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Unique cytologic and histologic features of a suspected cutaneous xanthoma in a dog

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ABSTRACT

A 4-year-old spayed female American Staffordshire terrier presented to the U-Vet Animal Hospital, Werribee, Australia, with a cutaneous mass that had been slowly growing over 12 months. Cytologic evaluation showed cohesive to individualized, vacuolated spindled cells often arranged in a perivascular pattern. The mass was completely excised, and the histopathologic examination demonstrated sheets of vacuolated spindled to round cells expanding the full thickness of the dermis. The cells demonstrated both Iba1 and CD18 antibody binding, leading to an initial interpretation of histiocytic sarcoma. Given the discordance with the clinical presentation, further immunohistochemistry (IHC) was performed. The cells demonstrated strong CD204 antibody binding and did not bind the E-cadherin antibody, consistent with dermal macrophage origin. Ki-67 antibody binding was regionally variable from <5-25%, with more regions that had low Ki-67 expression.

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Fasted serum biochemistry revealed hypertriglyceridemia and persistent hypercholesterolemia. Based on clinical, microscopic, biochemical, and IHC results, the final interpretation was an indolent dermal histiocytic proliferation of macrophage origin, with a preference for cutaneous xanthoma or reactive dermal fibrohistiocytoma.

KEYWORDS

Benign fibrohistiocytoma, foam cells, histiocytic, hyperlipidemia, immunohistochemistry, xanthogranuloma

CASE PRESENTATION

A 4-year-old female spayed 35.6kg American Staffordshire Terrier was presented to the U-Vet Animal Hospital, Werribee, Victoria, Australia, for routine vaccination and evaluation of a mass on the ventral thorax which had been slowly growing over the preceding 12 months. The dog had otherwise been clinically well but was overweight (BCS 8/9). Physical examination demonstrated an 8mm firm raised alopecic cutaneous mass in the right pectoral region. Fine-needle aspiration (FNA) of the mass was performed.

Cytopathology revealed highly to poorly cellular smears comprising apparently cohesive to individualized round to spindle cells often present in a striking perivascular arrangement, amongst a pale blue proteinaceous background with abundant fine, clear vacuoles (Figure 1A). The cells had round to oval nuclei with coarsely stippled chromatin and 1-3 variably distinct nucleoli. The cytoplasm was abundant, often highly vacuolated, and pale blue with indistinct cell borders. The cells displayed mild anisocytosis and mild to moderate anisokaryosis with occasional irregular nuclear borders. Occasional large irregular nucleoli were noted. Rare inflammatory cells comprising small lymphocytes, plasma cells, and eosinophils were also noted (Figure 1B).

The cytologic interpretation was soft-tissue sarcoma, likely liposarcoma, based on the high cellularity, striking perivascular pattern, spindle cell morphology, and marked vacuolation. Differentials included perivascular wall tumor (PWT) or sebaceous carcinoma. An excisional biopsy with wide margins was recommended.

Excision was performed 10 days later, and histopathology demonstrated a highly cellular, unencapsulated, mildly infiltrative proliferation of sheets of spindle to round cells, which infiltrated and expanded the full thickness of the dermis (Figures 2A and 2B). The cells had moderate amounts of vacuolated pink cytoplasm and indistinct cell borders. Vacuoles were small and numerous, clear, and had distinct borders. The nuclei were plump to ovoid with lacy chromatin with a single nucleolus (Figure 2C). Mitoses were two per ten 400x fields. There was mild to moderate anisokaryosis.

Multifocally, and throughout the mass, were small numbers of plasma cells and lymphocytes. Excision was complete with 6mm lateral margins and 12mm deep margins.

Immunohistochemistry with CD18 (mouse monoclonal, CA16.3.C10; Peter Moore, Davis, CA, USA) and ionized calcium-binding adapter molecule 1 (Iba1, rabbit polyclonal; Metagene, Brisbane, QLD, Australia) antibodies, using canine lymph nodes as positive controls, demonstrated moderate diffuse membranous binding of the CD18 antibody and strong diffuse membranous binding of the Iba1 antibody, reflecting a histiocytic origin (Figure 3). Given the histologic and IHC results, an initial diagnosis of histiocytic sarcoma was made, and screening for metastasis was recommended.

Thoracic radiographs and abdominal ultrasound were performed; both were unremarkable. Focused ultrasonographic examination of the right axillary lymph node, however, demonstrated moderate enlargement (width: 1.28cm), with normal echogenicity and margins. FNA was performed, and smears demonstrated an unremarkable lymph node cytologically; however, slides were noted to be of low cellularity, and thus the cause of the lymphadenopathy was not determined. Fasting serum biochemistry results, at this time, revealed a moderate hypercholesterolemia (348.0mg/dL; reference interval (RI) 150.8-301.6mg/dL). The CBC demonstrated mild hyperhemoglobinemia (19.2g/dL; RI 12.0-18.0g/dL) and moderate reticulocytosis ($209 \times 10^3/\mu\text{L}$; RI $10\text{--}110 \times 10^3/\mu\text{L}$) in the absence of anemia (HCT 52%; RI 37-55%) with moderately hemolyzed plasma.

