

Extreme neutrophilic leukocytosis in a dog

Contributors: Jelena Palić, Johannes Hirschberger
Clinic for Small Animal Medicine, Centre for Clinical Veterinary Medicine, Faculty of Veterinary Medicine, Ludwig Maximilian University Munich, Munich, Germany

Signalment: Mixed breed canine, 6 year old spayed female named Lusy

Specimen: Blood smear, Sysmex XT-2000iV scattergrams

History: Lusy was presented to the Clinic for Small Animal Medicine, Ludwig Maximilian University Munich with a one month history of reduced general condition, anorexia, vomiting once a week for the past month, melena of a few days duration, and marked neutrophilic leukocytosis. Prior to her presentation, she has been treated by the referring veterinarian with amoxicillin/clavulonic acid and enrofloxacin but no clinical improvement was observed. Lusy has been receiving nonsteroidal anti-inflammatory drugs (NSAIDs) from time to time due to orthopedic problems but has not been on NSAIDs or corticosteroids in recent months.

Clinical findings: Clinical examination revealed a thin, quiet patient with pale mucus membranes, strong regular pulse and 2/6 systolic heart murmur. Palpation of the abdomen showed prominent, soft, barrel-shaped liver. The rest of the clinical examination was unremarkable. Hematology results obtained on Sysmex XT-2000iV are listed in Table 1. Additionally, microscopic evaluation of the blood smear and a manual 500-cell differential count was performed as listed in Table 2. Total protein and albumin were decreased: TP 49.5 g/L (RI 55.5-77.6) and albumin 28.0 g/L (RI 31.3-43). Potassium was 3.5 mmol/L (RI 3.8-5.5). Liver enzyme activities were increased: ALT 266 IU/L (RI 18-110), ALP 849 IU/L (RI 13 – 152). Total bilirubin was 4.9 µmol/L (RI 0-5.26). RI means reference interval. PT and aPTT were within reference ranges. *Babesia canis* ELISA did not detect the presence of *B. canis* antibodies. Urine (obtained via cystocentesis) had specific gravity of 1.042 and pH of 6. Urinalysis was unremarkable with the exception of bilirubinuria ++, proteinuria + and rare bilirubin crystals.

Table 1. Hematology results (Sysmex XT-2000iV)

Parameter	Value	Reference interval	Unit
WBC	115.49 ↑	5-16	$\times 10^9/L$
RBC	3.24 ↓	5.5-9.3	$\times 10^{12}/L$
HGB	5.3 ↓	7.45-12.5	mmol/L
HCT	0.279 ↓	0.35-0.58	L/L
MCV	86.1 ↑	58-72	fL
MCH	1.636 ↑	1-1.4	fmol/L
MCHC	19	19-21	mmol/L
PLT	146 ↓	180-550	$\times 10^9/L$
NEUT#	96.8 ↑	3-9	$\times 10^9/L$
LYMPH#	9.47 ↑	1-3.6	$\times 10^9/L$
MONO#	8.5 ↑	0.04-0.5	$\times 10^9/L$
EO#	0.66 ↑	0.04.-0.6	$\times 10^9/L$
BASO#	0.06 ↑	0-0.04	$\times 10^9/L$
RET#	414.4 ↑	19-150	$\times 10^9/L$

Table 2. Hematology results obtained by a manual differential count

Parameter	Value	Reference interval	Unit
NEUT#	101.63 ↑	3-9	$\times 10^9/L$
BANDS#	3.46 ↑	0	$\times 10^9/L$
METAMYELOCYTES#	1.15 ↑	0	$\times 10^9/L$
LYMPH#	2.31	1-3.6	$\times 10^9/L$
MONO#	5.77 ↑	0.04-0.5	$\times 10^9/L$
EO#	1.15 ↑	0.04.-0.6	$\times 10^9/L$
BASO#	0	0-0.04	$\times 10^9/L$

Questions:

1. What are the diseases and conditions that cause marked neutrophilic leukocytosis in dogs?
2. How could you confirm the diagnosis?

