Neutrophil Counts and Morphology in Cats: A Retrospective Case-Control Study of 517 Cases

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ABSTRACT

Neutrophil count and morphological abnormalities are common in ill cats. This retrospective study examined the associations between these parameters and clinical and clinicopathologic findings, morbidity, mortality and the final diagnoses in a large population of ill cats, in a teaching hospital setting. The study included 517 cats, divided into three groups based on their neutrophil count; neutropenia (26 cats, 5%), within reference interval (WRI, 313 cats, 61%) and neutrophilia (178 cats, 34%). Occurrence of neutrophilic left shift and cytoplasmic toxicity was recorded. There were significant (P<0.05) group differences in concentrations of albumin, total protein, globulin, urea and bilirubin, aspartate aminotransferase and creatine kinase activities, and in frequencies of sepsis (P<0.0001), high rise syndrome (P=0.014), acute kidney injury (P=0.01), peritonitis (P=0.001), chronic kidney disease (P=0.023), pleural effusion (P=0.0002), pyothorax (P=0.012) and feline immunodeficiency virus (FIV) infection (P=0.02). The frequency of neutrophilia was unexpectedly high in FIV-infected cats (17/29, 59%). Neutrophil cytoplasmic toxicity and left shift occurred in 57% and 10% of the cats, respectively. Both were significantly more frequent in cats with neutrophilia or neutropenia compared to the group with neutrophil count WRI (P<0.0001). Mortality rate was higher (P<0.0001) in cats with neutropenia or neutrophilia. The area under the receiver operating characteristic curve of the neutrophil count as a predictor of mortality was 0.61 (95% confidence interval 0.55-0.68). Cost of treatment and hospitalization duration significantly differed between groups. Presence of left shift was significantly associated with mortality (P=0.004). Concurrent neutropenia or neutrophilia with cytoplasmic toxicity and left shift was significantly associated with mortality.

Key words: feline, hematology, leukocytosis, leukopenia, feline immunodeficiency virus, left shift.

INTRODUCTION

Neutrophils play a pivotal role in both the innate and adaptive immune responses, providing crucial defense against microorganisms, most importantly bacteria, and participate in responses to tissue trauma, inflammation and neoplasia (1-3). There are mature neutrophil reserves within the bone marrow, and in the peripheral blood central and marginated pools. In cats, the latter is three times larger than the former pool (4). Neutrophil counts are influenced by bone marrow production and release rates, and shifts between peripheral blood pools and the tissues (1-4). Since neutrophils constitute the majority of blood leukocytes in cats, leukocyte (WBC) count changes usually parallel changes in the absolute neutrophil counts (5).

Absolute neutropenia (neutrophils <2.5×10^3/μL) may result from increased tissue demand, decreased myelopoiesis and neutrophil release, distribution alterations and immune mediated destruction, although the latter has yet to be described in cats (3, 5, 6). Absolute neutrophilia (neutrophils >11.5×10^3/μL) may be physiologic (i.e., "pseudo-neutrophilia") or pathologic (3-5). The former, albeit temporary, is common, especially in young cats, resulting from excitement and epinephrine-

mediated neutrophil detachment from the endothelium into the central pool (5). Mature neutrophilia is also a hallmark of stress leukogram, a glucocorticosteroid-induced response, although it is less prominent in cats compared to dogs (5).

Pathologic neutrophilia occurs in response to inflammation, when bone marrow granulopoiesis and neutrophil release exceed tissue neutrophil demand. Its magnitude is determined by the etiology, location, severity and duration of inflammation (2-5, 7). Necrosis, infectious, immune-mediated and neoplastic disorders often result in pathologic neutrophilia (2-5, 8). Hyposegmentation (i.e., left shift), and infrequently the presence of early neutrophil precursors in the peripheral blood (i.e., metamyelocytes and myelocytes) may occur with pathologic neutrophilia due to dwindling bone marrow mature neutrophil reserves (5). Generally, the more acute and severe tissue neutrophil demand is, the greater is the left shift (4, 5). When segmented neutrophil counts exceed those of immature, mostly band neutrophils, left shift is termed 'regenerative'. When the band neutrophil count exceeds the mature neutrophil count, the condition is referred to as a 'degenerative' left shift. Typically, inflammation in cats results in neutrophil counts in the range of 10,000 to 30,000 cells/μL, although counts exceeding 30,000 cells/μL, and less frequently in excess of 75,000 cells/μL (i.e., leukemoid reactions), may be observed (4, 5, 8).

