CASE REPORT

Idiopathic myelofibrosis accompanied by peritoneal extramedullary hematopoiesis presenting as refractory ascites in a dog

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Abstract:

A 2.5-year-old spayed female American Pit Bull Terrier dog presented with a primary complaint of chronic refractory ascites. The dog's CBC displayed a moderate to severe macrocytic, hypochromic, non-regenerative anemia and a moderate leukopenia as result of a moderate neutropenia and monocytopenia. Microscopic examination of the blood smear showed marked anisocytosis, mild polychromasia, mild acanthocytosis and ovalocytosis, moderate schistocytosis and poikilocytosis, and 4 metarubricytes/100 WBC. Abdominal ultrasonography revealed a homogenous, mild to moderately hyperechoic appearing liver as well as marked amount of speckled anechoic to slightly hypoechoic peritoneal fluid. Cytology of the ascitic fluid demonstrated a sterile transudate, with evidence of a chronic inflammatory reaction as well as erythroid and myeloid precursor cells, and a few megakaryocytes with occasional micromegakaryocytes. Histologic sections of bone marrow, spleen and liver were examined, using routine H&E stains, as well as a variety of immunohistochemistry- and other special stains. Histopathology of the bone marrow and spleen revealed varying degrees of

fibrosis, erythroid and myeloid hyperplasia, as well as multiple small hyperplastic clusters of megakaryocytes. The megakaryocytes displayed many features of atypia such as increased cytoplasmic basophilia and occasional abnormal chromatin clumping with mitoses. Histopathologic examination of the liver presented evidence of mild extramedullary hematopoiesis. This case represents the first report of canine idiopathic myelofibrosis associated with peritoneal extramedullary hematopoiesis, resulting in refractory ascites. Although idiopathic myelofibrosis is a relatively rare condition in dogs, this case demonstrates that ascites caused by peritoneal implants of haematopoietic tissue may be the initial manifestation of myelofibrosis.

Key Words

Agnogenic, myeloid metaplasia, myelofibrosis

Case Presentation

A 2.5-year-old, spayed female American Pit Bull Terrier dog was referred to the Onderstepoort Veterinary Academic Hospital, University of Pretoria, South Africa with a primary complaint of chronic ascites. Therapeutic paracentesis was performed by the referring veterinarian, but the ascites re-occurred after 14 days. Physical examination revealed poor body condition (body condition score of 2/5), pallor of the mucous membranes, delayed capillary refill time, severe abdominal distension, and a fluid-wave upon abdominal ballottement. The rest of the physical examination was within normal limits. An initial diagnostic work up included fecal flotation, urinalysis and a CBC (Advia 2120, Siemens, Munich, Germany). The fecal flotation using Egg Flotation Fluid (Kyron Laboratories, Benrose, South Africa) was positive for *Ancylostoma* spp. ova. Urinalysis of a sample obtained through cystocentesis revealed adequately concentrated urine (SG >1.035) and urine dipstick analysis (Combur^{9} Test kit, Cobas, Roche, Basel, Switzerland), revealed a pH of 6 and a 4+ positive reaction on the heme-pad. The dog's CBC revealed a moderate to severe macrocytic, hypochromic, non-regenerative anemia and a moderate leukopenia as a result of a moderate neutropenia and monocytopenia (Table 1). Microscopic examination of the blood smear showed marked anisocytosis,

mild acanthocytosis and ovalocytosis, moderate schistocytosis and poikilocytosis and 4 metarubricytes/100 WBC (Figure 1).

Table 1. Presenting results of a complete blood count and serum biochemistry for a 2.5-year-old, spayed female American Pit Bull Terrier

	Result	Units	Reference intervals
HGB	50	g/L	120-180
RBC	1.83	x 10^{12}/L	5.5-8.5
HCT	0.18	L/L	0.37-0.55
MCV	97.5	fL	60-77
MCH	27.1	pg	
MCHC	27.8	g/dL	32-36
RDW	22.7	%	
WBC	2.67	x10^9/L	6-15
Segmented Neutrophil	1.12	x10^9/L	3-11.5
Band Neutrophil	0.05	x10^9/L	0-0.5
Lymphocytes	1.01	x10^9/L	1-4.8
Monocyte	0.05	x10^9/L	0.15-1.35
Eosinophil	0.43	x10^9/L	0.1-1.25
Basophil	0.00	x10^9/L	0-0.1
Platelet Count	275	x10^9/L	200-500
NRBC/100WBC	4		0-9
Reticulocyte Percentage	2.3	%	
Absolute Reticulocyte Count	42.0	x10^9/L	
Albumin	20.3	g/L	28-41
ALP	24	U/L	20-165
ALT	13	U/L	9-73
Urea Nitrogen	7.9	mmol/L	2.3-8.9
Creatinine	79	µmol/L	59-109

