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ORIGINAL ARTICLE

Malignant fibrous histiocytoma and fibrosarcoma of bone: a re-assessment in the light of currently employed morphological, immunohistochemical and molecular approaches

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Abstract Malignant fibrous histiocytoma (MFH) and fibrosarcoma (FS) of bone are rare malignant tumours and contentious entities. Sixty seven cases labelled as bone MFH (57) and bone FS (10) were retrieved from five bone tumour referral centres and reviewed to determine whether recent advances allowed for reclassification and identification of histological subgroups with distinct clinical behaviour. A panel of immunostains was applied: smooth muscle actin, desmin, h-caldesmon, cytokeratin AE1–AE3, CD31, CD34, CD68, CD163, CD45, S100 and epithelial membrane antigen. Additional fluorescence in situ hybridisation and immunohistochemistry were performed whenever appropriate. All cases were reviewed by six bone and soft tissue pathologists and a consensus was reached. Follow-up for 43 patients (median 42 months, range 6–223 months) was available. Initial histological diagnosis was reformulated in 18 cases (26.8 %). Seven cases were reclassified as leiomyosarcoma, six as osteosarcoma, three as myxofibrosarcoma and one each as

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embryonal rhabdomyosarcoma and interdigitating dendritic cell sarcoma. One case showed a peculiar biphasic phenotype with epithelioid nests and myofibroblastic spindle cells. Among the remaining 48 cases, which met the WHO criteria for bone FS and bone MFH, we identified five subgroups. Seven cases were reclassified as undifferentiated pleomorphic sarcoma (UPS) and 11 as UPS with incomplete myogenic differentiation due to positivity for at least one myogenic marker. Six were reclassified as spindle cell sarcoma not otherwise specified. Among the remaining 24 cases, we identified a further two recurrent morphologic patterns: eight cases demonstrated a myoepithelioma-like phenotype and 16 cases a myofibroblastic phenotype. One of the myoepithelioma-like cases harboured a *EWSR1–NFATC2* fusion. It appears that bone MFH and bone FS represent at best exclusion diagnoses.

Keywords Bone neoplasm · Fibrosarcoma of bone · Malignant fibrous histiocytoma of bone · Classification

Introduction

The 2002 WHO classification of tumours of soft tissue and bone significantly differs from the previous ones by integrating immunophenotypic as well as genetic information into tumour definition. Advances in the understanding of the genetics and biology allowed for a better separation of distinct clinical entities with specific clinical, biological and histopathological features. This has been extensively done for malignant fibrous histiocytoma (MFH) of soft tissue [1] and more recently also for adult fibrosarcoma (FS) of soft tissue [2] while no significant changes were introduced in the classification of FS and MFH arising primarily in bone [3]. Both are distinguished from osteosarcoma by the absence of malignant osteoid deposition [3]. According to the 2002 WHO classification, bone FS was defined as a 'malignant spindle cell neoplasm of bone in which the tumor cells are typically organised in a fascicular or herringbone pattern' [3]. Bone MFH was defined as a 'malignant neoplasm composed of fibroblasts and pleomorphic cells with a prominent storiform pattern' [3]. The recognition of these two entities is thus currently based on the mere presence of a growth pattern and not by the cellular differentiation. Remarkably, such patterns (i.e. herring-bone pattern and storiform pattern) have been shown—in soft tissue tumours—to be present in several different clinicopathologic entities and therefore their identification should not represent the only clue to diagnosis [3, 4]. We hypothesised that the absence of 'stringent criteria' might have resulted over the years in the inclusion of distinctively unrelated clinical entities under the diagnostic 'umbrella' of bone FS and bone MFH. To test this, we retrieved and reviewed cases previously diagnosed as bone FS and bone MFH from the archive of five European bone tumour referral centres. We reclassified a quarter of the cases and subdivided the rest into five subgroups and finally tested whether these subgroups may have any prognostic impact.

Materials and methods

Tumour samples

Formalin-fixed, paraffin-embedded samples from tumours originally diagnosed between 1990 and 2009 as MFH (n=57) and FS (n=10) of bone were collected in context of the research activities of EuroBoNeT, a Framework Program 6-funded network of excellence devoted to the study of the pathology and biology of bone tumours. Only paraffin blocks from cases with preserved antigenic properties (i.e. positivity of the internal positive controls) were included in the study.

The original pathology reports along with haematoxylin–eosin-stained original slides were reviewed by the local pathologist to identify representative blocks. New haematoxylin–eosin (HE)-stained slides were centrally prepared for morphological revision. Clinical information was reviewed and updated.

Histological revision was conducted in two steps: first, digitalised HE slides were allocated on a web repository to be accessed by a panel of pathologists trained in bone and soft tissue tumours pathology (SR, RT, PCWH, APDT, RS and MA). HE slides were scanned with the SCANSCOPE XT (automated high-throughput scanning—APERIO, San Diego, USA) 200× magnification and allocated on a web repository. Each file was 4–6 Gb in size.

A consensus joint microscopy session was subsequently held in order to reach a final diagnosis on each case (SR, JVMGB, NA, RT, PCWH and APDT). This included the review of the immunohistochemical stains as well as of radiological imaging. Incidentally, the members of the two sessions changed; nonetheless, every case was reviewed for at least one session by each pathologist.

All samples were handled in a coded fashion, and all procedures were performed according to the ethical guidelines of each contributing institution.

Radiological review

A radiological review was conducted in parallel with the pathological revision by a radiologist specifically trained in the radiological assessment of bone tumours (HMK). Radiological imaging, including plain radiography, CT scan, bone scans and MR, was available for 50 patients. Evaluated parameters included the margins of the lesion, extension in soft tissue, presence and extension of mineralisation, heterogeneity of the lesion and possible signs of Paget disease or bone infarct.

