and diagonal coefficients of stiffness matrix can be deduced using equation 6.

$$V_{ii} = \frac{V_{water}}{1 - \frac{\Delta t_{ij}}{\Delta L_B} V_{water}} \quad (7)$$

$$V_{ij} = \frac{\Delta L_B}{\frac{\Delta L_{alu}}{V_{alu}} - \Delta t_{ij}} \quad (8)$$

Assumptions to be checked

Even if the aim of ultrasound measurements is to identify changes in the bone over post-mortem time, many parameters can lead to differences between individuals studied, whether related to PMI or not, known or not. In order to limit the number of parameters monitored, several hypotheses are made about the mechanical behavior of the bone.

The first hypothesis states that local homogeneity of the bone allows us to consider all measurements on bone as equivalent regardless of the cubic sample chosen. The interest of this hypothesis is to question the human factor on the measurements. If the measurements strongly depend on the location of the sample in the cortical bone, then any conclusion between individuals must take into account the anatomical location. Theoretically, if discrepancies of the measured stiffness constants can be considered as low, then the hypothesis is verified.

The second hypothesis states that a transversely isotropic model makes it possible to reduce the number of stiffness coefficients compared to the orthotropic model. It allows firstly to associate C_{11} with C_{22} and C_{44} to C_{55} , and secondly to estimate the coefficient extra diagonal C_{12} . Theoretically, if $C_{11} = C_{22}$ and $C_{44} = C_{55}$ then the hypothesis is checked.

The last hypothesis states that the post-mortem mechanical evolution of the bone would be stronger than the pre-mortem parameters between individuals. This hypothesis suggests the possibility of grouping individuals with PMI of the same order of magnitude, and to find singular differences between these groups. The classification of bones should be possible according to their mechanical behavior. Theoretically, the use of a mechanical parameter that highlights groups of similar PMI should let to check this last hypothesis.

The hypotheses are accepted or rejected using Wilcoxon – Mann – Whitney statistical test. This test allows to compare membership of a sample according to two groups of data (low number of samples, <30). The significance level is $p < 0.01$ (very strong presumption against the null hypothesis) and $p < 0.05$ (strong presumption against the null hypothesis).

4. Results and discussion

In this section, the experimental results are presented and discussed: from the calculation of the diagonal coefficients of the stiffness matrix, followed by the calculation of the Trace of the stiffness matrix and the anisotropy ratios.

Diagonal coefficients

In this part we present the evolution of diagonal coefficients of the stiffness matrix as function of the post-mortem interval (PMI). The diagonal coefficients of the stiffness matrix were computed using the mass densities and compression and shear wave velocities measurements according to the three main directions of bone samples. Firstly, the last three coefficients resulting from the shear measurements were calculated using the averages of the corresponding velocities V_{ij} and V_{ji} . In order to ensure the comparability of individuals with each other, the two individuals presenting high porosity rates (> 10%) were removed from the data: the selected individuals then have an average porosity rate of 5.8% with a maximum standard deviation of 2.1%.

Figure 4 presents the diagonal coefficients measured for all individuals according to their PMI. For each group, two zones were delimited, letting to separate samples with high and low values of stiffness coefficients C_{ii} . For all C_{ii} , the highest values were obtained for the samples with PMI less than 50 years and is highlighted that C_{ii} may decrease between PMI ranging from 13 to 50 years (between G2 and G3). Ultrasound measurements on individuals of different PMI were therefore compared with each other without taking into account external data presented in the literature.

