Nanoindentation of trabecular bone in human vertebrae classified as normal, osteopenic and osteoporotic by ultrasonometry of the calcaneus
• The evaluation of microarchitecture resistance of the trabecular bone may contribute in determining the risk and preventing fractures associated to osteoporosis.
Motivation

Do the mechanical properties of trabecular bone microstructure allow us to indicate the bone quality of human vertebrae?
Is it possible to indentify (mechnically) osteoporosis in one single trabecula?
Evaluate the “quality" of trabecular bone microarchitecture of vertebrae (human cadavers), classified as normal, osteopenic and osteoporotic by ultrasonometry technique of the calcaneus, through nanoindentation test.
Introduction

Osteoporosis

- Loss of bone mass (density); Increased porosity and fracture risk;
- Specifically in trabeculae the **reduction of connectivity, number, thickness and deterioration of plate to rod format**.

**FIG. 1** - Trabecular bone of human vertebrae classified as: a) normal; b) osteopenic; c) osteoporotic
Introduction

Trabecular bone

- Composite
- Heterogeneous
- Orthotropic
- Anisotropic
- Viscoelastic

FIG. 2 – Trabecular bone of human vertebrae classified as normal
Introduction – Ultrasonometry of calcaneous

Classification of calcaneus ultrasonometry

FIG. 3 – Procedure for ultrasonometry analysis of calcaneus: a) Hygiene treatment with isopropyl alcohol; b) leg coupling in the Achilles Insight equipment; c) Results of an individual considered as normal (T-score -0.9) in function parameters such as gender, ethnicity, weight, age and bone stiffness of the calcaneus (elastic module).
Materials and Methods

Samples

• A total of 90 human trabecular vertebrae were dried (dehydrated), distributed proportionally by the regions $T_{12}$, $L_1$ and $L_4$ in a total of 30 vertebrae segments of each normal osteopenic and osteoporotic group extracted from individuals (human cadavers of Brazilian nationality).
Materials and Methods

Samples

- They are classified through bone quality index (BQI) as normal, osteopenic and osteoporotic bones through the ultrasonometry of the calcaneous bone.
Preparation of samples

FIG. 04 – a) Vertebral body penetration through the trephine drill; b) Cylindrical specimens of trabecular bone; c) Proof bodies (20 by 10) mm without bone marrow of L₁ region, classified as normal, osteopenic and osteoporotic

Bone marrow removal with physiological saline solution (NaCl 0.9%)
Preparation of samples

**FIG. 05** - Test samples without bone marrow, built into acrylic resin L1 region, classified as normal, osteopenic and osteoporotic
The nanoindentation technique allowed the evaluation of the elastic module (E) and nanohardness (H) in one single trabecula in each respective group: Normal, Osteopenic and osteoportic.
FIG. 6 - a) Typical nanoindentation curve; b) The surface profile and geometric parameters of an indented sample.
Nanoindentation test

FIG. 07 – a) Nanoindentation System (Nano Indenter XP), b) Realization of 24 indentations (6x4) in trabecular bone of the T12 region of the normal group.

Speed: 400MN/s;
Load cell: 50MN (5gf);
Resolution: 500nN
The vertebrae of the regions $T_{12}$ and $L_1$, were chosen because they have a higher rate of fractures and the $L_4$ has a better distribution of trabeculae and axial alignment to the cranial-caudal axis in the vertebrae body.
The minimum numbers of samples \( (N = 8.64) \) was determined as function of the quantitative variables for an infinite population and is defined by Equation.

\[
N = \left( \frac{Z_{\alpha/2} \text{SD}}{\text{SE}} \right)^2 \rightarrow N = 8.6
\]

Were \( Z_{\alpha/2} \) : critical value for the desired degree of confidence (usually: 1.96 (95%)); SD: standard deviation of the average; and SE: standard error of the average (SD = 1.80 MPa, SE = 1.2 MPa). The adopted sample size was ten \( (N = 10) \) for each group: normal, osteopenic and osteoporotic, totalizing thirty (30) subjects.
Statistical method

✓ Descriptive statistics: **Mean** (M), **Median** (Md), **Standard Deviation** (SD), **Standard Error** (SE), **Minimum Value** (Min), **Maximum Value** (Max), **Variational Coefficient** (VC) and **number of samples** (N).

✓ **Normality test by Shapiro-Wilk method**

✓ **Analysis of comparisons by ANOVA methods and multiple comparisons by Tukey HSD Kramer**
• Descriptive statistics of BQIs of 30 individuals classified as normal, osteopenic and osteoporotic bone by the ultrasonometry of the calcaneus.

• Descriptive statistics of mean E of trabecular bone between the regions $T_{12}$, $L_1$ and $L_4$ of 30 human vertebral segments, determined by mechanical testing of nanoindentation and classified as normal, osteopenic and osteoporotic bone by the QUS.

• Descriptive statistics of mean H of trabecular bone between the regions $T_{12}$, $L_1$ and $L_4$ of 30 human vertebral segments, determined by mechanical testing of nanoindentation and classified as normal, osteopenic and osteoporotic bone by the QUS.
Results

Ultrasonometry of calcaneal bone mineral density (BMD) in normal, osteopenic, and osteoporotic subjects, compared to the overall group. The BMD values are presented as bone quality index (BQI).
**Results**

**TAB. 1 –** Correlation of bone quality index (BQI), modulus of elasticity (E) and mechanical compression test USC (Ultimate Compressive Strength to Trabecular Bone = 2.48 ± 1.8 MPa) **maximum load supported by the bone** by the Spearman test. The value of r < than 0.5 → rejected.

<table>
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<th>Variable</th>
<th>Correlation</th>
<th>r</th>
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<tr>
<td>BQI</td>
<td>Age (years)</td>
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<tr>
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<td>E (MPa)</td>
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<tr>
<td>E</td>
<td>Age (years)</td>
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<td>Ultimate Compressive Strength To Trabecular Bone UCS = 2.48 ± 1.8 MPa</td>
<td>-0.684</td>
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• The nanoindentation test of vertebrae proof bodies (human cadaver) allowed the analysis of the mechanical properties in a single dehydrated trabecular. The suggested hypothesis was not confirmed, there were no significant differences between the normal osteopenic and osteoporotic groups.
Conclusion

• The tests demonstrated that the fracture resistance associated with osteoporosis is more closely linked to the architecture (macroscopic condition) of the set of trabeculae, than to the bone matrix (microscopic condition) of a single trabecula, because there were no significant differences between groups.
REFERENCES


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