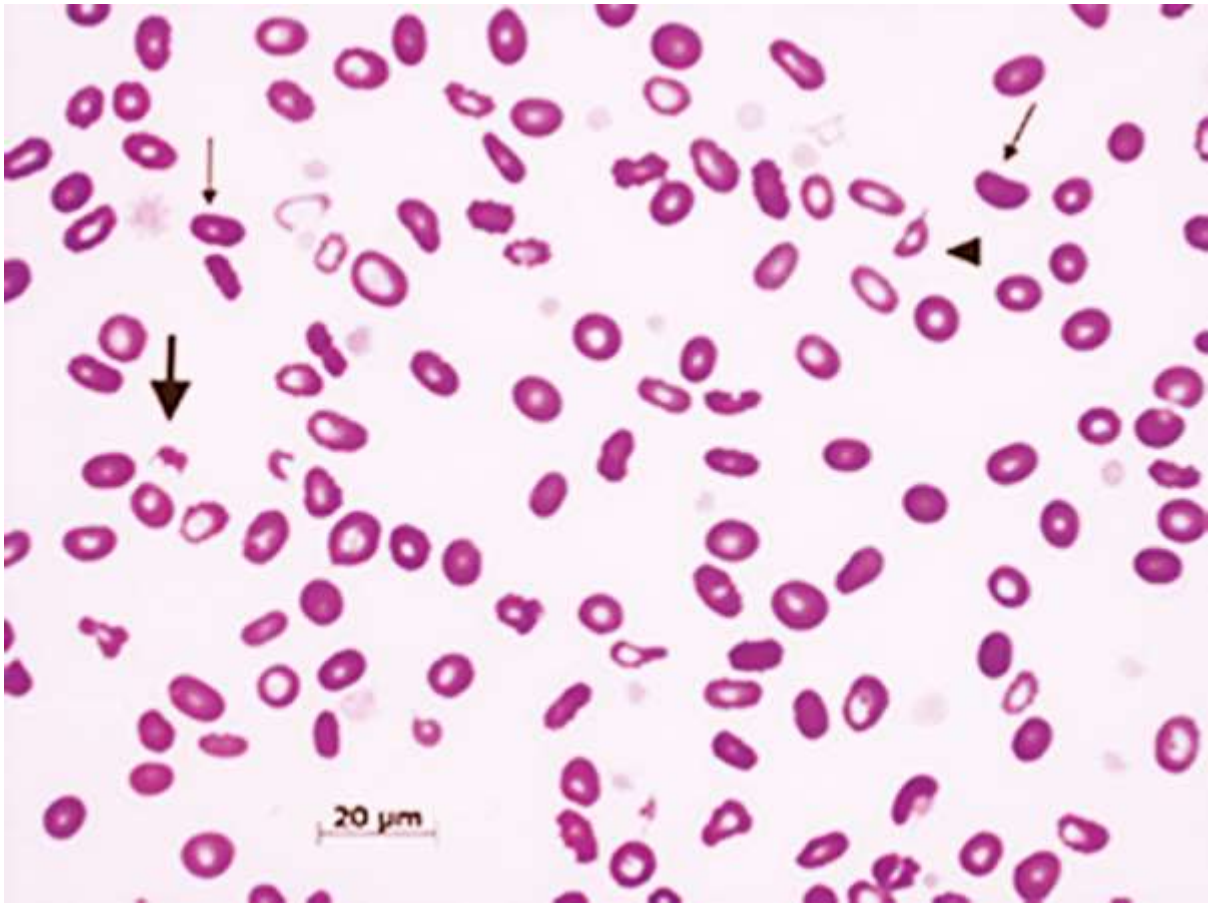

Figure 1. Blood smear of a 2.5-year-old spayed female American Pitt Bull Terrier dog showing marked anisocytosis, mild acanthocytosis *(arrow head)*, mild ovalocytosis *(thin arrow)*, moderate schistocytosis (*thick arrow)*, moderate poikilocytosis. Diff-Quik.

Further diagnostics included serum biochemistry assays (Cobas Integra 400, Roche, Basel, Switzerland), serology, an abdominal ultrasonographic examination, and a bone marrow aspirate. Mild hypoalbuminemia was the only serum biochemistry abnormality detected (Table 1). IgM and IgG titers for *Ehrlichia canis* were both negative, using the indirect fluorescent-antibody (IFA) technique. Abdominal ultrasonography revealed a homogenous, mild to moderately hyperechoic appearing liver. A marked amount of speckled anechoic to slightly hypoechoic peritoneal fluid was present.