Formalin-fixed samples were submitted to the University of California, Davis, CA, USA for further immunohistochemistry. The round to spindled cells demonstrated strong CD204 antibody binding, and no E-cadherin antibody binding, suggesting a dermal macrophage origin for the mass. Ki-67 antibody binding was highly and regionally variable, from below 5% to 25%, though the well-expressed regions were less frequent than the poorly-expressed regions.

A year later, fasted serum cholesterol and triglyceride testing demonstrated moderate hypertriglyceridemia (407.4mg/dL; RI 8.9-141.7mg/dL), and persistent moderate hypercholesterolemia (351.9mg/dL; RI 150.8-301.6mg/dL). The serum total thyroxine concentration was within normal limits ($2.03\mu\text{g/dL}$; RI $1.01\text{--}3.96\mu\text{g/dL}$). There was no evidence of recurrence at the surgical site, and no additional masses were noted. The dog remained clinically well, with only a single episode of self-limiting gastrointestinal upset eight months after biopsy. Given the clinical and immunohistochemical findings, the mass was diagnosed as an indolent dermal histiocytic proliferation of macrophage origin, with a preference for cutaneous xanthoma or, less likely, reactive dermal fibrohistiocytoma. The dog subsequently re-presented 18 months after initial biopsy for the presence of a 1cm diameter firm raised erythematous haired mass on the medial aspect of

the first digit of the left forepaw and right-sided otitis externa. Cytology of the digital mass was non-diagnostic due to poor cellularity, and the mass resolved spontaneously.

DISCUSSION

Cutaneous xanthomas have been described in a variety of veterinary species, most frequently in avian species^{1,2}, but also rarely in cats³⁻⁶, and very rarely in dogs^{7,8}, horses⁹, rabbits¹⁰. They are non-neoplastic masses composed of large foamy lipid-laden macrophages and are usually associated with defects in lipid metabolism or with metabolic disorders such as diabetes mellitus, hypothyroidism, or hyperadrenocorticism^{11,12}. Rarely, solitary xanthomas have been reported in the absence of abnormalities in lipid metabolism^{7,10}.

The dog, in this case, had persistent fasting moderate hypercholesterolemia and hypertriglyceridemia. Hyperlipemia in dogs may be primary or secondary to underlying nephrotic syndrome, hypothyroidism, cholestatic disease, or pancreatitis¹³. Hypothyroidism was considered unlikely based on normal serum thyroxine concentrations, and other secondary causes were considered unlikely given the lack of supportive biochemical or clinical findings; however, obesity might have been a predisposing factor¹³⁻¹⁵. As such, a low-fat diet and weight loss were recommended.

Initial moderate reticulocytosis in the absence of anemia was attributed to hemorrhage associated with surgical biopsy two weeks prior, post-operative carprofen administration, and/or excitement/splenic contraction; however, hemolysis could not be excluded. Given the normal red cell count ($8.4 \times 10^9/\text{dL}$; RI $5.5\text{-}8.5 \times 10^9/\text{dL}$), the hyperhemoglobinemia was likely an artifact of moderate sample hemolysis or could have reflected concurrent hyperlipidemia masked by hemolysis.

Hemogram monitoring was not performed; however, the dog remained clinically well, and there was no clinical evidence of hemorrhagic or hemolytic disease on subsequent follow-up examinations.

Commonly affected sites for cutaneous xanthomas are species-dependent, and in dogs, cutaneous xanthomas have been reported on the face, ears, and ventrum. In humans, the tuberous and eruptive forms characteristically form at sites of pressure and minor trauma¹⁶. This is postulated to be due to histamine release causing increased vascular permeability facilitating accelerated xanthoma formation¹⁷. While there was no history of trauma to the affected site, in this case, it is interesting to note that the single report of a solitary cutaneous xanthoma in a dog was also present on the ventral thorax. In that case, cutaneous mast cell tumors were also present⁷, and it could be

123 speculated that histamine-mediated mechanisms could have increased susceptibility to xanthoma
124 formation. There was no evidence of mast cell neoplasia in this case.

125 While the cytologic and histologic preparations demonstrated the characteristic and prominent
126 vacuolation observed in cutaneous xanthomas, there were several unique microscopic features in
127 this case. Firstly, the striking perivascular arrangement of cells noted on cytology, a common feature
128 of perivascular wall tumors, liposarcomas, and Leydig cell tumors on cytology¹⁸⁻²⁰, has not previously
129 been reported in xanthomas and led to the initial incorrect cytologic interpretation. This pattern has
130 been reported in diffuse normolipemic plane xanthoma in humans, a type of xanthoma that occurs
131 secondary to perivascular immunoglobulin deposition associated with plasma cell neoplasia,
132 monoclonal paraproteinemia, leukemia, and lymphoma²¹, but to the authors' knowledge, has not
133 been reported in other forms of xanthoma. Previous studies in humans have demonstrated that
134 endothelial cells appear to play a role in xanthoma development, by increasing von Willebrand
135 factor and E-selectin expression and undergoing proliferation to promote macrophage migration
136 into xanthomatous lesions²². If this also occurs in animals, it could explain the perivascular
137 arrangement observed in this case. Additionally, the frequent spindled morphology of histiocytes,
138 both cytologically and on histopathology, initially resulted in an erroneous interpretation of a
139 sarcoma. The additional finding of diffuse immunohistochemical binding of CD18 and Iba1
140 antibodies further confused the preliminary interpretation by suggesting a histiocytic sarcoma was
141 present, a more common malignant cutaneous neoplasm of dogs²³. While the high cellularity,
142 spindled morphology, and perivascular pattern on cytology could have supported a PWT; however,
143 these tumors do not typically bind the CD18 antibody²⁴.

