



Case Report

Chylous effusion in a dog related to malignant peritoneal mesothelioma: cytological diagnosis

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<p>Article history Received: 22 Feb, 2015 Revised: 21 Mar, 2015 Accepted: 28 Ma, 2015</p>	<p>Abstract A 12 years old female German Shepherd dog was presented to Veterinary Hospital with a history of vomiting, distended abdomen and the presence of fluid in the abdominal cavity. Abdominal fluid was pink/white in colour with opaque clarity and too lipemic that made difficult to estimate total protein. Value of triglyceride in the fluid was 438 mg/dl. Numerous clusters of cohesive cells in variable size amid many leukocytes and blood were observed in the smear slides which were made from the sediment of the fluid. Occasionally, cohesive cells were formed as an acinar-like structure around eosinophilic materials (collagen core). Cells were ovoid to polygonal with a round to ovoid nucleus, coarse chromatin, one to four variably sized nucleoli and a moderate to abundant volume of basophilic to deeply basophilic cytoplasm. A variable number of clear, round cytoplasmic vacuoles were observed in some cells. Moderate anisocytosis and anisokarosis, bi- and multi nucleation and prominent multiple nucleoli were observed in some cells. Some cells showed window interdigitating microvilli. These findings are consistent with a malignant mesothelioma.</p> <p>Keywords: Chylous effusion; mesothelioma; cytological diagnosis; dog</p>
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Introduction

Chylous ascites (CA) is an uncommon form of ascites, due to the accumulation of a milky or creamy fluid rich in triglycerides into the peritoneal cavity. Any obstruction or damage to the lymphatic channels results in chylous ascites. Lymphomas, cirrhosis, metastatic malignancies, abdominal surgeries, infections like tuberculosis are commonly causes of CA (Cardenas and Chopra, 2002; Talluri et al., 2011). The diagnosis of chylous effusion is usually based on gross examination, biochemical analysis (lipid studies in blood serum and

effusion; triglyceride and cholesterol levels, and lipoprotein electrophoresis) and cytological examination (Borku et al., 2006).

Mesothelioma is a rare malignant neoplasm arising from mesothelial cells that line the peritoneal and pleural surfaces. Spontaneous mesothelioma has been reported in humans as well as in many species of animals, including dogs, cattle, goats, horses, rats, and hamsters. This tumour is associated with exposure to asbestos in humans and potentially in dogs (Reggeti et al., 2005; Head et al., 2007). Like in humans, the pleura is the main site of mesotheliomas development after the

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pericardial and the peritoneal cavity in dogs (Kapakin et al., 2012).

Mesothelioma is an uncommon tumour in dogs and its diagnosis can be a challenge for veterinarians. Recent advances in therapy for patients with mesothelioma may result in an improved outcome if they are applied to stage I disease. Cytological evaluation of body cavity effusions is a fast and reliable diagnostic tool for establishing an etiologic diagnosis. Cytology is highly specific for malignant neoplasia, and also inflammatory and infectious processes can be recognized early in the diagnostic workup (Hirschberger et al., 1999). Nowadays, veterinary practitioners have increasingly sought cytology as an auxiliary and diagnostic tool for neoplasms (Martins et al., 2011).

In this report, we describe a rare case of canine peritoneal mesothelioma with chylous ascites presentation.

Case history

A 12 years old female German Shepherd dog was presented to Veterinary Hospital of Razi University with a history of vomiting and distended abdomen for one week. Blood specimens were collected from femoral artery and brachial vein for haematology. Also, ascites fluid was withdrawn with the syringe from abdominal cavity. Ascitic fluid was centrifuged at 3000 rpm for 5 minutes, sediment smears were prepared and then dry fixed for May-Giemsa staining. The morphological features of ascitic cytology were examined.

This study was performed under the approval of the State Committee on Animal Ethics, Razi University, Kermanshah, Iran. Also, the recommendations of European Council Directive (86/609/EC) of November 24, 1986, regarding the protection of animals used for experimental purposes were considered.

Results

Abdominal fluid was pink/white in colour with opaque clarity and too lipemic that made difficult to estimate total protein. Value of triglyceride in the fluid was 438 mg/dl. 2300/μl leukocyte and 60000/μl RBCs were estimated in fluid analysis.

Numerous clusters of cohesive cells (like mesothelial cells) amid many leukocytes and blood were observed in the smear slides which were made from the sediment of the fluid. Leukocytes were including 11% neutrophils, 67% small and intermediate lymphocytes, 17% macrophages, 4% eosinophils and 1% mast cells.