Blood smear images:

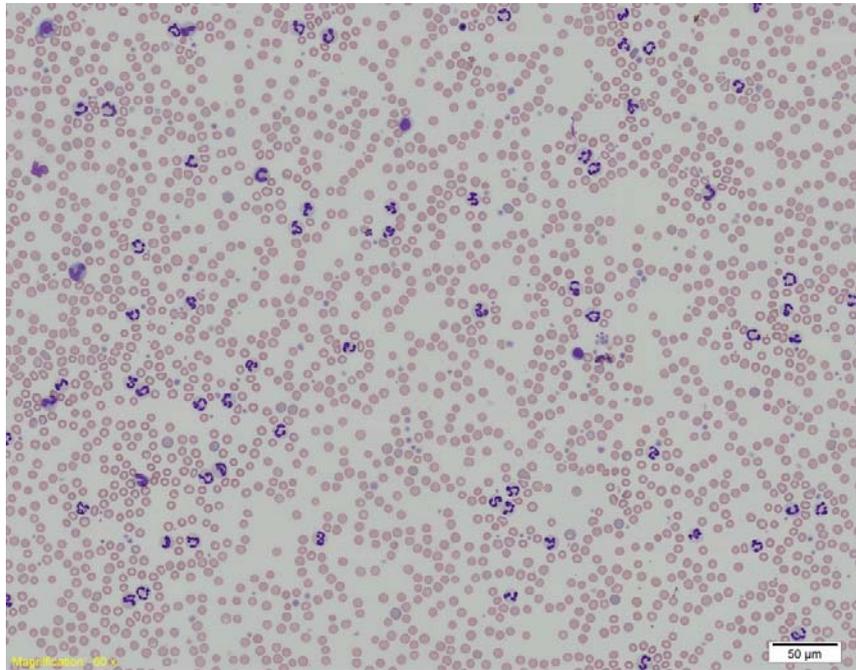


Figure 1. Blood smear from a mixed breed dog. Modified Wright's stain. Scale bar = 50μm.

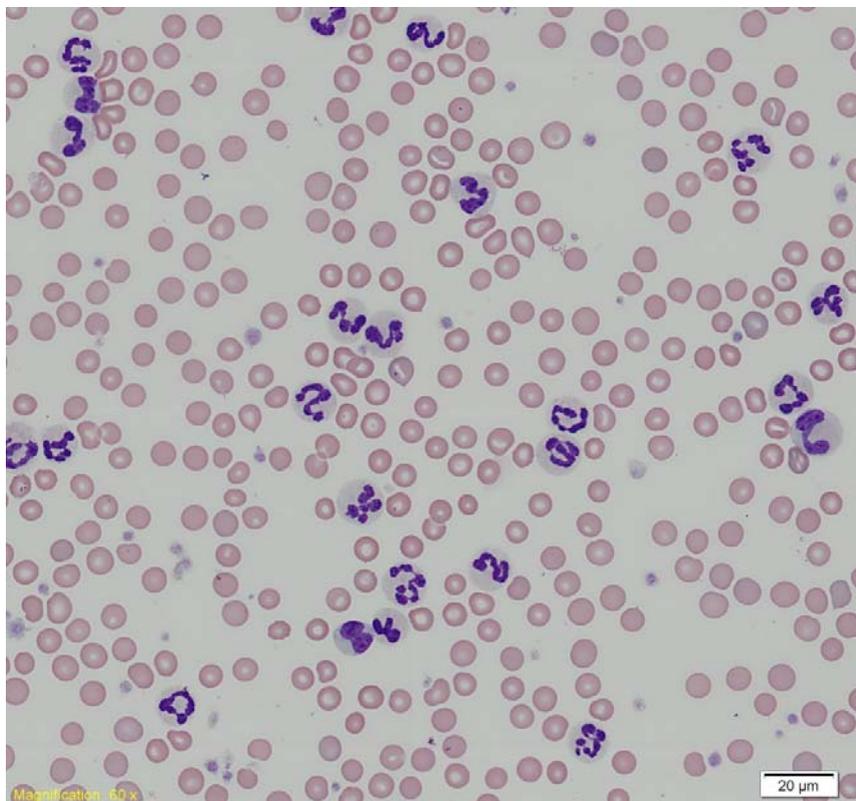
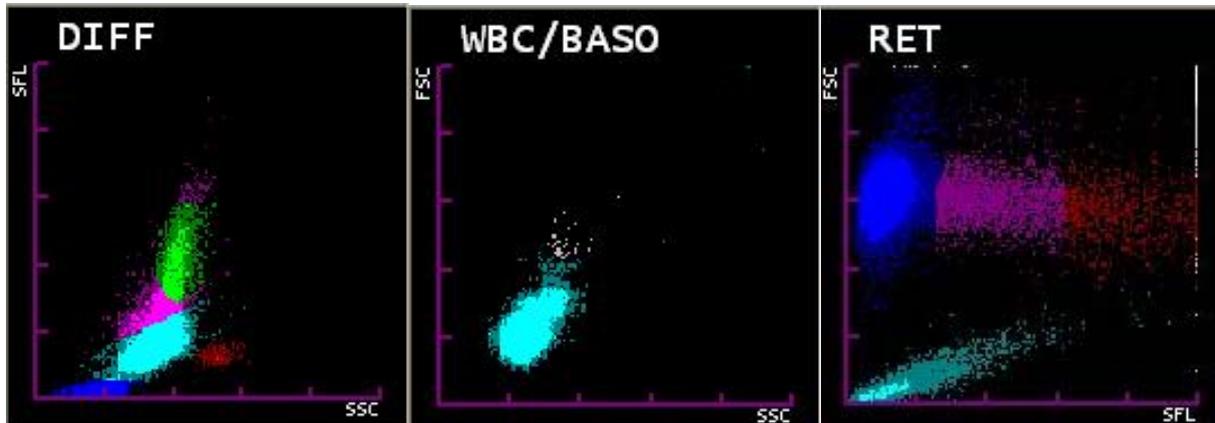


