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ORIGINAL ARTICLE

Primary Soft Tissue Lymphomas: Description of Seven Cases and Review of the Literature

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Abstract The present study describes a series of primary soft tissue lymphomas, including immunohistochemical characterization by tissue microarray and cytogenetic profiling. Formalin-fixed, paraffin-embedded tissue samples were collected from patients who underwent soft tissue biopsy. Cases were selected according to the definition of primary soft tissue lymphoma as a lymphoid malignancy arising in soft tissues without evidence of other nodal or extranodal localization for a period of at least 6 months. Our series comprised seven patients with a mean age of 72 years. There were three diffuse large B-cell lymphomas (DLBCLs); one B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma; one DLBCL derived from follicular lymphoma; one ALK-negative anaplastic large cell lymphoma; and one follicular lymphoma. Immunohistochemical and molecular profiles were consistent with the histological diagnoses. The present study contributes to our knowledge about uncommon presentation of lymphoid neoplasms and confirms previously published clinical-pathological data. We present, for the first time, the complete immunohistochemical profile and molecular cytogenetic studies of these lymphoid neoplasms. A rare case of a primary soft tissue ALK-negative anaplastic large cell lymphoma is described in detail.

Keywords Lymphoma · Soft tissue tumors · FISH · C-myc oncogene

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Introduction

A soft tissue initial presentation of a lymphoproliferative neoplasm is a very rare event, accounting for approximately 0.1 % of all lymphoid malignancies [1] and 0.01 % of all soft tissue tumors [2]. More commonly, the involvement of soft parts occurs by direct extension from lymph nodes or other extranodal structures, or by hematogenic dissemination. The limited number of cases described in the literature consist of non-Hodgkin lymphomas, and the most frequent histotype is diffuse large B-cell lymphoma (DLBCL) [1].

Clinically, soft tissue lymphomas generally present as painful subcutaneous masses in the thighs, trunk, and lower limbs that are fixed on deep tissues and rapidly increase in size. The main differential diagnosis is primary tumors of soft tissues, especially sarcomas derived from muscular, connective, or adipose tissues [3–9].

Imaging techniques, particularly computed tomography (TC) and magnetic resonance imaging (MRI), may reveal the presence of a neoplastic mass, and an incisional biopsy is necessary to reach a definitive diagnosis [10, 11].

In the present study, we describe a series of seven cases of primary soft tissue lymphoma, including immunohistochemical characterization by tissue microarray (TMA) and molecular cytogenetic characterization.

Materials and Methods

Formalin-fixed, paraffin-embedded tissue samples were collected from seven patients who underwent soft tissue biopsy for diagnostic purposes at the Division of Pathology of the Orthopedic Institute "Gaetano Pini" of Milan between 2004 and 2012.

The cases were selected according to the definition of primitive soft tissue lymphoma as a lymphoid malignancy arising in soft tissues without evidence of other nodal or extranodal localization for a period of at least 6 months [9].

The main clinical characteristics of the patients included in the study are summarized in Table 1.

Immunohistochemical Analysis

Routinely prepared formalin-fixed paraffin-embedded blocks were used to construct a paraffin-embedded tissue microarray. In this series, three tumor tissue cores were sampled for each case.

Cores with a diameter of 2 mm were generated using a semi-automatic arrayer (Alphelys Minicore2, Plaisir, France). Before the immunohistochemical (IHC) analyses, a section was cut from each tissue microarray block and stained with hematoxylin and eosin for morphological examination.

All the cases included in the tissue microarray were characterized by IHC with the following antibodies: Bcl 2 (clone 124), Bcl 6 (clone GI19E/A8), CD3 (clone 2GV6), CD10 (clone SP67), CD20 (clone L26), CD30 (clone Ber-h2), CD45, LCA (clone 2B11 & PD7/26), CD79a (clone SP18), Ki 67 (clone MIB-1), MUM1 (clone MRQ-43), CD4 (clone SP35), CD8 (clone SP57), Granzyme B (polyclonal), Perforin (clone MRQ-23), ALK (clone ALK), and c-myc (clone Y69).