Pathology review

Cases were re-classified based on the guidelines proposed in the 2002 WHO classification for soft tissue tumours [3]. Cases which met the WHO 2002 classification criteria for bone MFH and bone FS, therefore in the absence of a specific classic line of differentiation (i.e. leiomyosarcomas), were subdivided into five subgroups based on the presence/absence of pleomorphism, myoepithelioma-like features and myofibroblastic features according to the diagnostic algorithm outlined in Fig. 1. Myoepithelioma-like features were defined as presence of small/medium size epithelioid and/or spindle cells, featuring ovoid nuclei, sharp cytoplasmic borders and abundant pericellular collagen deposition. 'Myoepithelioma-like' terminology was used because none of the cases from this study fulfilled the minimum immunohistochemical criteria for myoepithelioma, i.e. the co-reactivity for EMA, with or without cytokeratin, together with either S100 or GFAP or both [5].

Myofibroblastic features were defined as presence of spindle cells, arranged in a storiform or fascicular pattern of growth, featuring tapering nuclei, pale cytoplasm with indistinct borders and set in stroma alternating myxoid and collagenised areas. Neoplastic cells featured immunopositivity for smooth muscle actin and tended to be negative for both desmin and h-caldesmon staining. Grading was done on a two-tiered scale (high and low grade, respectively).

Immunohistochemistry

The following panel of immunohistochemical stains was applied to each case: smooth muscle actin (SMA), desmin, h-caldesmon, cytokeratin AE1/AE3, CD31, CD34, CD68, CD163, CD45, S100 and epithelial membrane antigen (EMA) (details regarding these and second-line immunostains are shown in Table 1). Additional fluorescence in situ hybridisation (FISH) and immunohistochemistry were performed, when appropriate, to confirm/reject alternative diagnoses. All the tumours were scored using the sum of intensity of signal (possible range—0 = no expression; 1 = weak expression; 2 = moderate expression; 3 = strong expression) and the number of positive cells (% tumour cells—0=0 %; 1=1–25 %; 2=26–50 %, 3=51–75 %; 4= 76–100 %). The intracellular (nuclear, cytoplasmic and membranous) and the intra-tumoural (matrix-rich areas or cellular areas) localisation of immunoreactivity were noted.

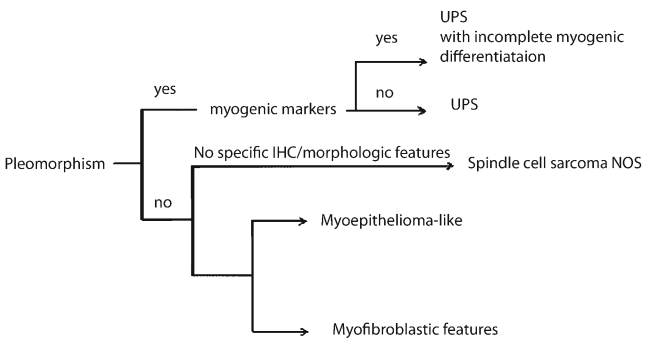

Fig. 1 Diagnostic algorithm used to subclassify cases which met the 2002 classification criteria for bone FS or bone MFH

Interphase FISH

Rearrangement of the *EWSR1* and *FUS* genes has been shown to be recurrent in myoepitheliomas of bone and soft tissue [5]. To assess this rearrangement, FISH using specific break-apart probes (Vysis LSI EWSR1 Dual Colour Break Apart Probe and Vysis LSI FUS Dual Color Break Apart Probe; Vysis, Downers Grove, IL, USA) was performed on all cases displaying myoepithelioma-like phenotype [5]. Furthermore, in order to rule out a diagnosis of synovial sarcoma, both cases displaying myoepithelioma-like phenotype as well as the cases grouped as spindle cell sarcoma NOS were tested for SS18 (previously known as SYT) rearrangement with specific break-apart probes (LSI SYT Dual Colour Break Apart Rearrangement Probe; Vysis). In brief, 5-μm paraffin-embedded tissue sections were used. Hybridisation was performed according to the manufacturer's protocol. Slides were mounted and counterstained with anti-fade DAPI (Vysis), visualised using an epifluorescence microscope (Olympus BX61) and analysed with a FISH Analysis software (Cytovision 4.5.1; Genetix, New Milton, UK); 200 interphase cells were analysed. One case (MFH19) showed a break-apart signal and amplification of the proximal *EWSR1* probe. As such pattern has been described to occur in association with *EWSR1–NFATC2* fusion, this rearrangement was tested via interphase FISH with a set of BAC probes, as previously reported [6]: RP5-994O24 for *NFATC2* and CTA-984 G1 for *EWSR1*.

Statistics

Follow-up was available for 43 patients (median 42 months, range 6–223 months).

Overall survival was evaluated with Kaplan–Meier curves. SPSS 15 software package was used. Given the limited numbers and the heterogeneity of the cases (i.e. treatments, size of the tumour and margins), only overall survival of cases without metastases at presentation were analysed.

Results

Clinical features

The median age of the patients in the entire cohort was 56 years (range 23–85), and men were more often affected