144 The spindled morphology of histiocytes has been reported in solitary reticulohistiocytoma and
145 cellular spindled histiocytic pseudotumor complicating fat necrosis in humans, and reactive
146 fibrohistiocytic nodules in humans and dogs^{17,25}. To the authors' knowledge, spindled histocytes
147 have not been reported in cutaneous xanthomas, which are typically characterized by the presence
148 of large round macrophages filled with lipid, sometimes termed "foam cells".

149 Reactive fibrohistiocytic nodules are uncommon in dogs and, as such, are poorly characterized. They
150 are described as solitary or occasionally multiple small cutaneous nodules that predominantly affect
151 dogs less than three years of age. Histologically these nodules comprise predominantly histiocytic
152 cells with round to polygonal to plump spindle morphology that contain variable numbers of
153 vacuoles. Fibroblasts and mixed lymphocytic, neutrophilic, and eosinophilic infiltrates might also be
154 present. These lesions are postulated to be reactive, with excision being curative.²⁵ While the
155 spindled morphology of the histiocytes, in this case, was most consistent with a reactive

fibrohistiocytic nodule, the clinical observation of hyperlipidemia and marked vacuolation of the histiocytes in the absence of significant inflammation was more consistent with cutaneous xanthoma, and as such was the favored interpretation.

Unfortunately, neither unstained smears nor frozen tissue samples were available in this case for Oil Red O or Sudan Black B staining for confirmation of lipid within macrophages. IHC, however, was essential for the identification of these cells.

CD18 is a β_2 integrin that is a major adhesion molecule for most leukocytes, however macrophages and granulocytes express ten-fold more CD18 than do lymphocytes¹². Iba1 is a protein involved in rearrangement of the actin cytoskeleton that is present in cells of the monocyte/macrophage lineage in mice, and has been demonstrated in canine histiocytic sarcomas; however, it has also been shown to label dendritic cells²⁶. CD204 is of the scavenger receptor superfamily and similarly labels cells of the monocyte/macrophage lineage; however, this molecule does not label dendritic cells. It has also been demonstrated in canine histiocytic sarcomas²⁷. E-cadherin is a member of the calcium-dependent cadherin family of adhesion molecules and is expressed in canine Langerhans cells and canine cutaneous histiocytoma²⁸. Thus, the IHC profile, in this case, supported monocyte/macrophage origin, rather than Langerhans cells (which comprise canine cutaneous histiocytoma)²⁸. Ki67 is a nuclear protein expressed in all phases of the cell cycle and is absent in non-cycling cells¹²; its low expression and regional variability, in this case, was supportive of a benign, reactive process rather than a proliferative malignant neoplasm (ie, histiocytic sarcoma), consistent with the clinical presentation.

In conclusion, this case report describes the unique cytologic and histologic features, including a perivascular arrangement and round to spindled morphology, of a suspected cutaneous xanthoma in a dog. The spindled to round morphology combined with positive CD18 and Iba1 immunohistochemical antibody binding, initially led to an erroneous interpretation of histiocytic sarcoma, which has markedly different clinical management and prognosis to both cutaneous xanthomas and reactive fibrohistiocytomas, which are reactive, rather than neoplastic, lesions. Further, IHC and biochemistry were helpful for accurate interpretation in this case.

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261 184.

262 263 264 **FIGURE LEGENDS**

265 **Figure 1.** Photomicrographs of a fine-needle aspirate from a cutaneous mass in a dog. Features
266 consist of a marked perivascular cellular arrangement and cytoplasmic vacuolation, with indistinct
267 cell borders. Wright-Giemsa stain. (A) x20 objective and (B) x50 objective.

268 **Figure 2.** Photomicrograph of histopathology of the excised cutaneous mass in a dog. Expansion of
269 the dermis is characterized by a highly cellular, unencapsulated, infiltrative proliferation of sheets of
270 spindled to round cells with vacuolated eosinophilic cytoplasm and indistinct cell borders. Mitoses
271 were two per ten high power fields (400x magnification). H&E stain, (A) x4 objective, and (B, C) x10
272 objective.

273 **Figure 3.** Photomicrograph of immunohistochemical antibody binding in the excised cutaneous mass
274 from a dog. Cytoplasmic binding with both (A) CD18 and (B) Iba1 antibodies. H&E stain, x10
275 objective.

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