# COMBINATION OF 3D NON-DESTRUCTIVE MICRO TOMOGRAPHY AND IMMUNOHISTOCHEMISTRY FOR THE CHARACTERISATION OF HUMAN BONES FROM MIDDLE AGES, EARLY AND LATE MODERN PERIOD: A PILOT STUDY

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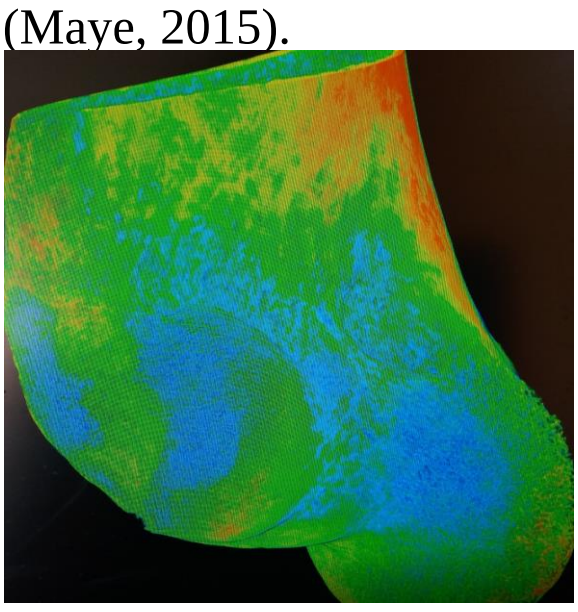
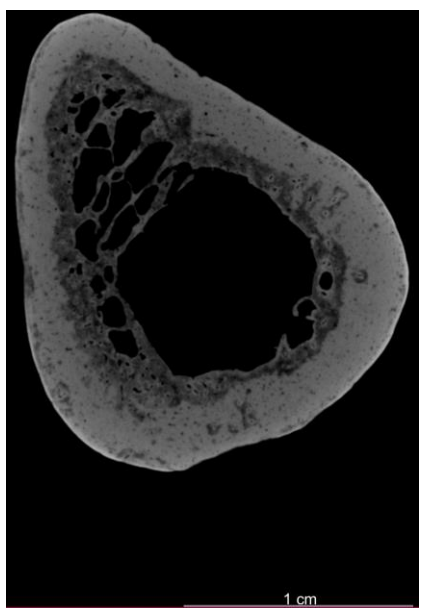
Bone quality encompasses correct architecture, porosity, and composition, including different hard tissue factors. There are specific studies about the bone quality in mediaeval skeletons, but they relate mainly to the distribution of different ion content (Rasmussen et al., 2017), diachronic changes in size and shape of bones, (Brzobohatá et al., 2016), dental and skeletal disorders (Novak et al., 2012), and osteoporosis (Maye, 2015).



Humerus anterio-lateral view with different optical density regions of bone.




Ulna withprominent internal demarcation zone.

## Aims of the project

**Thus, our aim was the research on the combination of hard tissue markers and 3D imaging indicators to reveal bone quality in human starting from the mediaeval period.**

