Peritoneal fluid from a dog

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Signalment: 5-year-old, male castrated, Shetland Sheepdog, "Twister"

Specimen: Peritoneal fluid, Wright-Giemsa stain

History: Twister was referred to the Atlantic Veterinary College Teaching Hospital due to penile necrosis of an undetermined cause. Three weeks prior to referral the patient presented to the referring veterinarian with a blue-tipped penis and subsequently demonstrated stranguria and mild epistaxis. The bladder had been catheterized or manually expressed several times to promote complete emptying of the bladder. The patient was being treated with prednisone (2 mg/kg q12 hours) for suspected immune mediated thrombocytopenia. Other therapies prior to referral included deracoxib (Deramaxx®), metronidazole, cephalexin and ranitidine.

Clinical findings: On presentation (day 1) Twister was quiet and depressed but responsive. On physical examination Twister had tacky pink mucus membranes, tachypnea, increased respiratory effort, tachycardia and a tense and painful abdomen on palpation. The rostral aspect (~3-5 cm) of the penis was devitalized and a smaller (1-2 cm) area of the proximal prepuce had devitalized skin.

Diagnostic procedures:
A complete blood count, serum biochemistry and urinalysis were performed. Results are in tables 1, 2 and 3.

Abdominal ultrasound (performed on day 1) revealed a large amount of echogenic free fluid throughout the abdomen. The bladder was mildly distended with echogenic fluid and a discontinuous segment of the caudal ventral bladder was noted. Within this region of the bladder wall, hyperechoic, distally attenuating foci were present and interpreted as free air. A positive contrast cystogram was performed using Omnopaque® and leakage into the peritoneum was not noted.

The peritoneal fluid obtained on day 1 via abdominocentesis was cloudy and slightly bloody. It was submitted for cytological evaluation (figures 1, 2 and 3). The nucleated cell count was 48.9 x 10^9/L, RBC count was 0.03 x 10^{12}/L and the protein concentration <25 g/L. Additional results from the abdominal fluid: creatinine = 1139 μmol/L, urea = 39.7 mmol/L and potassium = 11.2 mmol/L.

Table 1: Hematology results:

Analyte	Units	Day 1	Reference Interval
WBC	$x10^9/L$	**28.2**	5.4 - 14.3
RBC	$x10^{12}/L$	**4.6**	5.7 - 8.4
Hgb	g/L	**105**	135 - 198
Hct	L/L	**0.31**	0.40 - 0.56
Platelets	$x10^9/L$	231	218 - 470
Reticulocytes	$x10^9/L$	60	0 - 85
Segmented neutrophils	$x10^9/L$	**25.9**	2.8 - 10.1
Band neutrophils	$x10^9/L$	0.3	0.0 - 0.3
Lymphocytes	$x10^9/L$	**0.6**	0.9 - 4.6
Monocytes	$x10^9/L$	1.4	0.1 - 1.4

Table 2: Biochemistry results:

Analyte	Units	Day 1	Day 8	Reference Interval
Sodium	mmol/L	148	149	144 - 151
Potassium	mmol/L	5.2	4.9	3.9 - 5.3
Na: K ratio		28	30	
Chloride	mmol/L	107	108	105 - 117
Calcium	mmol/L	2.55	2.56	2.02 - 2.91
Phosphorus	mmol/L	**2.71**	1.13	0.84 - 1.83
Urea	mmol/L	**21.8**	5.2	2.8 - 9.8
Creatinine	µmol/L	**334**	**41**	54 - 122
Total Protein	g/L	64	58	56 - 71
Albumin	g/L	**24**	**28**	30 - 36
Globulin	g/L	**40**	30	25 - 38
A:G Ratio:		**0.6**	0.93	0.7 -1.50

Table 3: Urinalysis Results:

Analyte	Day 1	Analyte	Day 1
Collection	Catheterized	Ketones	Negative
Color	Yellow	Blood	Trace
Clarity	Clear	pH	5
Specific gravity	1.026	Protein dipstick	Negative
Glucose	Negative	Protein SSA	0 g/L
Clinitest	0 mmol/L	Urobilinogen	1.7 µmol/L
Bilirubin	Negative	Microscopic findings	1-3 WBCs/400x, 0-2 RBCs/400x

