Lymphoma with Mott cell differentiation and validation of immunohistochemical lymphoid markers in an African pygmy hedgehog (Atelerix albiventris)

Title: **Lymphoma with Mott cell differentiation and validation of immunohistochemical lymphoid markers in an African pygmy hedgehog (*Atelerix albiventris*)**

Running header: Lymphoma with Mott cell differentiation in a hedgehog

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Abstract

A 2 year old, female entire African pygmy hedgehog was presented for diagnostic investigation of a 2-month reduction in appetite, with weight loss, and recent vomiting. Clinical examination revealed a large, firm mass originating from the left cranial abdomen. Ultrasound guided fine needle aspirates of the mass, the liver and the mesenteric lymph nodes revealed a population of pleomorphic round cells, some of which contained variable numbers of round, clear vacuoles, consistent with a diagnosis of lymphoma with Mott cell differentiation. At post-mortem examination, there was marked diffuse splenic enlargement, with infiltration by a soft tissue mass. There were multiple coalescing masses in the liver, pallor of the kidneys, and enlargement of the mesenteric lymph nodes. On histological examination, the spleen, lymph nodes, and masses in the liver were extensively infiltrated by proliferating lymphoid cells with plasmacytoid and Mott cell differentiation. Cells with Mott cell morphology had accumulation of periodic acid-Schiff positive material in cytoplasmic inclusions, and were positive for cytoplasmic nucleic acids when stained with methyl green pyronin. In the population of neoplastic lymphoid cells, a majority of cells expressed the transcription factor Pax5, which drives B cell differentiation, and a minority expressed transcription factor IRF4/MUM-1, which drives plasma cell differentiation, consistent with a B cell lymphoma with plasmacytoid differentiation.

Case presentation

A 2 year old, female entire, African pygmy hedgehog (*Atelerix albiventris*) was presented to the Rabbit and Exotic Animal Practice at the Royal (Dick) School of Veterinary Studies,

Edinburgh, in February 2017, for diagnostic investigation of a 2-month period of weight loss and recent episodes of vomiting. On initial observations, the hedgehog was ambulatory, but with a subdued demeanor and exercise intolerance, when allowed to explore the mammal ward floor. The respiratory rate was elevated and there was increased respiratory effort and noise.

Clinical examination was performed under general anesthesia. The hedgehog weighed 275 g and had a reduced body condition score at 2 out of 5. Abdominal palpation revealed a large, well-defined mass in the cranial abdomen. On ultrasound examination, a large, heterogeneous mass was identified; the mass occupied most of the peritoneal cavity, causing a mass effect on all the abdominal organs. The mass appeared to be of splenic origin because of its location and appearance; no normal splenic parenchyma was visualized. The displaced abdominal organs, including the liver, both kidneys and the mesenteric lymph nodes, were also involved. The liver was moderately heterogeneous, with multiple hypoechoic nodules. Both kidneys were hyperechoic and had decreased definition of the corticomedullary junction. The abdominal lymph nodes were enlarged and heterogeneous. A moderate quantity of anechoic peritoneal effusion was evident.

The presumptive diagnosis from ultrasound investigation was splenic neoplasia, most likely lymphoma, with metastasis to the liver, kidneys and abdominal lymph nodes. Fine needle aspirates (FNAs) were taken from the abdominal mass, liver and lymph nodes, routinely stained by the May-Grünwald-Giemsa method, and examined microscopically. The abdominal mass FNAs were highly cellular and heavily hemodiluted. A population of pleomorphic round cells predominated. They ranged from large, round cells (approximately 20 µm in diameter), with a high nuclear to cytoplasmic ratio (N:C), deeply basophilic cytoplasm, occasionally with perinuclear clearing and some clear vacuoles, and a round to

irregular nucleus, with finely stippled chromatin, to smaller, oval cells, with a more abundant cytoplasm, filled with clear vacuoles, and a peripherally located nucleus with clumped chromatin (Figure 1 A & B). Mitotic figures were frequent and occasionally were bizarre (Figure 1B). Similar cells were present in the mesenteric lymph nodes and in the liver. Unstained lymph node smears were stained with periodic acid-Schiff (PAS), which indicates the presence of carbohydrates, and methyl green pyronin (MGP), a histochemical stain used to visualise RNA and DNA. The cytoplasmic inclusions of the neoplastic cells were strongly positive for PAS (Figure 1 C), suggesting the presence of antibodies that have been glycosylated in the endoplasmic reticulum,[1] while the cells had marked cytoplasmic pyroninophilia (Figure 1 D), demonstrating high cytoplasmic nucleic acid (RNA) content.