Table 1 Details of the antibodies used for immunohistochemistry

Antibody	Clone	Source	Dilution	AR
EMA	E29	Dako, Glostrup, Denmark	Prediluted	Flex (Dako)
CKAE1/AE3	Monoclonal AE1/AE3	Dako, Glostrup, Denmark	Prediluted	Flex (Dako)
SMA	Monoclonal 1°4	Dako, Glostrup, Denmark	Prediluted	Flex (Dako)
DESM	Monoclonal D33	Dako, Glostrup, Denmark	Prediluted	Flex (Dako)
CD34	QBend-10	Dako, Glostrup, Denmark	Prediluted	Flex (Dako)
CD31	Monoclonal JC70A	Dako, Glostrup, Denmark	Prediluted	Flex (Dako)
CD163	Monoclonal 10D6	Neomarkers	1/100	Flex (Dako)
Clusterin	Monoclonal NCL	Novocastra	1/100	Flex (Dako)
CD21	Monoclonal 1F8	Dako, Glostrup, Denmark	Prediluted	Flex (Dako)
Calponin	Monoclonal CALP	Dako, Glostrup, Denmark	1/200	Flex (Dako)
CD35	Monoclonal BER-MAC-DRP	Dako, Glostrup, Denmark	1/50	Flex (Dako)
CD10	56C6	Neomarkers	1/10	Flex (Dako)
CD68	PGM1	Dako, Glostrup, Denmark	Prediluted	Flex (Dako)
S100	Polyclonal	Dako, Glostrup, Denmark	Prediluted	Flex (Dako)
CD99	Monoclonal MIC2 clone 12E7	Dako, Glostrup, Denmark	Prediluted	Flex (Dako)
GFAP	Monoclonal 6F2	Dako, Glostrup, Denmark	1/1,000	Flex (Dako)
CD45	LCA-Monoclonal 2B11+PD7/26	Dako, Glostrup, Denmark	Prediluted	Flex (Dako)
INI1	Monoclonal BAF 47 (INI1)	BD	1/25	Flex (Dako)
TLE1	Monoclonal anti-TLE (M-101)	Santa Cruz Biotech	1/50	Flex (Dako)

(men/women 2.4:1) (Supplementary Table). Two cases, MFH 19 and MFH27, had received radiation therapy for a primary non-Hodgkin lymphoma of bone and endometrial carcinoma, respectively. The time interval between irradiation and bone tumour occurrence was 7 and 30 years, respectively.

Radiological review

The largest tumour diameter ranged from 2 to 25 cm. At the time of diagnosis, soft tissue extension was present in 82 % (28/34 radiologically evident) and metastasis in 14 % of the cases. A previous history of bone infarct and Paget disease was reported and radiologically confirmed for cases MFH89 and MFH72, respectively (Fig. 2). Previous fibrous dysplasia in the same bone occurred in case MFH75.

Pathology review

All the evaluated cases were morphologically high-grade sarcomas. Out of 67 cases, 18 were re-classified using existing WHO 2002 criteria for bone and soft tissue sarcomas as leiomyosarcoma (seven cases), osteosarcoma (six cases), myxofibrosarcoma (three cases), embryonal rhabdomyosarcoma (one case) and interdigitated dendritic cell (IDC) sarcoma (one case) (Table 2). MFH91 showed a unique biphasic morphology with epithelioid cells (Fig. 3a) positive for both CK AE1–3 staining (Fig. 3b) surrounded by elongated spindle cells positive for SMA (Fig. 3c).

The remaining cases met the WHO criteria for MFH or FS of bone. Among these 48 cases, five different patterns were distinguished: undifferentiated pleomorphic sarcoma (UPS), UPS with incomplete myogenic differentiation, spindle cell sarcoma not otherwise specified (NOS), myoepithelioma-like sarcoma and myofibroblastic-like sarcoma.

Leiomyosarcoma of bone

Seven cases were re-classified as primary bone leiomyosarcoma (Fig. 3d), one of which was previously diagnosed as FS; the remaining six cases were previously diagnosed as MFH. Median age at presentation was 63 years (range 29–84; male/female ratio 4:3) (Table 2). The lower limbs were most often affected, followed by the pelvis (ratio 2.5:1) (Table 2). One of the cases occurred in association with a previous bone infarction (MFH89) (Fig. 2). Histological features were overlapping with those of soft tissue leiomyosarcomas. More specifically, tumours were made up of a proliferation of intersecting elongated spindle cells with eosinophilic, fibrillary cytoplasm with blunt-ended nuclei (Fig. 3d). Diffuse nuclear pleomorphism was present in two cases and focal in the remaining cases. Necrosis was present in all samples in less than 50 % of the tumour. Immunohistochemistry showed, in all seven cases, positivity for SMA (Fig. 3e) (Supplementary Table) and at least one other myogenic marker; either desmin (Fig. 3f) or h-caldesmon (Supplementary Table). EMA and CK AE1–AE3

