

# Symposium 15

## Progress in bone pathology

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### Genetic instability in osteoblastic bone tumors

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#### Aims

At the histological level, the differential diagnosis of osteoblastic bone tumors is characterized by several problems that cannot be solved by conventional histological methods, including immunohistology.

Differentiating aneurysmal bone cyst from telangiectatic osteosarcoma or giant cell tumor from giant cell-containing highly malignant osteosarcoma are only two examples reflecting the complexity of this field. To develop a new approach to these diagnostic problems, we analyzed the genetic instability in a large number of bone-forming tumor-like lesions as well as in benign and malignant osteoblastic tumors.

#### Methods

Our research concentrated on genetic alterations in cell cycle regulator genes: mutations in the p53 and *ras* genes, loss of heterozygosity at the p53, 16 and Rb-locus and amplification of the *mdm2* gene and the *c-myc* gene. In addition to cell cycle regulators, telomerase activity was also analyzed.

#### Results

The data show that the number of genetic alterations increases with the malignancy of the tumors. The highest number of genetic alterations could thus be found in conventional intraosseous osteosarcoma. In tumor-like lesions, genetic alterations have rarely been observed. Rb-loss of heterozygosity could be found in more than 50% of the highly malignant osteosarcomas but in none of the cases of low malignant osteosarcoma.

#### Conclusions

The results of this study show that analyzing genetic instability probably contributes to improving the differential diagnosis of osteoblastic bone tumors. There seem to be considerable genetic differences between low and highly malignant osteosarcomas.

### The value of immunohistochemistry in the diagnosis of primary bone tumors

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Immunohistochemistry has a relatively restricted role in the diagnosis of bone tumors compared to some other areas of pathology. However, for certain categories of tumor such as malignant round cell tumor of/presenting in bone (MRCTB), it is absolutely essential in establishing a precise diagnosis. Other bone tumors where immunohistochemistry is routinely performed in a specialist bone tumor unit include chordoma, adamantinoma, spindle cell sarcomas other than osteosarcoma, tumors of vascular origin, eosinophilic granuloma/Langerhans' cell histiocytosis (LCH) and plasmacytoma/myeloma. It may also be helpful in tumors such as chondroblastoma and mesenchymal chondrosarcoma if the chondroid foci are sparse. Many specialist departments worldwide use needle biopsy routinely in the diagnosis of tumors presenting in bone. Immunohistochemistry often provides useful confirmatory evidence in achieving the diagnosis and may facilitate a diagnosis in a case where the biopsy sample is small and would otherwise need to be repeated.

It is a widely held belief that bone which has been decalcified will pose technical problems in achieving good quality immunostaining. If the bone is carefully decalcified, preferably in ethylenediaminetetraacetic acid (which is an agent used for antigen retrieval) and the end point of decalcification is accurately assessed, preferably by X-raying the sample, then any antibody which can be used on paraffin-embedded soft tissue can be used on bone. Initially, four levels are cut through the tissue block, one section is stained with hematoxylin and eosin (H&E) and two sections from each level are placed on vectabond-coated slides for immunostaining. In our Unit, the needle biopsies are brought sterile and unfixed to the histology laboratory by the radiologist performing the biopsy. Touch/imprint preparations are made, lightly fixed in alcohol and used to achieve a working or definitive diagnosis. If there is urgency in knowing the results of immunostains, immunocytochemistry can be performed on these imprint preparations. In this situation, the APAP method is used as the cytology slides usually contain a significant amount of blood so that the peroxidase method may be difficult to interpret.

#### Malignant round cell tumor (small blue cell tumor)

The differential diagnosis includes metastatic neuroblastoma, Ewing's/primitive neuroectodermal (PNET) tumors, metastatic rhabdomyosarcoma, lymphoma and metastatic small cell carcinoma. CD99 (Mic2) is known to react with lymphoid tissue, synovial

sarcoma and may be positive in osteosarcoma. However, if the tumor cells are negative for CD99, Ewing's/PNET can be excluded as a possible diagnosis. To establish a diagnosis of PNET tumor, two or more neural markers should be positive.

### **Chordoma**

The classical chordoma is positive for cytokeratin and S100 protein. A small number may be negative for S100.

### **Chondroid tumors**

Clear cell chondrosarcoma may be difficult to distinguish from metastatic clear cell renal carcinoma. Clear cell chondrosarcoma is strongly positive for S100 and negative for epithelial markers. Clear cell carcinoma is cytokeratin positive and may also be positive with S100. S100 may also be useful in confirming grade 3 chondrosarcoma and mesenchymal chondrosarcoma. S100 may assist in distinguishing chondroblastoma (positive) from giant cell tumor (positive).

