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DIFFUSE LARGE B-CELL LYMPHOMA PRESENTED AS BONE LESIONS. A STUDY OF 21 CASES AND REVIEW OF THE LITERATURE

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Diffuse large B-cell lymphoma is the most common lymphoma worldwide. Both morphologically and prognostically it represents a diverse spectrum of disease. Non-Hodgkin lymphoma of bone comprises approximately 7% of malignant bone tumors, 5% of extranodal lymphomas, and <1% of all non-Hodgkin lymphomas. Recent gene expression profiling studies have classified diffuse large B-cell lymphoma into 2 main subtypes, germinal center B-cell and activated B-cell, with the germinal center type showing an overall better survival. Twenty one patients with diffuse large B-cell lymphomas of bone were studied. The tumors were subclassified according to the criteria of the WHO standards and evaluated by immunohistochemistry for expression of antigens associated with germinal center (GC) and non-GC stages of B-cell differentiation. The panel included bcl-6, CD10, MUM-1, bcl-2, CD138. A survival analysis of 21 cases showed that GC-like tumors had longer overall survival duration compared with non-GC-like tumors. This review focuses solely on de novo DLBCL presenting with bone involvement without evidence of extraskeletal disease and do not include transformation from an underlying low-grade lymphoproliferative disorder.

Key words: *diffuse large B-cell lymphoma, bone involvement, immunohistochemistry, survival*

ПЕРВИЧНАЯ КРУПНОКЛЕТОЧНАЯ В-КЛЕТОЧНАЯ ЛИМФОМА КОСТЕЙ. ОПИСАНИЕ 21 БОЛЬНОГО С ОБЗОРОМ ЛИТЕРАТУРЫ

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Диффузная крупноклеточная В-клеточная лимфома повсеместно является наиболее часто встречающимся вариантом злокачественной лимфомы. С точки зрения морфологии и прогноза данная патология отличается большим количеством различных вариантов заболевания. На долю первичной неходжкинской лимфомы костей приходится около 7% всех злокачественных опухолей костей, 5% всех экстранодальных лимфом и менее 1% всех неходжкинских лимфом. Генетические исследования последних лет позволили выделить из общего числа первичных В-клеточных крупноклеточных лимфом две основные подгруппы: опухоли из клеток герминативных центров и опухоли из активированных В-клеток с более благоприятным прогнозом в группе опухолей из клеток герминативных центров. Изучение экспрессии ряда маркеров (bcl-6, CD10, MUM-1, bcl-2, CD138) иммуногистохимическим методом при обследовании 21 больного с первичной В-клеточной крупноклеточной лимфомой костей позволило выделить две основные подгруппы пациентов. В группе больных с опухолями из клеток герминативных центров отмечен более благоприятный прогноз. В исследование включены только случаи первичной В-клеточной крупноклеточной лимфомы костей, развившейся de novo без признаков экстраскелетного поражения и предшествовавшего лимфопролиферативного заболевания.

Ключевые слова: *диффузная крупноклеточная В-клеточная лимфома, поражение костей, иммуногистохимия, выживаемость*

Primary bone lymphoma is suggested when the patient remains free of extraskeletal disease for 6 months after diagnosis, it was first described by Oberling in 1928. Parker and Jackson in 1939 published their series under the designation «reticulum cell sarcoma of bone» and established primary bone lymphoma as a distinct entity. For several decades the lesion was considered to be of lymphoid nature and B-cell origin in the majority of cases [1, 2, 13]. Most primary bone lymphomas have similar morphology composed of large centroblasric cells. Presence of multilobated nuclei is common, the lesion is usually characterized by prominent sclerosis and classified as diffuse large B-cell lymphoma (DLBCL) in the WHO classification of malignancies [4]. Most primary bone lymphomas arise de novo with no evidence of underlying low-grade lymphoproliferative disorder or preexisting small B-cell proliferation. It has been hypothesized that centroblastic and immunoblastic morphologic variants of DLBCL might reflect germinal center (GC) and post-germinal center (post-GC) derivation of the tumor [14]. The rare occurrence of primary bone lymphoma make it difficult to recognize, the differential diagnosis include major list of small cell malignancies of bone as well as osteomyelitis and Langerhans cell histiocytosis [12].