The peritoneal fluid was collected via ultrasound-guided abdominocentesis and submitted for cytologic analysis. The fluid appeared slightly turbid pre-centrifugation and clear afterwards. The total nucleated cell count was 1270 cells/µL, the protein concentration was <25 g/L, and the specific

gravity was 1.016 as determined by refractometry. Cytological analysis of the sediment preparation revealed mild hemodilution and the nucleated cells consisted of 40% intact neutrophils, 18% mature lymphocytes, 16% active macrophages, some containing phagocytosed cellular debri, 14% eosinophils, 9% erythroid precursors, 1% myeloid precursors, 2% mast cells and the occasional Mott cell (Figures 2A, B). Activated mesothelial cells were present. Erythroid precursors present ranged from the prorubricyte stage to the metarubricyte stage, some undergoing mitosis. The myeloid precursors noted included myeloblasts, promyelocytes, and metamyelocytes. A small number of megakaryocytes were also present of which a few were micromegakaryocytes (dwarf) (Figure 2C). The cytological interpretation was a sterile transudate with evidence of a chronic inflammatory reaction as well as extramedullary hematopoiesis (EMH).

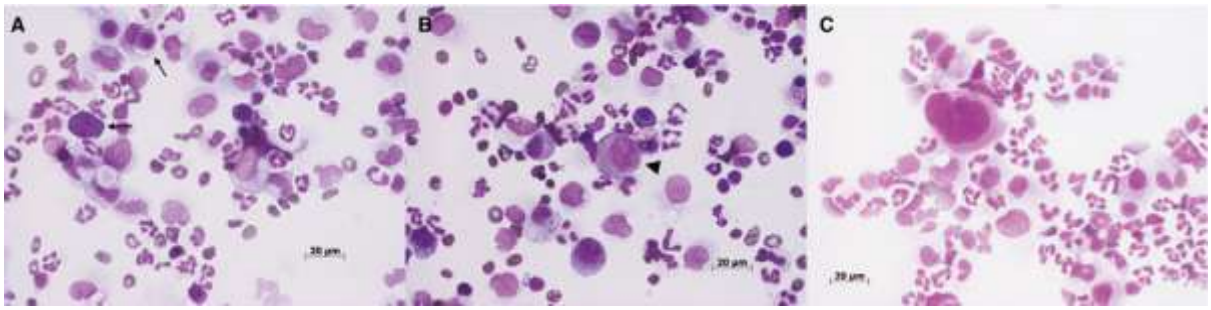

Figure 2. Cytologic sediment preparation of the peritoneal fluid of a 2.5-year-old spayed female American Pitt Bull Terrier dog. (**A**) Several erythroid precursors *(arrows)*. Diff-Quik. (**B**) Several myeloid precursors *(arrow head)*. Diff-Quik. (**C**) Few micromegakaryocytes (dwarfs). Diff-Quik.

The following day a bone marrow aspirate was obtained from the sternum and smears were prepared for cytological evaluation. The material consisted mostly of mature erythrocytes, occasional variable sized clusters of lipocytes and a few aggregates of mesenchymal type cells. Due to inadequate sample cellularity it was concluded that the submitted material was either indicative of bone marrow hypoplasia or not an adequate representation.

Due to poor quality of life and financial constraints that prohibited any further diagnostic investigation or therapeutic intervention, the dog was humanely euthanized later that same day and a necropsy was performed. The most significant macroscopic lesions included approximately three

litres of straw-coloured watery fluid in the abdominal cavity (severe ascites), approximately 250 mL of serosanguineous fluid in the pleural cavity (mild hydrothorax), and approximately 100 mL of serosanguineous fluid in the pericardial sac (moderate hydropericardium). No macroscopic abnormalities of the peritoneum were detected. There was also widespread subcutaneous fluid accumulation in the carcass (moderate anasarca). The spleen was macroscopically moderately enlarged due to a combination of white and red pulp hyperplasia. The bone marrow in the femur shaft was diffusely pink-red in color and appeared firmer than normal. The dog also had an incidental focal *Spirocerca lupi*-associated caudal esophageal nodule (with embedded adult worms) and there was evidence of moderate *Dipylidium caninum* and *Ancylostoma caninum* infections (a few worms were visible within the lumen of the small intestine and typical eggs were identified on fecal flotation).

