Diagnostic approach to malignant fibrous histiocytomas of soft tissue in dogs: a case report

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ABSTRACT: Malignant fibrous histiocytomas (MFHs), newly named as 'undifferentiated pleomorphic sarcomas' in 2002 by the World Health Organization, generally show an ambiguous origin. They have been described to fibroblastic or histiocytic in origin, while storiform-pleomorphic variants share a similar morphologic pattern with other sarcomas. For this reason additional diagnostic methods including immunology, ultrastructure analysis and molecular approaches are necessary for a more accurate diagnosis. We report three cases of MFH in individual dogs which presented histological characteristics of pleomorphic sarcomatoid tumours. Microscopic investigation, immunohistochemistry (vimentin, CD68, desmin, α-smooth muscle actin, and S-100) and electron microscopy were performed for diagnosis. One case was diagnosed as storiform-pleomorphic type and two were diagnosed as giant cell type MFHs. The present study demonstrates concepts of MFHs based on newly described categories and suggests useful diagnostic approaches for uncertain sarcomatoid soft tissue tumours in canines.

Keywords: canine; giant cell; malignant fibrous histiocytoma; storiform-pleomorphic; vimentin

Malignant fibrous histiocytomas (MFHs) are mesenchymal tumours which are generally found in older dogs, and are characterised by pleomorphic fibroblastic and histiocytic cells occasionally admixed with giant cells or inflammatory cells (Al-Agha and Igbokwe 2008). Over the decades, the concept and clear definition of MFHs has been challenging due to the uncertain histiocytic origin of the condition, as well as their morphologic patterns which are shared by a number of sarcomas (Al-Agha and Igbokwe 2008). Therefore, five subtypes of MFHs were newly categorised as (1) undifferentiated high-grade pleomorphic sarcoma, (2) myxofibrosarcoma, (3) undifferentiated pleomorphic sarcoma with giant cells, (4) undifferentiated pleomorphic sarcoma with prominent inflammation, (5) angiomatoid fibrous histiocytoma (2002 World Health Organization Classification) (Christopher et al. 2002). A diagnosis of MFHs based solely on morphology is largely insufficient (Ozzello et al. 1963; Kerlin and Hendrick 1996; Christopher et al. 2002; Al-Agha and Igbokwe 2008). The present study reports a diagnostic approach to MFH-like tumours from three dogs, using not only histological morphology, but also immunohistochemistry and ultrastructure analysis to investigate the origin of the tumour cells, and to differentiate them from other malignant sarcomas.

Case description

Three cases of dogs with soft tissue masses were presented to our laboratory for histological diagnosis. The first case involved a subcutaneous mass on the lumbar region of a 10-year-old spayed female schnauzer. The gross finding was a whitish poorly demarcated sarcomatoid mass with moderate hard-

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ness. The second case was that of a plaque-like cutaneous mass on the dorsal region of the trunk of a 10-year-old castrated male cocker spaniel. The third case was that of a slowly growing gingival mass of an 11-year-old intact male maltese. The three lesions had similar gross features.

Surgically removed tissue samples were fixed in 10% neutral buffered formalin (BBC, Mount Vernon, WA) for microscopic examination. Paraffin sections of each mass were stained with haematoxylin and eosin (HE), and Masson's trichrome. Immunohistochemical analysis was performed using the Vectastain® Elite ABC-Peroxidase kit (Vector Laboratories, Burlingame, USA) and mouse monoclonal antibodies against ki-67 (Dakocytomation, Glostrup, Denmark, 1 : 100), vimentin (Dakocytomation, Glostrup, Denmark, 1 : 400), CD68 (Dakocytomation, Glostrup, Denmark, 1 : 100), desmin (Dakocytomation, Glostrup, Denmark, 1 : 100), α-smooth muscle actin (Sigma, Saint Louis, USA, 1 : 400) and S-100 (abcam, Cambridge, UK, 1 : 100). The antibody reactions were visualised using a diaminobenzidine peroxidase substrate (Vector Laboratories, Burlingame, USA) and were counterstained with Mayer's haematoxylin. For transmission electron microscopy, sliced tissues (1 × 1 × 1 mm in dimension) were fixed in 2.5% glutaraldehyde (Sigma, Saint Louis, USA) at 4 °C for 24 h, post-fixed in osmium tetroxide (OsO_4, Merk, Darmstadt, Germany), and then dehydrated and embedded in Epon-812 (Electron microscopy sciences, PA, USA) according to standard procedures. Semi-thin sections were stained with 2% toluidine blue and were examined to locate areas of interest within the block. Ultrathin sections were cut with an Ultracut E microtome (Reichert-Jung, NY, USA) and were stained with 1.0% uranyl acetate and 1.0% lead citrate. Transmission electron microscopic (H-7650, Hitachi, Ontario, Canada) examination was performed with a standard square mesh (GG200-Ni, Electron microscopy sciences) for calibration.