### **Tumors arising in bone not derived from skeletal mesenchyme**

Appropriate antibodies are useful in confirming myelomalplasia and tumors of smooth muscle, vascular and neural origin.

### **Osteofibrous dysplasia and adamantinoma**

It is quite usual to identify scattered single cytokeratin positive cells in osteofibrous dysplasia. To diagnose coexisting adamantinoma, one needs to see strands, islands or glandular structures that are cytokeratin positive. Immunohistochemistry is helpful in confirming adamantinoma but cannot be used to distinguish it from metastatic carcinoma.

### **Langerhans' cell histiocytosis (eosinophilic granuloma)**

Confirmation of the Langerhans' cells with S100 and CD1a is helpful in confirming LCH presenting in bone and distinguishing it from osteomyelitis.

### **Osteosarcoma**

The use of immunohistochemistry in the diagnosis of osteosarcoma may be more confusing than helpful. Vimentin is positive and may be helpful in highlighting the branching pattern of osteoid. S100 is expressed by chondroblastic areas. Many osteosarcomas express smooth muscle actin and a small number show strong membrane staining with CD99. Markers such as osteopontin, osteonectin and osteocalcin require frozen sections. If unfixed tissue is available, a reliable fast and inexpensive method for confirming osteosarcoma is the use of a cytochemical stain for alkaline phosphatase on imprint preparations. If the malignant cells are positive, it confirms they have the potential to form bone, and it is particularly helpful if the osteosarcoma is of a histological subtype such as telangiectatic which produces little or no tumor bone.

### **Conclusion**

Immunohistochemistry plays an important but limited role in the diagnosis of primary bone tumors. The results should be carefully assessed taking into account the clinical and radiological features of the case and the morphology on the H&E stained sections. The prognostic value of antibodies such as the proliferation marker MIB1 and markers for multiple drug resistance may, in the future, become part of the standard panel for some primary bone tumors.

## **Core needle biopsy in the diagnosis of bone lesions**

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Core needle biopsy (CNB) has been recognized and used as a reliable method in the diagnosis of bone lesions. The use of needle aspiration smears was recommended by Coley *et al.* (1931) and Stewart *et al.* (1933) at the Sloan-Kettering Memorial Institute in New York. Ellis *et al.* (1947) in the United States and 1948, Valls, Ottolenghi and Schajowicz *et al.* in Argentina, reported the use of paraffin-embedded material for the histological study of bone lesions.

Despite these articles, very few institutions use CNB for diagnosis in patients with bone lesions. Ayala *et al.* reported that CNB and fine-needle aspiration (FNA), had not gained sufficient popularity among the bone and soft-tissue tumor specialists in the United States to be used on a routine basis and "this is in contrast to European and Argentinian specialists, who regularly use these procedures".

In 1976, Schajowicz and Hokama reported the results of 7,165 puncture biopsies during a 33-year period with about 73% positive results. In 1981, Schajowicz described more than 8,000 CNB, including approximately 2,200 vertebral punctures.

Since 1970, CNB has been used at the M.D. Anderson Cancer Center for the diagnosis and follow-up of bone lesions. In 1995, these workers reported their experience of over more than 800 CNB and FNAs performed from 1976 to 1992 on patients with bone and soft-tissue lesions.

The value of core needle biopsy (CNB) in the diagnosis of bone lesions has been widely discussed. In contrast to open biopsies that require a surgical excision to remove tissue for diagnosis, CNB do not and are referred to as 'closed biopsy procedures'. One of the main objections to the technique has been the degree of confidence that can be inspired by a diagnosis based on relatively small particles of tissue. In skilled hands, needle biopsy has a definite use, particularly in cases where the site of the lesion (such as the vertebra) or the treatment to be adopted makes open biopsy difficult or undesirable.

The application of CNB to a patient's bone lesion is a multidisciplinary approach that involves a team of medical specialist working together to evaluate and manage the patient. This team includes an orthopedic surgeon, a radiologist and a pathologist. Before performing a CNB procedure, the medical team makes a clinical and radiographic evaluation, determines the stage of the disease and explains the procedure to the patient. The biopsy site was determined in accordance with the planned subsequent resection surgery. Because limb saving surgery is the local treatment of choice in diverse malignant bone tumors, the biopsy site must be carefully thought about and the needle track should always be removed 'en bloc' in the surgical procedure.

Several different types of needle are used in a needle biopsy procedure. Needles with serrated ends produce fragmentation of bone dragging along the bone dust, which results in unsatisfactory specimens. In our experience, we prefer a 2-mm needle or a Jamshidi needle.

Using fluoroscopic guidance, and more recently computerized tomography, it is possible to reach small lesions in the spine or iliac bone.