Histologic sections of bone marrow, spleen, and liver were examined, using routine H&E stains, as well as a variety of special stains such as Masson's Trichrome and Gomori's reticulin for collagen fibres and immunohistochemistry stains (using the immunoperoxidase labelling technique) to detect CD3 (T-lymphocytes), CD18 (canine leukocytes, especially monocyte-macrophages), CD20 (B-lymphocytes and plasma cells), myeloid/histiocyte antigen (granulocytes, monocytes and tissue macrophages), and smooth muscle actin (vascular smooth muscle) (Table 2). All special stains were performed according to standard staining protocols in the Histopathology Laboratory, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria. Immunohistochemistry staining was performed manually following validated protocols.[1] The standard immunoperoxidase procedure included deparaffinization and hydration of slides, incubation with 3 % hydrogen peroxide for 15 minutes to quench endogenous peroxidase activity, enzymatic digestion or heat-induced epitope retrieval (Table 2) followed by non-specific immunoglobulin binding[a], incubation with the primary antibody, followed by application of the BioGenex Super Sensitive Polymer-HRP Detection System (QD420-YIKE, BioGenex, Fremont, CA, USA) in all cases (Table 2). Positive tissue controls included validated tissue sections with known reactivity for the tested antigen, and for negative reagent controls, buffer[b] was substituted for the primary antibody in each case. Negative-dog tissue

controls (normal spleen and bone marrow) were obtained from a 5-year-old healthy male Collie that died after a traumatic incident.

Table 2. Antibody reagents and antigen retrieval methods used in immunohistochemistry

Antibody Source (Product code)	Antibody Clone	Animal Source	Dilution	Incubation	Pretreatment		
					None	Enzyme*	HIER[8]
Dako, Denmark (A0452)	CD3	Rabbit	1:200	60 min			Citrate, pH 6.0
Dr. P. Moore, UC Davies, CA, USA	CD18 (CA16.3C10)	Mouse	1:100	90 min		X	
Dako, Denmark (M0755)	CD20cy (L26)	Rabbit	1:1200	60 min			Citrate, pH 6.0
Dako, Denmark (M0747)	Myeloid/Histiocyte antigen (MAC387)	Mouse	1:400	60 min			EDTA, pH 9.0
Dako, Denmark (M0851)	Smooth muscle actin (1A4)	Mouse	1:200	60 min	X		

* Protease XIV (code no. P5147-5G from Sigma Chemical Co, St. Louis, Missouri, USA)

[8] Heat-induced epitope retrieval (microwave heat in different buffer solutions at 96 °C)

Histopathology of the bone marrow revealed marked hypercellularity (Figure 3A), moderate fibrosis of the intertrabecular spaces, as seen with Masson's Trichrome for collagen (Figure 3B) and Gomori's reticulin (Figure 3C), and prominent angiogenesis, compared to normal bone marrow, using an antibody for vascular smooth muscle actin (Figure 3D). There was hyperplasia of the range of erythroid and myeloid precursors. The myeloid/histiocyte antigen marker (MAC387) that labels myeloid and monocytic precursors, as well as tissue macrophages revealed the myeloid/monocytic:

erythroid ratio to be in the region of 1-2:1 (Figure 3E). In addition, there were multiple small clusters (hyperplastic foci) composed of micromegakaryocytes, which represented approximately 50% of the megakaryocyte population. Many of these megakaryocytes displayed features of atypia, namely increased cytoplasmic basophilia and occasional abnormal chromatin clumping with mitoses (Figures 3F, G). There was also widespread hemosiderin accumulation in macrophages throughout the bone marrow specimen, but active erythrophagocytosis was minimal.

