Canine abdominal fluid with neoplastic mast cells

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Signalment and History

A male, 9 year-old Rottweiler had been diagnosed 2 months previously with an intestinal tumour. The location was not precisely known; the ultrasound examination suggested the ileocaecal valve. The tumour was 4x5 cm large and no other abnormalities were detected with abdominal ultrasound.
The veterinarian performed a laparotomy, resected the tumour and performed an enterectomy to achieve tumour-free margins. No other macroscopic abnormalities of abdominal organs were detected and the mesenteric lymph nodes were not enlarged. The resected tumour was submitted for histopathology. No cytology, haematology or other diagnostic investigations were performed at this time.
The histopathological diagnosis was a mast cell tumour grade III. The patient recovered uneventfully after surgery and no additional therapy was initiated.
Over the subsequent 2 months the dog became lethargic and inappetent. An abdominal ultrasound revealed free abdominal fluid.

Material and methods

The referring veterinarian performed an abdominocentesis and submitted one plain tube (without anticoagulant) with approximately 6 ml reddish, slightly turbid fluid. The TNCC was 24,210/µl, measured on the Advia 2120i (Siemens Diagnostics) with software setting for dogs. The total protein concentration was 26.6 g/l, measured by the biuret method on a Dimension RxL analyser (Siemens Diagnostics). Other biochemical tests performed where unremarkable.
Four slides were prepared for cytological examination – two from the non-concentrated sample and two from the cell pellet obtained after centrifuging the fluid for 5 min at 1200 G.
Analysis of the raw data was performed using the public-domain cytometric software WinMDI 2.8 (J. Trotter 1993) after export by Advia's Playback software.

Questions:

1. How would you classify the abdominal fluid according to the chemistry findings and the TNCC?
2. How would you interpret the cytograms: which two cell populations in the perox cytogram are well represented on the photomicrographs?
3. Can you identify the cell population shown on the two photomicrographs?

Answers

1) The ascitic fluid is classified as an exudate

2) The peroxidase cytogram (A) shows a large population of cells which fall into the area where canine eosinophils are found in haematological specimens (yellow).
On the left (dark blue, light blue and green) is a population of cells that shows a low to absent peroxidase activity, and a size ranging from that of a lymphocyte to a very large cell. This population occupies the regions where lymphocytes, monocytes and large unstained cells (LUCs) are gated in haematological specimens.
A second minor population of cells (dark blue) can be seen extending up from the bottom of the lymphocyte gating area and likely represents small lymphocytes.
The pink dots are consistent with a moderate number of neutrophils. Beneath that is the gate with tissue cells and possibly lysed cells (green and light blue).

The baso/lobularity cytogram (B) shows two populations in the lower region of the cytogram with mononuclear cells on the left and polymorphonuclear cells (PMN) extending towards the right. A second population with a predominance of mononuclear to slightly PMN extends up into the lysis-resistant portion of the cytogram.

The eosinophils seen on the perox cytogram can be clearly identified by cytology. The large population described above is represented by the neoplastic cells on the Romanowsky-stained smears.

3) The smear shows a mixed population of cells, with a predominance of round cells and eosinophils in a slightly proteinaceous background with moderate numbers of red blood cells. Moderate numbers of neutrophils, vacuolated macrophages and low numbers of small lymphocytes are also present.
The round cells display a marked anisocytosis and anisokaryosis. They have a moderately to deeply basophilic cytoplasm, occasionally with small vacuoles. Nuclei are round to multilobulated with coarse chromatin and multiple prominent, irregular nucleoli. Very few cytoplasmic granules were detected in the Romanovsky stained smears.
In addition, some unfixed smears were stained with toluidine blue stain. A small to moderate number of magenta-coloured granules were seen in the cytoplasm of a small fraction of the round cell population. A final diagnosis of an exudative effusion containing neoplastic mast cells with an accompanying eosinophilic inflammation was made.