Figure 2. Blood smear from a mixed breed dog. Modified Wright's stain. Scale bar = 20μm.

Sysmex XT-2000iV scattergrams:



Hematology interpretation: Anemia, regenerative, with mild appropriate rubricytosis, marked leukocytosis with marked neutrophilia, mild left shift, marked monocytosis, mild eosinophilia and mild thrombocytopenia.

Hematology description and comments:

Erythrocyte density was moderately decreased with moderate anisocytosis, moderate polychromasia and rare rubricytes (2 rubricytes were noted per 500-cell differential count). Leukocytosis was marked. There was marked neutrophilia with mild left shift (both bands and metamyelocytes were noted), marked monocytosis, and mild eosinophilia. Low numbers of neutrophils were hypersegmented. Rare reactive lymphocytes were seen. Platelet numbers were mildly decreased.

Sysmex XT-2000iV scattergrams description and comments:

There is a poor separation between neutrophil (light blue), lymphocyte (pink) and monocyte (green) cell clusters on Sysmex DIFF scattergram. It has been shown that neutrophils can be found higher and slightly left in the scattergram and can merge into the lymphocyte cluster in samples with left shifts¹. The discrepancy of the lymphocyte and monocyte counts between Sysmex and manual differential can also be explained by the fact that lymphocyte and monocyte counts on the Sysmex are higher than manual counts¹. Eosinophils (red) seem to be well separated. WBC/BASO scattergram increased density confirms leukocytosis. Increased density of reticulocyte clusters on Sysmex RET scattergram (purple and red) correlate well with moderate polychromasia observed on visual inspection of the slide.

Additional tests:

Ultrasound of the abdomen: The stomach was greatly expanded, liquid-and gas-filled. Liver, spleen, and both kidneys appeared unchanged in size and structure. There was a large inhomogeneous mass with a diameter up to 10 cm in the right cranial quadrant between liver and stomach. Due to the location of the mass, it was assumed to be gastric wall tumor. A fine-needle aspirate of the mass (Figs 3-6) was obtained and submitted for cytological evaluation to the Clinical Pathology Laboratory of the Clinic for Small Animal Medicine, Ludwig Maximilian University Munich.