Pathologic neutrophil nuclear and cytoplasmic morphological abnormalities (i.e., "toxic changes") often occur in diseases that lead to maturation defects within the bone marrow. Nuclear toxic changes include hyposegmentation, vacuolation and nuclear ring-forms. Cytoplasmic toxic changes include Döhle bodies and basophilia (due to endoplasmic reticulum aggregation, RNA and ribosomal retention), granulation (due to increased permeability of neutrophil granules to dyes or due to mucopolysaccharide accumulation) and vacuolation (due to auto-digestion or organelle and granule membrane lesions) (4, 5, 7). Toxic changes are often observed before leukogram changes or left shift occur, and are therefore early sensitive markers of inflammation, and may constitute the only hematological abnormality in ill cats (9, 10). These changes are semi-quantitated based on both the proportion of affected neutrophils, and subjective evaluation of the severity of the morphological abnormalities (9, 10). In cats, the severity of cytoplasmic toxicity is positively associated with longer hospitalization and higher disease severity, but not with increased mortality (10).

To date, there are no large-scale studies investigating the clinical significance of neutrophilia and neutropenia in ill cats in general. Previous studies have described neutrophil count alterations in certain diseases, including feline immunodeficiency virus (FIV) infection and sepsis (11-17). Etiologies and prognoses associated with absolute neutrophil counts have been investigated in a small number of studies, mostly focusing on marked neutrophil count abnormalities, thereby limiting analyses to a rather narrow set of conditions (8, 18). The aim of this study was therefore to examine the association of neutrophil count and morphology with clinical and clinicopathologic findings, morbidity, mortality and final diagnoses in a large population of ill cats in a hospital setting. Our main hypothesis was that neutrophil counts and morphology are useful diagnostic and prognostic tools in ill cats.

MATERIALS AND METHODS

Selection of cats and data collection

Medical records of cats presented to the Hebrew University Veterinary Teaching Hospital (HUVTH) between 1999 and 2005 were reviewed retrospectively. Cats with complete medical records were included if a complete blood count and blood smear examination were performed at presentation. Data collected included the signalment, history, clinical and clinicopathologic findings, diagnoses, hospitalization period, treatment cost and survival at 30-day post discharge.

Definitions

Cats were divided into three groups based on their neutrophil counts: within reference interval (WRI; 2.5-11.5×10^3/μL, based on the HUVTH Diagnostic Laboratory RI), neutropenia and neutrophilia. Left shift was defined as a neutrophil band count above 1,000/μL, when total neutrophil counts were within or above reference interval (RI), or when bands consisted of more than 10% of the neutrophils, in neutropenic cats. Left shift was defined as degenerative when the neutrophil band count was greater than the mature neutrophil count. When the band count was lower compared to the mature neutrophil count, left shift was defined as regenerative**.** Non-survivors included cats that died or were euthanized during hospitalization or within 30 day post discharge.

Laboratory tests

Blood samples for complete blood cell counts (CBC) and serum biochemistry were collected at presentation in potassium-EDTA and red-top tubes with gel-separators, respectively. CBC was performed within 30 minutes from collection (impedance hematology analyzers Abacus or Arcus, Diatron, Wien, Austria). Fresh blood smears were air-dried and stained (Modified Wright's stain, Bayer Hematek 2000 Slide Stainer, Bayer Diagnostics, Elkhart, IN, USA). A 100-200 manual differential leukocyte count was performed. Neutrophil cytoplasmic toxic changes were semi-quantitated as previously described (10). Samples for chemistry were allowed to clot, centrifuged, and either analyzed immediately or stored at 4°C, and analyzed within 24 hours from collection using a wet chemistry analyzer (Cobas-Mira, Roche, Mannheim, Germany, at 37°C).

