



## Current limitations to the histopathological diagnosis of some frequently encountered bone tumours

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The final diagnosis of a bone tumour comes in many cases like the last piece of a puzzle which requires integration of clinical, imaging and pathological data. However there are situations in which a discrepancy exists between histology and imaging studies and where histology alone cannot be decisive. This paper reviews such situations.

**Keywords** : bone tumour ; osteosarcoma ; chondrosarcoma.

### INTRODUCTION

The management of any musculoskeletal tumour requires a multidisciplinary approach in which clinical and imaging aspects are integrated. The biopsy, if required, is the final step in the diagnostic process. A definitive histopathological diagnosis is established with integration of clinical and imaging aspects. In most cases, histological findings are straightforward and leave no doubt about the diagnosis, which may be considered definitive. However, there are situations in which histology does not allow for a definitive diagnosis, even after the clinical data have been reviewed. In such circumstances, histology is confronted with its limitations.

The purpose of this paper is to review difficulties commonly encountered with respect to the histopathology of some common bone tumours, and the usual obstacles to a definitive diagnosis.

### Most frequent difficult lesions in orthopaedic oncology

#### *Osteosarcoma*

Osteosarcoma is a primary malignant tumour of mesenchymal origin in which the proliferating cells produce an immature bone matrix. The diagnosis of osteosarcoma requires the presence of two histological characteristics which must imperatively be present :

- a sarcomatous aspect of the cell population,
- production of an osteoid matrix (fig 1).

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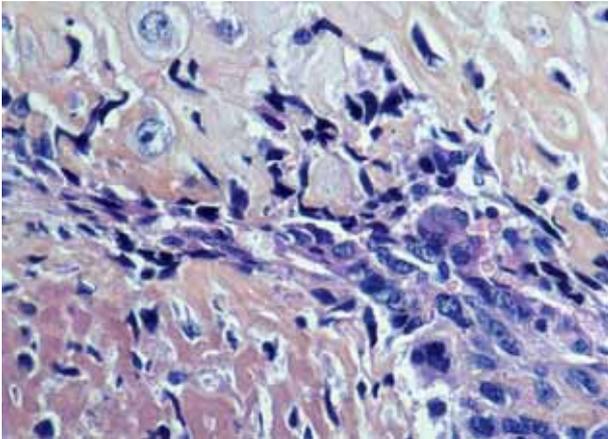
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**Fig. 1.** — Osteoid matrix surrounding atypical cells in an osteoblastic osteosarcoma (Haematoxylin-eosin[HE] stain, magnification  $\times 500$ ).

First of all, osteosarcoma is a sarcoma, and all the cytological criteria of malignancy have to be present in the specimen. Cells are atypical, displaying hyperchromatic and irregular nuclei and numerous mitoses. The presence of cell atypia alone is not sufficient to make a diagnosis of osteosarcoma, as atypical cells are also observed in non-osteogenic lesions involving bone, such as malignant histiocytofibroma, as well as in many metastatic lesions. Immunohistochemistry is very helpful to confirm a metastatic carcinoma in cases with positive epithelial markers. The second criterion which is required for a diagnosis of osteosarcoma is the presence of osteoid deposits. These

deposits consist of an eosinophilic extracellular network produced by the tumour (5,8). There is presently no reliable histochemical technique available to identify osteoid deposits. The pathologist must distinguish it from dense collagenous matrix. Moreover, osteoid deposits are also present in reactive bone formation associated with fracture callus or periosteal reaction, as well as in some benign tumours (11,12). Osteoid osteoma and osteoblastoma, for instance, are benign lesions in which there is production of an osteoid matrix, but the latter is well arranged and more structured than observed in an osteosarcoma (table I). The nidus, when it is identified, allows for an easy diagnosis (1,3,20,21).

The final diagnosis of osteosarcoma can be very difficult in cases with a sarcomatous cell population but without clear evidence of osteoid formation (6,18).

#### *Giant cell tumour and variants*

The epiphyso-metaphyseal giant cell tumour (GCT) is a benign tumour, characterized histologically by many osteoclast-type giant cells and numerous stromal cells (23). It is a highly vascularised lesion, which often shows haemorrhagic areas and haemosiderin-laden histiocytes (19).

Its tendency to recur locally is related to a high mitotic index in the stromal cells. In culture, only the stromal cells are able to proliferate, recruiting and stimulating the giant cells, with subsequent bone resorption. The histological diagnosis of the classical form does not raise any difficulties.