Cytology of canine stomach mass:

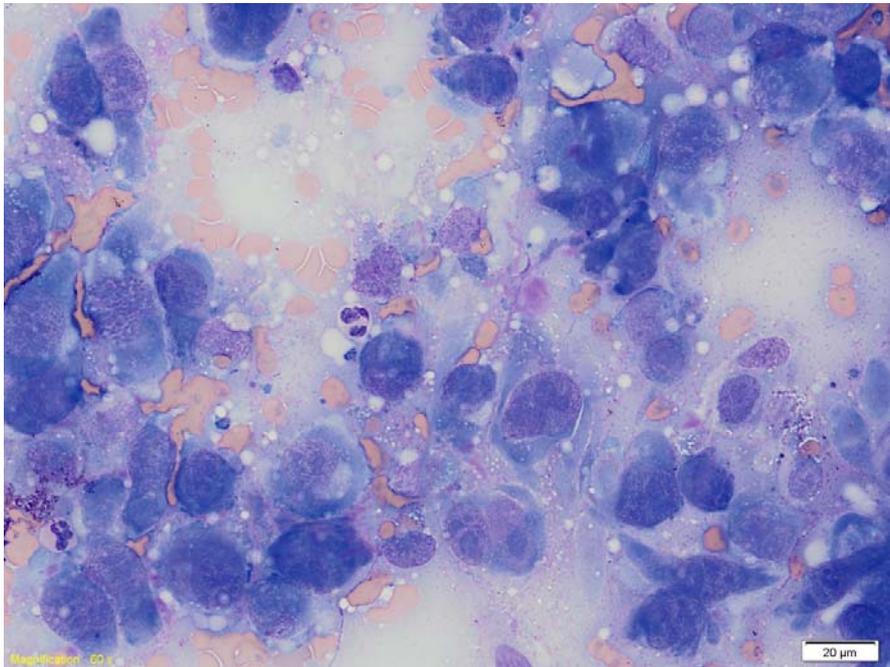


Figure 3. Fine-needle aspirate of a stomach mass from a mixed breed dog. Modified Wright's stain. Scale bar = 20 μ m.

