

Concomitant occurrence of hepatocellular carcinoma and benign anthracotic lymphadenopathy in a German Shepherd dog

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ABSTRACT

A nine-year-old male German Shepherd dog was presented with anorexia, lethargy and progressive weight loss. Clinical examination revealed emaciation, depression and dyspnoea. Haematological analysis showed leucocytosis and neutrophilia. The dog died following unsuccessful treatment and was submitted for necropsy. Gross examination revealed multiple tumour nodules in all liver lobes, lungs and lymph nodes (LN). The mesenteric lymph node (MSLN) appeared whitish-pink, whereas the mediastinal lymph node (MDSLN) showed black pigmentation on a greyish-white background. Cytology of hepatic nodules revealed clusters of neoplastic hepatocytes. Histopathology showed solid sheets of neoplastic cells consistent with hepatocellular carcinoma (HCC) with compression and invasion of adjacent hepatic parenchyma. Metastatic neoplastic cells were observed in the lungs and LNs. Additionally, the MDSLN showed macrophages containing black pigments consistent with benign anthracotic lymphadenopathy (BAL). Immunoreactivity of arginase-1 showed marked expression in the neoplastic cells. Hep-Par-1 showed minimal expression in neoplastic cells and strong expression in the normal hepatocytes. Mild expression of glypican-3 and AFP were noticed in the neoplastic cells. Both the MSLN and MDSLN showed strong expression of CD3 and moderate expression of CD20 in the lymphoid cells. Gross, cytological, histopathological and immunohistochemical findings were consistent in the diagnosis of HCC coexist with BAL in a dog.

Key words: BAL, Dog, HCC, Histopathology, Immunohistochemistry

Liver cancer in canines is rare, but a significant tumour, accounting for 0.6-1.3% of all neoplasms and primarily affecting dogs over 10 years of age^{1,2}. Primary liver tumours include hepatocellular adenoma, hepatocellular carcinoma (HCC), cholangiocellular carcinoma and neuroendocrine carcinoma. Among these, HCC is the most prevalent cancer in both humans and animals^{1,3}. The other less common hepatic tumours are bile duct carcinoma, sarcoma, lymphoma, malignant histiocytosis and mast cell tumours³. HCC in dogs usually occurs in solitary or massive, nodular and diffuse forms and mostly metastasizes within the liver by local infiltration or to extra-hepatic organs like lungs and local LNs⁴.

Anthraxis refers to the deposition of carbon particles following repeated inhalation of biomass smoke, coal dust, cigarette smoke and environmental air pollutants^{5,6}. It predominantly affects the lung parenchyma and airways, may also extend to extra-pulmonary sites including MDSLN and axillary LN^{5,6}. Enlargement of the MDSLN is an important diagnostic finding that may arise from aetiologies like tuberculosis⁷, sarcoidosis⁶ and malignancies⁸. Among the various causes, accumulation of carbon particles within lymphoid tissues, resulting in anthracotic lymphadenopathy, is rarely studied in animals. Benign anthracotic lymphadenopathy (BAL) in humans may occur as a primary finding or in association with inflammatory conditions and malignancies^{9,10}, whereas comparable observations in veterinary practice are meager. Therefore, the present report describes a unique case of HCC associated with BAL in German Shepherd dog.

A nine-year-old male German Shepherd dog was presented to the small animal clinic for treatment with a history of anorexia, lethargy and weight loss.

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The dog weighed about 23.7 kg and appeared emaciated, dull and depressed. Clinical examination revealed difficulty in breathing and congested visible mucous membranes. Haematological parameters, including total erythrocyte count ($5.38 \times 10^6/\mu\text{L}$), haemoglobin (10.5 g/dL), packed cell volume (36.4%) and platelets ($352 \times 10^3/\mu\text{L}$) were within the normal limits. A significant elevation in total leucocyte count ($33.9 \times 10^3/\mu\text{L}$) and neutrophils (78%) with unaltered eosinophils (7%), basophils (0), lymphocytes (11%) and monocytes (4%) was noticed.

