

The predictive role of Ki-67 protein in determining the aggressiveness of primary malignant bone tumors. A retrospective study

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Abstract

Introduction: In order to diagnose and stage malignant bone tumors, the pathologic examination of harvested pieces with immunohistochemistry test is necessary; they also provide information regarding the prognosis on a medium to long term. Among tissular biomarkers with potential predictive value, a raised Ki-67 protein level is used to determine the risk of local recurrence or metastasis.

Material and method: This study was performed on 50 patients with primary malignant bone tumors admitted in the Traumatology and Orthopedy Department of University Emergency Hospital, Bucharest. Patients repartition according to diagnosis was the following: 21 patients with osteosarcoma, 18 patients with chondrosarcoma, 6 patients with Ewing sarcoma, 3 patients with malignant fibrous histiocytoma, and 2 with fibrosarcoma. The follow-up period was between 12 and 72 months with a mean of 26 months.

Results: Patients were aged between 18 and 77 years old, with a mean age of 41,36. There were 22 women and 28 men. No sex or age difference was notable for the tumor outcome. After calculating the Ki-67 LI, 36 patients were included in the high-risk group (Ki-67 LI > 25%), while 14 had a low risk for metastasis and local relapse (Ki-67 < 25%). The low-risk patients had chondrosarcoma (8 patients), osteosarcoma (5 patients), and fibrosarcoma (1 patient). During the follow-up, 8 patients, all belonging to the high risk group, developed metastasis, while 5 patients developed local recurrences; 4 patients who relapsed belonged to the high risk group and 1 to the low risk group. Metastases developed in 3 patients with osteosarcoma, 2 with Ewing sarcoma, 2 with chondrosarcoma and 1 patient with fibrosarcoma. Most metastases occurred within one year after surgery. The other fibrosarcoma patient developed local recurrence after 6 months, while the other local recurrences occurred in osteosarcoma patients (2 cases) and 1 in a Ewing sarcoma patient and chondrosarcoma patient.

Conclusions: Our study concluded that while Ki-67 LI values are useful in determining the aggressivity of primary malignant bone tumors, it should always be used in conjunction with the clinical, imaging and anatomopathological diagnosis methods in order to accurately predict the patients' outcome.

Keywords: Ki-67, malignant bone tumor, metastasis, local recurrence, immunohistochemistry

Introduction

Primary malignant bone tumors are relatively rare compared to other neoplasms, with an annual incidence of nearly 10 cases in a million [1]. The most frequent primary malignant bone tumors are osteosarcomas (~35%), chondrosarcomas (~25%), Ewing sarcomas (~16%), followed, at great length by fibrosarcomas and malignant fibrous histiocytomas (under 5%); the malignant vascular bone tumors and adamantinomas are extremely rare [2]. In order to avoid diagnosis errors as well as to adopt an optimal therapy, the existence of specialized diagnostic and treatment centers is mandatory, as is a multidisciplinary team made up of orthopaedic surgeons, radiology specialists, and pathologists.

In order to diagnose and stage malignant bone tumors, the pathologic examination of harvested pieces with immunohistochemistry test is necessary; they also provide information regarding the prognosis on a medium to long term. Among tissular biomarkers with potential predictive value, a raised Ki-67 protein level is used in various malignancies, such as prostate, breast or brain cancer to determine the risk of local recurrence or metastasis. The Ki-67 antigen is probably associated with cellular proliferation, its expression being highest in the G₁, S-G₂ and M phases of the cellular cycle, while Ki-67 is absent in G₀-phase cells [3]. Thus, immunohistochemical stains using anti-Ki-67 monoclonal antibodies allow a rapid evaluation of the tumoral samples, without other preparations.

The authors of this article presented a retrospective study about the correlation between the Ki-67 antigen levels and the rate of post-surgery local recurrences or metastasis in treated primary malignant bone tumors.

Material and method

This study was performed on 50 patients with

primary malignant bone tumors admitted in the Traumatology and Orthopedy Department of University Emergency Hospital, Bucharest. The patients' repartition according to diagnosis was the following: 21 patients with osteosarcoma, 18 patients with chondrosarcoma, 6 patients with Ewing sarcoma, 3 patients with malignant fibrous histiocytoma, and 2 with fibrosarcoma. Following the clinical and imaging exams, the patients were diagnosed with limbs bone tumors, the tumoral samples being submitted to biopsy and pathological examination, which established the definite diagnosis. All patients underwent surgical treatment by resection-reconstruction technique followed by adjuvant oncological therapy according to the histological type and location of tumor. The follow-up period was between 12 and 72 months with a mean of 26 months.