IHC was performed using the automated system BenchMark XT (Ventana Medical Systems Inc., Tucson, Arizona, USA). Reactions were revealed using UltraView™ Universal DAB, a biotin-free, multimer-based detection system, according to the manufacturer's instructions.

In Situ Hybridization and Fluorescent in Situ Hybridization Analyses

In addition to the IHC profiling, EBV-DNA was analyzed by in situ hybridization (ISH) with the EBER probe (DAKO EBER PNA Probe/Fluorescein with the PNA ISH detection kit). Cases were further characterized by fluorescent in situ hybridization (FISH) using the protocol provided by the manufacturer of the multicolor probes LSI IGH/BCL2 Dual Color, Dual Fusion Translocation Probe Set, IGH/CCND1 Dual Color, Dual Fusion Translocation Probe Set, and MYC Dual Color, Break Apart Rearrangement Probe (Vysis Inc., Downers Grove, IL) for t(14;18), t(11;14), and MYC rearrangement, respectively.

Results

The tissue microarray included six cases (no. 1–6) and 19 spots (three for each case and one for the orientation spot with human placenta), without significant observable differences from a morphological and immunophenotypical point of view between different cores for each case. Case no. 7 was treated separately because of the paucity of material (insufficient thickness of the section), which prevented its addition to the TMA.

Morphological and Immunohistochemical Results

– DLBCL (cases 1, 4, 5, and 6)

All cases diagnosed with DLBCL showed a diffuse pattern of growth. The neoplastic population consisted of large and frequently discohesive tumor cells resembling centroblasts or large cells with abundant eosinophilic, sometimes clear,

Table 1 Clinicopathological data of the series

Case no.	Age	Sex	Histotype	Localization	Follow-up (months)
1	74	F	DLCBL	Right shoulder	Alive (192)
2	64	M	B-cell lymphoma unclassifiable, with features intermediate between DLBCL and BL	Right hip	Dead (12)
3	77	F	ALCL	Left back hand	Dead (12)
4	73	M	DLCBL	Left inguinal region	Dead ()[a]
5	66	M	DLCBL	Left tight	Alive (48)
6	88	F	DLCBL	Right wrist	Dead (24)
7	70	F	FL	Left arm	Alive (72)

Clinical and pathological features of seven primary soft tissue lymphomas

Abbreviations: *M* male, *F* female, *DLBCL* diffuse large B-cell lymphoma, *ALCL* anaplastic large cell lymphoma, *FL* follicular lymphoma, *BL* Burkitt lymphoma

