**Quantification of osteocyte lacunar density and anisotropy of vascular canals in compact bone**

**-Introduction, bone structure, remodelation, osteoporosis,**

Remodelling of Haversian bone may result in both spatial and temporal variability of osteocyte lacunar density and arrangement of vascular canals.

**-Parameters and methods in bone histomorphometry, tetracycline labeling, disector**

**The most commonly used histomorphometric parameters:** [1]

**1- Structural parameters**

**Measures that describe the biopsy specimen**

-Core width (mm)

C.Wi Overall size of the biopsy specimen, refers to the width of the entire biopsy specimen or core. It is the distance between the inner and outer periosteal surfaces

-Cortical width (μm)

Ct.Wi Distance between periosteal and endocortical surfaces, he sum of the widths of inner and outer cortices of the biopsy specimen.

-Bone volume / tissue volume (%)

BV/TV Space taken up by mineralized and unmineralized bone relative to the total size of a bone compartmen, is cancellous bone volume as a percentage of tissue volume. The numerator includes matrix, whether mineralized or not, and the denominator includes cancellous bone, marrow, and associated soft tissue.

**Measures that describe the configuration of trabeculae in space:**

Trabecular thickness (μm)

Tb.Th Is the mean distance *across* individual trabeculae

Trabecular number (/mm)

Tb.N Number of trabeculae that a line through a trabecular compartment would hit per millimeter of its length, (BV/TV)/Tb.Th

- Trabecular separation

Tb.Sp Is the mean distance between individual trabeculae

**2- Static formation parameters**

Osteoid thickness (μm)

O.Th Distance between the surface of the osteoid seam and mineralized bone

Osteoid surface / bone surface (%)

OS/BS Percentage of bone surface covered by osteoid

Osteoid volume / bone volume (%)

OV/BV Percentage of bone volume consisting of unmineralized osteoid

Osteoblast surface / bone surface (%)

Ob.S/BS Percentage of bone surface covered by osteoblasts

Wall thickness (μm)

W.Th Mean thickness of bone tissue that has been deposited at a remodeling site, is the mean distance between resting trabecular surfaces and cement lines

**3- Dynamic formation parameters**

Mineralizing surface / bone surface (%)

MS/BS Percentage of bone surface showing mineralizing activity

Mineral apposition rate (μm/d)

MAR Distance between two tetracycline labels divided by the length of the labeling interval

Mineralization lag time (d)

Mlt Time interval between the deposition and mineralization of matrix

Bone formation rate / bone surface (μm3×μm−2\*y−1)

BFR/BS Amount of bone formed per year on a given bone surface

**4- Static resorption parameters**

Eroded surface / bone surface (%)

ES/BS Percentage of bone surface presenting a scalloped appearance

Osteoclast surface / bone surface (%)

Oc.S/BS Percentage of bone surface covered by osteoclasts

**-Aims of the study**

The aim of the study was to assess locally specific numerical density of lacunes with a three-dimensional unbiased counting method and to analyze anisotropy of profiles of vascular canals in an embedded section of human tibia.

**-Material and methods**

**– optical disector**

**– Delaunay triangulation**

**- bone biopsy**

An undecalcified 150 micrometers thick transversal section was sawed from the diaphysis of tibia of a 70-year-old female, grinded to a 70-80 micrometers thick section, polished, stained with basic fuchsin, and observed with an optical microscope. We quantified numerical density of osteocyte lacunes in compact bone underlying the lateral, medial, and posterior surface. Image frames (n=42) were sampled in an systematic uniform random manner from cortical and medullar layer round the tibial circumference. Each of the layers comprised one half of the local thickness of the bone section. Series of seven optical sections registered with respect to the Z-axis were photographed in each image frame. The unbiased counting rule of optical disector was applied to these series in order to assess the number of osteocyte lacunes per unit volume. Area fraction of the profiles of vascular canals in the bone sections was estimated with the point-counting method. Disector and point-grid counting was performed with the Ellipse software (ViDiTo, Košice, Slovak Republic). The two-dimensional anisotropy of profiles of vascular canals was assessed with the Delaunay triangulation (Qhull software, The Geometry Center, Minneapolis MN, USA). We carried out a 3-D reconstruction of vascular canal and surrounding lacunes in a part of a randomly selected Haversian system (software Amira, Mercury Computer Systems SAS, Merignac Cedex, France).

**Results numerical density of lacunes – anisotropy of vascular canals**

Kruskal Wallis ANOVA did not prove significant differences (p=0.051) in lacunar density among the bone areas underlying the lateral (18088±2150 mm-3, mean±SD), medial (16655±3772 mm-3) and posterior surfaces (20222±4109 mm-3). Lacunar density was higher (p=0.032) in cortical (20356±3725 mm-3) than in medullar layer (16287±2274 mm-3). Area fraction of profiles of vascular canals was 10.5%. Delaunay triangulation of centres of gravity revealed considerable anisotropy in arrangement of profiles of vascular canals (n=1194). The 2-D arrangement of vascular canals was approximated by a network of vertices forming isosceles triangles with average basis (perpendicular to the bone surface) of 251±13 micrometers and height (parallel to the bone surface) of 258±24 micrometers.

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| Osteocyte lacunar density in individual bone areas underlying the lateral, medial and posterior surfaces of tibial shaft. ( mean±SD of 7 disectors) [mm-3] |
| layer: | facies lateralis | facies medialis | facies posterior |
| cortical | 18958±2409 | 19119±3488 | 22991±4017 |
| medulllar | 17218±1566 | 14191±2122 | 17453±1603 |

**Discussion**

**– biological interpretation of quantitative parameters**

**– časová naročnost**

**- disektor- obecne v MM jedinná metoda umožnujici 3D**

**- srovnání/přepočet 2D a 3D, vzorec**

**-Conclusion**

We suggest the combination of optical disector, stereological point-counting method, and Delaunay triangulation to be a set of complementary methods useful for description of local heterogeneities in lacunar density and arrangement of vascular canals in microstructure of compact bone.

**References:**

[1] RAUCH F, Watching bone cells at work: what we can see from bone biopsies, Pediatr Nephrol, 21: 457–462, 2006

[2] RAUCH F (2003) Bone histomorphometry. In: Glorieux FH,Pettifor J, Jueppner H (eds) Pediatric bone. Academic, San Diego, CA, pp 359–374

[3]RAUCH F, Travers R, Norman ME, Taylor A, Parfitt AM, Glorieux FH (2002) The bone formation defect in idiopathic juvenile osteoporosis is surface-specific. Bone 31:85–89

[3]GLORIEUX FH, Travers R, Taylor A, Bowen JR, Rauch F, Norman M, Parfitt AM (2000) Normative data for iliac bone histomorphometry in growing children. Bone 26:103–109

[4]PARFITT AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR (1987) Bone histomorphometry: standardization of nomenclature, symbols, and units.Report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res 2:595–610

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