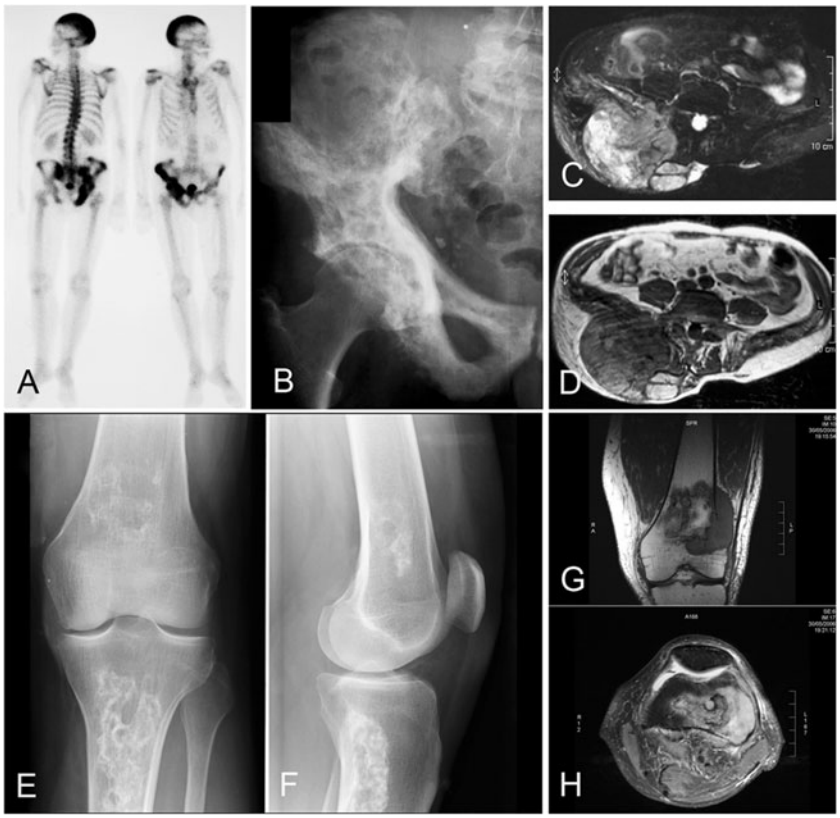

Fig. 2 Two tumours were radiologically confirmed to be secondary to Paget disease and bone infarct, respectively. **a** Bone scintigraphy showing markedly increased uptake of the radiopharmacon in the skull and right side of the pelvis. **b** Antero-posterior conventional radiograph of the right side of the pelvis. Structural changes are pathognomonic for Paget disease. Osteolytic lesion in the proximal part of the right iliac bone consistent with secondary sarcomatous degeneration. **c**, **d** Axial MR: T1-weighted (**c**) and T2-weighted (**d**) images demonstrating a large lesion arising from the right iliac bone with predominantly intermediate signal intensity on the T1-weighted image and predominantly high signal intensity on the T2-weighted image. Large accompanying posterior soft tissue mass is present, too. **e**, **f** Antero-posterior and lateral conventional radiographs of the knee demonstrating mixed osteolytic and sclerotic lesions in the distal part of the femur and proximal part of the tibia. The appearance is classic for areas of bone infarction. **g**, **h** Coronal T1-weighted (**g**) and axial T2-weighted images depicting the lesion in the distal femur. In addition to the centrally located bone infarct, there is a lesion with apparent cortical destruction and soft tissue extension with intermediate signal intensity on the T1-weighted coronal image, and predominantly high signal intensity on the axial T2-weighted image. These findings indicate secondary sarcomatous degeneration of the bone infarct

were found positive in five and four cases each (both positive in two) (Supplementary Table). The two pleomorphic cases were also tested for myogenin, to rule out pleomorphic rhabdomyosarcoma, which proved negative.

Table 2 Results of the reclassification of MFH and FS of bone

New diagnosis	No. of cases	Age range (median)	Gender (male/female ratio)	Location (axial/upper limbs/lower limbs)
Leiomyosarcoma	7	29–84 (63)	4/3	2/0/5
Osteosarcoma	6	25–70 (51)	4/0	0/0/4
Myxofibrosarcoma	3	39–68 (44)	2/1	0/0/3
Interdigitating dendritic cell sarcoma	1	30	Male	Unknown
Embryonal rhabdomyosarcoma	1	82	Female	Upper limb
Biphasic sarcoma	1	29	Male	Upper limb
Myoepihelioma-like	8	32–85 (70)	3/4	2/0/5
Myofibroblastic	16	36–78 (54)	13/3	3/2/11
Spindle cell NOS	6	51–76 (60)	4/1	2/0/4
UPS	7	49–68 (53)	3/2	0/1/3
UPS with myogenic	11	23–77 (43)	5/4	2/1/5

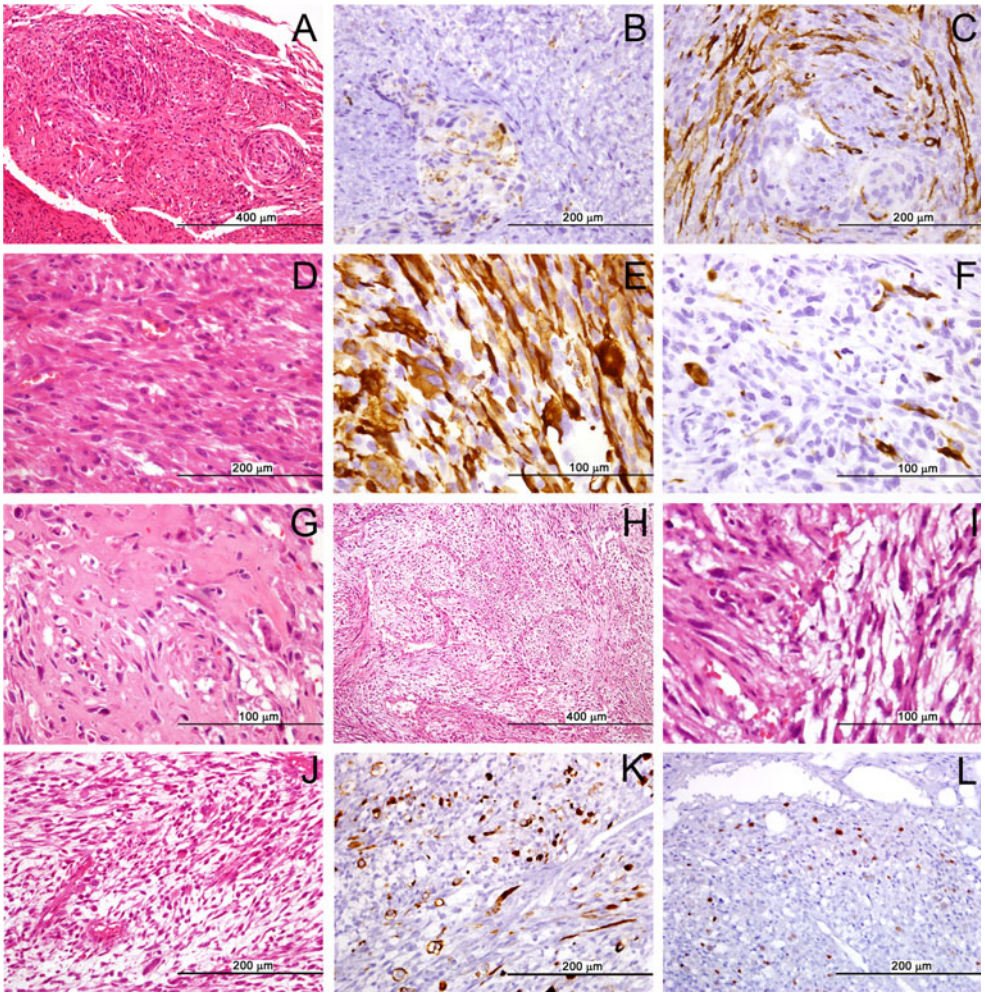

Fig. 3 One case was showing a unique biphasic phenotype, with epithelioid cells arranged in nests surrounded by elongated spindle cells (**a**). Epithelioid cells were positive for CK AE1–3 (**b**) while spindle cells were positive for SMA (**c**). More than a quarter of the cases were reclassified as leiomyosarcomas, due to their morphology (**d**) and the positivity for either desmin (**e**) and or heavy caldesmon (**f**), **g** osteosarcoma, **h**–**i** myxofibrosarcoma and **j** embryonal rhabdomyosarcoma, showing focal positivity for desmin (**k**) and myogenin (**l**)