Peritoneal fluid cytology:

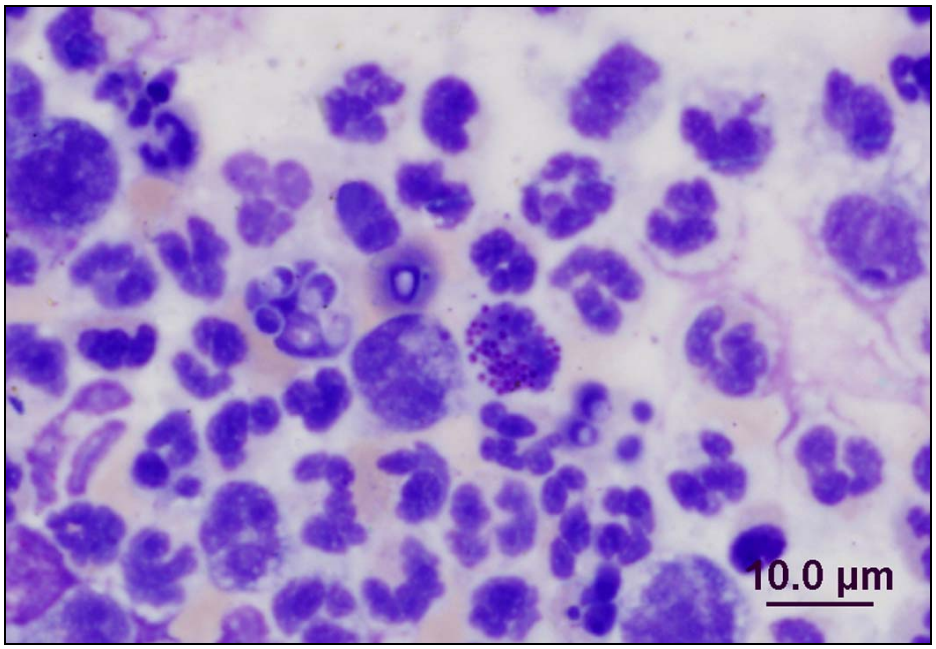

Figure 1: Cytocentrifuged preparation of canine peritoneal fluid. Wright-Giemsa Stain.

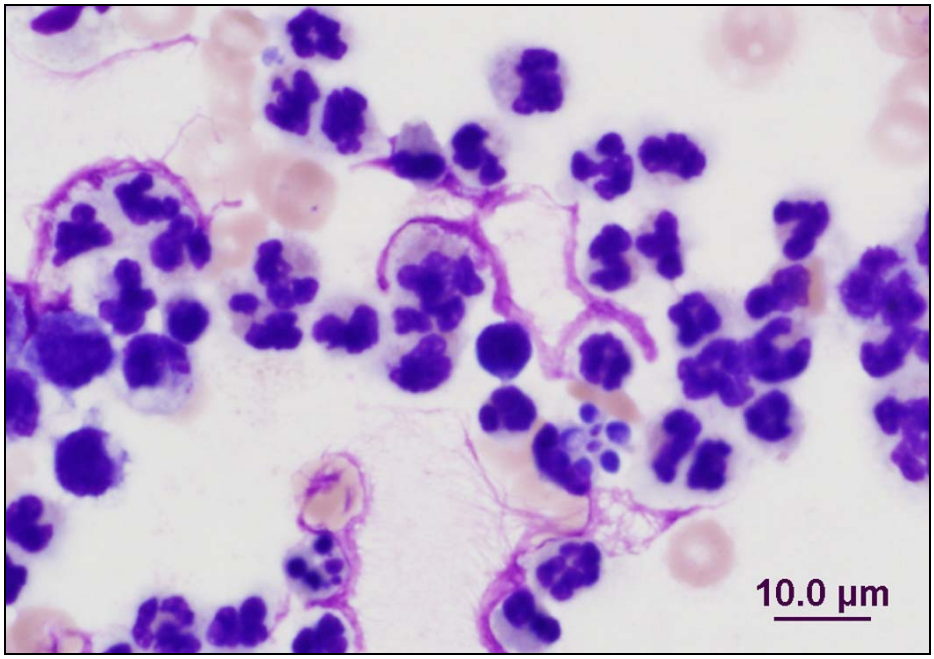

Figure 2: Sediment smear of canine peritoneal fluid. Wright-Giemsa Stain.