Histologically, the three soft tissue masses all showed sarcomatoid proliferation of pleomorphic cells (Figure 1A–C). The first case, especially, distinctly showed a storiform pattern of spindle-shaped tumour cells (Figure 1A). Masson's trichrome staining revealed collagen deposition through the cellular lesion (Figure 1D–F). However, the stained cells were collagen-producing stromal cells, and not tumour cells. Tumour cells were mostly characterised by abundant eosinophilic cytoplasm and large

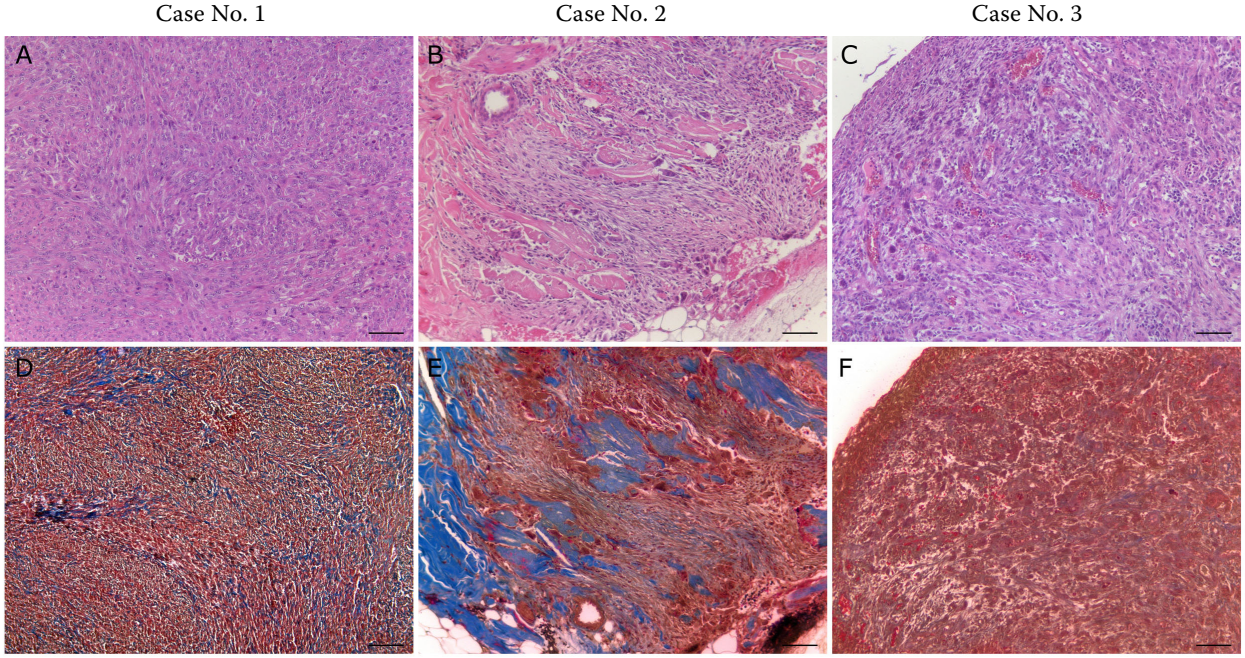

Figure 1. Histological features of MFHs under low magnification. Tumour cells revealed sarcomatoid proliferation and a storiform pattern was frequently observed in the histology of the first case (**A**). Together with the tumour cell growth, numerous giant cells had infiltrated in the second and the third case (**B**, **C**). All cases showed negative responses for collagen fibre staining (**D**–**F**). (A = haematoxylin-eosin, bar = 80 μm; B and C = haematoxylin-eosin, bar = 200 μm; D–F = Masson's Trichrome stain, bar = 200 μm)