A diagnosis of lymphoma with Mott cell differentiation was established. The hedgehog was euthanized on welfare grounds without additional work-up (e.g. hematology and biochemistry) and submitted for post-mortem examination. The spleen was markedly enlarged, weighing 76 g, and was mottled, dark red to purple, with multilobular, coalescent, pale yellow-brown masses; fibrin strands and haemorrhage were present on the surface (Figure 2). The liver was moderately enlarged, weighing 25 g, and was diffusely pale yellow-brown, with rounded borders and an accentuated lobular pattern. Multiple, raised, soft, pale yellow-brown nodules, approximately 5 mm in diameter, were present in the right lateral and right medial lobes of the liver. The mesenteric lymph nodes were enlarged and pale yellow-brown. The kidneys were slightly enlarged and pale brown. The brain was unremarkable on external examination; on sectioning, a 3 to 4 mm diameter white mass was evident, involving the ventral cerebellum and adjacent medulla oblongata. The lungs were moderately, diffusely oedematous. There were 6 mL of clotted blood in the peritoneal cavity. The bone marrow was pale yellow. The gross findings were consistent with infiltrative neoplasia of the spleen, liver, kidneys, and brain.

On histological examination, affected areas of the spleen, liver, kidneys, and lymph nodes were infiltrated, expanded and effaced by dense sheets of large, round, moderately pleomorphic, neoplastic lymphoid cells (Figure 3 A). The infiltrating cells had large, round to ovoid, sometimes indented, nuclei, clumped chromatin, large, prominent, eosinophilic nucleoli, and moderate amounts of eosinophilic to amphophilic cytoplasm; in some cells, there was a clear perinuclear area, consistent with a plasmacytoid morphology. Mott cells with eosinophilic cytoplasmic inclusions, consistent with Russell bodies, were scattered through the tissue. There were six to eight mitoses per high power field (400x magnification). Apoptosis was evident and there was multifocal, locally extensive necrosis and haemorrhage. Infiltrates of neutrophils were present in some areas. The bone marrow was hypercellular with a myeloid predominance. Mildly increased numbers of plasma cells and Mott cells were present; however, it was unclear if these and some of the larger immature cells present represented an early neoplastic infiltrate.

Immunohistochemistry was performed on the spleen; healthy splenic tissue from an unrelated African pygmy hedgehog was retrieved from our archive and used as a control for validation of the immunohistochemical stain. Sections (4µm thickness) of formalin-fixed, paraffin wax-embedded tissue were placed on SuperFrost® Plus coated slides (Thermo Electron, Runcorn, Cheshire, UK), dewaxed, hydrated, and rinsed in distilled water. To block non-specific endogenous peroxidase activity, sections were treated with blocking agent (Dako REAL blocking agent S202386) for 10 minutes at room temperature. Antibodies were diluted in antibody diluent (S0809, Dako) at 1/200 for detection of CD3 (mouse monoclonal anti-CD3; Novocastra, NCL-L-CD3), 1/40 for detection of PAX 5 (mouse monoclonal anti-PAX 5; Becton & Dickinson, P67320) and 1/40 for detection of MUM 1/IRF4 (interferon regulatory factor 4; rabbit polyclonal anti-MUM1; Thermofisher,

PA5-32511). Antigen retrieval was performed using 0.01 M citrate buffer pH 6.0 at 110 °C for 5 minutes (Histos 5 microwave processor), then sections were incubated with the primary antibody for 30 minutes at room temperature (RT) following antigen retrieval. Following incubation with primary antibody, the sections were incubated with secondary antibody (Envision anti-mouse HRP; Dako K4007) and visualized with DAB+ chromogen (Dako K3468). Sections were counterstained with Harris haematoxylin. All washings between steps were carried out using Tris-buffered saline with Tween (Thermo Fisher Scientific TA-999-TT).

On immunohistochemical examination of the splenic mass in the African pygmy hedgehog with lymphoma, a majority of neoplastic lymphoid cells (60-70%) expressed the transcription factor Pax5 (Figure 3 B), whilst a minority (10-20%) expressed the transcription factor MUM-1 (Figure 3 C). This was consistent with the majority of the neoplastic cells being morphologically compatible with lymphocytes, and with lower numbers being morphologically consistent with Mott cells. Moderate numbers of CD3 positive tumor infiltrating lymphocytes were present within the areas of neoplastic infiltration, representing 5% of the total cell population (Figure 3 D). In samples of spleen from a control African pygmy hedgehog that died of unrelated disease, there was positive immunostaining for Pax5, CD3, and MUM1 in expected lymphoid tissue zones (Supplementary Figures 4 – 6).