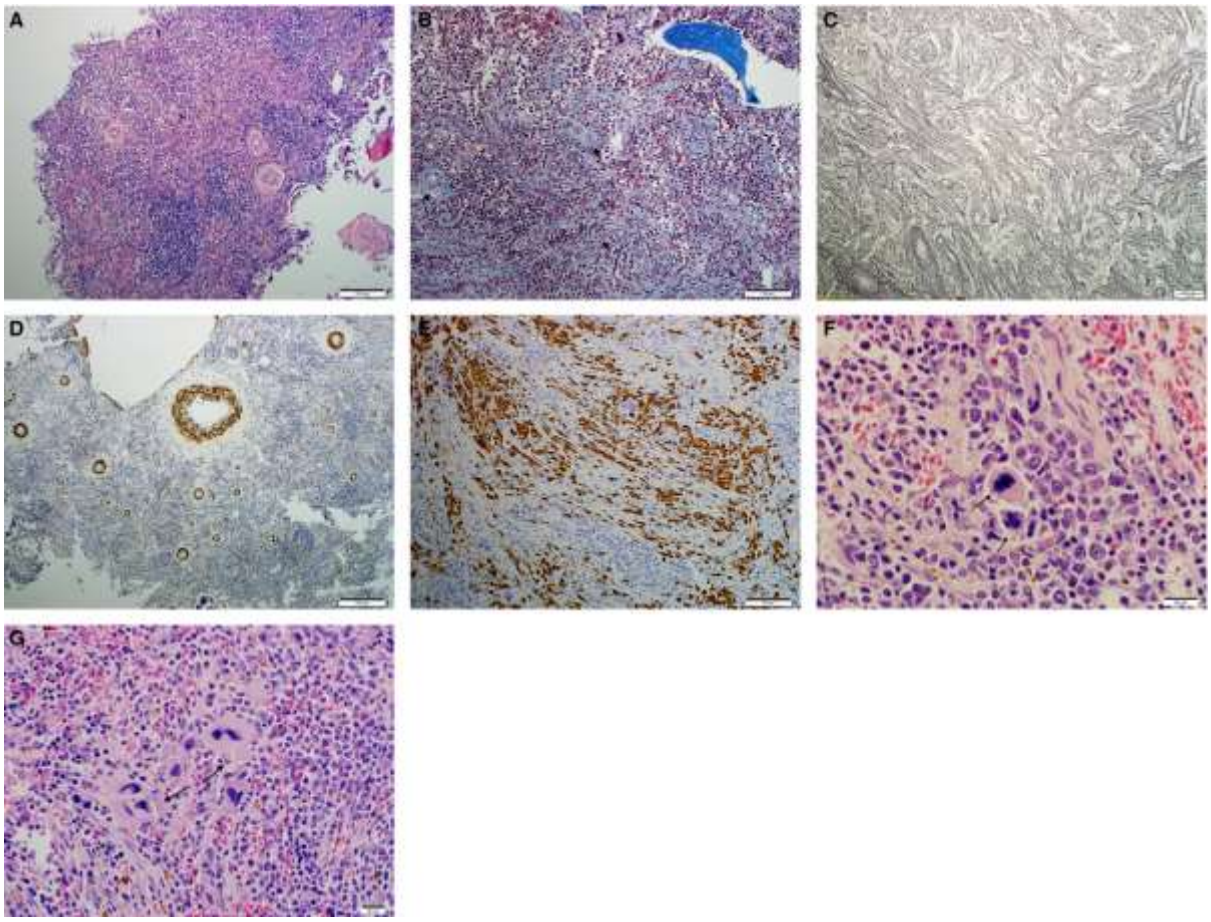

Figure 3. Histologic sections of the bone marrow in the dog. (**A**) Marked hypercellular bone marrow. H&E. (**B**) Moderate dissecting and perivascular fibrosis *(arrows)* of the intertrabecular spaces. Masson's Trichrome. (**C**) Diffuse network of both fine and coarse argyrophilic fibres involving more than 75% of the total marrow space, with extensive intersections and a few coarse bundles of collagen. Gomori's reticulin stain. (**D**) Vascular smooth muscle positive for alpha-smooth muscle actin. Diaminobenzidine immunohistochemistry with hematoxylin counterstain. ((**E**) Hyperplastic myeloid-monocytic cells positive for MAC387. Diaminobenzidine immunohistochemistry with hematoxylin counterstain. (**F**) Atypical megakaryocytes with hyperchromatic nuclei and diffuse chromatin clumping *(arrows)*. H&E. (**G**) Small clusters of hyperplastic, atypical megakaryocytes *(arrows)*. H&E.

Microscopic sections through the spleen revealed marked diffuse hypercellularity (Figure 4A) with little distinction between white and red pulp (Figure 4B), compared to normal spleen. The hypercellular red pulp contained clustered and singly dispersed megakaryocytes (also with nuclear atypia; Figure 4C), as well as numerous MAC387-positive myeloid and monocyte precursors (Figure 4D), and fewer erythroid precursors. Singly scattered and small clusters of hemosiderin-laden macrophages were dispersed throughout the red pulp. In addition, mild multifocal fibrosis was also present in the red pulp, compared to normal spleen (Figure 4E).