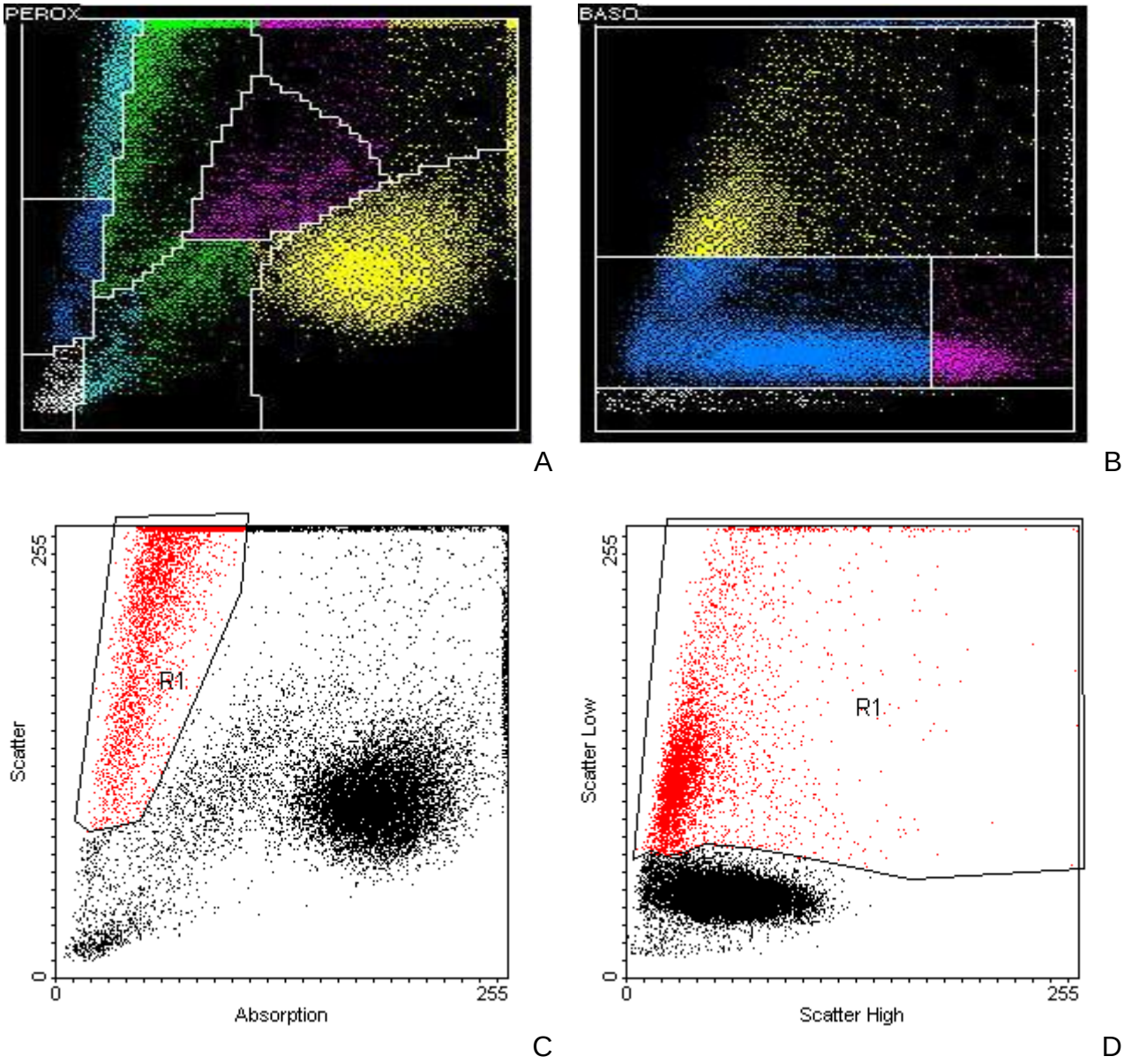

Fig. 1: (A) shows the Perox channel cytogram, (B) shows the Baso channel cytogram. The panels (C) and (D) display the corresponding cytograms with manual gating (R1) obtained by raw data analysis. Note that panel (D) is horizontally compressed compared to the original cytogram.