Osteosarcoma

Five cases previously diagnosed as MFH of bone were reclassified as conventional osteosarcoma (Fig. 3g) and one case previously diagnosed as FS was reclassified as teleangiectatic osteosarcoma. Median age at presentation was 51 years (range 25–70). All four osteosarcomas with known location were affecting the lower limbs. All patients were men. Main diagnostic clue was represented by the presence of unequivocal deposition of malignant osteoid, detected in all six cases (Table 2).

Myxofibrosarcoma of bone

Three cases originally diagnosed as bone MFH were reclassified as myxofibrosarcoma of bone (Fig. 3h and i). Median age at presentation was 44 years (range 39–68; male/female ratio 2:1). All cases were involving the lower limbs (Table 2). Histologically, tumours were composed by a spindle cell proliferation set in an abundant extracellular myxoid matrix alternating with more cellular areas with scant and denser extracellular matrix. In the myxoid areas, elongated curvilinear thin-walled blood vessels were found. Neoplastic cells were often pleomorphic with occasional pseudolipoblasts. All immunostains of the standard panel proved negative with the exception of scattered SMA positive neoplastic cells (Supplementary Table).

Other rare entities

Two cases were reclassified as IDC sarcoma and embryonal spindle cell rhabdomyosarcoma, respectively. The IDC sarcoma, previously diagnosed as bone MFH, was made up of elongated cells with eosinophilic cytoplasm interspersed with mononuclear inflammatory cells. The standard immunohistochemistry panel showed positivity only for S100 protein; second-line immunostains were all negative, including CD21, CD23, CD35, CD1a, clusterin, MITF1, HMB45, GFAP and MelanA. We favoured the diagnosis of IDC versus other 'dendritic' neoplasm based on the absence of any other specific markers. Melanoma and malignant peripheral nerve sheath tumour were excluded due to the absence of primary lesions and/or predisposing conditions. Furthermore, second-line melanoma markers were negative and GFAP was also negative.

One case diagnosed as FS was reclassified as embryonal spindle cell rhabdomyosarcoma. This tumour contained elongated and occasional 'tadpole' cells with densely eosinophilic cytoplasm with abundant myxoid extracellular matrix deposition (Fig. 3j). Immunostains showed widespread positivity for desmin and diffuse nuclear myogenin expression (Fig. 3k and l).

UPS with or without incomplete myogenic differentiation

Eighteen cases showed diffuse and severe pleomorphism with absence of a recognisable line of differentiation; they were grouped as UPS (seven cases) (Fig. 4m) or as UPS with incomplete myogenic differentiation (11 cases) (Fig. 4k) when showing at least patchy or focal positivity for one single myogenic marker (i.e. one marker among SMA, heavy caldesmon and desmin, usually SMA) (Fig. 4l).

Median age of occurrence for UPS and UPS with partial myogenic differentiation was 53 (range 49–68) and 43 years (range 23–77), respectively. Lower limbs and males were more often affected in both (Table 2).

Spindle cell sarcoma NOS

Three cases previously diagnosed as FS of bone and three previously diagnosed as MFH of bone were grouped as spindle cell sarcoma NOS. The median age was 60 years (range 51–76); men were more often affected (4:1). Four were located in the femur, one in the pelvis and one in a thoracic vertebra. They were made up of spindle cells, showing no marked pleomorphism (Fig. 4j). Focal storiform pattern and fascicular growth, but no herringbone growth pattern, were found. The standard panel of immunostains failed in demonstrating any line of differentiation. Interphase FISH for SS18 rearrangement and TLE1 staining were performed in all cases to exclude a synovial sarcoma and were negative.

Myoepithelioma-like sarcoma

These tumours were composed of small epithelioid and/or spindle cells, featuring a sharp cytoplasmatic border surrounded by abundant pericellular collagen (Fig. 4a). Immunostains for EMA and CK AE1–AE3 (Fig. 4b) were often