Cohesive cells were present in variable size clusters (cell balls) (Fig. 1) and occasionally were formed as an acinar-like structure around eosinophilic materials (collagen core) (Fig. 2). Cells were ovoid to polygonal with a round to ovoid nucleus, coarse chromatin, one to four variably sized nucleoli and a moderate to

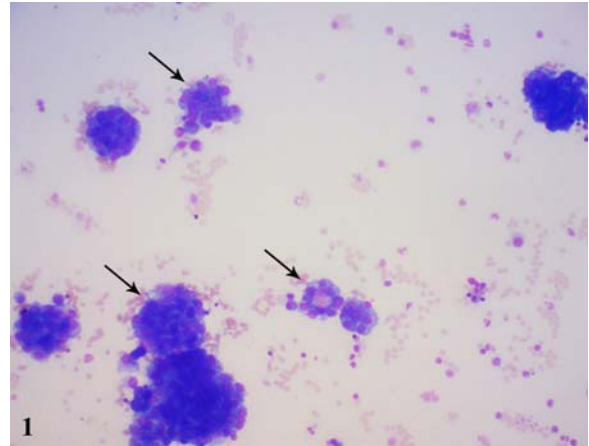


Fig 1: Cohesive cells in variable size clusters (cell balls) (arrows) (H&E; X750)

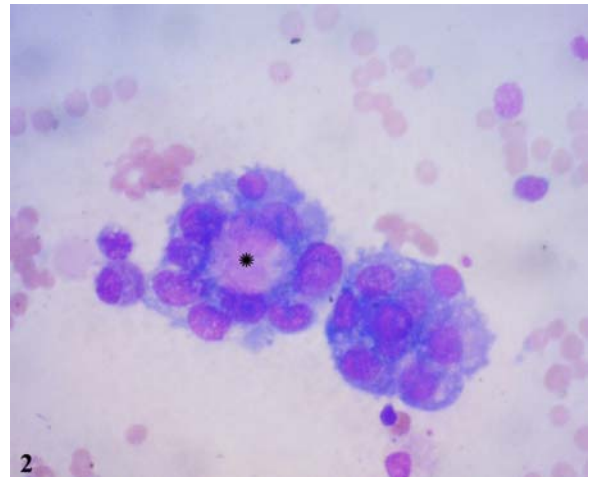


Fig 2: An acinar-like structure around eosinophilic cells (collagen core) (star) (H&E; X3000)

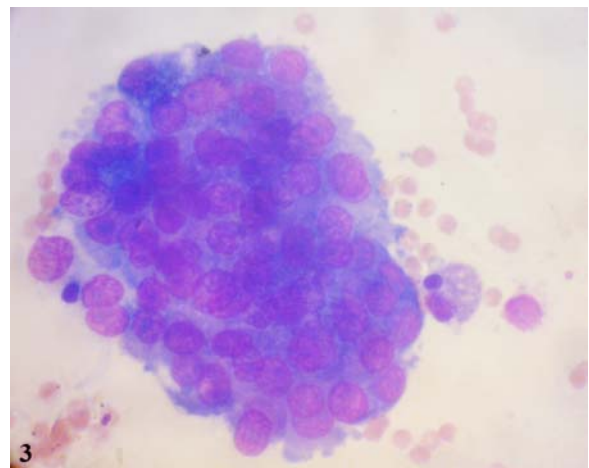


Fig 3: Ovoid to polygonal cells with a round nucleus, coarse chromatin, one to four variably sized nucleoli and basophilic cytoplasm (H&E; X3000)

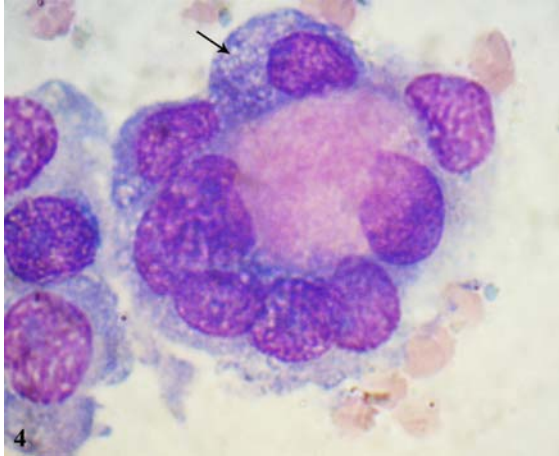


Fig 4: A variable number of clear, round cytoplasmic vacuoles were observed in some cells (arrow) (H&E; X3000)

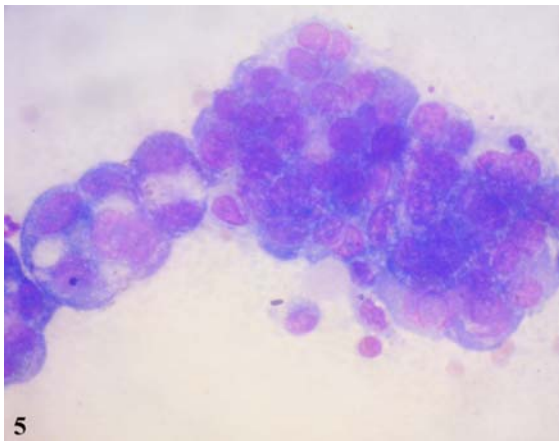


Fig 5: Smooth to more ragged and ruffled cytoplasmic borders with a window interdigitating microvilli (H&E; X3000)