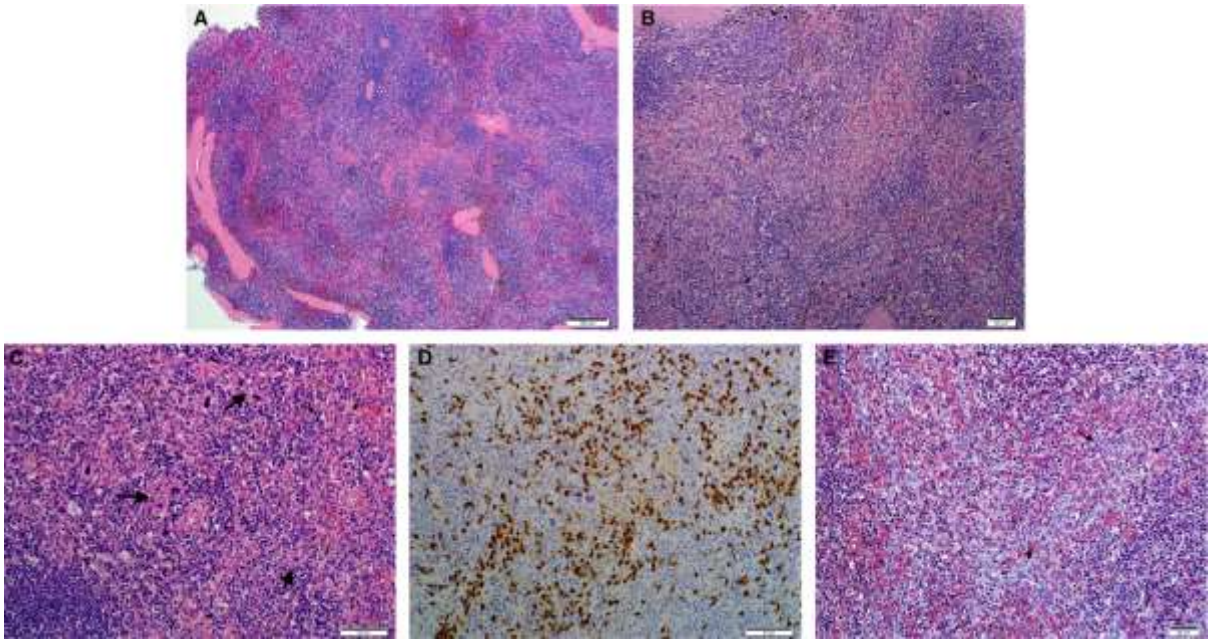

Figure 4. Histologic sections of the enlarged spleen of the dog. (**A**) Hypercellular red pulp. H&E. (**B**) Little distinction between the white and red pulp. H&E. (**C**) Clustered and singly dispersed megakaryocytes *(arrows)* displaying nuclear atypia in the hypercellular red pulp. (**D**) Hyperplastic myeloid-monocytes positive for MAC387. Diaminobenzidine immunohistochemistry with hematoxylin counterstain. (**E**) Mild multifocal fibrosis *(arrows)* in the red pulp. Masson's Trichrome.

Mild extramedullary hematopoiesis was also evident in the liver, which was especially prominent perivascularly in the portal tracts and periacinar areas, and to a lesser extent in the sinusoids. Given the histopathologic findings in conjunction with the clinical as well as the cytologic findings, a

diagnosis of idiopathic myelofibrosis (IMF) associated with peritoneal EMH resulting in refractory ascites was considered most likely.

Discussion

Canine myelofibrosis is a disorder that is characterized by an increase in collagen and other proteins of the extracellular matrix in the bone marrow, involving the increased proliferation and synthetic activity of marrow fibroblasts.[2,3] Myelofibrosis can be classified as either part of a distinct chronic idiopathic myeloproliferative disorder[4,5] or secondary to acute leukemia/lymphoma, bone marrow necrosis, immune-mediated hemolytic anemia, congenital pyruvate kinase deficiency, congenital non-spherocytic anemia, nonhemic tumors, whole-body irradiation,[2,6,7] infectious diseases, and drugs or toxicities.[8] Although most of the reported cases of canine myelofibrosis appear to be of secondary nature, at least four case reports seemed to correspond with the criteria for IMF (also known as primary myelofibrosis, myeloid metaplasia with myelofibrosis and agnogenic myeloid metaplasia) as described in the human literature.[4-6,9] IMF is classified as a chronic myeloproliferative disorder of erythroid, myeloid, and megakaryocytic lines,[6] that is characterized by marrow fibrosis and trilineage EMH in tissues throughout the body.[10] The fibroblast proliferation is a reactive, or secondary process and not a component of the underlying clonal hematopoiesis.[10] In humans, this clonal hemopathy is characterized by splenomegaly due to EMH (also referred to as myeloid metaplasia), immature granulocytes in blood, dacryocytosis (i.e., teardrop shaped erythrocytes), and severe myelofibrosis.[9,10] A prominent feature of IMF includes megakaryocyte hyperplasia and dysplasia.[6]

The dog reported in this case study had features consistent with idiopathic myelofibrosis as described in dogs[4,6], cats[4], marmosets[11], and human patients[9]. These features included splenomegaly, EMH, anemia, leukopenia, RBC anisocytosis and poikilocytosis, and bone marrow characterized by hypercellularity, moderate fibrosis and clustering of atypical megakaryocytes, indicating abnormal megakaryopoiesis. It should be noted that certain disorders, such as immune-mediated hemolytic anemia and/or immune-mediated thrombocytopaenia, lymphoma, drugs, and infectious diseases have