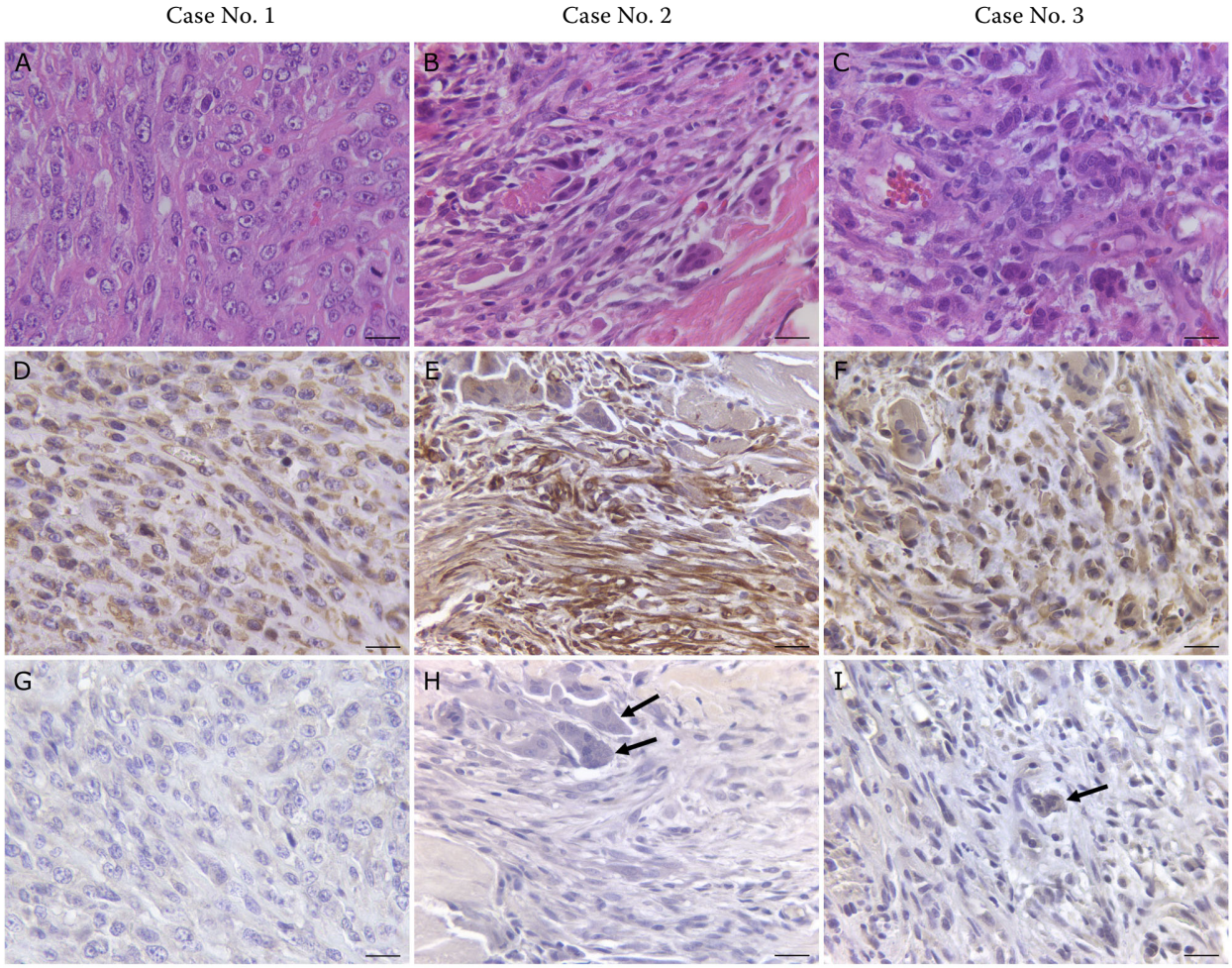

Figure 2. Histology and immunohistochemistry of MFHs under high magnification. Highly pleomorphic cells with mitosis, reflecting the high grade of the tumour, presented with positive immunoreactivity for vimentin in the cytoplasm, and negative immunoreactivity for CD68 (**A**, **D** and **G**). Tumour cells in the second and the third case showed marked cellular atypia with osteoclast-like giant cells (**B** and **C**). Immunohistochemistry revealed a strong positive reaction for vimentin in the cytoplasm of tumour cells (**E** and **F**) and a negative reaction for CD68, however; weakly positive reaction was limited to osteoclast-like giant cells (arrow, **H** and **I**). (A–C = haematoxylin-eosin, bar = 20 μm; D–F = immunohistochemistry for vimentin, bar = 20 μm, G–I = immunohistochemistry for CD68, bar = 20 μm)

