Case report

T CELL LYMPHOMA IN THE ATRIUM OF A YOUNG BULL

G. FARJANIKISH¹, O. DEZFOLIAN¹ & K. QEISARBEIGI²

¹Department of Pathobiology, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran; ²Veterinary Supervision of Khorramabad Region, Khorramabad, Iran

Summary


In this report, T-cell lymphoma in the atrium of a 1.5-year-old Holstein bull is described. Macroscopically, a large white to a yellow tumour mass with a size of 4.5×3.5×2 cm was observed in the left atrium. Histopathological examination revealed extensive infiltration of medium- to large-sized lymphocytic cells with round to oval nuclei and stippled chromatin, surrounded by a narrow rim of pale eosinophilic cytoplasm. Immunohistochemically, the tumour cells indicated positivity with CD3 and Ki-67, but negativity with CD79α, CD20 and S100. On the basis of the histologic and immunohistochemical findings, this tumour was diagnosed as a T-cell lymphoma.

Key words: atrium, cow, immunohistochemistry, lymphoma

Bovine lymphoma is one of the several common neoplasms identified in cattle causing considerable contention in terms of nomenclature and classification. Concerning pathogenesis and clinical pathology, this neoplasm in cattle is classified into enzootic bovine leukemia (EBL) and sporadic bovine leukemia (SBL) (Tani et al., 1997). EBL is most often associated with the enzootic leukemia virus and cattle affected with this form of lymphoma are usually 4–8 years of age. Among cattle, the tumour is commonly detected in the uterus, abomasum, heart, and peripheral lymph nodes. The SBL is not associated with the bovine leukaemia virus and can be classified into three further types with respect to the preferential sites of the neoplasia and age of the affected animal: calf type, thymic type, and skin type (Sasaki et al., 1997; Step et al., 2001; Harbo et al., 2004).

Although lymphomas are relatively uncommon in calves, the neoplasms are of interest due to their occurrence early in life (Meuten & Harvey, 2008; Step et al., 2001). Primary cardiac lymphomas are extremely rare and the most common gross appearance of tumours is as nodular or polypoid masses which predominantly involve the pericardium having variable
myocardial infiltration (Constantino et al., 1987; Holladay et al., 1992).

The present report describes the clinical, histopathological and immunohistochemical features of an atrial lymphoma in a young bull.

**Case presentation.** A Holstein bull 1.5 years of age, was presented to the clinic with a one-month history of progressive loss of appetite, decreased activity and loss of condition. The heart rate dropped down to 48 bpm, the respiratory rate was 28 breaths per minute, and the rectal temperature was 38°C. A moderate degree of dyspnea was observed; however, the animal was alert and did not cough. During the physical examination, no heart sounds could be auscultated and no jugular distention or pulsation was observed. The prefemoral and prescapular lymph nodes were not enlarged.

The bull had leukocytosis with neutrophilia (14,100 leukocytes/μL; normal 5,000 to 10,000 leukocytes/μL). The concentrations of blood urea (13.0 mmol/L; normal 2.4 to 6.5 mmol/L) and creatinine (119 μmol/L; normal 55 to 103 μmol/L) as well as the activities of glutamate dehydrogenase (GLDH; 30.5 U/L; normal 4 to 18 U/L) and sorbitol dehydrogenase (SDH; 67.8 U/L; normal 4 to 7 U/L) were increased. An ELISA for bovine leukaemia virus (CHEKIT® BLV-ELISA kit, IDEXX Switzerland) was negative.

The bull was euthanised because of a grave prognosis and necropsied. At necropsy, numerous bulging or polypoid masses with a diameter of about 4.5×3.5×2 cm were observed in the left atrium (Fig. 1). Neoplastic mass was white-to-yellow, infiltrated the left atrium but did not extend into the myocardium. The mass was granular and bulged from the cut surfaces. No evidence of neoplasm beyond the ventricle was found and no other gross abnormalities were also observed.

The appropriate tissues from the mass were fixed in 10% neutral buffered formalin, dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 5 μm were stained with haematoxylin and eosin (H&E) and studied microscopically.

Fig. 1. Heart. Note numerous bulging or polypoid masses in the atrium.
Immunohistochemical (IHC) analysis of the tumour sections was performed using monoclonal antibodies CD3 (Monoclonal Mouse Anti-Human CD3, Dako Cytomation), CD79a (Monoclonal Mouse Anti-Human CD79αcy Clone HM57, Dako Cytomation), CD20 (monoclonal mouse anti-human CD20cy, Dako Cytomation), Ki67 (Monoclonal Mouse Anti-Human Antigen Clone MIB-1, Dako Cytomation), S100 (polyclonal, 1:100; Dako Cytomation), vimentin (monoclonal mouse antibody clone V9, Dako Cytomation), and cytokeratin (Biogenex, AE1/AE3, USA.).

