Beyond the visible world: the role of microscopy in the study of past human conditions

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Paleopathology, summarily defined as the study of past diseases, has on the differential diagnosis a major challenge. Histological techniques offered the possibility to look inside the microstructure of both normal and abnormal body tissues to diagnose diseases that affected past populations, leading to the development of a new field of research - paleohistology or paleohistopathology. However, and contrary to paleopathology whose journey is well-established, in paleohistopathology there are many gaps that need to be filled. This occurrence is probably the result of a nonsystematic and non-standardized approach to the microscopic study of skeletal abnormalities, especially those of infectious origin involving periosteal new bone formation (PNBF). The aims of this work were: (1) to search for differences in the microstructure of PNBF with regard to the cause of death of the individual; (2) to infer differences between the macroscopic and microscopic proprieties of bone lesions, and (3) to ascertain the impact of diagenetic changes in the bone microstructure. For histological examination under transmitted and polarized light, a total of 34 dry bone specimens: 26 belonging to 23 individuals from the Human Identified Skeletal Collection from the Bocage Museum (Lisbon, Portugal), and eight from archaeological skeletons were prepared. The documented bone samples were collected from individuals who died from tuberculosis-TB (Group 1), non-TB infectious diseases (Group 2), and conditions other than those of TB and non-TB infectious origin (Group 3).

With regard to the diagnosis of pathological conditions, differences in the microstructure of PNBF were seen between Group 1 and Group 2 of cause of death and within groups. Multiple layers of "appositional bone" enclosing numerous primary vascular canals were the pattern most commonly observed (n=4) in the samples from Group 1. This type of PNBF seems to mimic the appositional growth that characterizes the modeling process of the periosteal and endosteal membranes (PEM) during rapid growth periods [1]. An abnormal stimulation of growth factors, especially of the vascular endothelial growth factor (VEGF) may eventually explain the extensive hypervascularization observed (Fig. 1 A-B). Periosteal lesions on ribs are normally associated with pulmonary infection (e.g. TB) disseminated from the lungs (via pleura) to the ribs [2]. However, three samples (one from Group 1, two from Group 2) presented a microstructure compatible with subperiosteal hematomas (Fig. 1 C-D) [3]. Repetitive microtrauma (e.g. chest wall vibration) that causes detachment of the periosteum may have leaded to subperiosteal bleeding and hematoma formation [4]. These observations suggest that beyond pulmonary diseases other mechanisms may stimulate PNBF on the visceral surface of ribs. Histological analysis was also fundamental in the description and characterization of bone changes. For example, of the five samples with "consolidated" fracture callus, only two presented a truly mature and remodeled microstructure. This means that the outer surface of a bone lesion may not give a complete picture of the tissues response (Fig. 2 A-B). In spite of the good preservation of some bone samples, massive diagenetic changes due to the action of bacteria and fungi were observed at microscopic level. This clearly suggests that gross inspection is not a good measure of the bone tissue quality. In contrast, microscopy is essential to differentiate between pseudopathology and physiological or pathological signs.

Microscopy revealed surprising results that reinforce the pertinence of applying histological techniques in the description and diagnosis of bone changes in human remains.

References
1. Maggiano, C. (2012). Making the mold: a microstructural perspective on bone modeling during growth and mechanical adaptation. In *Bone histology: an anthropological perspective*, Crowder, C. & Stout, S. (Eds.), pp. 45-90. Boca Raton: CRC Press.
2. Roberts, C. et al. (1998). Tubercle and Lung Disease **79**(1): 55-60.
3. Schultz, M. (2003). Light microscopic analysis in skeletal paleopathology. In *Identification of pathological conditions in human skeletal remains*, Ortner, D. (Eds.), pp. 73-107. Amsterdam: Academic Press.
4. Gorincour, G. et al. (2004). Archives of Disease Childhood **89**(11): 1078-1079.

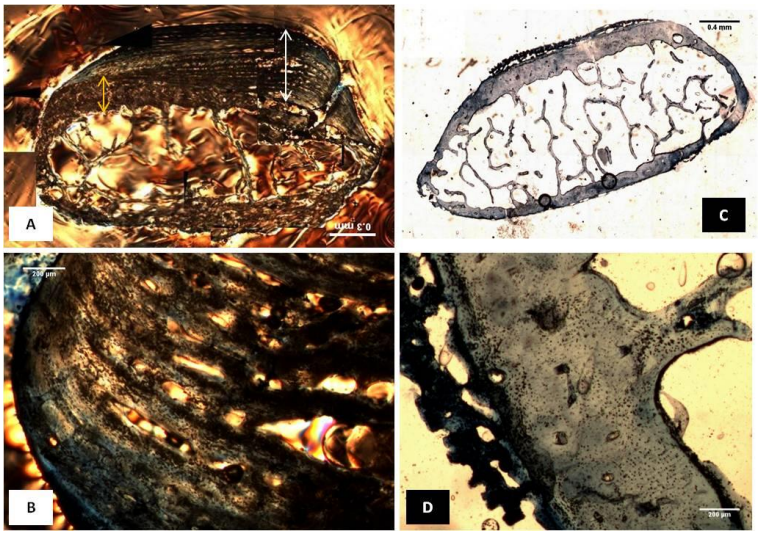

Figure 1 **A**: Thin section of a rib, under polarized light, from an adult male (Sk.154, 35 y.o.) who died from pulmonary TB. Increase thickness on the pleural surface due to new bone deposition (white arrow) in comparison with the underlying cortex (yellow arrow). Magn.10x. **B**: Detail of the PNBF showing multiple layers of appositional bone intercalated by primary vascular canals. Magn. 40x. **C**: Thin section of a rib, under transmitted light, of an adult female (Sk. 1383, 22 y.o.) who died from pulmonary TB. Note the presence of a thin rim of new bone upon the pleural surface. Magn. 10x. **D**: Close-up of the PNBF showing a ruffled rim of bone attached to the cortex by pedestals structures. Magn. 40x.

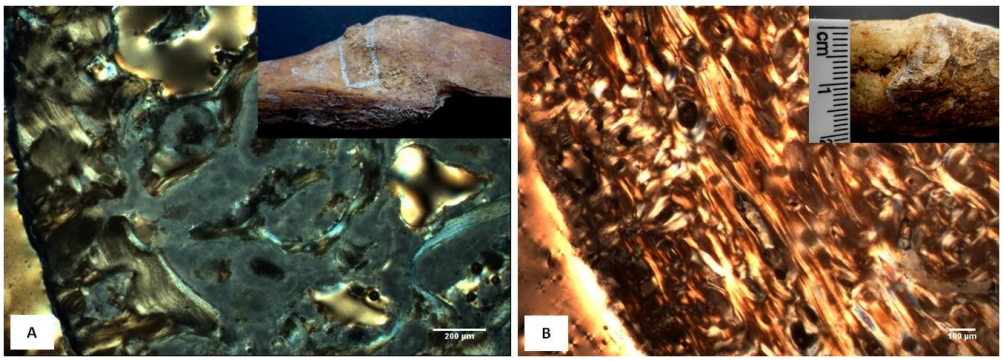

Figure 2 **A:** Bone callus from the right fibula of an adult male (Sk. 198, 68 y.o.) who died from urinary sepsis (upper right). Thin section under polarized light showing an immature structure composed by lamellae that surrounds the outer bone surface. Magn. 40x. **B:** Bone callus on a right tibia of an adult male (Sk. 54, 24 y.o.) who died from pulmonary TB (upper right).Thin section under polarized light showing a regular periosteal surface and a dense and mature cortical bone. Magn. 40x.

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