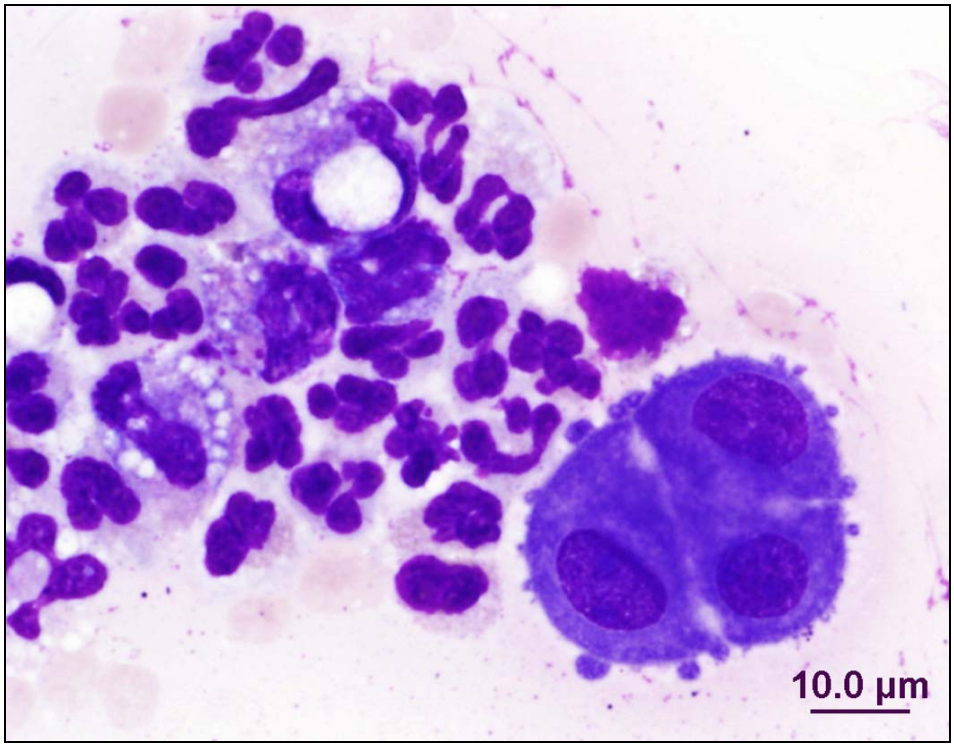

Figure 3: Sediment smear of canine peritoneal fluid. Wright-Giemsa Stain.

Questions:

1. What is your cytological diagnosis and interpretation for the peritoneal fluid?
2. What further tests would help confirm the cytological diagnosis?

Cytology description:

This sample contains a high number of nucleated cells and a low number of RBCs on a clear to pale pink background. The nucleated cells consist primarily of inflammatory cells/leukocytes (~98%) and low numbers of (~2%) of mesothelial cells. Based on a 300 cell count of the inflammatory cells/leukocytes there are 94% neutrophils and 6% macrophages. The neutrophils are a mixture of non-degenerate to degenerate cells. Neutrophils with pyknotic nuclei are often noted, as well as those undergoing karyorrhexis. Low to moderate numbers of coccoid to diplo-coccoid bacteria are noted in the background and within neutrophils. Low numbers of small (~2-3 x 3-4 µm), ovoid yeast-like organisms that are surrounded by a thin walled non-staining capsule and often contain a small (~0.5-1 µm) ovoid to elliptical basophilic central to peripherally located internal structure are present singly and in small groups. These are noted in the background, within neutrophils and rarely within macrophages. The mesothelial cells are present singly and occasionally in small clusters. These cells are ovoid and contain moderate amounts of blue cytoplasm that occasionally contains small round vacuoles. Nuclei are ovoid, have coarse granular chromatin and distinct single to multiple variably sized and shaped nucleoli. Cells are occasionally bi-nucleated or multi-nucleated. Low numbers of these cells have a fringed corona-like border. Overall, these cells have moderate anisocytosis and anisokaryosis.