[a] Patients were dead without knowledge of the follow-up

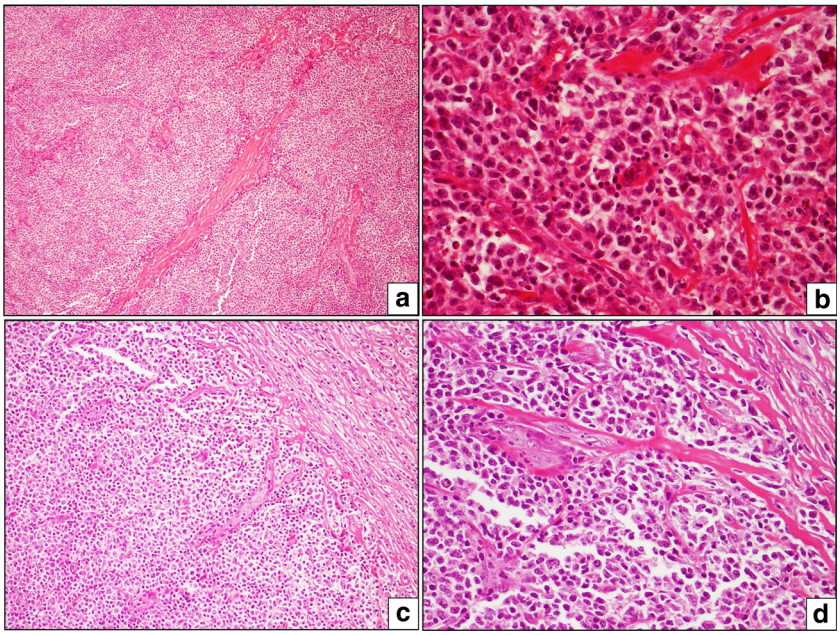

Fig. 1 Morphological results, cases 1 and 5. **Case no. 1.** Morphological features of a diffuse large B-cell lymphoma (DLBCL) case [**a**, 20× and **b**, 40× by hematoxylin and eosin (H&E), respectively] consisting of discohesive tumor cells, with abundant eosinophilic, sometimes clear, cytoplasm and with interstitial or broad bundles of sclerotic connective tissue. **Case no. 5.** Morphological features of a DLBCL case derived from FL (**c**, 20× and **d**, 40× by H&E, respectively), with a nodular and diffuse pattern, consisting of medium sized cleaved cells and large centroblasts, with interstitial sclerosis. Infiltration of adipose tissue is clearly evident

cytoplasm (Fig. 1a and b). The nuclei were round and vesicular, with small multiple nucleoli, frequently adherent to the nuclear membranes; in some cases, nuclei were irregular, folded, or multi-lobulated, with a polymorphic appearance (Fig. 1c and d). Numerous apoptotic bodies were also present in the neoplastic population. Interstitial or broad bundles of collagen were diffusely present within neoplastic elements, and extensive infiltration of the surrounding soft tissues was a consistent finding. In case no. 6, large areas of necrosis were also present.

Immunohistochemically (Table 2), all cases expressed CD45 (LCA), CD79a, and CD20. Based on the expression of three germinal center related-markers (CD10, BCL6, and MUM1) and according to the algorithm of Hans [12], the cases were divided as follows:

- One of three cases with germinal center-like immunophenotype (CD10+ and/or BCL6+, MUM1-);
- Two of three cases with non-germinal center-like immunophenotype (CD10-, variable expression of BCL6 and MUM1+).

C-myc expression was not observed in case no. 4, whereas case no. 1 showed immunoreactivity in less than 40 % of tumor cells, and case no. 6 was positive in more than 40 % of tumor cells.

- B-cell lymphoma with features intermediate between DLBCL and Burkitt lymphoma (case no. 2)

This peculiar case showed a neoplastic population composed of medium to large cells with blastic morphology, high mitotic activity, and numerous apoptotic bodies that diffusely infiltrated the scheletric muscle (Fig. 2c).

Neoplastic cells expressed BCL2 but not CD10 by IHC; these findings combined with MYC rearrangement detected by FISH and IHC led to the classification of this lymphoma as intermediate between DLBCL and Burkitt lymphoma.

C-myc immunoreactivity was detected in less than 40 % of the neoplastic population.

- ALK-negative Anaplastic Large Cell Lymphoma (ALCL) (case no. 3)

Table 2 Immunohistochemical results

Case no.	CD45	CD20	CD79a	CD10	BCL6	MUM1	BCL2	CD30	CD3	CD5	Ki-67
1	+	+	+	+	+	−	−	+/−	−	−	85 %
2	+	+	+	−	+	+	+/−	−	−	−	>90 %
3	−/+	−	−	−	−	−	−	+	−	+	80 %
4	+	+	+	−	+	+	−	−	−	−	65 %
5	+	+	+	+	+	−	+	−	−	−	40 %
6	+	+	+	−	+	+	+/−	+	−	−	65 %
7	+	+	+	+	+	−	+	−	−	−	20 %