been associated with both secondary myelofibrosis and megakaryocytic dysplasia.[12,13] Considering the lack of molecular testing for an extensive panel of infectious diseases as well as a Coomb's test, due financial constraints, secondary myelofibrosis potentially due to non-regenerative immune-mediated or infectious disease could not be definitively excluded. However, secondary myelofibrosis due to infectious disease was unlikely in this case based on the patient history, as well as the normal platelet count which is unusual for the infectious agents common to this particular demographic area, e.g. ehrlichiosis, babesiosis, and infrequently theileriosis.[13] Other differential diagnoses for this dog included acute myeloid leukemia of megakaryocytic lineage and primary myelodysplastic syndrome (MDS). The percentage of nonlymphoid blast cells (myeloblasts, rubriblasts, or megakaryoblasts) in the bone marrow in relation to all nucleated marrow cells can serve as a differentiating feature: in acute megakaryoblastic leukemia (AMegL), they exceed 20% of which ≥50% of the blasts are of megakaryocytic lineage,[13,15] and in MDS they are less than 20% based upon recommendations by the World Health Organisation,[13] whereas in IMF the corresponding figure is also less than 20%.[4] For this case we were unable to perform a differential cell count due to the low cellularity of the bone marrow aspirate. However, the overall blast percentage was <5% and the combined percentage of megakaryoblasts and promegakaryocytes in the histologic sections of bone marrow was estimated to be less than 1%. These findings excluded a diagnosis of AMegL in our case. MDS was included as a differential in our case due to the findings of bicytopenia (severe non-regenerative anemia and neutropenia) as well as hypercellular marrow with evidence of (unilineage) dysplasia. MDS can be classified into three subtypes based on blood and bone marrow findings.[16] Cases where the myeloblasts are increased (5%-19% of the bone marrow nucleated cells), are classified as the myelodysplastic syndrome-excess blasts (MDS-EB) subtype.[16] Based upon the low overall nonlymphoid blast percentage in our present case this subtype could be excluded. MDS with erythroid predominance in the bone marrow (MDS-ER) has a myeloid: erythroid (M:E) ratio below 1[16], and could thus be excluded based upon the myeloid/monocytic: erythroid ratio of 1-2:1 in our current case. Patients with refractory anemia and an M:E ratio above 1, with other refractory cytopenias, are described as myelodysplastic syndrome-refractory cytopenias (MDS-RC).[13,16] Based upon the absence of dysplastic changes within the two cell lineages associated with cytopenias, the

erythroid and myeloid lineages, MDS-RC could be excluded in our case. Occasionally variant forms of MDS are seen and primary dysmegakaryopoiesis, MDS with megakaryocytic predominance (MDS-Meg), is the most common variant form.[15,17] However, although dysplastic features were reported within the megakaryocytic lineage, the automated platelet count was well within the reference interval, indicating effective thrombopoiesis, which is in contrast with chronic moderate to severe thrombocytopenia seen in dogs with MDS-Meg.[17]

When bone marrow is destroyed or replaced by neoplastic cells, resulting in severely reduced intramedullary hematopoiesis, blood cell production can be seen outside the medullary spaces of the bone marrow.[18] The most frequent sites of EMH are the reticuloendothelial system, i.e. the liver, spleen, and lymph nodes.[18] EMH in adult animals involves pathophysiological alterations in stem cells and their microenvironment, which include the extracellular matrix, stromal cells, and local and systemic chemokine production.[8] IMF is the prototypical example of bone marrow failure associated with EMH.[8] In human pathology, EMH as a result of IMF or other myelofibrotic/myelodysplastic syndromes is sometimes referred to as "myeloid metaplasia" or "agnogenic myeloid metaplasia".[8] However, the use of these terms have recently been rejected by the International Working Group on Myelofibrosis Research as their meaning is no longer viewed applicable within the context of the disease.[8] In humans, EMH is most commonly associated with IMF[18] and has been reported to frequently affect the spleen, liver, lymph nodes,[19] and less frequently the peritoneum,[20-22] skin,[23] lungs,[19] kidneys,[18,19] and adrenal glands.[19] EMH associated with canine IMF has been reported to affect the spleen, liver, lungs[5], and testicles.[4]

The cytological examination of the peritoneal fluid in our case revealed megakaryocytes along with erythroid and myeloid precursors. Findings of ascites, hydrothorax as well as anasarca similar to our case have previously been described as post-mortem findings in a case of canine IMF; however, no cytological analysis of the fluid was performed and no peritoneal/pleural abnormalities were documented in that reported case.[4] Similar ascitic cytology has been reported in humans with IMF associated with peritoneal EMH.[20-22] Peritoneal biopsy is considered to be the gold standard procedure for the diagnosis of peritoneal EMH, whereas cytology of the ascitic fluid is also

considered to be a reliable diagnostic means.[21] No peritoneal biopsies were taken in our case. In humans the longstanding course of refractory ascites, together with the presence of various hematopoietic precursor cells in the fluid in the absence of blasts, and no other apparent cause for the ascites strongly suggests EMH in the peritoneum as the cause. Moreover, in human medicine it has been shown that karyotypical analysis of the cells in the cavitatory effusions of patients with IMF might be a useful additional diagnostic procedure for the confirmation of the presence of clonal EMH secondary to IMF[21], as abnormal karyotypes have been concurrently identified in the bone marrow cells as well as the effusion cells of these patients.[24] The prognostic implications of an abnormal karyotype in human IMF is, however, controversial at this stage.[25,26]