Serum biochemical values such as total protein (6.69 g/dL), albumin (2.50 g/dL), blood urea nitrogen (19.81 mg/dL), creatinine (1.44 mg/dL), calcium (7.44 mg/dL) and phosphorus (5.37 mg/dL) did not show any alterations. The dog was treated for five days, but died following unsuccessful treatment and was subsequently submitted for post mortem examination.

At necropsy, numerous nodules of varying sizes (3-24 mm) were noticed in all liver lobes (Fig. 1). The liver along with nodules weighed about 185 g. The cut surface of the nodules appeared grayish-white in colour. Cytology of hepatic nodules revealed a cluster of neoplastic hepatocytes with features of malignancy like anisocytosis, anisokaryosis and increased nuclear to cytoplasmic ratio (Fig. 2). Metastatic tumour nodules were also noticed in the lungs, MSLN and MDSL. The MSLN appeared hard and whitish-pink in colour, whereas, MDSL appeared

in shades of black against a greyish-white background. Representative tissue pieces from liver, lungs and lymph nodes were collected, fixed in 10% neutral buffered formalin and processed for histopathology.

Microscopically, the hepatic nodules showed solid sheets of neoplastic cells surrounded by fine fibrous stromal tissues. The neoplastic cells arranged in trabecular and acinar patterns were surrounded with collagenous stroma and angiogenesis (Fig. 3). The neoplastic cells were round to polygonal in shape with eosinophilic cytoplasm, vesiculated nuclei and prominent nucleoli. High mitotic activity (15-20 mitoses per 10 high-power fields) and moderate degrees of anisocytosis and anisokaryosis were noticed. Hepatic parenchyma around the tumour nodules were invaded by neoplastic cells. The invaded areas showed necrosis with infiltration of lymphocytes and a few plasma cells. The metastatic neoplastic cells observed in lungs (Fig. 4), MSLN (Fig. 5) and MDSL also revealed features similar to those noticed in hepatic neoplastic cells. In addition, MDSL revealed deposition of black pigments in the cytoplasm of macrophages consistent with benign anthracotic lymphadenopathy (BAL) (Fig. 6).

A few paraffin-embedded tissue sections were subjected to immunohistochemical (IHC) staining. The sections were incubated with primary antibodies (Table 1) followed by a secondary antibody using the PolyExcel HRP/DAB detection system (M/s