Discussion

Neoplasia is reported commonly in African pygmy hedgehogs (*Atelerix albiventris*).[2] In a study on captive African hedgehogs, 53% of animals between 2 and 5.5 years of age had at least one type of tumor, while 8.6% of animals had more than one type of tumor.[2] In another retrospective study of 14 African hedgehogs, the prevalence of neoplasia was 29%.[3]

154 The most commonly reported neoplasms are carcinomas and lymphomas, and malignant
155 neoplasia is more frequent.[2]
156
157 Mott cells are plasma cells which have retained immunoglobulins packed in vesicles, known
158 as Russell bodies, giving these cells a distinctive appearance.[4, 5] Due to the carbohydrate
159 component of the immunoglobulins, Russell bodies are also positive when stained with PAS
160 (Figure 1 C).[1, 6, 7] Plasmacytoid cells producing immunoglobulin and immunoblasts contain
161 high quantities of rough endothelial reticulum and their cytoplasm is therefore positive on
162 staining with methyl green pyronin, which highlights nucleic acid (RNA) content (Figure 1
163 D).[6, 8]
164
165 As described in dogs[1] in our case the neoplasia was composed of many lymphoid cells, which
166 occasionally contained some vacuoles (Russell bodies), and by lower numbers of Mott cells,
167 in the absence of cells with a morphology consistent with well differentiated plasma cells.
168 For this reason, a plasma cell tumor, including a multiple myeloma, was considered less
169 likely, and the neoplasia was considered morphologically consistent with a lymphoma with
170 Mott cell differentiation. Immunohistochemistry was consistent with the morphologic
171 diagnosis. The transcription factor Pax5 is expressed in all pre-B and mature B cell stages.[9]
172 Pax5 expression is downregulated when B cells undergo plasma cell differentiation.[10] In our
173 case, as in similar previous cases in other species,[7, 11] the majority of neoplastic cells
174 expressed the transcription factor Pax5, indicating a B cell origin (Figure 3 B). Some plasma
175 cell tumors, including multiple myeloma, can express Pax5, as well as other B cell markers,
176 such as CD20.[12] Therefore, the diagnosis of B cell lymphoma with Mott cell differentiation,
177 in the present case, was based on the morphological appearance of the population of
178 neoplastic cells, supported by demonstration of Pax5 expression. MUM1 is a transcription
179 factor expressed in plasma cells.[13] In our case, as in previously reported cases,[7, 14] cells with

Mott cell differentiation expressed nuclear immunohistochemical positivity for MUM1 (Figure 3 C). The presence of scattered CD3 positive T-cells (Figure 3 D) was interpreted to be due to the presence of tumor infiltrating T lymphocytes. Negative control samples from an African pygmy hedgehog without neoplasia provided validation for the immunohistochemical markers in this species (Supplementary Figure 4 – 6).

Analogous B cell lymphomas have been described in dogs,[1, 11, 14, 15] in a cat,[7] and in a ferret.[16] Similar to the case reported here, previous cases were characterized by a bi-phasic population of larger, more immature lymphoid B cells that differentiate to Mott cells filled with characteristic Russell bodies. While plasma globulins can be within the reference interval, and no obvious monoclonal peak may be detected using electrophoresis,[16] immunofixation demonstrated the presence of monoclonal bands in the IgM and IgA proteins in two dogs.[15] Circulating neoplastic cells have been described in two dogs with B cell lymphomas with Mott cell differentiation.[1] Unfortunately the overall low number of reported cases is insufficient to reach any definitive conclusions on the prognosis for this neoplasia.

In conclusion, this is the first case report of B cell lymphoma with Mott cell differentiation in a hedgehog, with validation of Pax5, CD3, and MUM1 immunohistochemical markers in this species.

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Figures

Figure 1. Fine needle aspirate of a splenic mass of an African pygmy hedgehog. (A and B) Note the presence of a pleomorphic population of round cells that often have clear cytoplasmic vacuoles; (B) mitotic figures are also present. May-Grünwald-Giemsa stain. (A) 60x objective. (B) 100x objective.