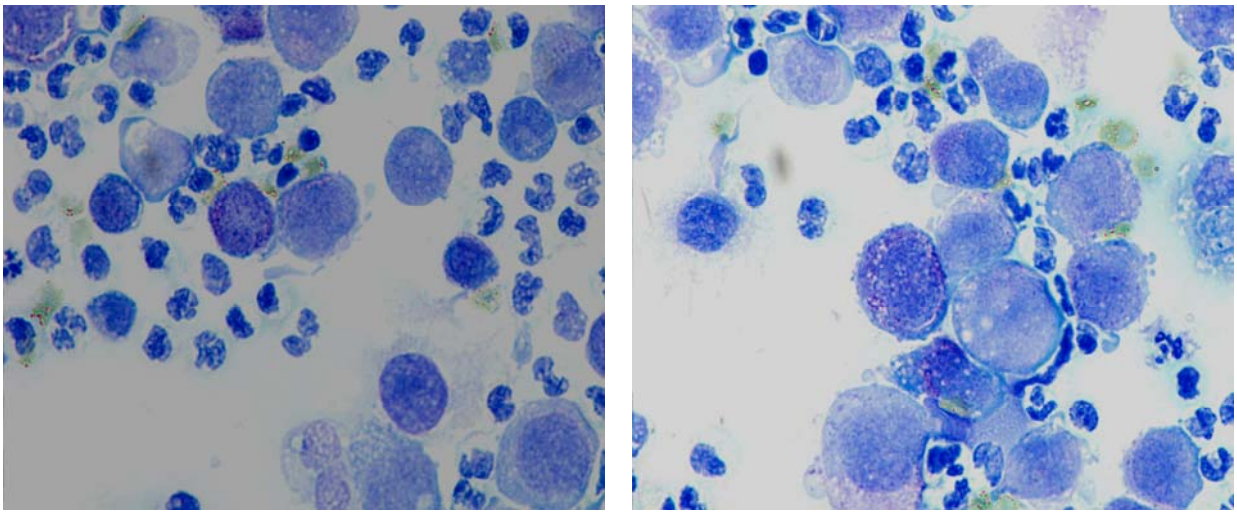

Fig. 2: Photomicrographs of a smear of the ascitic fluid, toluidine blue stain, left 600x, right 1000x.

Outcome

The peroxidase channel showed two major cell populations. One population clearly consisted of eosinophils. The second cell population lay in the LUC and monocyte area, mixed in size from lymphocyte size to very large, blast cell size. The Baso-channel also showed two major populations: one consisting of cells with non-lobulated nuclei which extended into the polymorphonuclear lysis-resistant region. The second one lay lower down and extended from the mononuclear region to the PMN region. This population was considered to be consistent with the eosinophils and neutrophils.

A population of large round cells and high numbers of eosinophils were noted on the Romanovsky-stained smear.

Based on the morphologic characteristics of the round cell population and paucity of metachromatic granulation in the round cells by modified Romanowsky stain, the presence of mesothelial cells could not be excluded. However, results of the toluidine blue staining confirmed the suspicion of a mast cell population.

Manual gating by the WinMDI software showed that identical proportions of cells, presumably representing the mast cells, could be identified in both channels (22.4% in the Perox channel and 22.7% in the Baso-channel respectively).

The final diagnosis of the ascitic fluid was an exudate with neoplastic mast cells and eosinophils accompanied by a minor mixed inflammatory reaction. The dog was euthanized after the diagnosis of the mastocytosis in the ascites.

Discussion

Tumours of primary visceral location in dogs are rare – in the thoracic cavity as well in the abdominal cavity (1). In a survey of insured dogs in the UK an incidence rate of 210/100,000 dogs was reported for alimentary tumours (2). The most common primary intestinal tumours in dogs were lymphomas (3). In one review from 1977 from 10,270 canine necropsies, 55 neoplasms of the stomach and 64 of the small and large intestines were diagnosed. There was a predominance of adenocarcinomas, no mast cell tumours (MCT) were reported in this study (4).

There are only a few reports of intestinal MCT in dogs; these tumours appear to be more common in cats (2). In a study from 118 dogs diagnosed with mast cell tumours, only 10 met the criteria for visceral mast cell tumours (2). There was a

higher incidence observed in small breed dogs, especially Maltese. The prognosis is extremely guarded as metastasis can occur early in disease and tumour-free surgical margins are not easy to achieve (4). To the best of our knowledge only one author has described the presence of mast cells in abdominal fluid (5).
Criteria to distinguish primary visceral tumour from metastasis from another primary mast cell tumour are described in one study. The diagnosis of primary visceral MCT was confirmed by histologic, cytologic and blood smear examination, with no evidence of past or present cutaneus MCT (1).
The mast cells found in the visceral tumours differed cytochemically as well histochemically from the skin MCT (1, 3). In addition, the characteristic granulation of mast cells in those tumours was highly decreased compared to the mast cells commonly found in skin tumours (4).
The reason for this is assumed to be the microenvironment. All mast cells share a common bone marrow precursor. Those mast cells that subsequently migrate to the skin are called cutaneous mast cells and the tumor derived from these cells is called a cutaneous mast cell tumour (CMCT). The mast cells which migrate to the intestines are called mucosal mast cells and can give rise to mucosal mast cell tumours (MMCT, 3).
The cytograms of the body fluids performed on the Advia may be helpful in evaluation of the cell type in the ascitic fluid (6). It can provide clues to cell morphology such as size, peroxidase activity and mononuclear or polymorphonuclear cell type, but cannot replace the microscopic examination of the body fluid.
The raw data analysis offered the advantage of manual gating in comparison to the partly arbitrary gating performed by the Advia.
Based on the history of the previous diagnosis of a grade III intestinal, the authors conclude that the observed ascitic mastocytosis was most likely a consequence of the exfoliation of cells from a metastasis from the primary intestinal mastocytoma.

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