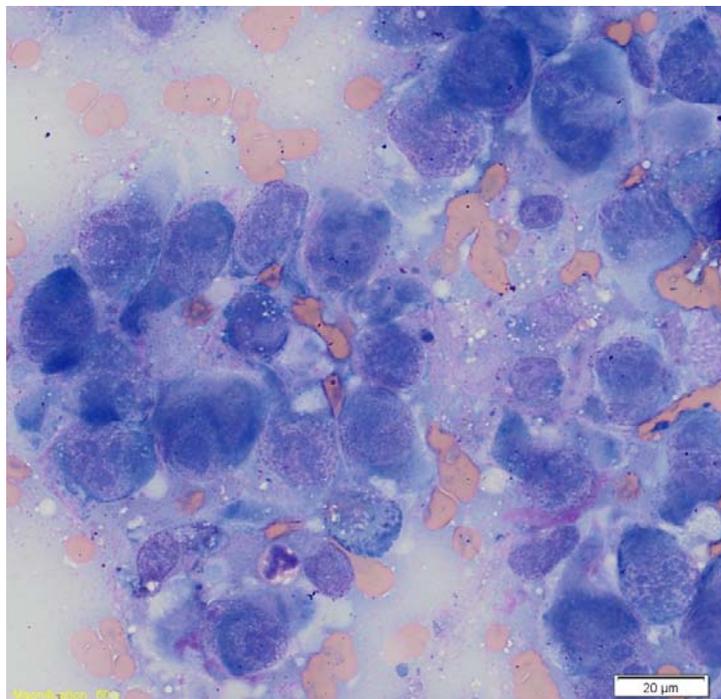
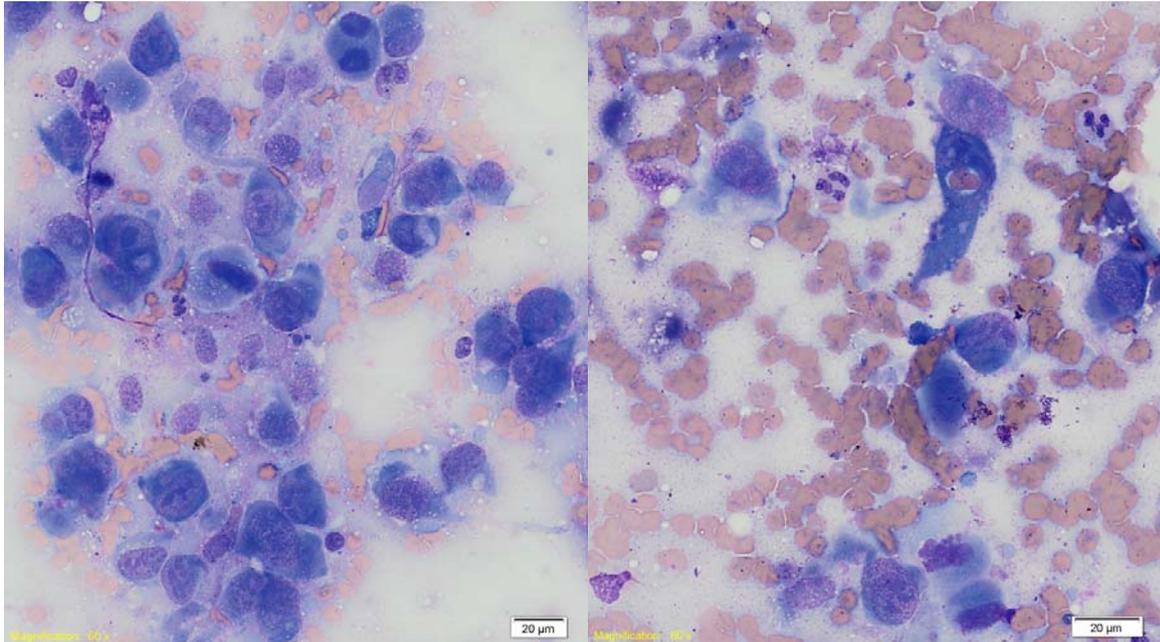


Figure 4. Fine-needle aspirate of a stomach mass from a mixed breed dog. Modified Wright's stain. Scale bar = 20 μ m.



Figures 5 and 6. Fine-needle aspirate of a stomach mass from a mixed breed dog. Modified Wright's stain. Scale bar = 20µm.

Cytology interpretation: Sarcoma

Cytology description and comments: FNA preparations of the stomach wall mass were of high cellularity with low numbers of RBC in the background. Spindle cells present had marked anisocytosis and anisokaryosis. They had round to oval nuclei with finely stippled chromatin and 1-5 prominent round to oval to linear to bizarre shaped nucleoli. Macronucleoli were often noted. N:C ratio was high but variable. Cytoplasm varied from light to dark basophilic and sometimes contained small clear vacuoles and small eosinophilic granules. Nuclear moulding was present. Moderate numbers of binucleated cells were seen and they had moderate to marked anisokaryosis. Cells occasionally associated with homogenous eosinophilic extracellular matrix. High numbers of mitotic figures were present. Erythrophagocytosis by some neoplastic cells was also noted.

Clinical follow-up: Lusy was placed on intravenous fluids, omeprazole, sucralfate, amoxicillin/clavulonic acid and buprenorphine, and remained in the hospital for two days; her clinical condition did not improve. Due to the poor prognosis, the owner has chosen euthanasia. Necropsy was declined and body was transported for private cremation.