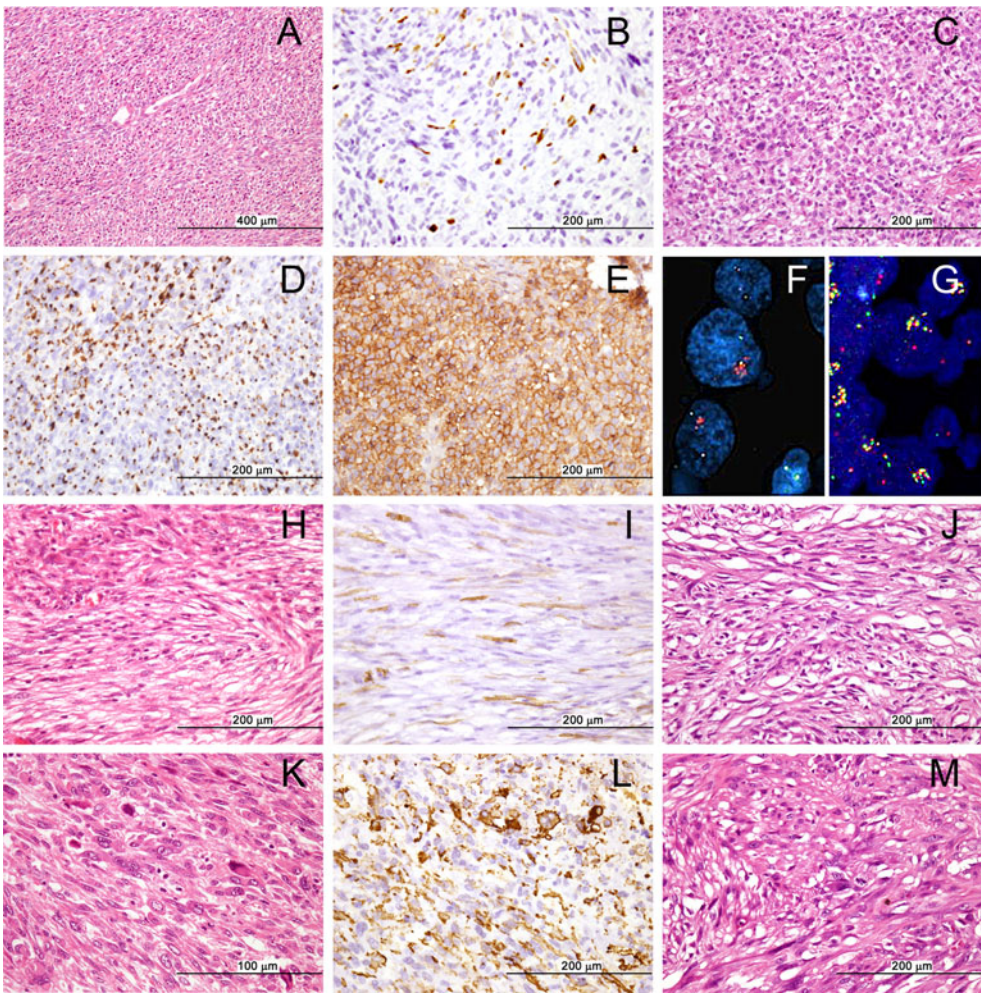

Fig. 4 Some cases not showing any specific lineage of differentiation were reclassified as **a**–**b** myoepithelioma-like sarcoma. Among myoepithelioma-like sarcoma case, MFH19 (**c**–**f**) showed diffuse positivity for CK AE1–3 (**d**) and CD99 (**e**) and rearrangement of EWSR1 with amplification of the proximal 5′ probe shown by interphase FISH with a split-apart probe set for EWSR1 (**f**) and a characteristic fusion and amplification with NFATC2 shown by interphase FISH with a co-localisation probe set for EWSR1–NFATC2 (**g**). The remaining cases were classified as **h**–**i** myofibroblastic sarcoma, characterised by positivity for SMA (**i**), **j** spindle cell sarcoma NOS, **k** UPS with partial myogenic differentiation due for the positivity for SMA (**l**) and UPS without partial myogenic differentiation (**m**)

positive but no staining for S100 was observed. To confirm/rule out a full-blown myoepithelioma, second-line immunohistochemistry was performed including immunostains for GFAP and calponin. The former stain was negative in all cases while calponin was focally positive in two cases (MFH1 and MFH19).

Furthermore, the *EWSR1* gene was assessed by interphase FISH in seven cases. One case (MFH19) (Fig. 4f) showed a split signal of the two probes flanking the *EWSR1* locus and multiple signals for the telomeric probe reflecting a genomic amplification of 5′ part of *EWSR1* (Fig. 4f). Such an amplification pattern has been previously observed [6] to be associated with *NFTAC2* gene rearrangement resulting in an in-frame *EWSR1–NFATC2* fusion. This case showed diffuse positivity for CK AE1–3 (Fig. 4d) and EMA. Second-line immunostains showed diffuse positivity for CD99 (Fig. 4e), patchy positivity for calponin and negativity for chromogranin, CD56, CD20 and p63. The presence of *EWSR1–NFATC2* fusion was confirmed also in our case (Fig. 4g).

This patient had previously (7 years before) received radiotherapy for a non-Hodgkin lymphoma of bone and was alive with no evidence of disease at 64 months' follow-up.

None of the remaining cases showed rearrangements of either *EWSR1* or *FUS*.

Prompted by the results in MFH19, CD99 staining was performed also in all the seven remaining myoepithelioma-like sarcomas and found to be diffusely positive in three of them.

Epithelioid morphology together with the positivity for epithelial markers (CK AE1–3 and EMA) prompted us also to rule out a possible synovial sarcoma or an epithelioid sarcoma. Neither *SS18 (SYT)* rearrangement, as assessed by interphase FISH, nor loss of INI1 nuclear staining, as assessed by immunohistochemistry, was found in 6/6 and 8/8 cases, respectively. The presence of other primary epithelial cancers was excluded in all patients.

Myofibroblastic-like sarcoma

The myofibroblastic-like sarcomas were made up of spindle cells, more often arranged in a storiform pattern or with a tissue culture-like appearance (Fig. 4h). Pleomorphism was mostly absent or only focally present. Immunostaining showed varying patterns of SMA positivity, ranging from isolated, weakly stained neoplastic cells to diffuse positivity (Fig. 4i). Desmin and h-caldesmon were negative.

None of the reviewed cases showed neoplastic cells positive for either CD68 or CD163 or LCA. However, as highlighted by these stains, a moderate, often intense, intra- and peri-tumoural inflammatory infiltrate was always present together with CD68 and CD163 positive multinucleated giant cells. ALK was negative in all cases.

Clinical features and overall survival data

The present study cohort was heterogeneous with regard to original tumour burden, stage at presentation, margin status at surgery and therapeutic regime. Furthermore, follow-up was not available for all the cases (Supplementary Table). Analysis of the survival curves in patients without metastasis at presentation did not reveal any significant differences.

Discussion

Bone MFH and FS are contentious entities and few extensive or recent reports are available in the literature. Nishida et al. [7] reported a series of 81 cases of bone MFH diagnosed at the Mayo Clinic between 1910 and 1993. Our study differs from previous ones both in reviewing cases previously diagnosed either as bone MFH or as bone FS and for the application of the 2002 WHO classification criteria, as well as using immunohistochemistry and molecular genetics to further analyse the tumours. This resulted in reclassification of a substantial (>25 %) number of cases.