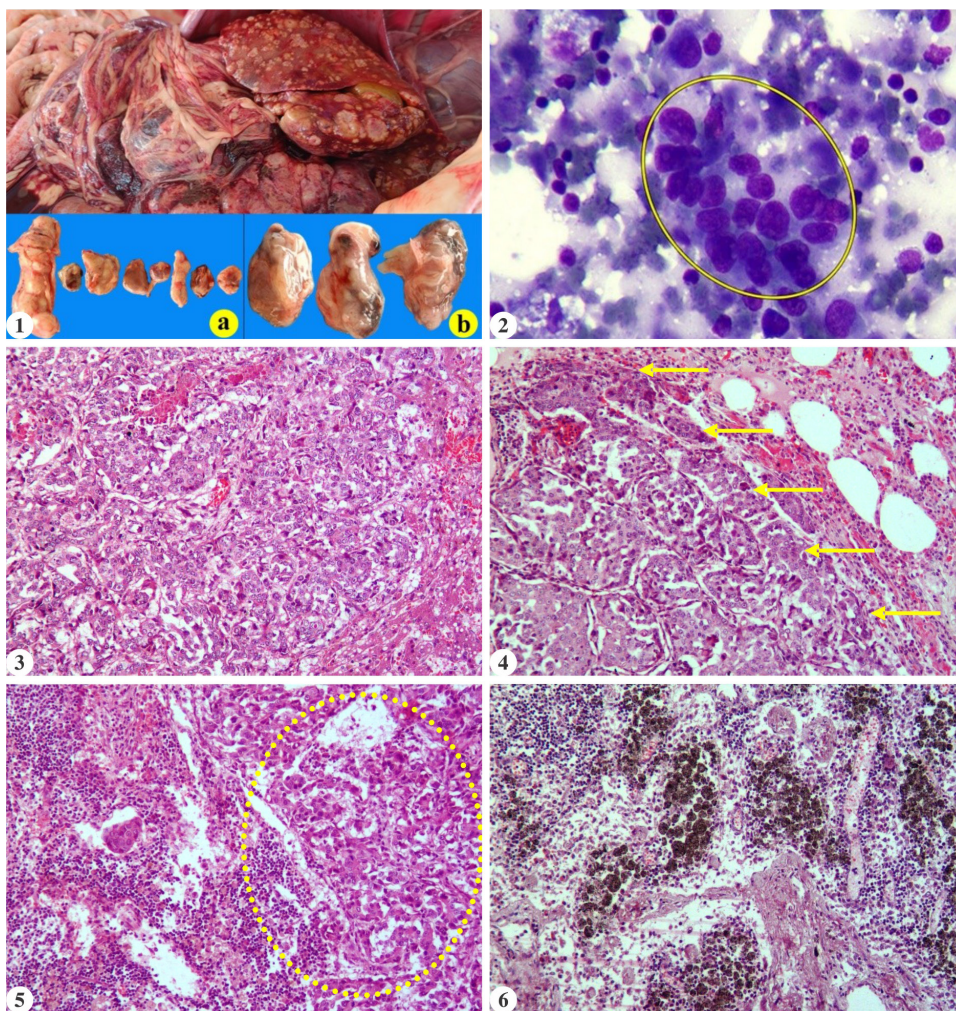


Fig. 1. German Shepherd dog: Liver showing numerous nodules of varying sizes in the surface of all lobes. a). Mesenteric lymph node appearing whitish-pink in colour. b). Mediastinal lymph node showing shades of black in greyish-white background; **Fig. 2.** Cytology of hepatic nodules showing a cluster of neoplastic cells with anisocytosis, anisokaryosis and increased nuclear to cytoplasmic ratios. Wright's-Giemsa x1000; **Fig. 3.** Hepatocellular carcinoma showing solid sheets of neoplastic cells arranged in trabecular and acinar patterns surrounded by collagenous stroma and angiogenesis. H&E x100; **Fig. 4.** Lung showing solid sheets of neoplastic cells occupying most of the alveoli (arrows). H&E x100; **Fig. 5.** Mesenteric lymph node showing the sheets of neoplastic cells (circle) replacing the lymphoid cells. H&E x100; **Fig. 6.** Mediastinal lymph node showing the deposition of carbon particles in the cytoplasm of macrophages consistent with anthracotic lymphadenopathy. H&E x100.

Table 1. The details of primary antibodies used for immunohistochemistry.

S. No.	Primary antibody	Host	Clone	Dilution	Location of expression
1.	Arginase-1	Mouse	Monoclonal(ARG1)	1:25	Cytoplasmic
2.	Hep-par-1	Mouse	Monoclonal(EP265)	1:50	Cytoplasmic
3.	Glypican-3	Mouse	Monoclonal(GPC3)	1:50	Cytoplasmic
4.	Alpha fetoprotein (AFP)	Rabbit	Monoclonal(EP209)	1:50	Cytoplasmic
5.	CD3	Rabbit	Polyclonal(PP160)	1:25	Nuclear, Membranous
6.	CD20	Mouse	Monoclonal(L26)	1:50	Nuclear, Membranous

PathnSitu Biotechnologies, Hyderabad, India). Slides were counter stained with Mayer’s haematoxylin. Immunoreactivity in tumour tissues showed marked cytoplasmic expression of arginase-1 in neoplastic cells and mild expression in normal

hepatic cells and stromal tissues (Fig. 7). Hep-Par-1 showed minimal cytoplasmic expression in neoplastic cells, strong expression in normal hepatocytes and mild expression in stromal tissues (Fig. 8). Glypican-3 revealed mild cytoplasmic expression in neoplastic cells, minimal expression in normal hepatic cells and moderate expression in stromal tissues (Fig. 9). AFP showed mild cytoplasmic expression in neoplastic cells and minimal expression in normal hepatic cells and stromal tissues (Fig. 10). The metastatic neoplastic cells in the lungs, MSLN and MDLNL exhibited IHC expression patterns comparable to those of the hepatic neoplastic cells for Arginase-1, Hep-Par-1, Glypican-3 and AFP.