(C) Cytoplasmic vacuoles are PAS positive, supporting their identification as Russell bodies. Periodic acid-Schiff stain, 60x objective. (D) The presence of cytoplasmic red staining suggests a high RNA content. Methyl green pyronin stain, 100x objective.

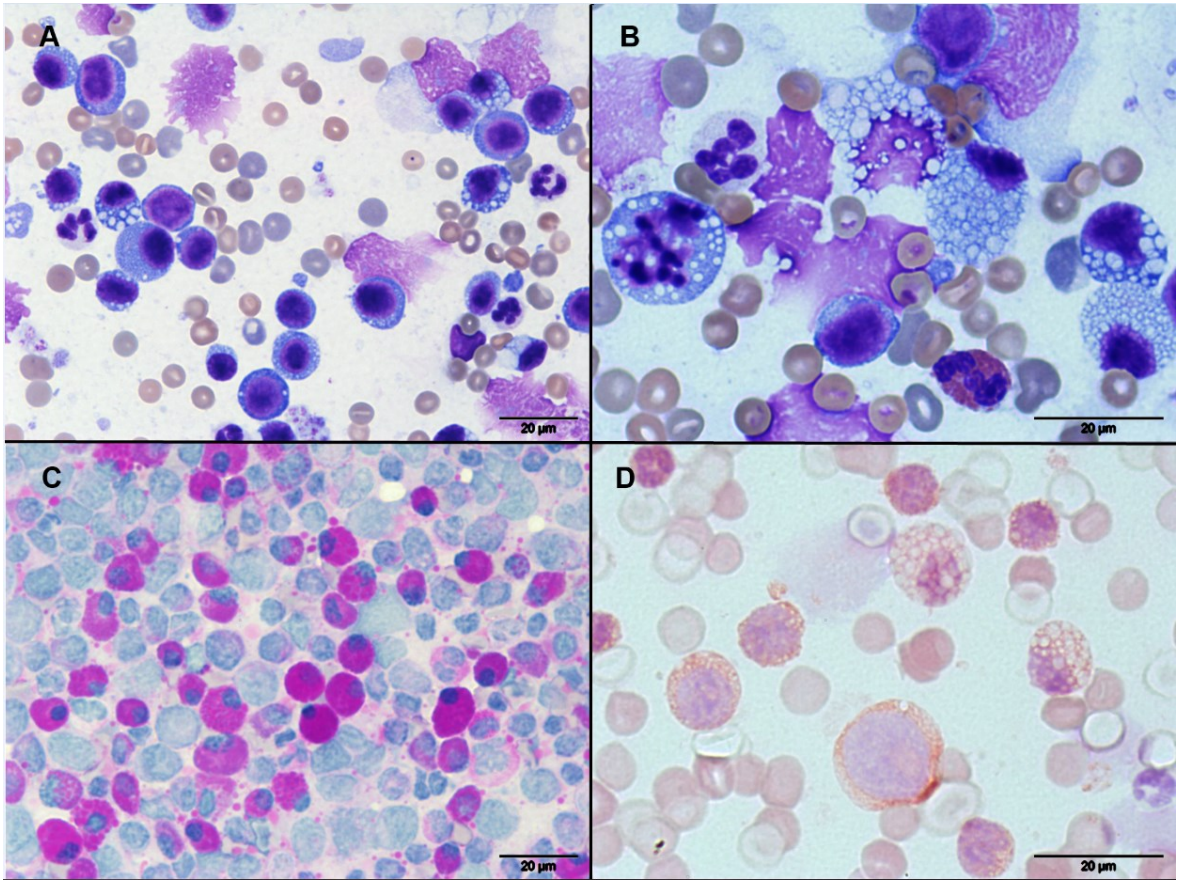


Figure 2. Gross findings at post-mortem examination of a hedgehog. The spleen is enlarged, mottled, and covered with a fibrin exudate. The liver is pale and has rounded borders. There is a fluid effusion in the pleural cavity.