In this report we studied 21 cases of primary DLBCLs presenting with bone involvement. All secondary cases of bone lymphoma were excluded. The tumors were subclassified

Table 1

CLINICAL FEATURES AND OUTCOME OF 21 PATIENTS WITH DDLBCL PRESENTING AS A BONE LESION

Case	Sex/age	Location/Path. fracture	Unifocal/Multifocal	Treatment	Follow-up
1	m/4	Pelvis	Multifocal	CT	Alive 64 mo
2	f/75	Pelvis	Multifocal	CT/RT	Died 2 mo
3	f/41	Femur/Fracture	Unifocal	S/CT/RT	Alive 68 mo
4	f/83	Sternum	Unifocal	No data	Died 3 mo
5	m/44	Femur/Fracture	Unifocal	CT/RT	Alive 78 mo
6	f/84	Humerus/Fracture	Unifocal	None	No data
7	f/56	Femur/Fracture	Unifocal	S/CT/RT	No data
8	f/86	Femur	Unifocal	S/prosthesis	No data
9	f/52	Femur	Unifocal	S/CT	Alive 72 mo
10	m/58	Femur/Fracture	Multifocal	S/CT/RT	Alive 24 mo
11	f/73	Scapula	Unifocal	RT	Died 1 mo
12	f/75	Sacrum, spine	Multifocal	CT/RT	Died 10 mo
13	m/82	Femur	Unifocal	S/prosthesis	Died 2 mo
14	m/56	Femur	Unifocal	S/CT	Alive 46 mo
15	f/57	Jaw bones	Multifocal	CT	Alive112 mo
16	m/65	Spine/Fracture	Multifocal	CT	Alive 24 mo
17	m/28	Femur	Unifocal	S/CT	Alive 24 mo
18	f/83	Humerus/Fracture	Unifocal	S/prosthesis	Died 1 mo
19	f/58	Femur/Fracture	Unifocal	S/CT/RT	Died 56 mo
20	m/27	Femur	Unifocal	CT/RT	Alive 26 mo
21	f/83	Humerus/Fracture	Unifocal	S	Died 12 mo

S – sergery, CT – chemotherapy, RT – radiation therapy

morphologically according to the criteria of the WHO classification and evaluated by immunohistochemistry for expression of antigens that are expressed in germinal center (GC) and post germinal center (post-GC) stage of B-cell differentiation. The spectrum of antigens including bcl-6, CD10, MUM-1, CD 138, bcl-2 and p53 helps to investigate the possible relationship of DLBCL of bone to stages of normal B-cell differentiation, confirm the accurate classification of DLBCL into prognostically relevant subgroups.

MATERIALS AND METHODS

We studied 21 case of DLBCL with diverse clinical and immunohistochemical characteristics. Clinical information was obtained from the patient's medical records. All cases for which paraffin blocks were available and sufficient tissue remained were selected for study, all cases had regular x-ray, CT scan and MR images. Additional immunohistochemical tests were performed. Cases were considered as primary bone lymphoma if disease was restricted to bone and adjacent soft tissue at the time of the diagnosis and were further stratified as unifocal or multifocal.

For each case, sections of formalin-fixed, paraffin embedded tissue stained with hematoxylin and eosin were reviewed. The morphology was classified as centroblastic if more than 20% of the malignant cells were large, had scant cytoplasm and 1-3 peripherraly located nucleoli. Immunoblastic morphology was classified if more than 90% of malignant cells were immunoblasts with centrally located nucleoli and abundant eosinophilic cytoplasm. Polymorphous cases were sorted out with borderline morphology between centroblastic and immunoblastic variants. The presence of cells with multilobated nuclei was a characteristic feature in most of the cases and was included in the diagnosis when more than 10% of malignant cells showed the evidence of that feature. The decalcification procedure of the core biopsies was performed in EDTA solution. Total morphological examination of the tumor was performed in most surgical cases.

Immunohistochemistry

The routine diagnostic workup included the evaluation of B-cell (CD20) and T-cell (CD3) antigens. All antigens (bcl-6, CD10, MUM-1, bcl-2, CD138) (DAKO Corporation), were checked with tonsil control tissue and used in recommended dilutions. Immunostains were performed using a two-step avidin-biotin-peroxidase standard technique after antigen retrieval. For all antigens tumors were considered positive if >10% cells stained appropriately positive. For bcl-2 and MUM-1, positive cases were scored as 1+ (<30% positive cells), 2+ (30–60% of positive cells), 3+ (>60% of positive cells) [11].

Table 2

IMMUNOHISTOCHEMICAL FEATURES OF 21 DLBCLS OF BONE

Case	Morphology	bcl-6	CD10	MUM-1	bcl-2	CD138	p53
1	CB/PM	3+	+	0	0	0	10%
2	CB/ML	2+	+	ND	0	0	25%
3	CB/ML	3+	+	0	+	1+	10%
4	CB	0	0	2+	0	ND	30%
5	IB	3+	0	ND	+	0	5%
6	CB	1+	+	+	+	0	ND
7	CB	1+	+	+	0	0	ND
8	UC	2+	+	ND	0	0	ND
9	CB	2+	0	2+	+	ND	5%
10	CB	0	0	2+	+	0	0%
11	CB/PM	0	0	0	+	0	20%
12	CB/PM	2+	+	0	+	ND	20%
13	PM	0	0	1+	+	0	25%
14	CB/ML	0	0	2+	+	0	0%
15	IB	3+	+	+	+	0	20%
16	CB	1+	+	ND	+	ND	5%
17	CB	3+	+	+	0	0	0%
18	CB/ML	0	0	+	0	0	30%
19	CB/ML	0	0	ND	+	0	20%
20	CB/ML	1+	+	0	+	ND	0%
21	CB/ML	0	0	ND	+	ND	15%