Statistical analysis

The data distribution pattern of quantitative variables was analyzed using the Shapiro-Wilk test. Comparison of normally distributed quantitative variables between the three groups was done using the ANOVA, and when significant, *post hoc* Student's *t*-tests with Dunnett's correction were used to compare each two-group pair. Non-normally distributed variables were compared between three groups using the Kruskal-Wallis test, and when significant, *post hoc* comparisons of each group-pair were made using the Mann-Whitney *U*-test with Bonferroni's correction. Chi-square and Fisher's exact tests were used to compare categorical variables between groups. Receiver Operator Characteristic (ROC) analysis, with its area under the curve (AUC) and 95% confidence interval ($CI_{95\%}$), was performed to assess several variables as predictors of mortality. To further assess the relationship between neutrophilia and mortality, the neutrophilia group was divided into quartiles, which were then treated as categorical variables. Logistic regression analysis was then performed, using the cats with neutrophil count WRI as a reference category. All tests were two-tailed. A $P \leq 0.05$ was considered significant. Statistical analyses were performed using a statistical software package (SPSS 17.0, SPSS Inc., Chicago, IL).

RESULTS

The study included 517 cats, with a mean age of 6.2 years (standard deviation (SD) 4.8). Sex was documented in 515 cats. There were 290 males (56.3%; 167 neutered, 57.5%) and 225 females (43.7%; 131 neutered, 58.2%). There were no significant age and sex differences between groups. There were 313 cats (60.5%) with neutrophil counts WRI, 178 (34.4%) with neutrophilia and 26 (5%) with neutropenia. The WBC count was WRI in 324 cats (62.9%), while 154 (29.9%) had leukocytosis, and 37 (7.2%) had leukopenia. There was a significant ($P<0.0001$) group difference in the WBC, with significant ($P<0.017$) *post hoc* differences between each pair of groups. The highest WBC was observed in the neutrophilia group, followed by the group with neutrophil count WRI and the neutropenia group (Table 1). There were no significant group differences in other hematological parameters.

There were significant ($P<0.05$) group differences in concentrations of albumin, total protein (TP), globulin, urea, total bilirubin (TB), the albumin to globulin (A/G) ratio, activities of aspartate aminotransferase (AST) and creatine kinase (CK), and in frequencies of hyperbilirubinemia, hyperglobulinemia, hypoalbuminemia, hypoproteinemia, hypophosphatemia, hypocalcemia, decreased A/G ratio, and increased AST and CK activities (Table 1). Median albumin and TP concentrations were significantly lower in the neutropenia group, while median globulin concentration and frequency of hyperglobulinemia and decreased serum A/G ratio were significantly higher in the neutrophilia group. Hypoalbuminemia was significantly more common in the neutrophilia and neutropenia groups compared to the group with neutrophil counts WRI (Table 1).

There were significant differences in the frequencies of certain diagnoses between groups, including sepsis ($P<0.0001$), high rise syndrome (HRS, $P=0.014$), acute kidney injury (AKI, $P=0.01$), FIV infection ($P=0.02$), peritonitis ($P=0.001$), chronic kidney disease (CKD, $P=0.023$) and pleural effusion ($P=0.0002$) (Table 2). The frequency of pyothorax was similar in the neutropenia and neutrophilia groups (3.8% and 3.3%, respectively), but was significantly ($P=0.012$) higher compared to the group with neutrophil counts WRI (Table 2).

The frequencies of neutrophil cytoplasmic toxicity and left shift in the whole study population were 57% and 10%, respectively. Both were significantly more common in cats with neutrophilia or neutropenia compared to the group with neutrophil counts WRI (Table 3). Left shift was almost always (95%) regenerative; three cats had degenerative left shift (two with pyothorax and one with hepatic lipidosis and pancreatitis), and only one of them survived. The mortality rate among cats with

Table 1: Serum biochemistry and hematological results* of 517 cats grouped by their absolute neutrophil count