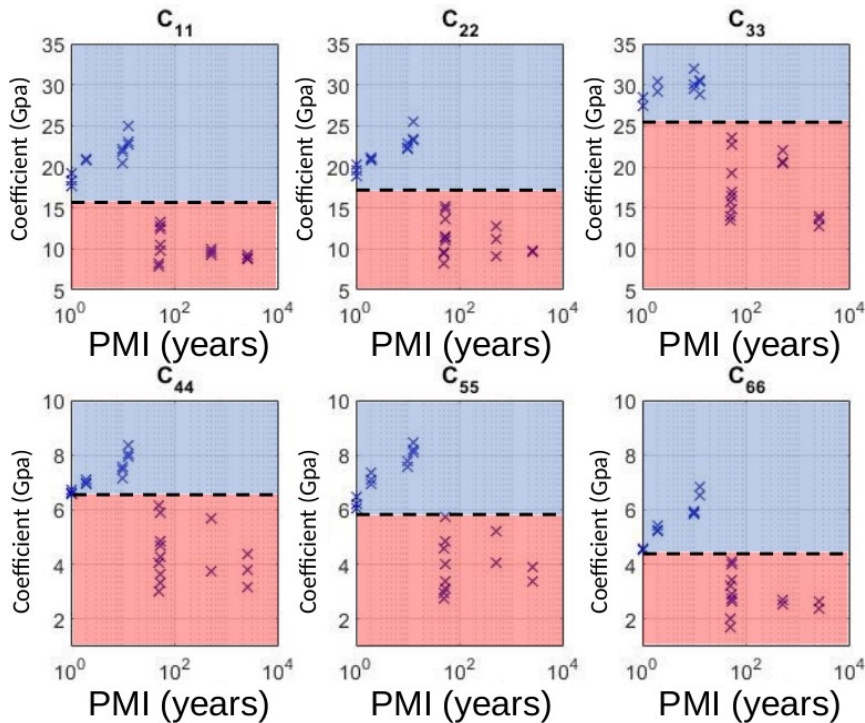


Figure 4: Measured diagonal coefficients as function of PMI.

The coefficient values presented show a slight increase between groups G1 and G2 (from 1 to 3 GPa), then a drastic drop between groups G2 and G3 (close to a factor 2). Groups G3 and G4 do not seem to differ. These differences between groups are highlighted by the WMW statistical test. The 0.05 threshold makes it possible to distinguish groups G1 // G2 and G2 // G3 as different populations (p -value < 0.04 for all C_{ii}). On the other hand this threshold does not allow the G3 // G4 groups to be dissociated (agreement with the null hypothesis, p -value > 0.21 for all C_{ii}).

The precision of measurement of the diagonal coefficients per individual is shown in Table 2. Overall precision per diagonal coefficient ranges between 3% (C_{11}) to 15% (C_{44}). However, the standard deviation of the measurement of C_{44} , C_{55} and C_{66} can be greater than 20% according to the individual. The average precision per individual ranges from 2% to 20%, with the lowest values for the individuals of groups G1 and G2 (with PMI lower than 50 years). Therefore, this leads to validate local homogeneity assumption for individuals of G1 and G2, whereas is less valid for G3 and G4. For these last, the measurement of the shear waves and therefore the associated coefficients may be impacted due to the heterogeneity of those samples.

Table 2: Precision of diagonal coefficients measurement.

	Sample	C_{11}	C_{22}	C_{33}	C_{44}	C_{55}	C_{66}	Average per individual	Average per PMI group
G1	Ind 1	4%	4%	2%	1%	3%	1%	3%	2.5%
	Ind 3	1%	0%	2%	1%	3%	2%	2%	
G2	Ind 1	4%	2%	4%	3%	1%	1%	3%	3.5%
	Ind 3	5%	5%	3%	3%	2%	3%	4%	
G3	Ind 1	2%	8%	8%	36%	29%	35%	20%	13%
	Ind 2	3%	5%	11%	28%	10%	10%	11%	
	Ind 3	4%	2%	7%	15%	13%	4%	7%	
G4	Ind 1	4%	17%	4%	29%	18%	3%	12%	9.5%
	Ind 2	3%	2%	5%	16%	8%	6%	7%	
	Average	3%	5%	5%	15%	10%	7%		

Table 3 presents the average of these coefficients for the four different PMI groups as well as their standard deviations. The standard deviations presented show repeatable measurements for groups G1 and G2 both between individuals of the same PMI, but also between the three samples from the same individual (maximum standard deviation of 8.6% for these two groups). This remark is not valid for groups G3 and G4 whose standard deviations can reach 38%. We recall that the individuals retained for these four groups are assumed comparable: neither their porosity rates nor their densities allow them to be distinguished. The values of measured coefficients for groups G3 and G4 are comparable, not allowing us to differentiate them. The PMI is the only known parameter that can differentiate these individuals. Unlike groups G1 and G2, the measurements of the coefficients depend on one hand, on their different post-mortem alterations, between individuals and on the other hand, between samples from the same individual.