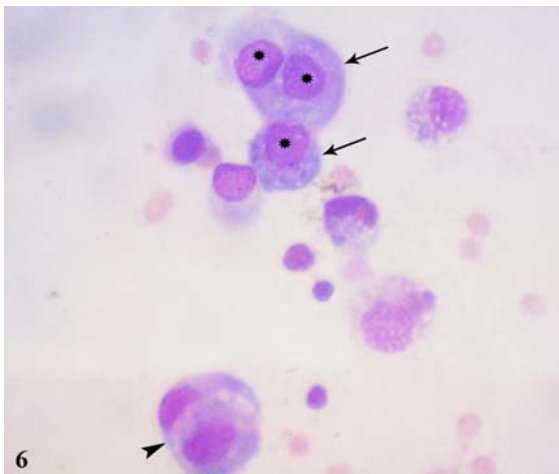


Fig 6: Anisocytosis (arrows), anisokarosis (stars) and bi- and multi nucleation (arrow head) (H&E; X750)

abundant volume of basophilic to deeply basophilic cytoplasm (Fig. 3).

A variable number of clear, round cytoplasmic vacuoles were observed in some cells (Fig. 4). Cytoplasmic borders varied from smooth to more ragged and ruffled (Fig. 5). Some cells displayed moderate anisocytosis and anisokarosis, bi- and multi nucleation (Fig. 6) and prominent multiple nucleoli (Fig. 4). Mesothelial cells showed window interdigitating microvilli (Fig. 5). No infectious agents were identified in this case.

Discussion

In a normal animal, the peritoneal space contains small amount of fluid (less than 1mg/kg bodyweight), which moistens the opposing surface and serves to reduce friction between the abdominal organs. The normal peritoneal fluid is clear to slightly yellow with specific gravity less than 1.016 containing about 2g/dl protein (mainly albumin) and WBC (2000- 2500/ml, 50% macrophages, eosinophils, mast cells, and few polymorph neutrophils) without fibrinogen (does not clots on standing) and fibronectin (Harbison and Godleski, 1983).

Chylous ascites is an uncommon form of ascites, due to accumulation of chylous fluid (lymph and chylomicron) in the pleural or peritoneal cavity. The triglyceride levels in ascitic fluid are very important in defining chylous ascites. Triglyceride values are typically above 200 mg/dl, although some authors use a cut off value of 110 mg/dl. The WBC count is elevated in all inflammatory condition and malignant ascites. Malignant cells may be seen in ascites due to malignancy (Cardenas and Chopra, 2002; Borku et al., 2006).

Mesotheliomas are the most frequent primary malignant tumours of serosal cavities with a poor prognosis. Recent advances in therapy for patients with mesothelioma may result in an improved outcome if they are applied to stage I disease. Hence, a definitive and early diagnosis on effusion samples is very important. The cytological characteristics of malignant mesothelioma have been well described (Pomjanski et al., 2008; Kinoshita et al., 2013).

The cytological diagnosis of diffuse malignant mesothelioma of the epithelial type is a two-step process. First, the cells in the fluid must be recognized as being of mesothelial lineage, and second, they must be recognized as neoplastic cells. The first step is usually not difficult because mesothelioma cells of the epithelial type retain the morphologic features of benign mesothelial cells. In smears, the morphologic features of non-neoplastic mesothelial cells, recapitulated in their neoplastic form, are those of shape; cytoplasmic staining reaction; nuclear position, size, and shape; nucleolar prominence; and types of intercellular articulation, as well as the formation of small or large mosaic sheets of cells and irregularly

shaped clusters with knobby contours. Malignant mesothelioma cells should show these morphologic characteristics to be recognized as mesothelial, but they should also be different enough from the normal or hypertrophic mesothelial cells to appear neoplastic. Usually it is not difficult to perceive their mesothelial lineage, but it may be difficult to conclude that they are malignant because the standard nuclear feature of malignancy may be poorly represented (Bibbo and Wilbur, 2008).

In contrast to the picture of mesothelial hypertrophy and hyperplasia, the cells of mesotheliomas tend to be more profuse, often extremely so, and to form large cohesive clusters accompanied by many smaller clusters and isolated cells; all exhibiting distinct morphologic characteristics of benign mesothelial cells. Collectively, the individual mesothelioma cells are larger than benign mesothelial cells, although their nucleocytoplasmic ratio is often lower. Their nuclei also tend to be smoothly round or oval, in contrast to the more angulated nuclear outline typical of adenocarcinoma. Nucleoli are prominent, and the nuclear chromatin is slightly increased in density and more coarsely granular than in benign mesothelial cells (Bibbo and Wilbur, 2008). Patel et al (2007) explained cytomorphologic features of primary peritoneal mesothelioma in effusions similar to our findings.

Therapeutic options for mesothelioma include surgical resection, radiotherapy, and chemotherapy. Because of the distribution of the lesions and tissue toxicity, chemotherapy is usually the treatment of choice, but no single agent or combination of drugs has yielded satisfactory results in patients (Reggeti et al., 2005).

In summary, this is a rare case of chylous ascites with peritoneal mesothelioma. Cytological assessment of fluid is a primary investigation in determining the cause of an ascitic effusion. Cytological evaluation is a useful diagnostic tool for malignant mesothelioma.

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