HIGH GRADE LYMPHOMA IN A SIX-YEAR OLD BOERBOEL: A CASE REPORT

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Summary

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A case of high grade large cell lymphoma was diagnosed in a dog at the Veterinary Teaching Hospital, University of Ibadan. The animal was a male Boerboel over six years old. The case was monitored clinically until necropsy where cytological, gross and histological techniques were used for detailed examination of morphological features of the tumour. The morphological pattern and classification schemes of lymphoma were generally reviewed. The relevance of other diagnostic methods was emphasised.

Key words: canine, diagnosis, lymphoma, pathology

Lymphoma is one of the haematopoietic malignancies encountered in small animals. It accounts for 7% to 24% of all canine tumours and up to 83% of all canine haematopoietic malignancies (Vail, 2010; Zandvliet, 2016). However, apart from reports of splenic lymphoma in a local bitch (Oni *et al.*, 2004) and another CD33 positive lymphoma in an Alsatian, very little is known on the epidemiology of canine lymphoma in Nigeria.

Clinical signs and physical examination are often suggestive of lymphoma or

lymphosarcoma (LSA). The definitive diagnosis requires cytology, histopathology and/or molecular diagnostics (Couto, 2009).

A number of classification schemes have been developed over the years, however, both the revised European American (REAL) and the very similar WHO systems are appropriate for the classification of animal haematopoietic neoplasms. The morphological presentation of the neoplastic cells still remains crucial in diagnosis of lymphoma in animals (Ponce *et*

al, 2010). Other factors to consider include history and physical examination, complete blood count and serum chemistry, urinalysis, and/or radiography. The clinical picture and pathological features observed in this case were described to further understand the subtle characteristic presentation and epidemiology of canine lymphoma in our environment.

The dog, a male Boerboel over six years old with brown fur was presented to the Small Animal Clinic of the Veterinary Teaching Hospital, University of Ibadan with a complaint of inability to bear weight on the right hind limb. The dog had been recumbent since the condition was first noticed. Clinical signs observed included: swelling of the inguinal and anal regions, bilateral muco-purulent ocular discharges, and marked distention of the abdomen. The dog was also unable to urinate (stranguria) due to compression in the inguinal region. There was pedal oedema, dyspnoea, weak pulse and faint heart sound on auscultation. The 6 lead ECG report showed cardiac arrhythmia and prolonged Q-T wave (Fig. 1) indica-

tive of cardiac abnormality. The haematological and serum biochemical values were within the normal range for the species. Fine needle aspiration (FNA) cytology was equally carried out as well as coprology which revealed presence of 2–3 *Isospora canis* per high power field.

Ultrasonography of the abdominal region revealed a few abdominal masses. The weight of the animal dropped from 75 kg on presentation to 52 kg before death in space of two weeks. A tentative clinical diagnosis of multi-organ failure was made.

On necropsy, the carcass was fresh. There were several ticks on the body of the carcass, it was moderately emaciated. The oral and ocular mucous membranes were pale. There was widespread enlargement of superficial lymph nodes (submandibular, axillary, popliteal and pre-femoral) (Fig. 2A). The abdomen was markedly distended (caudally from the position of the last set of ribs). There were multiple raised dark spotted nodules of <2 cm diameter on the lung lobes. The mediastinal lymph nodes were enlarged

Fig. 1. ECG record showing prolonged QT- interval and ventricular premature complexes.

Fig. 2. A. Lymph node with effacement of medulla (asterisk) and haemorrhagic periphery (arrow).
B. Liver with cream coloured nodules (asterisk) on cut surface.

Fig. 3. Large lymphoid cells (arrows) from lymph node (A) and heart nodule (B) with anisocytosis, prominent nucleoli and high nuclear-cytoplasmic ratio. Giemsa, scale bar=18 µm.

and haemorrhagic on cut surface. An irregular spherical large nodule (>2.5 cm diameter) was seen on the base of the heart while the left ventricular wall was enlarged and distended with irregular greyish areas (infiltrative nodules). The muscular part of the diaphragm was markedly thickened with foci of neoplastic infiltrates and nodules. The abdominal cavity contained about 3.5 L of straw coloured fluid.