Parameter (units)		Neut[1]<RI[2]	Neut[1] WRI[3]	Neut[1]>RI[2]	All cats	P value	RI[2]
Albumin (g/dL) ^	n	20	183	106	309		
	Median	2.5[a]	2.9[b]	2.6[a,b]	2.8	0.015	
	(range)	(1.1-3.8)	(1.4-3.9)	(1.5-6.7)	(1.1-6.7)		3.0-4.6
	%<RI[2]	75	56	70.8	62.5		
	%>RI[2]	0.0	0.0	0.9	0.3	0.019	
Total Protein (g/dL) ^	n	20	181	106	307		
	Median	6.3[a]	7.3[b]	7.0[b]	7.1	0.004	
	(range)	(3.4-9.3)	(4-10)	(4.2-11)	(3.4-11)		5.2-8.0
	%<RI[2]	20.1	3.3	7.5	5.9		
	%>RI[2]	10.0	22.1	23.6	21.8	0.042	
A:G[4]	n	20	181	106	307		
	Median	0.7[a]	0.7[a]	0.6[b]	0.7	0.011	
	(range)	(0.4-1.2)	(0.3-1.4)	(0.2-7.4)	(0.2-7.4)		0.5-1.7
	%<RI[2]	0.1	13.3	29.0	18.6		
	%>RI[2]	0.0	0.0	2.0	0.6	0.020	
Total bilirubin (mg/dL)	n	20	181	107	308		
	Median	0.82[a]	0.25[b]	0.35[a,b]	0.29	0.011	0.0-0.4
	(range)	(0.11-17.05)	(0.04-18.0)	(0.07-43.4)	(0.04-43.4)		
	%>RI[2]	60.0	30.4	42.0	36.4	0.011	
Urea (mg/dL)^	n	22	234	141	397		
	Median	46.17[a]	49.54[a,b]	57.80[b]	51.50	0.027	
	(range)	(6-193)	(6-679)	(12-630)	(6-679)		25.00-70.00
	%<RI[2]	18.2	5.6	5.0	6		
	%>RI[2]	18.2	26.5	35.5	29.2	0.060	
AST[5] (U/L)	n	20	182	106	308		
	Median	70[a]	39[b]	58[c]	45	<0.001	
	(range)	(14-390)	(3-735)	(1-1500)	(1-1500)		10-50
	%<RI[2]	0.0	0.5	1.9	1.0		
	%>RI[2]	70.0	36.8	61.3	47.4	<0.001	
Creatine kinase (U/L)	n	20	181	105	306		
	Median	781[a]	315[b]	692[a,b]	437	0.028	
	(range)	(6-36100)	(36-330900)	(40-506870)	(6-506870)		50-400
	%<RI[2]	5.0	2.2	1.9	2.3		
	%>RI[2]	55.0	45.0	65.0	52.3	0.010	
Total Calcium (mg/dL)^	n	20	180	104	304		
	Median	8.6	9.2	8.9	9.1	0.200	
	(range)	(7.1-13.4)	(5.3-13.0)	(3.9-13.2)	(3.9-13.4)		8.6-11.9
	%<RI[2]	50	21.7	26.9	25.3		
	%>RI[2]	5.0	1.7	2.9	2.3	0.033	
Phosphorus (mg/dL)^	n	20	179	102	301		
	Median	4.1	4.3	5.0	4.5	0.11	
	(range)	(2.0-6.8)	(0.8-27.0)	(0.42-27.0)	(0.42-27.0)		4.0-7.2
	%<RI[2]	50.0	36.9	23.5	33.2		
	%>RI[2]	0.0	11.7	15.7	12.3	0.04	
Leukocytes ($\times 10^3/mm^3$)	n	26	313	178	517		
	Median	3.6[a]	9.2[b]	20.9[c]	11.5	<0.001	
	(range)	(0.44-23)	(2.9-31.6)	(12.8-150)	(0.44-150)		5.0-16.0
	%<RI[2]	88	5.1	0.0	7.2		
	%>RI[2]	4.2	2.6	81.5	29.9	<0.001	

*) analytes presented if significant ($P \leq 0.05$) frequency or medians differences were observed among groups; 1) absolute neutrophil count; 2) reference interval; 3) within reference interval; 4) albumin to globulin ratio; 5) aspartate aminotransferase; ^, normal distribution; a b c) groups designated by different letters differed significantly ($P<0.017$) in *post hoc* analysis.