Additionally, both MSLN and MDLNL exhibited strong nuclear and membranous immunoreactivity for CD3 in lymphoid cells (Fig. 11), indicating a predominance of T-lymphocytes, whereas CD20 expression was moderate with similar nuclear and membranous localization (Fig. 12), denoting a comparatively lower proportion of B-lymphocytes.

A rare case of HCC with BAL was diagnosed in male German Shepherd dog. The clinical signs recorded in this case could be attributed to

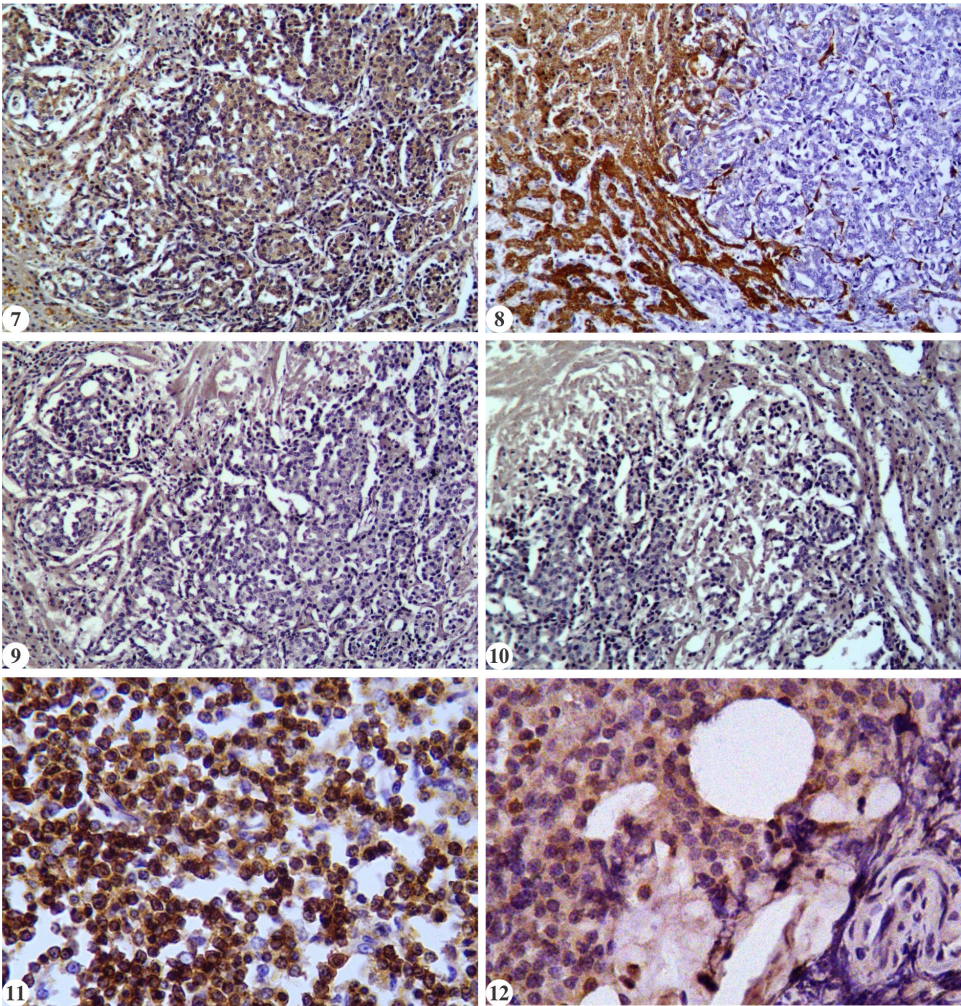


Fig. 7. Tumour section showing marked cytoplasmic expression to arginase-1 in the neoplastic cells. (IHC, Arginase-1 x100); **Fig. 8.** Tumour section showing minimal cytoplasmic expression in neoplastic cells and strong expression in normal hepatic cells to Hep-Par-1. (IHC, Hep-Par-1 x100); **Fig. 9.** Tumour section showing mild cytoplasmic expression to glypican-3 in the neoplastic cells. (IHC, Glypican-3 x100); **Fig. 10.** Tumour section showing mild cytoplasmic expression to AFP in the neoplastic cells. (IHC, AFP x100); **Fig. 11.** Mesenteric lymph node showing strong nuclear and membranous expression to CD3 in the lymphoid cells. (IHC, CD3 x400); **Fig. 12.** Mesenteric lymph node showing moderate nuclear and membranous expression to CD20 in the lymphoid cells. (IHC, CD20 x400).

paraneoplastic syndromes that might be the reason for death of the dog⁴. Blood parameters revealed leucocytosis and neutrophilia in this case, similar to the previous workers^{2,4}, who also recorded anaemia and thrombocytosis in dogs affected with hepatic tumours. Serum biochemical values of this dog did not show any alterations. However, increased levels of total bilirubin, total protein, alanine transaminase, aspartate transaminase, alkaline phosphatase and lactate dehydrogenase were reported in dogs with liver tumours^{2,4}.

Grossly, the entire surface of all liver lobes showed numerous nodules, similar to the observations of previous workers, who also noticed higher frequency of tumour nodules in the left lateral lobe^{4,11}. Cytological features of this case concur with the findings of earlier investigators¹². Histological examination of tumour nodules showed features of HCC that are in agreement with the findings of