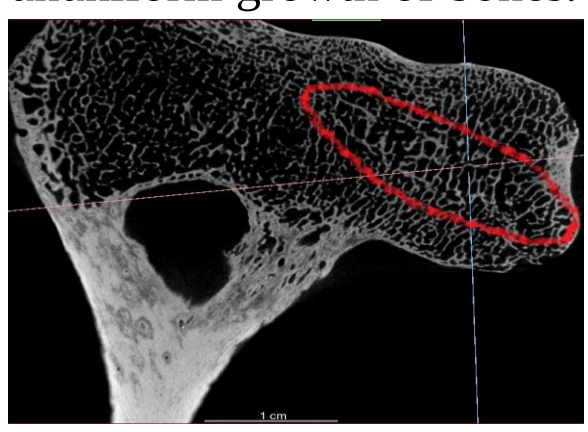
## Materials and methods

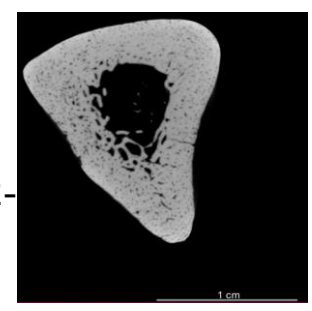
Two human ulna from Middle Ages and humerus from Early Modern Period obtained during archeologic excavations (1983-1985) in the Saint George castle of the Order of the Brethren were used simultaneously to the two control ulna from the late Post Modern period (property of the Institute of Anatomy and Anthropology). Bone measurements and 3D pictures were done by ZEISS Xradia 510 Versa X-Ray Microscopy, while tissue were detected for osteocalcin (OC), osteopontin (OP), osteoprotegerin (OPG), Runt-related transcription factor 2 (RunX2), matrix metalloproteinase 2 (MMP2), tissue inhibitor of matrix metalloproteinase 2 (TIMP2), basic fibroblast growth factor (bFGF), transforming growth factor beta (TGFß), bone morphogenetic protein 2/4 (BMP2/4), Interleukin 1 (IL-1), Interleukin 10 (L-10), human beta defensin 2 (def2), apoptosis.

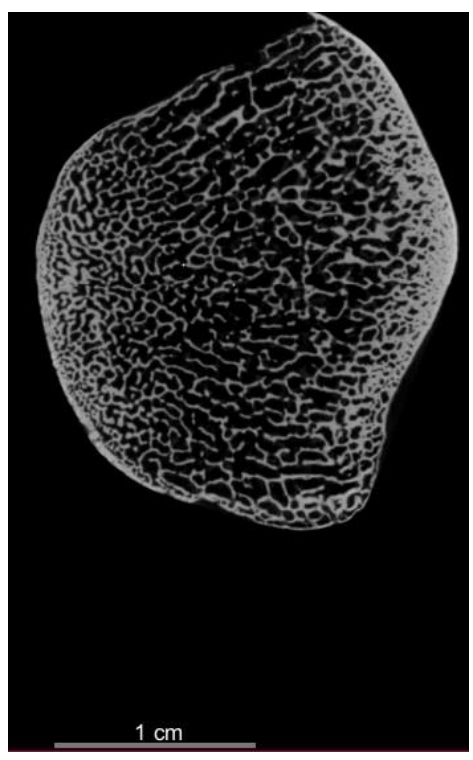
## Results (all the pictures are 3D done by ZEISS Xradia 510 Versa X-Ray Microscopy)

Results revealed variations in bone volume from 28.82-33.85 (%/0.5cm³), trabecular thickness from 180,85±20,71 to 222,26±29,01 µm, and thickness of cortical bone from 3320,23 to 3555,34 µm. The diameter of pores was the most variable and showed from 313,12±91,60 to 447,79±105,71 µm even within the frame of one Age.

The bones of Middle Ages showed from very occasional to occasional number of positive tissue factors structures. Early Modern Period demonstrated an absence of apoptosis, BMP2/4, TIMP2, RunX2 in the bone, while the number of OC, OP, OPG, MMP2, bFGF, TGFß, IL-1, IL-10 and def2 positive osteocytes varied from few to moderate. Finally, the two controls showed totally opposite distribution of the tissue factors. So, one bone demosntrated few to moderate number of almost all factors, except RunX2 and IL-1, but the other possessed occasional to few number of OC, OP, OPG, MMP2, TGFß, BMP2/4, IL-10, and def2 with an increase of TIMP2 until the numerous positive structures, and the lack of IL-1 and RunX2. Interestingly, 3D imaging revealed ununiform growth of bones.




Figs. 1-3. 1) Humerus with ununiform growing zone; 2-3) ulna with pores and internal surface.

## Conclusions

The bones of Middle Ages demonstrate the common degradation and cant be representatives for the evaluation of bone homeostasis of that time. Early Modern Period bones and controls of Post Modern period are similar with different bone factors expression, thus differences here may indicate the individual chages rather than the century changes. The bone architecture doesn't show statistically significant century differences between bone volume, trabecular thickness and cortical thickness, while variable diameter of pores seems again to characterises the individual bone structure. Finally, 3D imaging excellently reveals the growth disordered zones in bones starting from the Middle Ages.