To our knowledge, there are no studies carried out using ultrasound on the question of post-mortem bone degradation. However, the results suggest a late evolution of the coefficients for PMI at least greater than 10 years. This remark suggests that the results obtained on recent individuals are comparable to studies carried out in the medical field: to approach the conditions of living bone, these studies were carried out on fresh bones. The results of groups G1 and G2 of PMI between 1 and 13 years are in agreement with adult bone studies in the medical field [6,15,20].

Table 3: Evolution of diagonal coefficients as function of PMI group.

	C_{11}	C_{22}	C_{33}
G1: 1 – 3 years	19.6 GPa ± 7.2%	20.2 GPa ± 6.3%	28.6 GPa ± 7.0%
G2: 10 – 13 years	22.5 GPa ± 6.4%	23.2 GPa ± 5.4%	30.2 GPa ± 3.6%
G3: ~50 years	10.4 GPa ± 22.7%	11.7 GPa ± 23.5%	17.4 GPa ± 22.1%
G4: Archeo	9.3 GPa ± 5.6%	10.4 GPa ± 8.7%	17.2 GPa ± 31.1%
	C_{44}	C_{55}	C_{66}
G1: 1 – 3 years	6.8 GPa ± 3.9%	6.7 GPa ± 7.6%	4.9 GPa ± 8.6%
G2: 10 – 13 years	7.7 GPa ± 5.6%	8.0 GPa ± 4.1%	6.3 GPa ± 7.5%
G3: ~50 years	4.1 GPa ± 37.3%	4.0 GPa ± 38.4%	2.9 GPa ± 38.8%
G4: Archeo	4.5 GPa ± 17.7%	4.0 GPa ± 17.6%	2.6 GPa ± 4.7%

Trace of stiffness matrix

The sum of the diagonal coefficients of the stiffness matrix is an interesting parameter to follow: this sum is called the Trace of the matrix ($\text{Tr}(C_{ij})$). The Trace is a global parameter independent of the study axis allowing an averaged view of the matrix: the study is not then made on six independent coefficients but on a single parameter.

Figure 5 presents the Trace of each sample from each individual according to their PMI and their mass densities. A color code is used to link the figure on the left and that on the right: the group G1 (1 – 3 years) is represented in blue, the group G2 (10 – 13 years) in red and groups G3-G4 (over 50 years later noted G3+4) in black. As for the diagonal coefficients of the matrix, two zones can be delimited for samples with high and low values of the Trace. The results show a margin of distinction of around 10 GPa, letting to separate samples with PMI lower and higher than 50 years (Figure 5A).

The precision of measurement of the diagonal coefficients has an impact on the Trace of the stiffness matrix. In the case of G1, the precision per individual is around 2.5% and two groups of blue points (between 80 and 90 GPa) can be distinguished from two groups of red points of G2 (between 95 and 105 GPa), where precision per individual is around 3.5%. Taking into account the high precision, the Trace representation can lead to possibility to distinguish groups G1 and G2. However, for PMI over 13 years, the precision per individual (and therefore per PMI group) is lower, and is not possible to assign a belonging of PMI group to a range of values of the Trace.

The Figure 5B presents the evolution of the Trace of stiffness matrix with respect to mass density. This representation let us again to define a delimitation zone whose margin of distinction is around 10 GPa. For the upper zone, the results show that for PMI lower than 50 years, the Trace is linearly related to mass density ($R^2 = 0.92$). Therefore, elastic and homogeneous hypotheses can be checked

where mass density is predominant, since the Trace of the matrix is proportional to the mass density (common factor for all diagonal coefficients). However, for the lower zone, the results show that PMI over 50 years, the Trace follows less a linear behavior ($R^2 = 0.56$), for which a nonlinear trend would favor multi-parameter influence hypothesis (not only mass density).

