Acral congenital superficial dermal lymphatic malformations in two unrelated cats: clinicopathological, dermoscopic and ultrastructural findings

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Background –Cutaneous vascular malformations (CVM) represent a spectrum of human diseases identified at birth or in paediatric patients and classified according to the type of vessel affected. Confusing classification inhuman medicine has led to misdiagnoses and frequent nomenclature revision. Cutaneous lymphatic malformations (CLM) are reported sporadically in humans. Objective –To describe the clinicopathological findings of superficial dermal cutaneous lymphatic malformations(SDCLM) in two cats. Animals –Two unrelated adult domestic short hair cats. Methods –The two cats were evaluated clinically and with a dermoscope for recurrent swelling and presence of vesicles oozing serosanguineous fluid affecting the right and left hind foot, respectively, since birth. Skin biopsy specimens were collected for histopathological and immunohistochemical evaluation and electron microscopy. Results –A CVM was suspected based on the age of onset, clinical signs, results of diagnostic imaging and histopathological findings. Dermoscopy was used to describe the alterations of the skin surface. The involvement of the lymphatic vessels was confirmed using immunohistochemical findings and electron microscopy. Conclusion and clinical importance –To the best of the authors' knowledge, this is the first description of the clinical, dermoscopic, histopathological and ultrastructural characteristics of SDCLM in cats resembling the human counterpart. SDCLMs are rare conditions and appropriate histopathological and immunohistochemical confirmation is required to avoid misdiagnosis and mistreatment

Introduction

Cutaneous vascular malformations (CVM) in humans are rare heterogeneous disorders believed to be errors in vas-cular development, classified according to the affected vessel type and flow characteristics.1,2Due to significant histological overlap, confusing classifications have led to misdiagnoses and frequent nomenclature revision. Cutaneous lymphatic malformations (CLM),

slow-flow developmental anomalies of the lymphatic system, are sporadic in humans and manifest most often at birth or by two years of age.1,2 They can be classified by their location in superficial dermal or deep subcutaneous forms. Depending on the size of the dilated vessels CLM can be divided in microcystic (<1 cm diameter), macrocystic(>1 cm diameter) or mixed.2,3Superficial dermal cutaneous lymphatic malformations (SDCLM) previously were referred to with the biologically inaccurate term of "lymphangiomas", with the microcystic form known as lymphangioma circumscriptum.1–3The clinical manifestations of SDCLM consist of hyperkeratotic papules or vesicles, yellow-orange to red in colour that can be associated withswelling.2To the best of the authors' knowledge, developmental abnormalities of the cutaneous lymphatic vessels have not been reported in cats. We herein describe the clinical, dermoscopic, histopathological and ultrastructural findings in two unrelated cats, affected by a SDCLM resembling the human counterpart.

Case reports

Case 1

An 8-year-old, male neutered, domestic short hair (DSH)cat was presented with a history of swelling of the left hind foot associated with intermittent oozing of transparent to haemorrhagic fluid and lameness, since birth. On physical examination swelling of the left hind foot and mild left popliteal lymph node enlargement were apparent. On dermatological examination of the skin of the distal metatarsal area, the fourth and fifth digits and the interdigital skin between the third and fifth digits, numerous small translucent to reddish vesicles were apparent (Figure 1a). Dermoscopic examination with a handheld polarized light dermoscope (Handyscopeâ,FotofinderâSystems GmbH; Bad Birnbach, Germany)connected to a smartphone (iPhone SEâ, Apple; Cuper-tino, CA, USA) was performed. Images were obtained and stored through a dedicated iPhone app (Handyscopeâapp). At920 magnification, pale pink to dark red,