previous workers^{1,2,4}. HCC metastasis to lungs, MSLN and MDSLN concur with the findings of previous workers^{2,4,13} who reported frequent metastasis to peritoneum along with these organs. The metastatic MDSLN also revealed the presence of anthracotic lymphadenopathy in the present case. Earlier reports are available on mediastinal lymphadenopathy due to metastasis of HCC and anthracosis in humans^{5,6,9,10,14}, whereas similar reports are lacking in dogs.

Immunoreactivity of arginase-1 showed marked expression in neoplastic cells in this case, which is in agreement with the observations of previous researchers in humans and animals^{15,16,17}. Arginase-1 has been reported to exhibit 100% sensitivity in well-differentiated HCC in humans¹⁵, with immunoexpression characteristically observed in the cytoplasm, along with variable nuclear and membranous localization within neoplastic cells¹⁶. Hep-par-1 showed minimal expression in neoplastic cells and strong expression in normal hepatic cells, which is in agreement with the findings of previous workers^{1,15,16,17}. Hep-Par-1 has been documented to exhibit diminished cytoplasmic immunoreactivity along with relatively lower diagnostic sensitivity in well-differentiated HCC in humans^{15,16}. Mild expression of glypican-3 and AFP in this study concurs with the findings of earlier workers^{2,15,16}.

In the present study, both the MSLN and MDSLN demonstrated strong CD3 expression and moderate CD20 expression in lymphoid cells, which is in agreement with previous workers¹⁸. This immunophenotypic pattern suggests a predominance of T-cells with a comparatively lesser proportion of B-cells, indicative of active lymphoid cell turnover, which may be associated with conditions such as anthracosis, inflammation or malignancy.

The study results revealed that the primary tumour originated from liver which metastasized to lungs and

regional LNs. The MDSLN revealed mixed population of metastatic neoplastic cells and carbon particles laden macrophages. Considering the rarity of co-occurrence, the present case is reported to highlight the diagnostic challenges and clinical significance of concurrent HCC and BAL in dogs.

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