As with the study of isolated diagonal coefficients, the evolution of the trace makes it possible to divide the groups of individuals into three distinct populations: this division is supported by the WMW test with a threshold of 0.01. The same test can be performed for density. density. The G1 // G2 and G2 // G3+4 groups can be divided into two populations (p-values < 0.002), this is not the case for the G1 // G3+4 groups whose p-value associated with density (p-value = 0.021), is distinct from that associated with trace (p-value < 0.001).

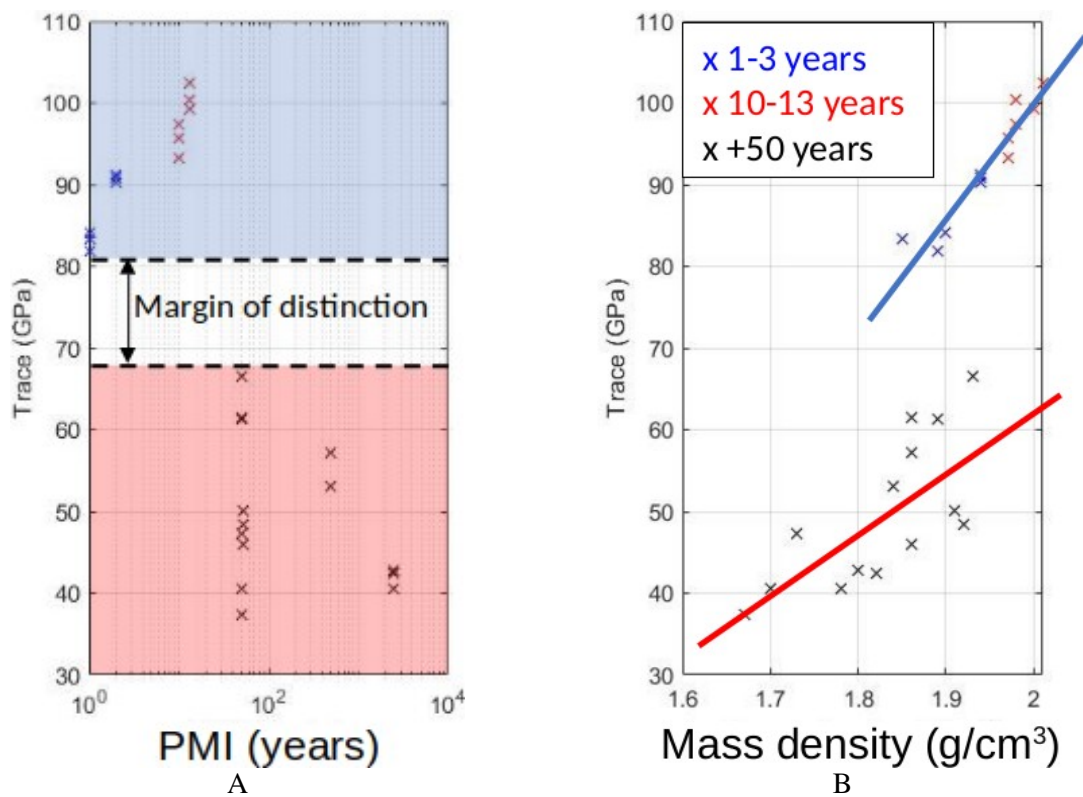


Figure 5: Trace of stiffness matrix per individual as function of: A) PMI and B) Mass density.

Anisotropy ratios

The anisotropy ratios C_{11} / C_{22} and C_{44} / C_{55} were calculated for each sample from each individual and are shown in Figure 6. The average value of these ratios per PMI group as well as their standard deviations are listed in Table 4.

In general, the estimates of the ratios from the G3 and G4 groups are less repeatable than those of groups G1 and G2: the two youngest groups have standard deviations less than 6% while the two oldest groups have standard deviations included between 5% and 28%. These differences may be associated with the heterogeneity between individuals belonging to the same group and/or to differences between samples from the same individual.