smooth bordered, round to oval structures demarcated by a paler border were visualized (so-called lacunae). A colour transition from dark red to light pink within the same lacuna sometimes was observed (Figure 1b). Some of these structures were protruding from the skin surface in small clusters and ruptured on touch with discharge of serosanguineous fluid. The diameter of the lacunae was evaluated with measurement software (Rasband, ImageJ, US NIH; Bethesda, MD, USA) and varied from 0.3 to 1.6 mm.A CVM was suspected. Ultrasonographic evaluation detected focal heterogeneous echogenicity with tubular hypoechoic structures without colour and power flow-Doppler signals, suggesting lymphangiectasia, see DataS1. A total body computer tomography scan (Light Speed, GE Medical System; Milano, Italy) and angiography of the affected limb were performed, and no arteriovenous mal-formations were detected. Cytological examination of the oozing fluid showed erythrocytes, scarce neutrophils and occasional small lymphocytes. Fine-needle aspirate of the enlarged popliteal lymph node showed reactive hyperplasia. Multiple skin biopsy specimens were collected for histopathological examination, routinely processed and stained. Histological findings revealed the superficial der-mal presence of large (≤100 nm diameter) welldefined, cystic-ectatic vascular spaces characterized by empty lumens and lined by a single row of well-differentiated endothelial cells lacking atypical features. Occasionally, endothelial endoluminal papillary projections were visible. Papillary ingrowths were supported by dense bundles of collagen containing a variable number of small mature lymphocytes and lesser numbers of mature plasma cells and occasional Mott cells. The mid- and deep dermis contained elevated numbers of dilated lymphatic vessels with irregular contours in association with thick collagen bundles. Vessels did not efface or alter adnexal structures. In the deep dermis and subcutis, vessels with vari-ably thick walls characterized by an elevated amount of collagen and segmental presence of smooth muscle cells were observed (Figure 1c).Immunohistochemical (IHC) investigation to detect Factor VIII-RA (dilution 1:400 Dako; Glostrup, Denmark)demonstrated cytoplasmic immunoreactivity of endothelial cells with

higher intensity for those in well-differentiated pre-existing vessels. Mild multifocal anti-lymphatic vessel endothelial receptor-1 (rabbit anti-human LYVE-1polyclonal 1:50 dilution, Abcam; Cambridge, MA, USA)positivity of endothelial cells was observed (Figure 2a,see Data S1). A portion of the lesion from the paraffin block was dewaxed in xylene overnight and routinely processed for transmission electron microscopy (TEM).Ultrathin sections were observed by TEM after double contrast with 2% uranyl acetate and lead citrate. Vascular spaces lined by endotheliocytes directly attached to colla-gen without an intermediate basal membrane were seen and considered to be of lymphatic origin. Pericytes along the abluminal surface of these vessels were not seen. A definitive diagnosis of microcystic SDCLM was made

.Case 2

A 5-month-old, male intact, DSH cat was presented with a history of swelling of the right hind foot. On physical examination moderate swelling of the right hind foot was present. On dermatological examination, on the skin of the fourth and fifth digits, small translucent to red vesicles were apparent (Figure 1d). Dermoscopic examination was performed as described for Case 1. At920 magnification, pink to dark red, round to oval lacunae, as well as serpiginous and elongated ones (with similar colour) were visualized (Figure 1e). The width of the lacunae was eval-uated (Rasband, ImageJ) and measured atc.0.4 mm; the serpiginous tracts were a few millimetres long. A skin biopsy specimen was collected for histopathological examination, routinely processed and stained. Histological findings showed superficial dermal dilated vessels, as in Case 1, although the dilation was less severe. A single thrombus, in a small superficial dermal small cystic vessel, was observed. In the dermis, findings closely paralleled those of the previous case with superficial, mid- and deep dermal vessels with irregular contours in association with increased collagen, sparing adnexal structures. Mild dermal accumulation of mature lymphocytes and plasma cells with occasional mast cells was present. The subcutis

contained multiple, dilated, irregular to misshapen lymphatic vessels with variably thick walls; these were lined by well-differentiated endothelial cells lacking atypical features admixed with increased collagen, and segmental but prominent presence of irregular and variably thick smooth muscle cells layers. In one of the largest malformed vessels, abnormal endoluminal projections of endothelial cells supported by minimal stroma and interpreted as malformed vascular walls or valvular structures, were observed (Figure 1f).IHC investigation for Factor VIII-RA (dilution 1:400Dako) demonstrated cytoplasmic positive endothelial cells lining most dilated vessels and mature preexisting vessels. Mild multifocal LYVE-1 (rabbit anti-human LYVE-1 polyclonal 1:50 dilution, Abcam) positivity of endothelial cells was observed (Figure 2b). As for the first case, the overall morphology of the lesion together with IHC was considered consistent with microcystic SDCLM. TEM was performed as for Case 1 and ultrastructural findings were similar (Figure 2c). A definitive diagnosis of micro-cystic SDCLM was made.