Not surprisingly, the vast majority of reclassified cases (13 out of 18) were osteosarcomas and bone leiomyosarcomas. Bone leiomyosarcomas may be easily overlooked if smooth muscle differentiation is not morphologically suspected and subsequent appropriate immunostaining is not performed: misdiagnosis as either bone MFH or bone FS, depending on the degree of cytological pleomorphism, may occur [8]. Cases occurring as secondary to either radiation or bone infarct have been reported [8, 9], and we here report an additional leiomyosarcoma of bone secondary to bone infarct [9].

Osteosarcoma is the most common primary malignant bone tumour. Its diagnosis is based on the microscopical identification of osteoid produced by malignant cells in a distinct clinical and radiological setting. Since osteoid deposition may be focal and scant, it may be overlooked and therefore the proper diagnosis is not rendered, most often when dealing with needle biopsies [10]. Particular attention should be paid to the presence of mineralisation on the imaging, therefore confirming that radiological evaluation still plays a major role in the diagnostic workout of bone lesions [10]. Finally, it is worthy to remark that since only focal osteoid deposition may be found, sampling errors may occur and therefore the diagnosis of osteosarcoma may not be rendered [10]. This may occasionally have occurred in our case series, too.

We were able to identify three examples of primary myxofibrosarcoma of bone. Since myxoid MFH has been widely used in the past as an alternative label for myxofibrosarcoma [11–13], it is very likely that the case reported by Nishida et al. [7] as myxoid MFH would now be reclassified as myxofibrosarcoma of bone. As for myxofibrosarcoma of soft tissue, the

bone cases in this study showed a broad intra-tumoural heterogeneity with respect to cellularity and pleomorphism, with morphologically low-grade malignant areas juxtaposed to high-grade malignant areas [1]. It is important to emphasise that reclassification of myxoid MFH into myxofibrosarcoma is not just a renaming exercise since the metastatic rate of soft tissue high-grade myxofibrosarcoma is lower than that observed in other subtypes of pleomorphic sarcoma [14]. Two of the presented three cases, for which metastases were not present at diagnosis, showed a longer overall survival when compared to the other groups. Larger numbers should be evaluated to test whether there is a significantly better prognosis for this group of tumours, but in consideration of the extreme rarity, such a goal appears considerably difficult to attain.

Cases that did not match with conventional clinicopathologic entities and met the 2002 WHO criteria for MFH or FS of bone were subclassified in five subgroups. One subgroup included lesions that we agreed to label descriptively as 'myoepithelioma-like neoplasms'. These cases morphologically showed a striking resemblance to malignant myoepithelioma. Major differences were the absence of a myxoid background and the fact that none matched the most typical immunohistochemical criteria for myoepithelioma, i.e. the coreactivity for EMA ± cytokeratin and either S100 or GFAP [5]. Remarkably, one of the cases (MFH19) showed rearrangement of the *EWSR1* gene. The positivity for CD99 and the particular rearrangement of *EWSR1*, namely amplification of the 5′ part of *EWSR1*, prompted us to evaluate the presence of a *EWSR1–NFATC2* fusion [6]. Using interphase FISH, we confirmed the *EWSR1–NFATC2* fusion in this myoepithelioma-like tumour, therefore widening the range of phenotypes reported for this specific translocation in bone tumours [6], and further supporting the relationship of this group of lesions with typical primary bone myoepithelial lesions. In the study from Bahrami et al. [2], some of the cases previously diagnosed as soft tissue fibrosarcomas were reclassified as either monophasic synovial sarcoma or solitary fibrous tumour. No such cases were encountered here. Remarkably, Verbeke et al. [15] in a study focused on the reappraisal of cases previously diagnosed as primary bone haemangiopericytoma actually reclassified several cases as examples of bone monophasic synovial sarcomas and bone solitary fibrous tumour. Cases with prominent HPC-like vascular organisation were not present in our series.

Pleomorphic undifferentiated sarcomas represented less than half of the unclassified tumours, a remarkable proportion of which showed incomplete myogenic differentiation. Expression of smooth muscle markers is known to occur in cases previously labelled as bone MFH [16] and also electron microscopic examination shows that myofibroblasts are often present in this tumour [17–19]. As UPS with myogenic differentiation of soft tissue are known to be associated with a worse prognosis [14], we investigated a possible correlation between the presence of incomplete myogenic differentiation and outcome. In contrast with what has been reported in soft tissue, we actually found an opposite trend. Whether this difference may be related to a different mesenchymal cell of origin (i.e. mesenchymal stem cells, the supposed cells of origin of sarcoma, have different plasticity according to the tissue they have been isolated from [20]) and/or distinct microenvironment cannot currently be established due to the low number bias and the absence of a uniform treatment regimen.

For a subset of the cases of the present study (n=11), karyotypes based on conventional chromosome banding analysis have been reported [21]. They all showed complex karyotypes and neither the overall pattern (ploidy level, degree of complexity) nor specific cytogenetic features distinguished any of the subtypes [21]. This indicates that potential genotype–phenotype correlations between morphologic subtypes among so-called MFH of bone are beyond the resolution level of chromosome banding. Further, more detailed genetic studies are warranted.

In the 2002 WHO classification, it was stated that based on its rarity further subclassification of bone MFH is substantially useless [3]. Until now, both so-called MFH and FS of bone have been treated similarly to osteosarcoma. In our series, a substantial proportion of cases previously diagnosed as bone MFH and bone FS were reclassified as another type of sarcoma (i.e. leiomyosarcoma and myxofibrosarcoma). Since both leiomyosarcoma and UPS may be treated systemically with alternative drugs (i.e. gemcitabine alone or in combination with taxotere) [22, 23], a more precise classification is reasonably expected to impact also on the choice of the most appropriate therapeutic option. In this light, it should be emphasised that the correct classification requires a certain skill set and expertise; therefore, bone tumours should be sent to specialist clinics for the most accurate diagnosis and treatment. We identified two groups of lesions (myoepithelial-like and myofibroblastic-like) that may well represent distinctive subgroups that will deserve further characterisation through accrual of a larger number of cases.