Immunophenotypic features of seven primary soft tissue lymphomas

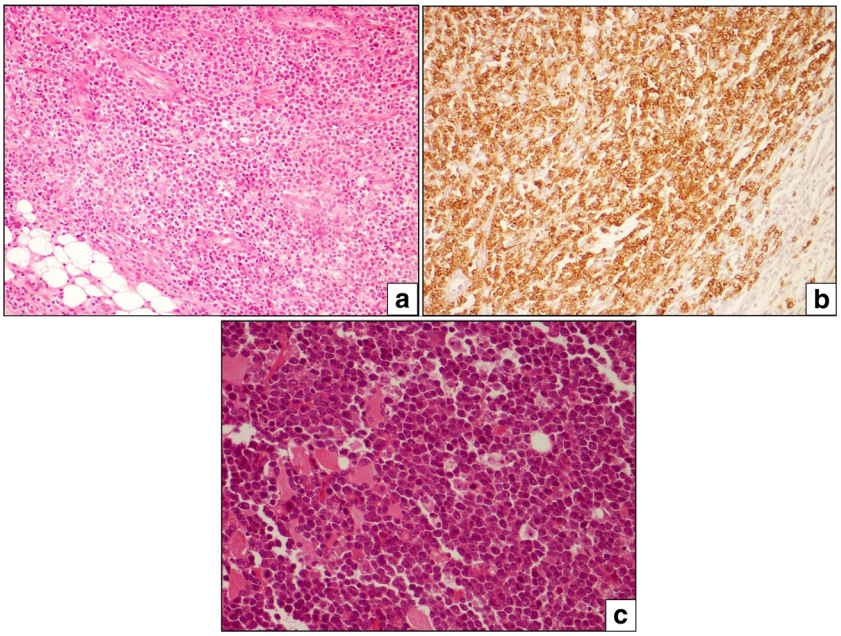

Fig. 2 Morphological results, case 2 and 3. **Case no. 3.** Morphologic features of ALK-negative anaplastic large cell lymphoma of the back hand. (**a**, 20×). The tumor shows a diffuse pattern of growth and is composed of large cells with a high degree of anaplasia and diffuse CD30 (**b**, 20×) expression. **Case no. 2.** H&E features of B-cell lymphoma intermediate between DLBCL and Burkitt lymphoma, showing a neoplastic population composed of medium cells with blastic morphology, high mitotic activity, and apoptotic bodies (**c**, 40×). **Case no. 5.** FISH analysis of the t(14;18) translocation: one red signal for the LSI BCL2 probe (SpectrumOrange Probe), one green signal for the LSI IGH probe (SpectrumGreen Probe) representing the normal homolog, and two red/green fusion signals representing the two derivative chromosomes resulting from the reciprocal translocation were detected. FISH analysis of the MYC rearrangement in the positive case no. 2: one red signal for the 5' LSI MYC probe (SpectrumOrange Probe), one green signal (SpectrumGreen Probe) for the 3' LSI MYC probe detecting a breakpoint, and one red/green fusion signal were observed (**d**)

This case was characterized by a diffuse pattern of growth and a high degree of anaplasia, round or oval large cells, with abundant cytoplasm and horseshoe or embryo-like nuclei ("hallmark cells"), which tended to cluster typically around large vessels (Fig. 2a). Neoplastic elements showed CD30 expression in more than 80 % of cells (Fig. 2b), and weak and focal expression of the leukocyte common antigen CD45. Cells were negative for ALK expression. CD5 positivity indicated a T-cell phenotype, while other T and NK markers (such as CD3, CD4, CD8, Perforin, and Granzyme B) were negative.

– Follicular lymphoma (case no. 7)

This case showed closely packed follicles containing small cleaved cells without nucleoli (centrocytes) and larger non-cleaved cells with moderate cytoplasm, open chromatin, and multiple nucleoli (centroblasts). No apoptotic cells or tangible body macrophages were observed. Tumor tissues contained dense fibrous bands; necrosis was absent.

Neoplastic cells expressed LCA (CD45) and showed strongly positive expression of B-cell markers (CD20 and CD79a). CD10, BCL6, and BCL2 showed strongly positive expression by IHC. C-myc expression was negative by IHC in neoplastic cells.