After the removal of the tumors, the tissue fragments were sent for processing and examination to our Department of Pathology. Samples of tissue were fixed with 10% buffered formalin and sent for histopathological processing by conventional method using paraffin inclusion and Hematoxylin-Eosin (H&E) staining. Also, immunohistochemical (IHC) tests were performed. The paraffin blocks acquired by histopathological processing were sectioned at microtome resulting 3- μ m-thickness sections mounted on slides covered with poly-L-lysine. After that, the sections were deparaffinized in toluene and alcohol successive baths, one hour, 15 minutes by bath and rehydrated (three successive alcohol baths with decreased concentration: 96%, 80% and 70%, 10 minutes in each bath, followed by a bath with distilled water, the sections being held for 10 minutes). These steps were followed by washing in phosphate saline buffer (PBS), incubation with normal serum, for 20 minutes, incubation with primary antibody overnight, Dako LSAB kit, washing in carbonate buffer and development in 3,3'-diaminobenzidine (DAB) hydrochloride/ hydrogen peroxide and nuclear counterstaining with Mayer's Hematoxylin.

The following antibodies from NeoMarkers LabVision were used: Ki67, s100, D2-40, CD99, Top2alpha, vimentin, and P53.

The percentage of Ki-67 positive cells in each sample was quantified in 1000 cells and expressed as Ki-67 labeling index (Ki-67 LI) with a value over 25% being considered significant.

Results

50 cases with primary malignant bone tumors were admitted in our department and included in this study. Patients were aged between 18 and 77 years old, with a mean age of 41,36. There were 22 women and 28 men. No sex or age difference was notable for the tumor outcome. 21 patients were diagnosed with osteosarcoma, 18 patients with chondrosarcoma, 6 patients with Ewing sarcoma, 3 patients with malignant fibrous histiocytoma and 2 with fibrosarcoma. After calculating the Ki-67 LI, 36 patients were included in the high-risk group (Ki-67 LI > 25%), while 14 had a low risk for metastasis and local relapse (Ki-67 < 25%). The low-risk patients had chondrosarcoma (8 patients), osteosarcoma (5 patients), and fibrosarcoma (1 patient).

All patients with chondrosarcomas with an increased risk of relapse were all grade 2 or 3 (Fig. 1). The grading of these tumors was based on cellularity and nuclear changes of the malignant chondrocytes: well, moderate or poorly differentiated correspond to grades 1-3; grade 4 was spindled tumor representing either chondroblastic osteosarcoma or dedifferentiated chondrosarcoma. Also, all the chondrosarcomas, which at the time of initial diagnosis had invasion of the skeletal muscle (Fig. 2) and frequent hemorrhagic areas (Fig. 1,2), were considered to have a high risk of metastasis or recurrence. From the immunohistochemical point of view, all high-risk tumors had a Ki67 index above 25% (Fig. 4,5).

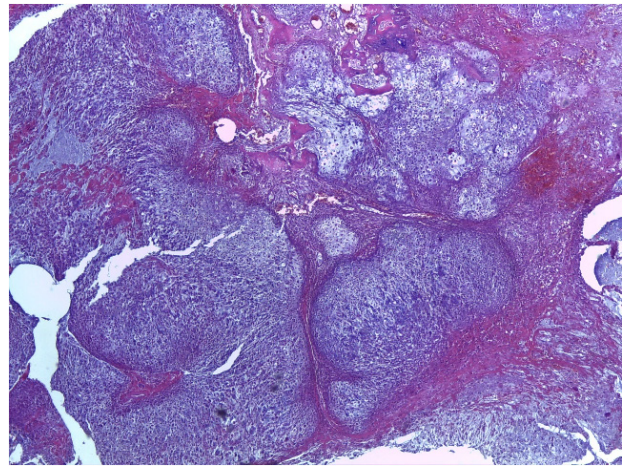


Fig. 1 Grade 3 chondrosarcoma with haemorrhagic areas. Note the invasive lobulated mass, the marked hypercellularity, extreme pleomorphism and bizarre tumor giant cells and frequent mitotic figures. H&E staining, ob. 4x

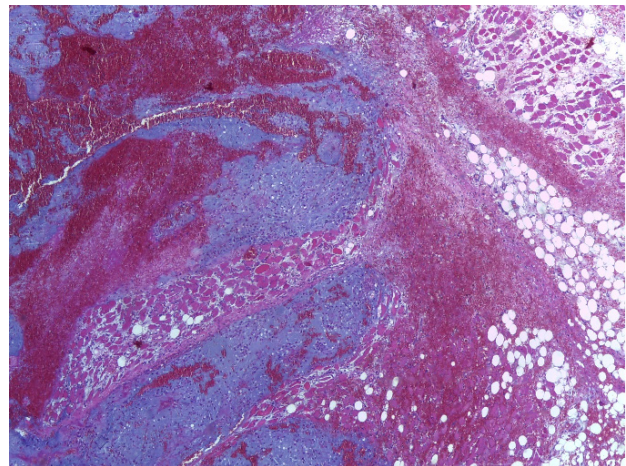


Fig. 2 Grade 2 chondrosarcoma with many haemorrhagic areas. Note the deep soft tissue invasion (skeletal muscle and adipose tissue). H&E staining, ob. 4x