The liver was dark red and markedly enlarged with numerous irregular sized creamy coloured deep seated nodules on the hepatic lobes (Fig. 2B). The right lobe

of the liver was adhered to the diaphragm and loops of the pancreas. Multiple dark coloured nodules (<2 cm) were also present in the pancreas. A firm irregular and large sized tissue mass of firm consistency (7×6 cm) was found in the retroperitoneal fat attached to the right kidney and in the inguinal region (inguinal lymph node). Another similar tissue mass was also found extensively attached to the liver, duodenum, pancreas and muscular diaphragm. The mesenteric lymph nodes were enlarged with thin cortex and distended capsule and mottled medulla. The spleen was dark red, moderately enlarged

with a few raised circumscribed nodules. The gastric mucosa was haemorrhagic with multiple ulcers (0.5×1 cm) in the fundus. The intestinal serosa had raised opaque nodules of various size. The entire length of the intestinal mucosa was haemorrhagic (suffusive) with ulcers at the jejunal region. The prostate gland had nodules of various size which were haemorrhagic on cut surface. Similar nodules were also present on the kidneys. The urinary bladder mucosa was thickened and had areas of suffusive haemorrhages with ulcers of variable sizes. The bone marrow from the diaphysis of the femur was diffusely cherry red in colour.

Cytological smears obtained by FNA and impressions from nodal or extranodal masses (liver, pancreas, lungs, spleen, and prostate, intestinal serosa, kidney) were air-dried, fixed, and stained using the May Grunwald Giemsa technique. Microscopic examination of Giemsa stained smears from the liver nodules showed high cellularity, marked infiltrates of predominantly large to small discrete round (lymphoid) cells. The cells had high nuclear-cytoplasmic (N:C) ratio, marked anisocytosis, thin rim of bluish cytoplasm, and prominent to multiple nucleoli. There

were also a few vacuolated hepatocytes, neutrophils and macrophages. Other nodal masses showed preponderance of large lymphoid cells (lymphoblast) having high N:C, narrow/thin rim of cytoplasm, nuclear moulding, 3–5 mitotic figures/HPF and also anisocytosis. The lymphoblasts accounted for >50% of all nucleated cells present in a lymph node (Fig. 3A). Similar discrete round cells were present in the smears from the muscles, prostate, lungs, peripheral blood, bone marrow, pancreas and intestinal nodules (Fig. 3B).

The cytological diagnosis was metastatic round cell tumour (lymphoma), based on the preponderance of lymphoblasts accounting for more than 50% of all nucleated cells present in nodal and extranodal masses.

Histological evaluation of biopsy specimens from enlarged nodal and extranodal masses were fixed in 10% neutral buffered formalin at room temperature for 48 h and embedded in paraffin wax. Tissue sections 5 µm in thickness were stained with haematoxylin and eosin (HE) and examined using light microscope. Morphologically, the involved lymph nodes showed a diffuse effacement of normal architecture with marked thinning

Fig. 4. **A.** Lymph node showing loss of architecture with marked expansion of the medullary region (asterisk). HE, scale bar=180 µm. **B.** Lymph node showing preponderance of large cells (arrow) in the lymphoid follicle. HE, scale bar=45 µm.

Fig. 5. Liver (A) and heart (B) showing diffuse infiltration of neoplastic cells (arrows) and distortion of normal architecture. HE, scale bar=45 µm.

of the peripheral capsule and focal colonisation of the capsule and perinodal structure (Fig. 4A). Marked atrophy and coagulation necrosis of lymphoid follicles, apoptotic bodies, infilling of the paracortex extending into the medullary region with numerous tangible body macrophages, expansion of the medullary cords and compression of the medullary sinuses were noticed (Fig. 4B). There were also lighter areas at a higher level of cellular proliferation. The neoplastic cells had chromocentres separated by parachromatin areas in the nucleus. The chromatin was densely stained with prominent nucleolus. Similar neoplastic cells were diffusely infiltrating the liver causing compressional atrophy of hepatocytes (Fig. 5A), heart (Fig. 5B) and diaphragmatic muscles, lungs, spleen, intestine and kidneys. Morphological diagnosis of lymphoma (LSA) was made.

Grading was determined by the size of cells (majority of large-sized cells) and by mitotic index (MI). Thus, considering the cytological details and morphological criteria based on the updated Kiel histological and cytological classification, a case of high grade large cell lymphoblastic lymphoma was identified.

Lymphoma or lymphosarcoma arises due to the proliferation of malignant lymphoid cells – usually in lymphoid tissue, such as lymph nodes, liver or spleen, but the tumour may originate in any tissue (Couto, 2009; Vail, 2010). This origin from solid organs distinguishes LSA from lymphoid leukaemia, as the latter arises from bone marrow (Couto, 2009). The etiology of LSA is considered to be multifactorial. However, several environmental, infectious, immune-mediated and genetic factors are associated with a higher risk of developing LSA in other climes (Mortier *et al.*, 2012).