ISH and FISH Results

ISH analysis of EBV-DNA showed negative results in all cases.

B-cell lymphomas were also characterized by FISH analysis for the presence of the chromosomal translocations t(11;14) and t(14;18) and Myc rearrangements (Table 3):

- No cases were positive for t(11;14) translocation, confirming the morphological and immunophenotypical diagnosis.

Table 3 Molecular results

Case no.	Histotype	T(11–14)	T(14–18)	c-myc
1	DLCBL	Negative	Negative	Negative
2	B-cell lymphoma unclassifiable, with features intermediate between DLBCL and BL	Negative	Negative	Positive
4	DLCBL	Negative	Negative	Negative
5	DLCBL	Negative	Positive	Negative
6	DLCBL	Negative	Negative	Negative
7	FL	Negative	Positive	Negative

Cytogenetic studies of six out of seven primary soft tissue lymphomas with B-cell phenotype

Abbreviations: *DLBCL* diffuse large B-cell lymphoma, *FL* follicular lymphoma, *BL* Burkitt lymphoma

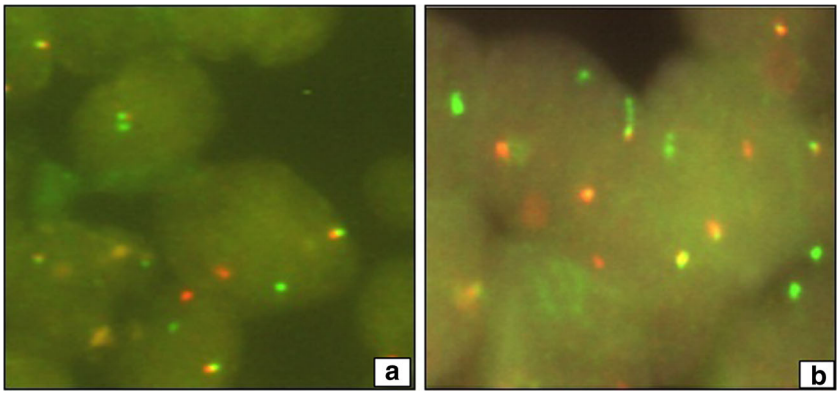

Fig. 3 Cytogenetic analysis, cases 2 and 5. **Case no. 2.** FISH analysis of the MYC rearrangement: one red signal for the 5' LSI MYC probe (SpectrumOrange Probe), one green signal (SpectrumGreen Probe) for the 3' LSI MYC probe detecting a breakpoint, and one red/green fusion signal were observed (**a**). **Case no. 5.** FISH analysis of the t(14;18) translocation: one red signal for the LSI BCL2 probe (SpectrumOrange Probe), one green signal for the LSI IGH probe (SpectrumGreen Probe) representing the normal homolog, and two red/green fusion signals representing the two derivative chromosomes resulting from the reciprocal translocation were detected (**b**)

- One case (case no. 2) displayed MYC gene rearrangement (Fig. 3a).
- Two cases (cases 5 and 7) were positive for the t(14;18) translocation. These data allowed us to confirm the morphological and immunohistochemical diagnosis of follicular lymphoma in case no. 7 and to confirm the hypothesis of the derivation from a previous follicular lymphoma in case no. 5 (DLCBL) (Fig. 3b).

Discussion

In the present study, we described the clinical, pathological and molecular features of a series of primary soft tissue lymphomas. Soft tissue localization of a lymphoproliferative neoplasm is a rare finding that should be distinguished from extranodal extension of lymphoproliferative disease, metastatic lymphoproliferative disease, and soft tissue sarcoma.

The AFIP series of soft tissue tumors included approximately 40,000 cases recorded between 1980 and 1989, of which approximately 470 were lymphomas, accounting for approximately 0.01 % of all cases [2].