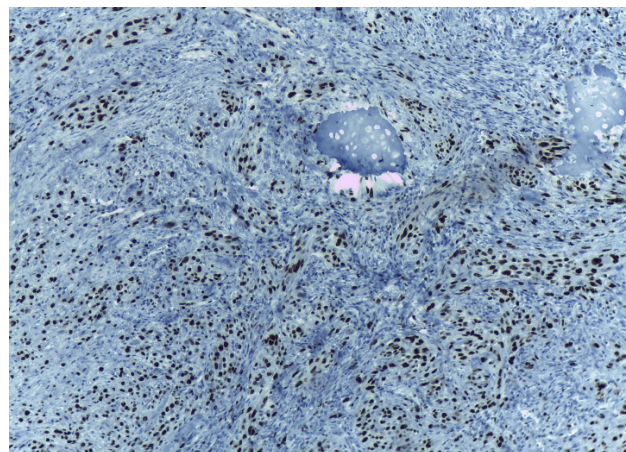


Fig. 3 Intense nuclear immunodepression for Ki67 (MIB1) in approx 40% of the tumor cells in a G3 chondrosarcoma. IHC staining with DAB chromogen, ob 10x

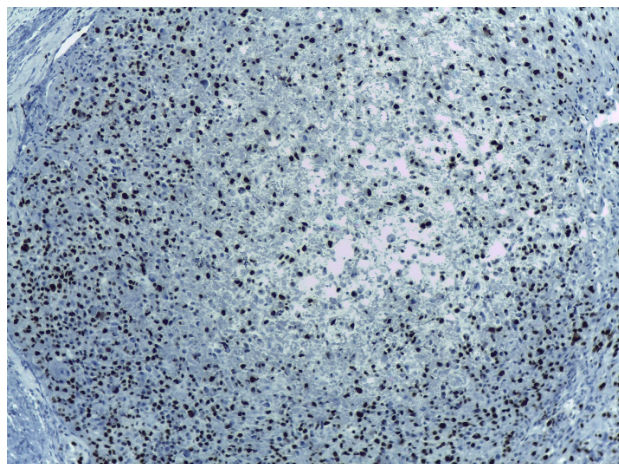


Fig. 4 G3 chondrosarcoma. Intense nuclear immunodepression for KI67 (MIB1) in approx 50% of the tumor cells, especially on the periphery of invasive proliferation. IHC staining with DAB chromogen, ob 10x

Table 1. Postoperative relapses

	Recurrence	Metastasis
Osteosarcoma	2	3
Chondrosarcoma	1	2
Ewing sarcoma	1	2
Malignant fibrous histiocytoma	-	-
Fibrosarcoma	1	1

Discussions

Although no definite conclusion could be drawn from our data, due to the lack of a statistical significance, a certain pattern could be observed regarding the Ki-67 LI significance. In patients with Ewing sarcoma, a tumor well known for its aggressivity, all Ki-67 values were high and 3 out of 6 patients relapsed. On the other hand, while having relatively low Ki-67 LI values, the 2 patients with fibrosarcoma both developed recurrences or metastases and the malignant fibrous histiocytoma patients had good long-term outcomes, despite their high-value Ki-67 LI. Thus, we theorized that Ki-67 LI is a more useful predictive statistical factor rather than an individual predictive factor, i.e. Ki-67 LI should be used as an aid to histopathologic evaluation particularly in those instances in which the pathologic criteria are not very well defined, and a long-term prognosis is

hard to postulate. In any case, Ki-67 staining should always be used in conjunction with the other diagnosis methods and should never be employed as a single predictive factor for the patient's outcome.

During the follow-up, 8 patients, all belonging to the high risk group, developed metastasis, while 5 patients developed local recurrences; 4 patients who relapsed belonged to the high risk group and 1 to the low risk group. Metastases developed in 3 patients with osteosarcoma, 2 patients with Ewing sarcoma, 2 patients with chondrosarcoma and 1 patient with fibrosarcoma. Most metastases occurred within one year after surgery. The other fibrosarcoma patient developed local recurrence after 6 months, while the other local recurrences occurred in osteosarcoma patients (2 cases) and 1 in a Ewing sarcoma patient and chondrosarcoma patient (Table 1).

In our study, there were only 3 patients with malignant fibrous histiocytoma and all underwent chemotherapy, which can account for the favorable outcome.

Five out of 21 patients with osteosarcoma (25%) developed recurrences or metastases, all of them having a high Ki-67 LI value, thus confirming our theory that for larger groups of patients the Ki-67 levels correlate with the aggressivity of the tumor.

All metastasis cases were pulmonary metastases and had a high Ki-67 LI, which correlated with other studies in literature [4]. Also, most metastases occurred within one year after surgery, confirming previous observations that a high Ki-67 LI value correlates with early metastasis [5].

Conclusions

Our study concluded that while Ki-67 LI values are useful in determining the aggressivity of primary malignant bone tumors, it should always be used in conjunction with the clinical, imaging and anatomopathological diagnosis methods in order to accurately predict the patients' outcome.

Conflict of Interest statements

Authors state no conflict of interest.

Informed Consent and Human and Animal Rights statements

Informed consent has been obtained from all individuals included in this study.

Authorization for the use of human subjects

Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies, is in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

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