CB – centroblastic, PM – polymorphous, IB – immunoblastic, ML – multilobated nuclei, ND – not done

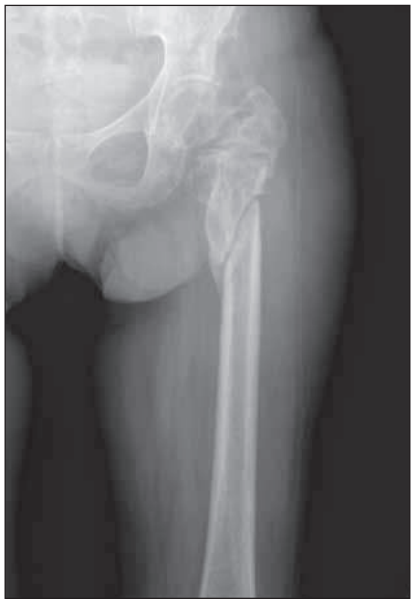

Figure 1. *Radiograph. Primary lymphoma of the proximal femur. Lytic-destructive pattern. Pathologic fracture*

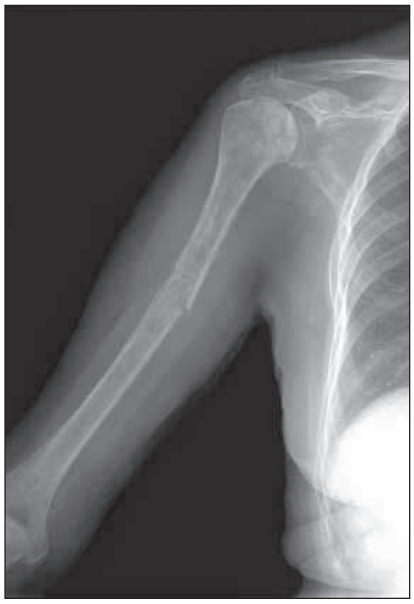

Figure 2. *Primary lymphoma of the humerus. Moth-eaten pattern of destruction and aggressive periosteal reaction. Pathologis fracture*

RESULTS

Clinical features

All 21 patients had disease limited to long bones 14/21 (67%), axial skeleton 5/21 (23%), multiple sites 2/21 (10%). The tumors occurred in 8 males and 13 females, 23 to 86 years of age (median 43 years) (Table 1). Bone involvement presented as a solitary lesion in 15/21 (71%) and multiple in 6/21 (29%) cases, with femur as the most common location 11/21 (62%). A pathologic fracture occurred in 9/21 (44%) of the patients with an episode of broken bone after chemotherapy in 4/21 (47%) of all cases with pathologic fractures.

Treatment modalities were known for 20 patients, 1 female 84 years old patient did not receive any surgery, radiation, or chemotherapy. The majority of patients were treated with different surgical procedures in combination with chemoтtherapy and radiation therapy 18/21 (86%).

Follow up was available in 18 cases, 8 patients died in 1 to 56 months after the diagnosis, 10 patients were alive in 24 to 112 months, in 2 patients the cause of death was not disease related.

Radiologic features

The radiographic appearances of primary bone lymphoma are variable and a second modality such as computer tomography (CT) or magnetic resonance (MR) imaging should be used [8]. The presence of a solitary, permiative, metadiaphyseal lesion with a layered periosteal reaction on plain radiographs especially in older patients is highly suggestive of lymphoma. The case for a diagnosis of primary bone lymphoma is further strengthened if the soft-tissue mass and marrow changes are associated with little cortical destruction. The lytic-destructive pattern is the most common radiographic appearance of primary bone lymphoma, as it was reported in 18/21 (86%) of our cases. The lytic pattern may be permiative – characterized by numerous small, elongated rarefactions that are parallel to the long axis of the bone and uniform in size, or moth-eaten – a pattern of many medium to large areas of radiolucency in a poorly marginated area of bone. Cortical breakthrough, pathologic fractures and soft-tissue masses represent a more aggressive pattern of involvement and a poorer prognosis. Periosteal reaction may be either lamellated or layered, parallel to the long axis, or broken when interrupted periosteal new bone is seen. The latter appearance of disrupted periosteal bone is believed to be a helpful radiographic sign that indicates a poorer prognosis.