Cytology interpretation/diagnosis:
Exudate - marked neutrophilic septic inflammation with presence of intracellular bacteria and yeast
Uroabdomen
Reactive mesothelial cell population

Additional findings:
An exploratory celiotomy was performed (day 1) and a defect in the bladder wall was repaired. The necrotic portion of the penis was resected. The abdomen was lavaged thoroughly with sterile saline and a closed suction drain was placed.

Culture results:
Peritoneal fluid: *Candida glabrata* and a scant growth of *Staphylococcus* species.
Urine: *Candida glabrata* and *Staphylococcus epidermidis*

Histopathology Description:
Penis: At the proximal margin of the resection there is viable tissue which transitions distally to complete coagulative necrosis of all the tissue. Relatively large areas of hemorrhage, fibrin exudation and aggregates of mostly degenerate inflammatory cells (apparent neutrophils) are scattered throughout many areas. In some sections medium sized arteries, at the junction between viable and necrotic tissue, contain fibrin thrombi with some showing early organization.

Urinary Bladder (from area of defect repair): Some sections show widespread ulceration/erosion with a layer of fibrin admixed with neutrophils overlying the surface. In some of these latter areas bacteria and small to moderate numbers of yeast-like bodies, occasionally in aggregates are present (figure 4). These fungal organisms are round to slightly oval, ~ 2 to 3 μm in diameter and show occasional budding. The underlying submucosa shows patchy to widespread hemorrhage and in several areas fibroplasia and fibrosis (granulation-like). In some sections this hemorrhage and fibroplasia extends through the muscle layers (presumably these tissue fragments are from an area of perforation).

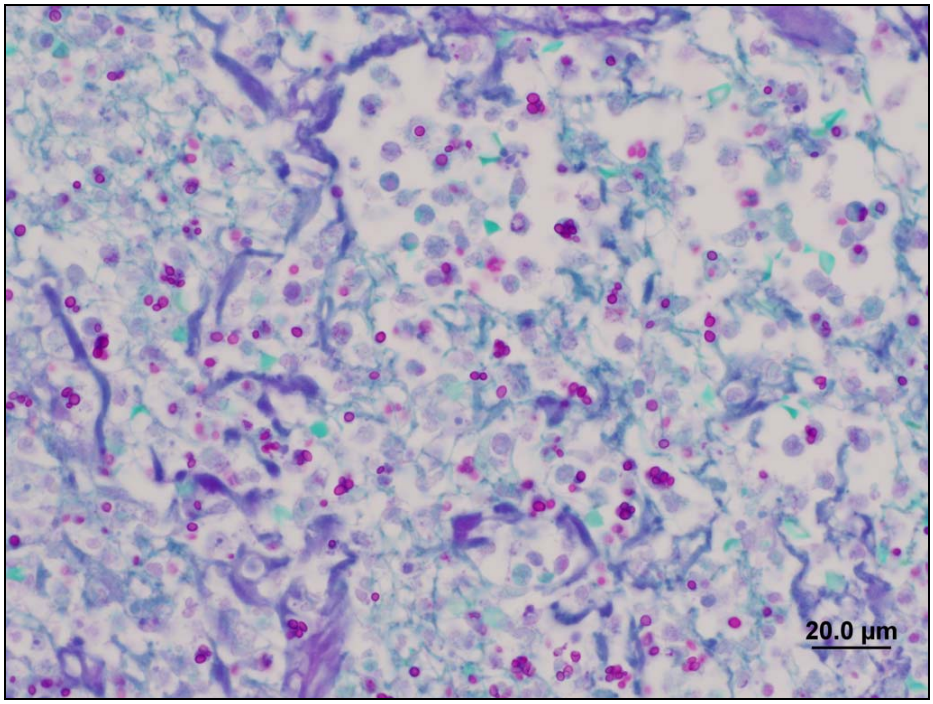

Figure 4: Numerous yeast forms are scattered throughout the exudate which is adhered to the ulcerated surface of the urinary bladder. Periodic acid-Schiff.