There are several case reports in the literature that include one or more cases with histological and immunophenotypical characterization, and their therapeutic approach and survival data overlap with ours. In particular, the most frequent histological type is confirmed to be DLBCL [1, 4, 5, 13]. These neoplasms most commonly affect the thigh, trunk, arm, and leg [13]. Clinical presentation is often characterized by the presence of a painful swelling that is fixed on deep layers of the limbs [2].

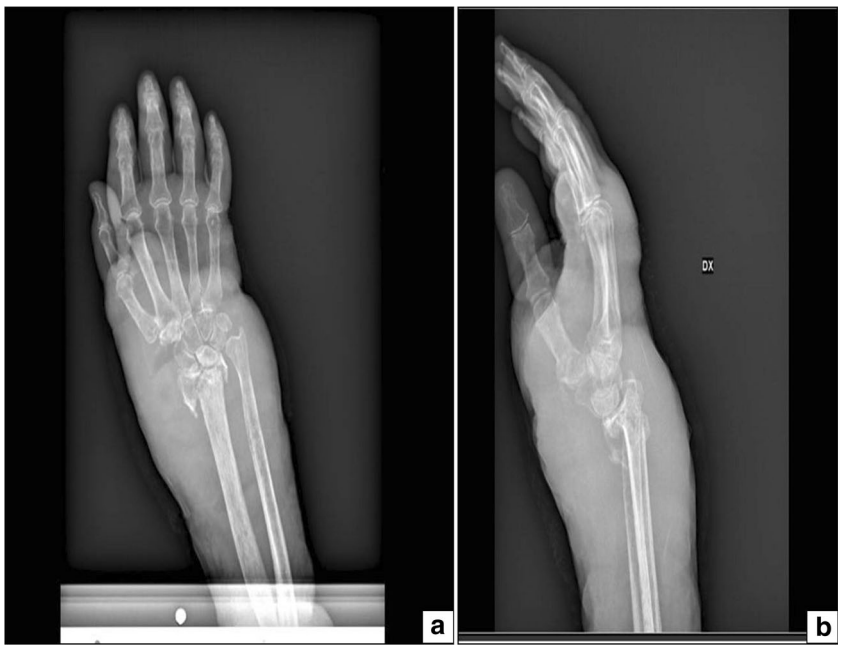

Fig. 4 Radiological findings **Case no. 6.** DLBCL of the wrist. Radiological findings show marked swelling and thickening of the soft tissues of the distal third of the forearm, wrist, and hand (**a**: antero-posterior, **b**: latero-lateral)

Routine X-ray examination may only show thickening and swelling of the affected region (Fig. 4). The most accurate imaging technique for the differential diagnosis of soft tissue masses is nuclear magnetic resonance (MR), which allows the radiologist to differentiate lymphoid tumors from other soft tissue tumors and tumor-like lesions [14].

The MR characteristics of soft tissue lymphoma include homogeneity on T1- and T2-weighted imaging, multicompartment involvement, and entrapment of neural and vascular structures [15].

Differential diagnosis with soft tissue sarcomas is important for the surgeon because of differences in the therapeutic approach, as sarcomas and non-neoplastic soft tissue masses are treated by excisional surgery, whereas lymphoma is best treated by chemotherapy and/or radiotherapy [2].

Accordingly, all the cases described in the literature were treated with chemotherapy combined with radiotherapy, and the most common chemoterapeutic protocol is CHOP [5].

The prognosis of patients affected by DLBCL of the soft tissues is generally poor, confirming the aggressiveness of this histological type, in particular when it arises in extranodal sites [1].

One peculiar case in our series was the ALK-negative ALCL of the soft tissue of the back of the hand. Only four cases of this type of lymphoma are described in the literature [16, 17, 18] and the main differential diagnosis in these cases is the so-called “blue round cell tumor of the soft parts”.

In conclusion, the present study contributes to our knowledge of rare lymphoid neoplasms and confirms previously published clinical-pathological data. We describe, for the first time, the complete IHC profile of these tumors, including cytogenetic characterization with the molecular FISH technique. Considering that the therapeutic strategy for non-lymphoid primary soft tissue tumors usually consists of demolitive surgery, the accurate diagnosis of this rare neoplastic entity could avoid unnecessary surgeries and improve patient prognosis.