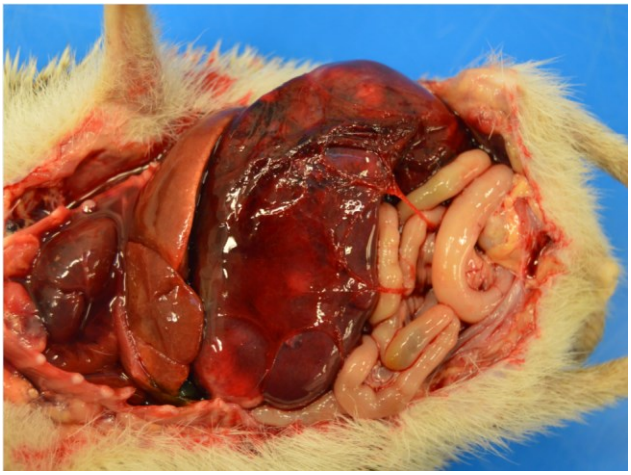
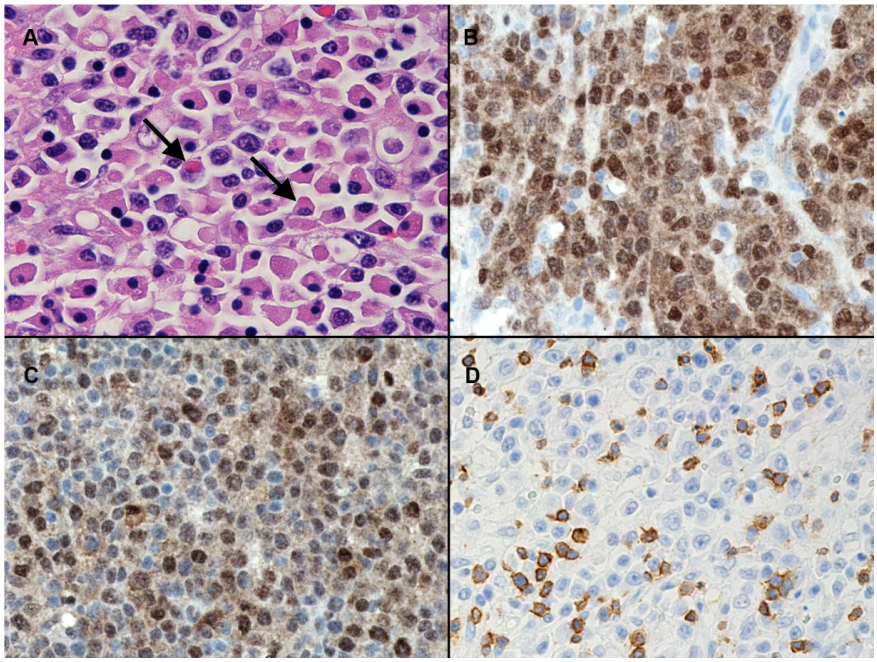
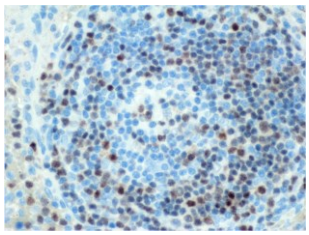


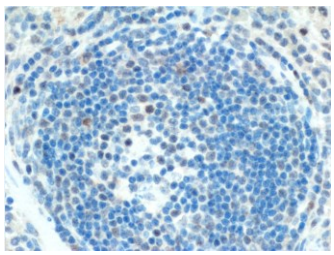
Figure 3. Histologic sections from an abdominal mass in a hedgehog. (A) Histological appearance of the spleen showing diffuse infiltrates of neoplastic cells with plasmacytoid morphology and several Mott cells containing Russell bodies (arrows). 40x objective. Scale bar = 50 µm. (B) Immunohistochemistry for Pax5, (C) MUM1, and (D) CD3 in the spleen. 40x objective. Scale bars = 50 µm.



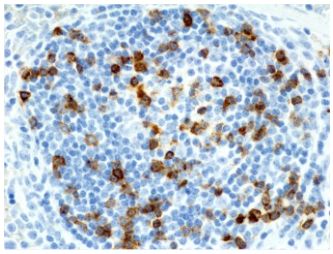
Supplementary figure 4. Immunohistochemistry for Pax5 in lymphoid tissue in the white pulp of a normal spleen in a control African pygmy hedgehog. 40x objective. Scale bars = 50 µm.



Supplementary figure 5. Immunohistochemistry for MUM in lymphoid tissue in the white pulp of a normal spleen in a control African pygmy hedgehog. 40x objective. Scale bars = 50 µm.



Supplementary figure 6. Immunohistochemistry for CD3 in lymphoid tissue in the white pulp of a normal spleen in a control African pygmy hedgehog. 40x objective. Scale bars = 50 µm.



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