ovoid or polygonal nuclei. There was accompanying infiltration of various numbers of giant cells and a few inflammatory cells (Figure 2A–C). The tumour cells were positive for vimentin (Figure 2D–F), weakly positive for CD68 (localised by multi-nucleated giant cells) (Figure 2G–I), and negative for desmin, α-SMA and S-100 in immunohistochemical staining (data not shown).

As an additional diagnostic method, electron microscopic investigation revealed various kinds of tumour cells. Fibrocytic giant cells, characterised by nuclei with prominent nucleoli and abundant rough endoplasmic reticulum (Figure 3A), and fibril-forming fibroblast-like cells were detected (Figure 3B). A small number of round cells had phagosomes and lysosomes which suggested histiocytic features (Figure 3C). Osteoclast-like giant cells had multiple nuclei, numerous intracytoplasmic vesicles, and ruffled borders (Figure 3D).

In conclusion, the first case of a lumbar lesion which presented a storiform pattern of pleomorphic histiocytoid cells accompanying abundant atypical mitoses and focal giant cell infiltration was diagnosed as storiform-pleomorphic MFH (newly named undifferentiated high-grade pleomorphic sarcoma). The other two cases featured the emergence of osteoclast-like giant cells over the lesion, and were diagnosed as giant cell MFH