Final diagnosis: Sarcoma of the gastric wall with paraneoplastic leukocytosis

Discussion:

This report describes 6 year old spayed female mixed breed dog that initially presented for reduced general condition, anorexia, vomiting, melena, and marked neutrophilic leukocytosis. Diseases and conditions that cause marked neutrophilia include focal suppurative lesions (such as pyometra, pleuritis or pyothorax, peritonitis, prostatitis, pneumonia, and abscesses), hemolytic anemia (especially immune-mediated), canine babesiosis, canine hepatozoonosis caused by *Hepatozoon americanum*, chronic neutrophilic leukemia, paraneoplastic neutrophilia, neutrophilia of LAD (leukocyte adhesion deficiency), and G-CSF administration². Marked neutrophilic leukocytosis typically ranging from 50 000 to 100 000 cells/ μ L of blood with a concurrent, orderly left shift that may extend to myelocytes or promyelocytes is termed leukemoid reaction since it resembles granulocytic leukemia but is caused by some other process³. Leukograms that are characterized by neutrophilic leukocytosis with >100 000 cells/ μ L of blood with a left shift and no evidence of hematopoietic neoplasia are also referred to as extreme neutrophilic leukocytoses; toxic changes may or may not be present³.

The diagnosis of paraneoplastic syndrome associated with extreme neutrophilic leukocytosis has been made in this case by exclusion of other possible causes of extreme neutrophilic leukocytosis. Based on the clinical exam, laboratory findings and the ultrasound examination, the presence of focal suppurative lesions, infectious agents, immune-mediated causes, chronic myelogenous leukemia and drug administration has been ruled out. It is possible that chronic inflammation and necrosis contributed to neutrophilia as well, however they would likely not cause the increase of this magnitude. Visual evaluation of the blood smear revealed the presence of mild left shift, normal neutrophil morphology and low numbers of hypersegmented neutrophils. The presence of hypersegmented neutrophils in this case can be attributed to severe stress (due to endogenous corticosteroid release) since the dog did not show any signs of hyperadrenocorticism and did not receive exogenous glucocorticoids.

A paraneoplastic syndrome is an abnormality associated with a tumor, but not due to the local presence of the tumor, that resolves after removal or control of the neoplasm and returns when the tumor recurs⁴. Paraneoplastic leukocytosis has been reported in dogs with pulmonary papillary carcinoma⁴, rectal adenomatous polyp⁶, adenomatous tubulopapillary polyp⁷, renal transitional cell carcinoma⁵, renal carcinoma^{8, 9, 10}, and metastatic fibrosarcoma¹¹. Neutrophilia in these patients was extremely high, ranging from 74 347 to 202 522 cells/ μ L, and was often accompanied by a left shift, monocytosis and eosinophilia¹², similar to our patient.

Paraneoplastic leukocytosis is thought to be caused mainly by overproduction of granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF). Production of CSF was demonstrated so far in only three cases in dogs: by PCR in a pulmonary papillary carcinoma producing GM-CSF and G-CSF⁴ and by immunohistochemical staining in a renal transitional cell carcinoma⁵ and renal carcinoma producing GM-CSF¹⁰. Unfortunately, we were not able to perform any additional testing since the dog has been euthanized and necropsy declined.

Histopathology of the gastric wall mass has also not been performed in this case due to owner's wishes. The most likely differential for primary gastric neoplasm of

mesenchymal origin was leiomyosarcoma. Smooth muscle tumors (leiomyomas and leiomyosarcomas) are the second most frequently reported primary gastric tumors¹³. Leiomyosarcomas are usually large and solitary tumors causing clinical signs of GI dysfunction (vomiting and/or diarrhea which may contain blood) because of their size and position¹³. These tumors are frequently described as locally invasive but slow to metastasize; however occurrence of metastasis may be underestimated due to lack of follow-up information. Leiomyosarcoma is histologically described by the presence of pleomorphic cells that vary from spindle shaped to round¹⁴. Their nuclei are variable in size, some are hyperchromic, and giant nuclei as well as many typical and atypical mitotic figures can be seen¹⁴. Our cytological findings correlate well with the histopathological characteristics described above. Erythrophagia in dogs has not been reported in leiomyosarcoma but has been recently reported in osteosarcoma and hemangiosarcoma in addition to hemophagocytic histiocytic sarcoma¹⁵.