Histopathological examination revealed the extensive infiltration of medium to large-sized lymphocytic cells. The
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neoplastic cells predominantly had round to oval nuclei with stippled chromatin as well as a nucleolus, surrounded by a narrow rim of pale eosinophilic cytoplasm (Fig. 2). There were moderate numbers of mitotic figures, some of which were atypical. The tumour cells were surrounded by a sparse fibrovascular stroma. No invasion into the myocardium was identified in tissue sections.

Immunohistochemical staining was positive for CD3 in neoplastic cells (Fig. 3). The proliferation marker Ki-67 was immunohistochemically expressed by the majority of neoplastic cells (95%), reflecting a high proliferative rate (Fig. 4). Neoplastic cells were not stained with all other immunostains, including CD 79α, CD20, S100, cytokeratin and vimentin.

Bovine lymphoma associated with bovine leukaemia virus infection usually occurs in adult cattle 4 to 8 years of age. The tumours have a predilection for the abomasum, uterus, right heart, and peripheral lymph nodes (Thurmond, 1996; Johnson, 1999; Welker, 1999). Based on epidemiological and clinicopathographical criteria, this neoplasm in cattle is classified into enzootic bovine leukosis and sporadic bovine leukosis. EBL is endemic across the USA and occurs as multicentric lymphadenopathy in older cows; whereas the SBL is the predominant type of leukosis in Europe (Grünberg & Eisenberg, 2013). The age of the affected also plays a critical role in presenting a classification: viral enzootic bovine leukosis occurs in adult cattle and SBL is detected in young cattle (Jacobs et al., 2002; Radostits et al., 2007; Grünberg & Eisenberg, 2013). In the present report, T-cell lymphoma was detected in the atrium of a 1.5-year-old Holstein bull. Lymphomas are also subdivided into three further groups in calves: a) spontaneous tumours of the congenital type, occurring in foetuses, newborns and relatively young calves, b) spontaneous tumours of the juvenile type, occurring in older calves (aged 2–12 months), and c) iatrogenic tumours, such as skin papillomas caused by the papilloma virus after tattooing or dehorning (Misdorp, 2002).

The most obvious clinical symptom of lymphoma is the enlargement of peripheral lymph nodes (Johnson, 1999). Superficial lymph node enlargement occurs in

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**Fig. 4.** The majority of neoplastic cells express the proliferation marker Ki-67 (arrows) (IHC, Bar=25 µm).
75–90% of cases (Malatestinic, 2003). A strange finding in this regard was the absence of this lesion in peripheral lymph nodes.

Histopathologically, the neoplasm was characterised by extensive infiltration of medium to large-sized lymphocytic cells being consistent with those reported by other researchers (Tani et al., 1997; Braun et al., 2015). The neoplastic cells predominantly had round to oval nuclei with stippled chromatin and one nucleolus surrounded by a narrow rim of pale eosinophilic cytoplasm. Similar histological features have frequently been reported for bovine lymphomas in previous studies (Step et al., 2001; Harbo et al., 2004). These histological features are compatible with those of large T-cell lymphoma, as previously reported in calves (Tani et al., 1997; Harbo et al., 2004; Braun et al., 2015).

CD3 and CD79 antibodies have been documented as effective reagents for the immunohistochemical assessment of T-/B-cell lineage lymphomas in animals, as in humans. CD3 (T-cell lymphocytes marker) and CD79a (B-cell lymphocytes marker) immunohistochemistry were used to classify the tumour as lymphoma. One type of lymphoma caused by bovine leukaemia virus is B-cell lymphoma (Jacobs et al., 2002), whereas sporadic bovine leukosis is more likely to be linked with T cells (Step et al., 2001; Jacobs et al., 2002). In this case, immunostaining for CD3 was observed while immunolabelling of tumour cells for the CD79 antibody was not detected. Positivity with CD3 and negativity with CD20 and CD 79a indicated that tumour cells might originate from the immature T-lymphocytes, as documented in earlier studies (Kebriaei et al., 2003).

The proportion of proliferating cells can also be estimated by immunohistochemical assay of Ki-67 antigen expression, which has proven to be of effect in human oncology (Fournel-Fleury et al., 1997). Ki-67 is a highly conserved non-histone nuclear antigen protein involved in maintaining chromosomal stability during mitosis (Phillips et al., 2000). In dogs with highly malignant T-cell lymphoma, Ki-67 index ranged from 50% to 70% (Fournel-Fleury et al., 1997). Ki-67 index was 95% in the present case, which was in agreement with the high mitotic index.

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Correspondence:

Ghasem Farjanikish, DVM, PhD
Department of Pathobiology,
Faculty of Veterinary Medicine,
Lorestan University, Khorramabad, Iran
e-mail: farjanikish.gh@lu.ac.ir