Compliance with Ethical Standards

Conflict of Interest The authors have no conflicts of interest to declare.

References

1. Derenzini E, Casadei B, Pellegrini C, Argnani L, Pileri S, Zinzani PL (2013) Non-hodgkin lymphomas presenting as soft tissue masses: a single center experience and meta-analysis of the published series. Clin Lymphoma Myeloma Leuk 13:258–265
2. Kransdorf MJ (1995) Is it necessary to routinely use gadolinium in the MR imaging evaluation of soft-tissue tumors of the extremities? AJR Am J Roentgenol 165:1545
3. Yang J, Zhang F, Fang H, et al. (2010) Clinicopathologic features of primary lymphoma in soft tissue. Leuk Lymphoma 51: 2039–2046
4. Travis WD, Banks PM, Reiman HM (1987) Primary extranodal soft tissue lymphoma of the extremities. Am J Surg Pathol 11: 359–366
5. Salamao DR, Nascimento AG, Lloyd RV, et al. (1996) Lymphoma in soft tissue: a clinicopathologic study of 19 cases. Hum Pathol 27: 253–257
6. O’Neill JK, Devaraj V, Silver DA, et al. (2007) Extranodal lymphomas presenting as soft tissue sarcomas to a sarcoma service over a two-year period. J Plast Reconstr Aesthet Surg 60:646–654
7. Damron TA, Le MH, Rooney MT, et al. (1999) Lymphoma presenting as a soft tissue mass. A soft tissue sarcoma simulator. Clin Orthop Relat Res 360:221–230
8. Hudacko R, Rapkiewicz A, Berman RS, et al. (2011) ALK-negative anaplastic large cell lymphoma mimicking a soft tissue sarcoma. J Cytol 28:230–233
9. Knowles B, Serpell JW (2003) Extra-nodal lymphoma presenting as a mimic of soft-tissue sarcoma. ANZ J Surg 73:26–30
10. Suresh S, Saifuddin A, O’Donnell P (2008) Lymphoma presenting as a musculoskeletal soft tissue mass: MRI findings in 24 cases. Eur Radiol 18:2628–2634
11. Chun CW, Jee WH, Park HJ, et al. (2010) MRI features of skeletal muscle lymphoma. AJR Am J Roentgenol 195:1355–1360
12. Hans CP, Weisenburger DD, Greiner TC, et al. (2004) Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 103:275–282
13. Lanham GR, Weiss SW, Enzinger FM (1989) Malignant lymphoma. A study of 75 cases presenting in soft tissue. Am J Surg Pathol 13:1–10
14. Chun CW, Jee WH, Park HJ, Kim YJ, Park JM, Lee SH, Park SH (2010) MRI features of skeletal muscle lymphoma. AJR Am J Roentgenol 195:1355–1360
15. Carroll G, Breidahl W, Robbins P (2013) Musculoskeletal lymphoma: MRI of bone or soft tissue presentations. J Med Imaging Radiat Oncol 57:663–673
16. Pant V, Jambhekar NA, Madur B, Shet TM, Agarwal M, Puri A, Gujral S, Banavali M, Arora B (2007) Anaplastic large cell lymphoma (ALCL) presenting as primary bone and soft tissue sarcoma–a study of 12 cases. Indian J Pathol Microbiol 50:303–307
17. Driss M, Abbes I, Mrad K, Sassi S, Oubich F, Barsaoui S, Romdhane KB (2009) Primary CD30/ALK-1 positive anaplastic large cell lymphoma of the skeletal muscle in a child. Pathologica 101:97–100
18. Ishii E, Honda K, Nakagawa A, et al. (2000) Primary CD30/ki-1 positive anaplastic large cell lymphoma of skeletal muscle with der; 17t(1;17)(q11;p11). Cancer Genet Cytogenet 122:116–120