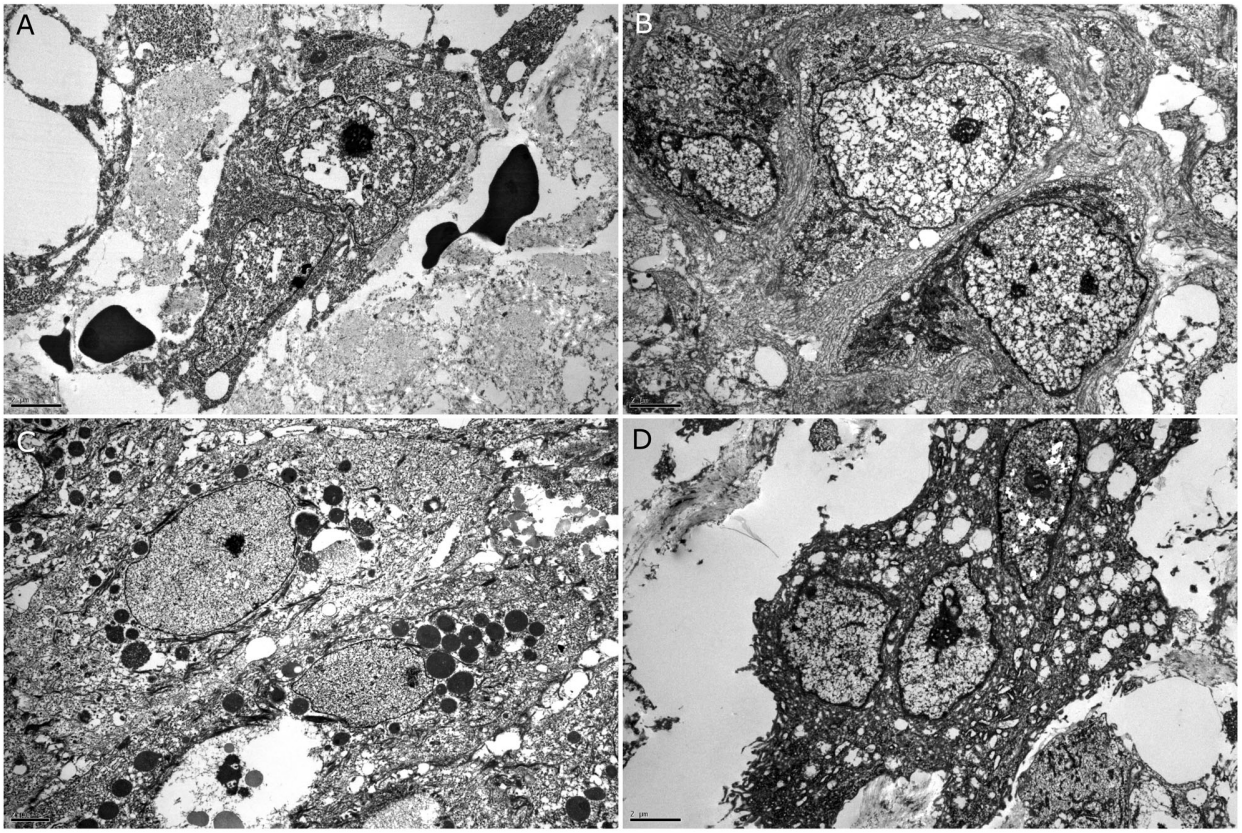

Figure 3. Ultrastructure findings of tumour cells and osteoclast-like giant cells. Fibrocytic or fibroblastic cells were observed with abundant rER and pleomorphic nuclei with prominent nucleoli (**A** and **B**). Phagocytic vacuoles and lysosomes in the cytoplasm showed histiocytic characters within a few tumour cells (**C**). Osteoclast-like giant cells exhibited multiple bizarre nuclei with various intracytoplasmic vesicles and ruffled borders (**D**). (A–D, bar = 2 μm)

(newly named undifferentiated pleomorphic sarcoma with giant cells).

DISCUSSION AND CONCLUSIONS

MFHs are mesenchymal tumours which occur relatively frequently in canines. MFHs arise in the skin or spleen as a single, expansile tumour, or are often found in multisystemic organs such as the lungs, lymph nodes or bone marrow (Hendrick et al. 1992; Kerlin and Hendrick 1996). In human cases, MFH has been divided into subtypes based on the pattern and predominance of the cell types; storiform-pleomorphic, giant cell, inflammatory, myxoid and angiomatoid. However, only the first three types have been reported in domestic animals (Morris et al. 2002; Al-Agha and Igbokwee 2008).

The term MFH was first described based on its morphological features indicating fibroblastic and histiocytic origin; however, the pattern of MFHs, especially the storiform-pleomorphic variant, shares similar histological features with other lines of tumours (Ozzello et al. 1963; Al-Agha and Igbokwe 2008). In fact, high numbers of tumours of epithelial or mesenchymal origin have been misdiagnosed as MFH (Fletcher 1992). Moreover, the origin of the tumour cells has been debated over the decades (Ozzello et al. 1963; Fletcher 1987). More sensitive diagnostic techniques, such as immunohistochemistry, electron microscopy, and molecular techniques have suggested a fibroblastic or undifferentiated mesenchymal cell origin. These can then differentiate into fibroblast and histiocytic cells, and histiocytes are no longer the accepted origin of tumour cells (Hoffman 1983; Fletcher 1987; Al-Agha and Igbokwe 2008). Therefore, diagnosis of MFHs can occur when no line of differentiation is identified (Al-Agha and Igbokwe 2008).

According to the latest WHO classification, MFHs are still within the "fibrohistiocytic" category, but three subtypes of MFH; storiform-pleomorphic,

Table 1. Nomenclature and histological features of malignant fibrous histiocytoma (MFH) subtypes (World Health Organization Classification 2002)

Old nomenclature	Current nomenclature	Tumor category	Histological features
Storifrom-pleomorphic MFH	undifferentiated high-grade pleomorphic sarcoma	fibrohistiocytic	marked cytological and nuclear pleomorphism, often bizarre tumor giant cells, admixed with spindle cell, often rounded histiocyte-like cell
Giant cell MFH	undifferentiated pleomorphic sarcoma with giant cells	fibrohistiocytic	atypical spindle-shaped and more epithelioid cells admixed with prominent osteoclastic giant cell
Inflammatory MFH	undifferentiated pleomorphic sarcoma with prominent inflammation	fibrohistiocytic	pleomorphic spindle cells with numerous inflammatory cells including neutrophils, eosinophils and minor component of lymphocytes and plasma cell

giant cell and inflammatory subtypes, have been re-categorised as "undifferentiated pleomorphic sarcomas" (Table 1) (Christopher et al. 2002). In the same vein, the diagnosis of MFHs in domestic animals has a number of requirements: (1) determination of the character of MFH-like tumours via diverse diagnostic approaches, (2) revamping of the terminology and categories, and (3) retrospective studies in order to determine the most effective clinical approaches and to aid in the prediction of prognosis.