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Conflict of interest statement We declare that we have no conflict of interest.

References

1. Dei Tos AP (2006) Classification of pleomorphic sarcomas: where are we now? Histopathology 48(1):51–62

2. Bahrami A, Folpe AL (2010) Adult-type fibrosarcoma: a reevaluation of 163 putative cases diagnosed at a single institution over a 48-year period. Am J Surg Pathol 34(10):1504–1513
3. Fletcher CDM, Unni KK, Mertens F (2002) WHO Classification of tumours. Pathology & genetics of tumours of soft tissue and bone. IARC, Lyon
4. Fletcher CDM (1992) Pleomorphic malignant fibrous histiocytoma: fact or fiction? A critical reappraisal based on 159 tumors diagnosed as pleomorphic sarcoma. Am J Surg Pathol 16:213–228
5. Antonescu CR, Zhang L, Chang NE, Pawel BR, Travis W, Katabi N et al (2010) EWSR1–POU5F1 fusion in soft tissue myoepithelial tumors. A molecular analysis of sixty-six cases, including soft tissue, bone, and visceral lesions, showing common involvement of the EWSR1 gene. Gene Chromosomes Canc 49(12):1114–1124
6. Szuhai K, Ijszenga M, de Jong D, Karseladze A, Tanke HJ, Hogendoorn PC (2009) The NFATc2 gene is involved in a novel cloned translocation in a Ewing sarcoma variant that couples its function in immunology to oncology. Clin Cancer Res 15 (7):2259–2268
7. Nishida J, Sim FH, Wenger DE, Unni KK (1997) Malignant fibrous histiocytoma of bone. A clinicopathologic study of 81 patients. Cancer 79(3):482–493
8. Antonescu CR, Erlandson RA, Huvos AG (1997) Primary leiomyosarcoma of bone: a clinicopathologic, immunohistochemical, and ultrastructural study of 33 patients and a literature review. Am J Surg Pathol 21(11):1281–1294
9. Petra M, Gibbons CL, Athanasou NA (2002) Leiomyosarcoma of bone arising in association with a bone infarct. Sarcoma 6(1):47–50
10. Raymond AK, Ayala AG, Knuutila S (2002) Conventional osteosarcoma. In: Fletcher CDM, Unni KK, Mertens F (eds) World Health Organization classification of tumours. Pathology and genetics of tumours of soft tissue and bone. IARC, Lyon, pp 264–270
11. Angervall L, Kindblom LG, Merck C (1977) Myxofibrosarcoma. A study of 30 cases. Acta Pathol Microbiol Scand A 85A(2):127–140
12. Weiss SW, Enzinger FM (1977) Myxoid variant of malignant fibrous histiocytoma. Cancer 39:1672–1685
13. Mentzel T, Calonje E, Wadden C, Camplejohn RS, Beham A, Smith MA et al (1996) Myxofibrosarcoma. Clinicopathologic analysis of 75 cases with emphasis on the low-grade variant. Am J Surg Pathol 20(4):391–405
14. Fletcher CD, Gustafson P, Rydholm A, Willen H, Akerman M (2001) Clinicopathologic re-evaluation of 100 malignant fibrous histiocytomas: prognostic relevance of subclassification. J Clin Oncol 19(12):3045–3050
15. Verbeke SL, Fletcher CD, Alberghini M, Daugaard S, Flanagan AM, Parratt T et al (2010) A reappraisal of hemangiopericytoma of bone; analysis of cases reclassified as synovial sarcoma and solitary fibrous tumor of bone. Am J Surg Pathol 34(6):777–783
16. Ueda T, Araki N, Mano M, Myoui A, Joyama S, Ishiguro S et al (2002) Frequent expression of smooth muscle markers in malignant fibrous histiocytoma of bone. J Clin Pathol 55(11):853–858
17. Erlandson RA, Antonescu CR (2004) The rise and fall of malignant fibrous histiocytoma. Ultrastruct Pathol 28(5–6):283–289
18. Antonescu CR, Erlandson RA, Huvos AG (2000) Primary fibrosarcoma and malignant fibrous histiocytoma of bone—a comparative ultrastructural study: evidence of a spectrum of fibroblastic differentiation. Ultrastruct Pathol 24(2):83–91
19. Montgomery E, Fisher C (2001) Myofibroblastic differentiation in malignant fibrous histiocytoma (pleomorphic myofibrosarcoma): a clinicopathological study. Histopathology 38(6):499–509
20. Bernardo ME, Emons JA, Karperien M, Nauta AJ, Willemze R, Roelofs H et al (2007) Human mesenchymal stem cells derived from bone marrow display a better chondrogenic differentiation compared with other sources. Connect Tissue Res 48(3):132–140
21. Mertens F, Romeo S, Bovée JVMG, Tirabosco R, Athanasou NA, Alberghini M et al (2011) Reclassification and subtyping of so-called malignant fibrous histiocytoma of bone: comparison with cytogenetic features. Clin Sarcoma Res 1(1):10
22. Maki RG, Wathen JK, Patel SR, Priebat DA, Okuno SH, Samuels B et al (2007) Randomized phase II study of gemcitabine and docetaxel compared with gemcitabine alone in patients with metastatic soft tissue sarcomas: results of sarcoma alliance for research through collaboration study 002. J Clin Oncol 25(19):2755–2763 [corrected]
23. Hensley ML, Maki R, Venkatraman E, Geller G, Lovegren M, Aghajanian C et al (2002) Gemcitabine and docetaxel in patients with unresectable leiomyosarcoma: results of a phase II trial. J Clin Oncol 20(12):2824–2831