Discussion

As described in humans, the SDCLMs in these two unrelated cats were present from birth and remained stable idistribution and severity, supporting the diagnosis oCVM. Swelling of the affected areas, oozing of serosanguinous fluid and the location in one area of the body alsare commonly reported in humans. Human SDCLMs caoccur on any cutaneous surface although they mainly arreported involving the limbs, gluteal region or oral cav-ity;1,2in both of our cases, a hind limb extremity was affected. Dermoscopy is a noninvasive technique that allows the magnified visualization of superficial cutaneoustructures. Dermoscopic findings of human SDCLMs have been described and, combined with clinical findings and behavioural observations, they can support the diagnostic process.4,5Dermoscopic findings in our cases were consistent with frequently described features in humans where dilated vascular spaces, called lacunae, and vascular structures are observed in 89% of human patients. Lacunae often are

reported as being red or dark in colour and, less frequently, yellowish to whitish or multicoloured. The colour is supposed to vary depending on the presence of erythrocytes in the dilated lymphatic structures. Moreover, the sedimentation of erythrocytes at the bottom of the dilated lymphatics is thought to create the colour transition from light to dark (from top to bottom) of some lacunae that defines the so called hypopyon sign (two-tone lacunae); similar findings were observed in our cases.4–6Correct identification of the vessel type involved could help in the development of a clear classification and pro-vide the basis for different therapeutic approaches. Blood and lymphatic endothelial cells cannot be distinguished unequivocally with haematoxylin and eosin staining.7Fac-tor VIII-related antigen can be used to confirm the vascular nature of the condition but does not allow the differentiation between blood and lymphatic endothelial cells, and, therefore, the use of specific lymphatic endothelial cell markers is recommended. The LYVE-1 molecule, a receptor for hyaluronan absent in blood vessels, is considered aspecific marker for lymphatic endothelial cells and was positive in both cats, confirming the lymphatic nature of the vascular malformation.7–11These findings were fur-ther supported by the TEM visualization of a discontinuous or absent basement membrane.7,12In conclusion, SDCLMs are rare conditions that should be suspected in feline patients with supportive history, clinical and dermoscopic findings. Appropriate histopathological and immunohistochemical confirmation is required to avoid misdiagnosis.

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FIGURE LEGENDS

Figure 1.Clinical presentation, histopathological and dermoscopic findings in two cats with cutaneous vascular malformations (cases 1 & 2).(a–c) Left hind foot; Case 1. (a) Macroscopic view of the lesion: small translucent to red vesicles oozing serosanguineous fluid. (b) Dermoscopeimage (polarized light) of dorsal aspect: translucent to reddish lacunae demarcated by paler borders are visible. Colour transition from dark red to yellowish and white visible within the same lacuna (hypopyon-like sign, black arrows). (c) Haired skin, histopathological results. Multiple, large superficial dermal cystic-vesicular lymphatic dilations. Presence of variably dilated and irregular lymphatics in superficial dermis. Haematoxylin andeosin,910 magnification. (d–f) Right hind foot: Case 2. (d) Macroscopic view of the lesion: small translucent to red vesicles oozing sanguineous fluid. (e) Dermoscope image (polarized): pink to dark red elongated to serpiginous structures (lacunae and vascular spaces). (f) Haired skin, histopathological results. Presence of superficial, mid- and deep dermal dilated and irregular lymphatic vessels. In the deep dermis at the limit with the panniculus a large lymphatic vessel with occasional smooth muscle cells in the vascular wall. In the lumen abnormal multiple ramifying projections interpreted as abnormal valves are evident. H&E,910 magnification

Figure 2.Photomicrographs of the histopathological, immunohistochemical and ultrastructural findings in two cats with cutaneous vascular mal-formations.(a) Left hind foot; Case 1. Haired skin. Photomicrograph of immunohistochemistry for Lyve-1 in a cystic and distorted lymphatic vessel with an irregular endoluminal valvular projection. Endothelial cells show positivity for Lyve-1 (ABC method with Carbazole and Haematoxylin counterstain,940 magnification). (b,c) Right hind foot: case 2. (b) Haired skin. Photomicrograph of immunohistochemistry for Lyve-1 in an area with intersecting lymphatics. Endothelial cells show positivity for Lyve-1 (ABC

method with carbazole and Haematoxylin counterstain,940 magnification). (c) Elec-tron microscopy (uranyl acetate-lead citrate staining): empty space (asterisk) surrounded by plump endothelial cells (arrowheads) without a visible basal lamina, thus referable to lymphatics.91,000 magnification.