Three cases were analysed in the present study using macro/microscopic exploration, immunohistochemistry and investigation of the ultrastructure of the tumour cells. In H&E staining, proliferating cells were pleomorphic, and showed a streaming pattern of proliferation with poorly demarcated boundaries. Mesenchymal tumours, such as fibrogenic, myogenic, adipogenic and neurogenic sarcomas, usually present the similar histological features as MFHs, as well as anaplastic carcinomas. The origin of tumour cells can be determined using special staining, immunological procedures or by electron microscopy. Generally, immunological detection for vimentin has been widely used to confirm the mesenchymal origin of soft tissue tumours in both humans and dogs (Enzinger and Weiss 1995; Perez et al. 1996; Williamson and Middleton 1998; Morris et al. 2002). All three tumours showed strong immunoreactivity for vimentin, which indicates sarcomas. Fibrogenic sarcomas contain various amounts of collagenous fibres which can be detected by Masson's trichrome staining, whereas collagen production is not unusual in MFHs (Schneider et al. 1999). Myogenic sarcomas, such as leiomyosarcomas and rhabdomyosarcomas with osteoclastic giant cells are identified through myogenic markers, desmin or α-smooth muscle actin, and by the large number of myofilaments in their ultrastructures. Desmin can be applied for the differential diagnosis of both canine rhadomyosarcoma and leiomyosarcimas, while α-smooth muscle actin antibody is only applied for leiomyosarcoma (Andreasen and Mahaffey 1987; Morris et al. 2002). One of the cases presented here, which was diagnosed as a storiform-pleomorphic MFH, showed focal positive immunoreactivity for α-smooth muscle actin. Focal positive reactions for α-smooth muscle actin in MFH have been previously reported in several case studies (Enzinger and Weiss 1995; Morris et al. 2002; Kijima et al. 2007). As a neuronal marker, S-100 staining was not observed in any of the cases in our study. S-100 is known to be found in melanomas, and 50% of malignant peripheral nerve sheath tumours (MPNST) (Nonaka et al. 2008). A hitiocytic marker, CD68, exhibited weakly positive immunoreactivity in giant cells in the three cases cases. Regardless of the nomenclature of "MFH", histiocytic markers (CD68, α1-antitrypsin, α1-antichymotrypsin, lysozyme, and factor XIII) no longer have useful roles in the diagnosis of MFHs (Leader et al. 1987; Cassidy et al. 1994; Christopher et al. 2002; Morris et al. 2002; Szollosi et al. 2005). To summarise, "vimentin only" immunoreactivity without any other specific or distinct expression of cell line markers can indicate a diagnosis of MFH.

The ultrastructure of tumour cells in these case series mostly showed undifferentiated cellular features, accompanying partial fibroblast-like, or histiocyte-like characteristics. Pleomorphic cells containing nuclei of various morphologies with

distinct nucleoli were presented as expanded rER or occasionally contained phagosomes and lysosomes, which parallels previous cases of human MFHs (Thomas and Koshi 2012; Phui-Ly et al. 2012). Thus, these vimentin-positive tumour cells were considered to be undifferentiated mesenchymal cells, which were undergoing differentiation to histiocytes or fibroblasts.

MFHs within the soft tissue are considered to be locally aggressive, especially storiform-pleomorphic MFH, and the giant cell variant of MFH was previously reported to be associated with poor prognosis (Waters et al. 1994; Morris et al. 2002). The metastatic rate of MFH is relatively lower than that of other pleomorphic sarcomas, including high grade myxofibrosarcomas or pleomorphic myogenic sarcomas (leiomyosarcoma or rhabdomyosarcoma) (Morris et al. 2002).

The present study demonstrates the concepts of MFHs based on newly described categories, and suggests useful diagnostic approaches for the clarification of uncertain sarcomatoid soft tissue tumours in canine cases. In dogs, the prognostic factors of MFHs which are of clinical significance are still unclear, and so, the described diagnostic methods should also serve to establish the proper clinical approach based on further accurate subclassification.

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