The most frequently observed clinical signs are non-specific including peripheral lymphadenopathy. All the lymph nodes including mandibular and prescapular ones, were affected in this case. The difficulty in micturition was due to compression from the nodal, prostatic masses and ensued polypoid cystitis. Haematologic and serum biochemical changes were within normal range in this case, though usually they are not diagnostic (Couto, 2009; Mizutani *et al.*, 2016). Anaemia and thrombocytopaenia, if present, are usually related to bone marrow infiltration, paraneoplastic immune-mediated destruction,

splenic infiltration and/or chronic disease. Regenerative anaemia may also be associated with concomitant blood loss (Couto, 2009; Vail, 2010). The ECG abnormality suggested that the tumour induces cardiomyopathy, weakness and exercise intolerance of the dog *ante mortem*. This nature of metastasis to distant organs and tissues confirmed the high grade of the neoplasm.

The defining strategy of the revised European American (REAL) and the very similar WHO systems is that each entity is a fully characterised scheme based on all information including cell type, tumour architecture, topography, age, gender, and phenotype and in some cases genotype (Valli *et al.*, 2013). Both systems recognise and separate lesions that may have similar morphology, but different phenotypes and rates of biological progression (Morton *et al.*, 2007). Furthermore, the REAL system can be applied very largely on the basis of routine histochemistry to define B- and T-cell lymphocyte derivation on paraffin embedded tissues (Jaffe *et al.*, 2008). The application of these systems however, to reviews of lymphomas in animals and their response to therapy provide major impetus to the understanding of lymphomas in animals from the standpoint of research on spontaneous lymphoid neoplasms of outbred species, as well as treatment of lymphoid tumours in companion animals, which may be very useful in this environment.

In general, lymphoproliferative diseases form a spectrum with lymphoma and lymphoid leukaemia at their extremes, and it is not always possible to determine whether the disease in a particular animal is primarily peripheral (lymphoma), or of bone marrow origin (leukaemia). The major distinction between both is in the tissue area with the largest mass of tumour

cells. It is now understood that, in malignancies of the lymphoid system, there are always tumour cells in the circulation whether or not they are recognised in the peripheral blood. However, the operative factors in the dissemination of tumours of the lymphoid system are the presence and type of intercellular cytoplasmic adhesion molecules (ICAMs) on the surface of the tumour cells that mediate adhesion to endothelium and the ability to emigrate to new tissue areas (Rezuke *et al.*, 1997; Atizadeh *et al.*, 2000). Malignancy in an ontogenically primitive cell is likely to occur in the bone marrow, thus presenting as leukaemia in a young animal or individual with the disease. In contrast, clonal autonomy in a mature lymphocyte is likely to occur in the peripheral tissues in a mature animal or individual with presentation as a lymphoma.

Moreover, leukaemias present some degree of marrow failure characterised by anaemia, thrombocytopaenia, or neutropenia, which occur when 50% or more of the bone marrow is involved by the tumour. Under these circumstances, the blood and bone marrow are always diagnostic. In contrast, the lymphomas which involve peripheral tissues tend to leave the bone marrow relatively uninvolved and, at the time of diagnosis, these animals usually have normal haemoglobin, platelet and leukocyte counts, as observed in this case.

Lymphoma of small lymphocytic cell type (SLL) needs to be differentiated from peripheralising lymphoma of larger lymphocytes and chronic lymphocytic leukaemia which are distinguished by their cytoplasmic granules (Erdman *et al.*, 1995). Another major differential diagnosis is prolymphocytic leukaemia (PLL). SLL must have less than 10% prolymphocytes whereas disorders with 10 to 50%

prolymphocytes are considered chronic lymphocytic leukaemia (CLL) and PLL when greater than 50% of prolymphocytes in the peripheral blood (Zwiebel & Cheson, 1998).

Once a definitive diagnosis is achieved, clinical staging can be done to allow more accurate prognosis. Immunophenotyping of canine lymphoma can classify tumours as T-cell (CD3) or B-cell (CD79); the latter being more common and the former associated with a poorer prognosis. The monoclonality of a population of neoplastic lymphoid cells support a diagnosis of lymphoma. Both B- and T-cells have cognate receptors which enable them to take part in the immune response. PCR amplification of either the T-cell receptor (TCR) or immunoglobulin chains on B-cells will demonstrate either a mixed population (i.e. with a reactive lymphadenopathy) or a clonal population of cells (Vernau & Moore, 1999). Other diagnostic and prognostic markers have been suggested including serum levels of alpha 1-acid glycoprotein (AGP) and matrix metalloproteinases 2 and 9 as indicators of relapse. However, new markers and sophisticated molecular techniques (such as micro and tissue arrays) are developed to provide information for the clinician in the management of canine lymphomas. Hopefully these would be adopted even in poor resource setting like ours to further characterise the epidemiology of this neoplasm in dogs.

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