Pathologic features

All cases were classified as either centroblastic, centroblastic polymorphous, centroblastic with multilobated nucleus, or immunoblastic. Multilobation of the nuclei was a common and prominent morphologic feature. There was no evidence of a follicular, or small B-cell component admixed or adjacent to the large cell lymphoma in any of the cases [7].

Immunohistochemical studies

No correlation between morphology and immunophenotype was found. The tumors were subdivided into three categories: tumors with CD10+, bcl-6+ or germinal center phenotype 11/21 (53%), tumors with CD10-, bcl-6+, or intermediate phenotype 2/21 (10%) and negative CD10 and bcl-6, or post germinal center-like phenotype 8/21 (37%). The intensity of the staining was variable from case to case, bcl-6 was 3+ positive in 5 cases, 2+ patchy positive in 4 cases and 1+ positive in 4 cases. All CD10 positive cases coexpressed bcl-6. MUM-1 was detected in 10 cases. The bcl-2 marker was positive in the majority of tumor cells and the frequency of bcl-2 expression did not differ significantly in the three immunophenotypic categories [6].

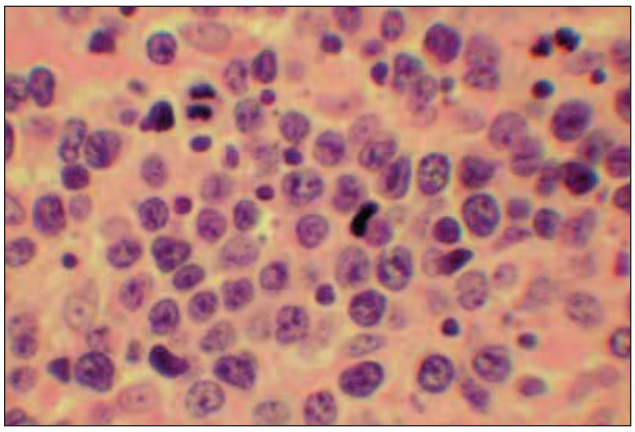

Figure 3. *Typical case of primary bone lymphoma showing centroblastic cells with some multilobated nuclei (original magnification ×200)*

DISCUSSION

We report a series of 21 primary bone lymphoma cases with female predominance 8/21 (13%), which do not correspond to the literature. The median age of the patients overlaps with that of other clinical series. In our study, the femur was the most common site of involvement and axial skeleton the second most common location. Most tumors were centroblastic, or centroblastic with multilobated nuclei 15/21 (71%).

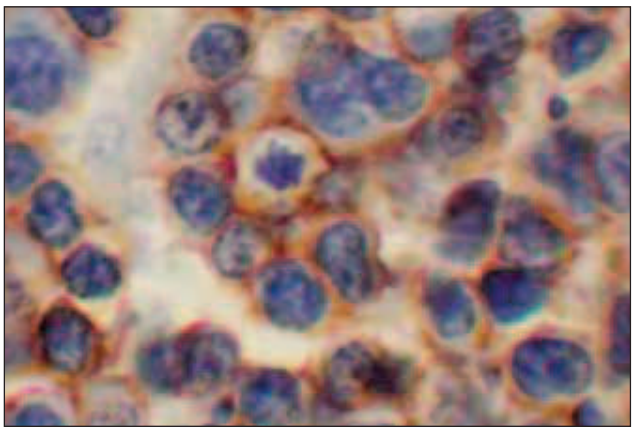

Figure 4. *Immuynohistochemical findings in primary diffuse large B-cell lymphoma of bone. CD20 positive membrane staining in the majority of the tumor cells (original magnification ×400)*

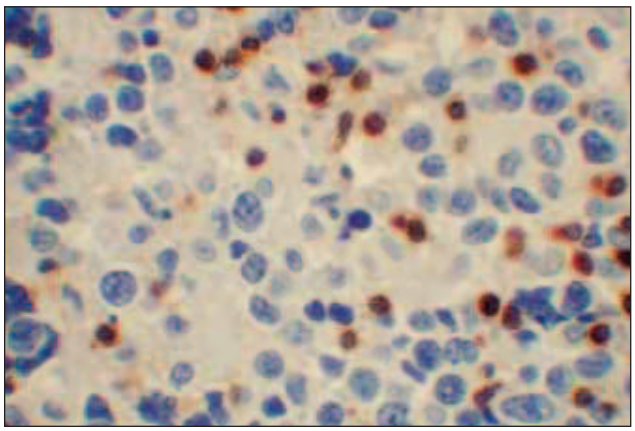

Figure 5. *Immuynohistochemical findings in primary diffuse large B-cell lymphoma of bone, CD3 positive nuclear staining in small lymphocytes. Tumor cells are negative (original magnification ×200)*