Table I. — Histological differential diagnosis of a bone lesion based on the major cellular component

		Main differential diagnosis	Other differential diagnosis
Bone component	<i>Osteosarcoma</i> : immature bone deposits, atypical osteoblastic cells	<i>Malignant fibrous histiocytoma, metastasis</i> : absence of osteoid	<i>Callus, periosteal reaction, osteoid osteoma</i> : absence of atypia
Giant cell component	<i>Giant cell tumour, aneurysmal cyst</i> : numerous giant cells, reactive osteoid	<i>Giant cell-rich osteosarcoma</i> : presence of atypia	<i>Hyperparathyroidism, repairing giant cell granuloma</i> : different clinical context
Cartilage component	<i>Chondrosarcoma</i> : chondroid or myxoid matrix, atypia	<i>Chondroma</i> : chondroid matrix, absence of atypia or less pronounced atypia	<i>Chondromyxoid fibroma</i> : myxoid matrix without atypia

However, giant cells may be very scarce in some "giant-cell tumours" and the histological differential diagnosis with a non ossifying fibroma then becomes very difficult. The anatomico-radiological confrontation is essential in such cases.

However any lesion rich in giant cells does not necessarily correspond to a giant cell tumour. Numerous other lesions, benign or malignant, may feature the presence of giant cells (2). The differential diagnosis can be very difficult between a giant cell rich osteosarcoma and a GCT (10). The different topography of the lesion, the detection of osteoid deposits, combined with the presence of cellular atypia, will usually make the distinction. Various other benign lesions show an identical histological picture, such as the brown tumour of hyperparathyroidism, aneurysmal bone cyst (ABC) and giant cell granuloma (table I).

The brown tumour develops in a context of hyperparathyroidism ; it is usually observed in the diaphyseal region and it appears more fibrous at histology. ABC will be the most difficult diagnosis to exclude. Secondary aneurysmal modifications can also be present in giant cells tumours, and it is not always easy to make the distinction with ABC.

### *Chondroma*

Chondroma is a benign tumour characterized by its mature hyaline cartilage containing chondrocytes without nuclear anomalies. The cartilaginous lobules have well-defined limits, and are surrounded by conjunctive tissue with abundant small capillary vessels. Within the nodules, the chondroid matrix is hyaline, and there is no vascularisation. Staining with periodic acid-Schiff stain and alcian blue shows variable positivity, depending on the amount of mucopolysaccharides (glycoaminoglycans ?) in the cartilage (8). The cytological features of the cartilaginous cells are fundamental to establish the benign nature of the lesion. The cellular density in a benign lesion is low, although a greater cellular density may be observed at the periphery of the nodules, in the zones of growth. The chondrocytes are small and the nuclei are round, with even sizes (table I). Some binucleated cells can be present.

### *Chondrosarcoma*

An accurate diagnosis of chondrosarcoma remains a true challenge for the pathologist (8). Careful assessment of clinical and imaging aspects is required, as well as a significant experience on the part of the pathologist. The distinction between benign enchondroma and low grade chondrosarcoma is particularly challenging (15).

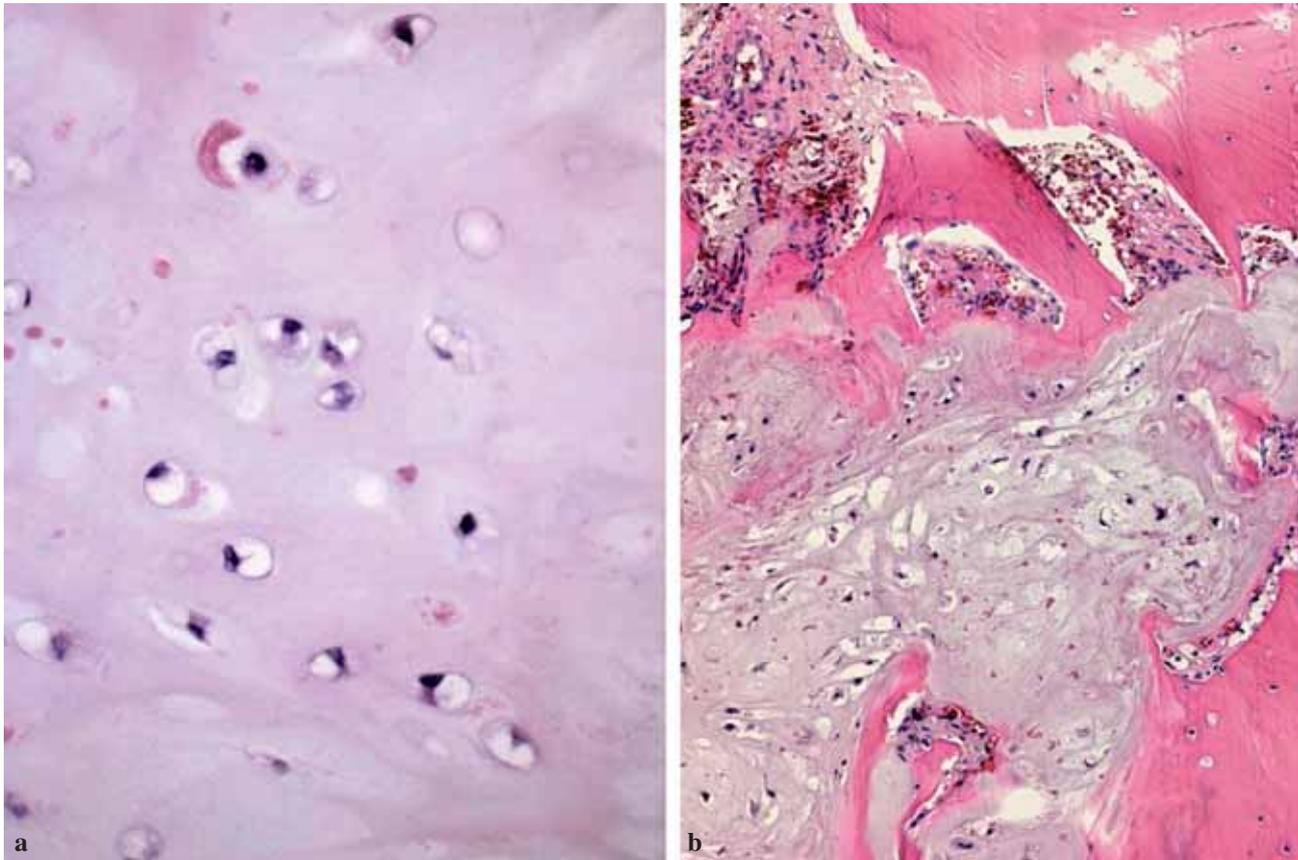
Malignancy can be assessed only on large surgical fragments and it only relies on the importance of the nuclear anomalies of the chondrocytes. The cells exhibit more dense nuclei, there is a higher number of binucleated cells and a higher cellular density than in a benign chondroma.