The C_{11} / C_{22} and C_{44} / C_{55} ratios provide information on the anisotropy model applied to the bone. The average of the results per group shows that the bone is generally transversely isotropic to within 10%: the average ratios have values between 0.90 and 1.11. However this transverse isotropy hypothesis seems to evolve with the PMI: groups G1 and G2 have ratios closer to 1 (difference less than 0.03 for standard deviations less than 6%) than the ratios of groups G3 and G4 (difference greater than 0.07 for standard deviations between 5% and 28%). Considering these results, the post-mortem mechanical evolution of the bone would be stronger than the pre-mortem parameters between individuals. Therefore, elastic homogeneous transversely isotropic model could be used to highlight belonging of a bone to PMI lower than 50 years (even close to pre-mortem situation). However after 13 years, the post-mortem evolution (multi-parameter) makes it difficult to distinguish and classify bones according to their mechanical behavior. This last situation requires to take into account heterogeneity in fiber direction and another anisotropy model might be necessary.

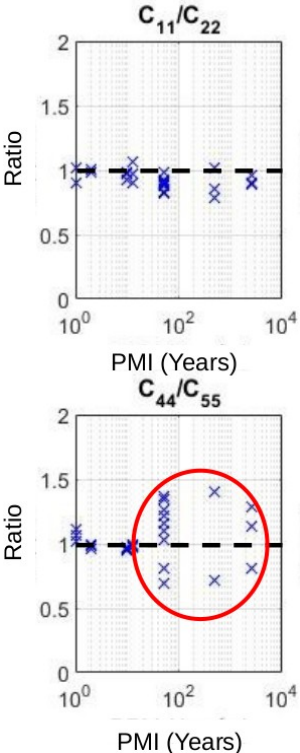


Figure 6: Measured anisotropy ratios for individuals as of PMI.

Table 4: Mean and standard deviation of anisotropy ratios as function of PMI group.

	C_{11} / C_{22}	C_{44} / C_{55}
G1: 1 – 3 years	0.97 ± 6%	1.02 ± 6%
G2: 10 – 13 years	0.97 ± 6%	0.97 ± 2%
G3: ~50 years	0.90 ± 5%	1.11 ± 21%
G4: Archeo	0.90 ± 9%	1.07 ± 28%

5. Conclusion

The technique presented here is a nondestructive experimental method for the characterization of post-mortem interval (PMI) of human bones. In order to validate the feasibility, an experimental technique was implemented based on test bench for the measurement of stiffness coefficients already used for bone ultrasonic assessment in medical applications. The study addressed the questions about the mechanical behavior of post-mortem bone and the relevance of elastic homogeneous transversely isotropic model and its validity according to PMI. For this, physical measurements and extraction of diagonal coefficients of the stiffness matrix were carried out. On one hand, the analysis of measurement precision and the use of the Trace let to validate the elastic and homogeneous hypotheses, where mass density is predominant. On the other hand, the study of anisotropy ratios let to study the behavior of this parameter during post-mortem alteration. Overall, the results of this work permitted to carry out a first classification of post-mortem bone relating physico-mechanical parameters and PMI. The agreement with literature between stiffness coefficients of short PMI close to pre-mortem condition allowed to validate the experimental approach.

In conclusion, this study contributes a new set of elasticity values for post-mortem human cortical bone, providing insights into the evolution of stiffness coefficients with post-mortem alteration. Moreover, it offers the first complete analysis of diagonal coefficients and the Trace of stiffness matrix, giving some indication of how bone anisotropy is related to time after death. Future perspectives may include studying the bone samples with PMI between 13 and 50 years and the use of complementary techniques. Wave attenuation might be a parameter of interest for further studies on scattering and viscoelasticity of post-mortem human bone, and techniques such as Resonant Ultrasonic Spectroscopy (which allows full stiffness matrix characterization) or opto-acoustic measurements (in order to reduce bone conditioning), could be considered.

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