Diagnosis:

1. Penile vascular thrombosis with infarction (dry gangrene)
2. Cystitis, ulcerative and fibrinosuppurative, locally extensive, chronic-active, severe with intralesional bacteria and yeast (*Staphylococcus epidermidis* and *Candida glabrata*)
3. Bacterial and fungal peritonitis (*Staphylococcus* species and *Candida glabrata*)
4. Uroabdomen

Clinical outcome:

Twister recovered well post-surgery and was initially treated with intravenous fluids, fentanyl (4µg/kg/hr), ampicillin (22 mg/kg IV q8 hours), enrofloxacin (5 mg/kg PO q 24 hours) and famotidine (0.5 mg/kg IV q 12 hours). Fluconazole (100 mg PO q 12 hours) was subsequently initiated and antibiotic therapy switched to Doxycycline (100 mg PO q 12 hours) due to sensitivity testing of the bacteria. Twister had an indwelling urinary catheter for 4 days post-operatively and after removal of the catheter Twister was started on prazosin (1 mg PO q 12 hours) and bethanechol (5 mg PO q 8 hours) due to urine dribbling and incomplete bladder emptying. Twister continued to improve clinically and was discharged on day 12.

Discussion:

Yeast forms of *Candida* species are normal commensals of mammalian gastrointestinal, upper respiratory and genital mucosa and are considered opportunistic pathogens that reproduce by budding.[1,2] Infections involving *Candida glabrata* have rarely been reported involving the urinary tract in cats[3,4] and dogs,[4] but in humans infections with *C. glabrata* are often the second to third most common cause of candidiasis with *C. albicans* being most common.[5] *Candida*

peritonitis has been recently reported in dogs, but most often *C. albicans* was isolated[6-8] and less often *C. glabrata*.[6] In general, patients at increased risk for candidiasis include those that are immunosuppressed or those receiving chronic antibacterial therapy.[1,5] In humans, *C. glabrata* nosocomial infections are important with two major risk factors being prior antimicrobial usage and prolonged hospitalization.[9] Identification of the species of *Candida* is important as resistance to fluconazole therapy has also been reported with some species, including *C. glabrata*.[10,11]

Candida glabrata differs from *C. albicans* in that it has a haploid genone, is not dimorphic and is therefore present as blastoconidia in both the commensal and pathogenic form.[1,5] Blastoconidia from *C. glabrata* are also smaller (1 - 4 μm) compared to those from *C. albicans* (4 - 6 μm).[1,5] In addition, *Candida* species form pseudohyphae at temperatures above 37˚C with the exception of *C. glabrata*.[5]

Candidiasis can be diagnosed using culture of various samples, including blood, urine or other tissues.[1] Arterial blood samples are preferred over venous samples as organisms often are effectively filtered by various tissues.[1] If *Candida spp.* are cultured from cutaneous or mucosal surfaces this does not confirm the presence of an invasive infection. Rather, histological evidence of tissue invasion and host reaction must be sought.[1,12] Polymerase chain reaction (PCR) has also been used to detect and identify *Candida spp.* in veterinary patients.[13,14]

In this patient the source of the bacterial and fungal peritonitis was most likely the urinary tract given the uroabdomen and culture results from the urine. The initiating cause of the penile vascular thrombosis is not known in this case, but given the difficulties with urination that occurred as a result of this, it is not surprising that the urinary system became compromised, thus predisposing it to both a bacterial and fungal cystitis. Also, the glucocorticoid therapy may also have contributed by altering cellular immunity of the patient.[15]

The neutrophilia and lymphopenia were most likely due to a stress or corticosteroid response, as well as an inflammatory component for the neutrophilia. The non-regenerative anemia was most likely due to decreased erythropoiesis secondary to inflammation. An increase in inflammatory proteins likely contributed to the mild hyperglobulinemia. The hypoalbuminemia may have been due to decreased protein synthesis as it is a negative acute phase protein, although other contributing factors cannot entirely be excluded. The uroabdomen was the cause of the post-renal azotemia and the hyperphosphatemia. The urinalysis was unremarkable despite significant positive fungal and bacterial culture results, which may also suggest an immunosuppressed state for the patient.

Although relatively uncommon, candidiasis is an important disease that warrants attention in ill and immunosuppressed patients. It is important to determine the species of *Candida* in patients for both epidemiological and therapeutic reasons.

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