The regenerative anemia present in the patient was due to ulceration and hemorrhage because of the associated sarcoma that likely extended to the mucosal surface. Mild thrombocytopenia has also been attributed to platelet loss and consumption due to gastric bleeding. Increased ALT activity is due to hepatocellular damage, likely because of the compression of the liver by neoplastic mass and subsequent hepatocellular swelling and leakage. Increased activity of ALP, bilirubinuria and bilirubin crystals in the urine are due to cholestasis, either intrahepatic or posthepatic. Hypoproteinemia and hypoalbuminemia have been attributed to gastrointestinal loss, and/or decreased liver albumin production. Hypokalemia is likely due to anorexia. 1+ proteinuria with adequate urine specific gravity and lack of UTI cannot be readily explained and would require further testing.

In summary, this is the first description of the extreme neutrophilic leukocytosis likely due to paraneoplastic syndrome caused by a gastric sarcoma in a dog.

References:

1. Lilliehöök I, Tvedten H. Validation of the Sysmex XT-2000iV hematology system for dogs, cats, and horses. II. Differential leukocyte counts. *Vet Clin Pathol* 2009;38(2):175-82.
2. Stockam SL, Scott MA. Leukocytes. In: Stockam SL, Scott MA, editors. *Fundamentals of veterinary clinical pathology*. 2nd ed. Ames (IA): Iowa State Press; 2008. p. 53-106.
3. Shultze AE. Interpretation of canine leukocyte responses. In: Weiss DJ, Wardrop KJ, editors. *Schalm's veterinary hematology*. 6th ed. Philadelphia: Blackwell Publishing; 2010. p. 321-34.
4. Sharkey LC, Rosol TJ, Grone A, Ward H, and Steinmeyer C. Production of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor by carcinomas in a dog and a cat with paraneoplastic leukocytosis. *J Vet Intern Med* 1996;10 (6):405-8.
5. Peeters D, Clercx C, Thiry A, Hamaide A, Snaps F, Henroteaux M, Ogilvie GK, Day MJ. Resolution of paraneoplastic leukocytosis and hypertrophic osteopathy after resection of a renal transitional cell carcinoma producing granulocyte-macrophage colony-stimulating factor in a young bull terrier. *J Vet Intern Med* 2001;15:407-411.
6. Thompson JP, Christopher MM, Ellison GW, Homer BL, Buchanan BA. Paraneoplastic leukocytosis associated with a rectal adenomatous polyp in a dog. *J Am Vet Med Assoc* 1992;201(5):737-8.
7. Knottenbelt CM, Simpson JW, Chandler ML. Neutrophilic leucocytosis in a dog with a rectal tumour. *J Small Anim Pract* 2000;41(10):457-60.
8. Lappin MR, Latimer KS. Hematuria and extreme neutrophilic leukocytosis in a dog with renal tubular carcinoma. *J Am Vet Med Assoc* 1988;192(9):1289-92.
9. Madewell BR, Wilson DW, Hornof WJ, Gregory CR. Leukemoid blood response and bone infarcts in a dog with renal tubular adenocarcinoma. *J Am Vet Med Assoc* 1990;197(12):1623-5.
10. Petterino C, Luzio E, Baracchini L, Ferrari A, Ratto A. Paraneoplastic leukocytosis in a dog with a renal carcinoma. *Vet Clin Pathol* 2011;40(1):89-94.
11. Chinn DR, Myers RK, Matthews JA. Neutrophilic leukocytosis associated with metastatic fibrosarcoma in a dog. *J Am Vet Med Assoc* 1985; 186(8):806-9.
12. Childress MO. Hematologic abnormalities in the small animal cancer patient. *Vet Clin North Am Small Anim Pract* 2012;42(1):123-55.
13. Cooper BJ, Valentine BA. Tumors of muscle. In: Meuten DJ, editor. *Tumors in domestic animals*. 4th ed. Ames (IA): Iowa State Press; 2002. p. 319-63.
14. Head KW, Else RW, Dubielzig RR. Tumors of the alimentary tract. In: Meuten DJ, editor. *Tumors in domestic animals*. 4th ed. Ames (IA): Iowa State Press; 2002. p. 401-81.
15. Barger AM, Skowronski MC, MacNeill AL. Cytologic identification of erythrophagocytic neoplasms in dogs. *Vet Clin Pathol* 2012;41(4):587-89.