Excessive production or intramedullary release of fibrogenic cytokines by dysplastic megakaryocytes or platelets play a pivotal role in the pathogenesis of myelofibrosis in IMF.[10] Changes in the synthesis of various chemokines that stimulate collagen synthesis and decrease collagenolysis result in the cellular abnormalities and marrow disorders that characterize IMF.[8] Increased production of thrombopoietin, collagenase inhibitors[8] and fibrogenic cytokines such as transforming growth factor β , platelet derived growth factor , and epidermal growth factor have been identified as important modulators of proliferation and synthesis in marrow fibroblasts and endothelial cells.[10] A direct correlation between serum thrombopoietin concentration and the amount of bone marrow fibrosis, as well as a direct relationship between the cytokines required for fibrogenesis and the α-granules of megakaryocytes and platelets have been shown.[8] Aberrant proliferation of hematopoietic stem cells characterize IMF, however it has recently been documented that certain molecular alterations in microenvironment alone can result in myeloproliferative disease.[8] A recent important molecular discovery, with regards the underlying pathogenesis of IMF in humans, was that patients with IMF carried a Janus kinase 2 (*JAK2*) V617F mutation.[27,28] The Janus family of kinases (JAK) include JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2), and are necessary for the physiologic signalling of cytokines and growth factors through receptors that intrinsically lack kinase activity (for example; erythropoietin, granulocyte–macrophage colony stimulating factor, interleukin [IL]-3, IL-5, thrombopoietin, growth hormone and prolactin-mediated signalling).[28] Functionally,

JAK2 is important for hematopoietic growth factors signalling such as thrombopoietin, IL-3, IL-5, growth hormone and prolactin-mediated signalling.[28] Additional mutations have been identified in human IMF patients and these mutations fall into two main classes, which include the signalling mutations that activate the thrombopoietin receptor (MPL) and mutations in epigenetic regulators of DNA methylation (*TET2, DNMT3A,* and *IDH1/2*)17-20 or chromatin structure (*EZH2* and *ASXL1*).[27,29] Further studies are underway in order to determine the pathogenetic relevance of these mutations.[29]

In humans, symptomatic ascites associated with myelofibrosis is rare and usually develops in the context of well-established disease.[22] Ascites has been reported to occur in 2-10% of human cases with IMF,[20,21] with myelofibrosis only rarely manifesting as marked ascites.[20] The etiology of ascites in IMF is assumed to result from three major sources, which include: 1) portal hypertension (PH) secondary to EMH in the liver and spleen, 2) thrombosis of the portal vein, and 3) ectopic metastasis (seeding) of hematopoietic tissue in the peritoneum.[20,21] The formation of ascites secondary to peritoneal EMH is the result of increased permeability of capillaries or obstruction of the lymphatic ducts.[22] The exact mechanisms leading to PH in IMF are still unclear.[30] Two theories have been proposed in the absence of portal and/or hepatic vein thrombosis.[30] The first theory postulates that PH develops due to sinusoidal narrowing and intrahepatic obstruction secondary to EMH and hepatic infiltration by myeloid cells resulting in increased intrahepatic resistance.[22,30] The second theory states that the increased portal blood flow secondary to increased splanchnic blood flow, as a result of the splenomegaly, might result in the formation of PH.[22,30] Routine abdominal ultrasound examination in our case did not reveal obstruction of the hepatic veins. PH was considered unlikely to have been significant in this case, based on the lack of compatible histomorphologic findings, including the presence of only a mild degree of hepatic EMH in the periportal and periacinar areas, and only moderate splenomegaly, as was observed during the gross necropsy examination. Although the lack of further ultrasonographic evaluation of the portal blood flow, as well as the absence of wedged hepatic venous pressure determination, prevented definitive exclusion of PH, the cause of the ascites was primarily attributed to peritoneal EMH in this case.To the authors' knowledge this case

represents the first report of canine myelofibrosis, most likely idiopathic, associated with peritoneal EMH, resulting in refractory ascites. Although IMF is a relatively rare condition in dogs,[6] this case demonstrates that ascites caused by peritoneal implants of hematopoietic tissue may be the initial manifestation of myelofibrosis. Therefore EMH should be included in the differential diagnosis of ascites.

[a] Normal goat serum (code no. G9023, Sigma Chemical Co, St Louis, Missouri, USA), diluted 1:5 with PBS buffer, pH 7.6 containing 0.1 % bovine serum albumin/BSA for 20 minutes in a humidified chamber at room temperature

[b] 0.1 molar phosphate buffered saline/ PBS, pH 7.6 containing 0.1 % bovine serum albumin/BSA

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