



## Technical aspects: how do we best prepare bone samples for proper histological analysis?

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Résumé en anglais

Histological analysis of bone is a critical step for the diagnosis of malignancies. It allows direct identification of malignant cells inside marrow spaces in case of bone metastases or hematological disorders. Bone biopsy is superior to marrow aspiration because the microarchitecture of the bone marrow is preserved, a parameter that is especially important in hematological disorders. Because marrow cells are in direct contact with bone cells (lining cells, osteoblasts, osteoclasts, and their precursors), an abnormal bone remodeling rate has been described in a variety of malignant cell proliferations when developing and expanding inside marrow spaces. Bone cells elaborate and synthesize a variety of cytokines acting on hematological precursors (e.g., M-CSF)<sup>1</sup> and malignant cells release other cytokines active on bone remodeling<sup>2-4</sup>: it is likely that bone changes are almost always associated with bone marrow alterations and vice versa. Histomorphometric analysis is a powerful tool in the evaluation of bone remodeling in metabolic bone diseases and was also successfully applied to hematological disorders and metastases from solid tumors<sup>5,6</sup>. Bone histomorphometry is a powerful method in the early diagnosis of B-cell malignancies, and smoldering myeloma or lymphomas can be characterized in patients with a monoclonal gammopathy of undetermined significance (MGUS) several years before the tumor has shown clinical expression. Bone histomorphometry is also useful in animal models of cancer bone lesions, since it permits a precise evaluation of the bone remodeling changes induced by tumor cells<sup>7-9</sup>. However, bone histomorphometry must be done on undecalcified bone sections which allow a perfect identification of osteoid tissue (the unmineralized bone matrix recently synthesized by osteoblasts), a precise identification of osteoclasts (by using histoenzymatic detection) and histodynamic analyses (after a double tetracycline labeling in humans or using a variety of other fluorochromes in the animal). These methods cannot be used on decalcified and paraffin embedded bone, since decalcification abolishes the osteoid/bone matrix differential staining and removes the fluorochrome labels, and hot paraffin embedding destroys enzyme activities. However, decalcification and paraffin remain useful for immunohistochemistry, which is difficult and hazardous on plastic sections. The main disadvantage of polymer embedding was formerly the prolonged time for preparing bone specimens (several months when polyester resins were used). With the development of histological techniques, it is now possible to have polymer embedding methods that are as fast as conventional paraffin methods. The following techniques have been developed and improved in our laboratory during the last two decades and used on more than 3000 human bone biopsies and a large number of animal studies performed in a variety of animal species (for example, mouse, rat, chicken, dog, goat, sheep, pig).

Notes

In: Heymann D, editor. "Bone cancer: progression and therapeutic approaches". London: Acad. Press. Elsevier Inc. 2nd edition

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