However, these anomalies may be fairly subtle in a well-differentiated grade I chondrosarcoma (fig 2a), and the malignant nature of the lesion is sometimes only established by the extension of chondroid lobules into soft tissue or in the aponeurotic structures after destruction of the cortical bone. A good histological sign to ascertain chondrosarcoma is the destruction of normal trabecular bone structures (fig 2b) (16).

The presence of a predominantly myxoid rather than hyaline matrix is usually observed in grade II chondrosarcoma. However, this predominance does not automatically reflect malignancy, as some benign lesions such as chondromyxoid fibroma can also display such a matrix. These benign entities must be identified in order to avoid misdiagnosis (table I).

### **The contribution of genomic analysis**

Most neoplastic lesions show very marked chromosomal abnormalities. Conventional osteosarcomas are high-grade lesions, and caryotype analysis shows numerous abnormalities : hyperploidy, with various chromosomal gains and losses. Chromosomal abnormalities are highly diverse and variable, including gains of chromosome 1p, 2p, 3q, 5q, 5p, and 6p and losses of 14q, 15q, 16p and 21q (7). Low-grade osteosarcoma on the other hand presents a more simple genetic profile. Ring chromosomes have been described in these tumours as well as the specific amplification of



**Fig. 2.** — a : Slight atypia in a grade -1 chondrosarcoma (HE stain, magnification  $\times 500$ ), b : Disruption of lamellar bone by a chondrosarcoma (HE stain, magnification  $\times 240$ ).

12q. The 12q region includes oncogenes MDM2 and CDK4 (14,22). Abnormalities discovered in low-grade osteosarcoma present high similarities with those of well-differentiated liposarcoma, suggesting a similar histogenesis. For chondrosarcoma, the *CDKN2A* (p16) tumour suppressor gene, located in chromosome 9p21, was shown to be important for tumour progression (4,9). Its inactivation is restricted to high-grade tumours. The progression from low-grade towards high-grade central chondrosarcoma is also characterised by *P53* alterations. Several translocations involving chromosome 17 have been reported in aneurysmal bone cyst. A fusion gene is formed by the translocation, including the oncogene *USP6* (13,17).

Genetic analysis of the specimen is now part of the routine investigations which lead to the final diagnosis of any tumour.

#### **Need for a global assessment**

Difficulties in the histological diagnosis of bone lesions are frequent. First of all, no diagnosis should be made without taking into account relevant clinical data such as age and localisation of the lesion. For example, a chondrosarcoma in a child is very uncommon, as well as a giant cell lesion originating in the metaphyseal region of a long bone. Moreover, a multidisciplinary approach is mandatory for every bone lesion, starting with

the radiologist. In the radiological analysis, the precise localisation of the lesion, its extension the nature of its matrix and its vascularisation are important criteria which have to be correlated with the histological aspects.

### CONCLUSIONS

Bone tumours represent a particularly difficult field in pathology and they require a global assessment taking into account clinical and imaging data. Even under optimal conditions including representative biopsies and adequate imaging documents that allow an optimal anatomopathological confrontation, diagnostic difficulties can persist.

Difficult cases should be referred to review groups for bone pathology. Such experts groups are very useful to achieve a definitive diagnosis. Histological assessment by a panel of experts will probably be required before treatment in the future for certain diagnoses such as osteosarcoma. Finally, molecular markers and genetic analysis should soon offer a decisive help in difficult cases such as the distinction between chondroma and grade 1-chondrosarcoma, but further research is still required before routine clinical application. These biomolecular markers should be more systematically included in the multidisciplinary diagnostic workup of skeletal tumours and they will play a role in the management of bone tumours in the future.

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