Table 2: Frequency of several diagnoses* in 517 cats grouped by their absolute neutrophil count

Diagnosis	Neutropenia n (%)	Neutrophils WRI[1] n (%)	Neutrophilia n (%)	All cats n (%)	*P* value
Sepsis	4 (15.4%)	3 (0.9%)	0 (0%)	7 (1.3%)	<0.0001
Chronic kidney disease	0 (0%)	25 (8%)	25 (14%)	50 (9.7%)	0.023
FIV[2] infection	1 (3.8%)	11 (3.5%)	17 (9.5%)	29 (5.6%)	0.02
Acute kidney injury	3 (11.5%)	4 (1.3%)	5 (2.8%)	12 (2.3%)	0.01
High rise syndrome	4 (15.4%)	8 (2.6%)	5 (2.8%)	17 (3.2%)	0.014
Peritonitis	0 (0%)	0 (0%)	7 (3.9%)	7 (1.3%)	0.001
Pleural effusion	0 (0%)	3 (0.9%)	14 (7.8%)	17 (3.3%)	0.0002
Pyothorax	1 (3.8%)	1 (0.3%)	6 (3.3%)	8 (1.5%)	0.012

*) diseases included if diagnosed in ≥13 cats (2.5% of the study population), and if significant ($P \leq 0.05$) frequency differences were observed among groups; 1) within reference interval; 2) feline immunodeficiency virus.

cytoplasmic toxicity was higher compared to cats with no cytoplasmic toxicity (25%, vs. 19%, respectively), but this did not reach statistical significance ($P=0.12$). The mortality rate among cats with left shift was significantly higher compared to cats in which left shift was absent (38%, vs. 20%, respectively, $P=0.04$). There was a significant ($P<0.0001$) positive association between the occurrence of left shift and cytoplasmic toxicity.

Cost of treatment was significantly different amongst groups. It was significantly ($P<0.017$) higher in the neutropenia group (2002±1214 New Israel Shekel (NIS)) compared to the neutrophilia group, and was significantly ($P<0.017$) higher in the neutrophilia group compared to the WRI group with neutrophil counts (1764±1053 NIS vs. 1451±1011 NIS, respectively). The hospitalization period was also significantly different among study groups, mostly attributable to differences between the neutrophilia group and the group with WRI neutrophil counts ($P<0.017$) (Table 4).

The hospitalization period was significantly ($P=0.0004$) longer in cats with neutrophil cytoplasmic toxicity (n=289; 2.6±2.7 days) compared to cats in which toxicity was absent (n=218; 1.7±2.7 days). Similarly, it was significantly ($P=0.006$) longer in cats with left shift (n=53; 3.4±3.3 days) compared to cats in which left shift was absent (n=454; 2.1±2.6 days). Cost of treatment in cats with neutrophil cytoplasmic toxicity (n=291) was significantly ($P=0.0001$) higher compared to cats in which it was absent (1740±1121 NIS vs. 1389±909 NIS, respectively) and in those with left shift (n=54; 2080±1195 NIS) compared to cats in which it was absent (n=455; 1535±1019 NIS; $P=0.003$).

The mortality rate was similar in the neutrophilia and neutropenia groups (32.0% and 30.7%, respectively), and was significantly ($P<0.0001$) higher compared to the group with neutrophil count WRI (15.7%). All four septic cats in the neutropenia group either died or were euthanized, while all three septic cats in the group with neutrophil count WRI survived.

The area under the ROC curve of the neutrophil count serving as a predictor of outcome was 0.61 ($CI_{95\%}$ 0.55-0.68). The optimal cut-off point, which is associated with the fewest misclassifications, was 9.6×10^3cell/μL, corresponding to sensitivity and specificity of 58% and 59%, respectively.

Table 3: Occurrence of neutrophil cytoplasmic toxic changes and left shift in 517 cats grouped by their absolute neutrophil count

Group	Neutropenia n (%)	Neutrophils WRI[1] n (%)	Neutrophilia n (%)	All cats n (%)	*P* value
Toxicity[2]	18 (69.2%)	155 (49.5%)	124 (69.7%)	297 (100.0%)	
No Toxicity[2]	8 (30.8%)	158 (50.5%)	54(30.3%)	220 (100.0%)	
Left Shift[3]	6 (23.1%)	16 (5.1%)	32 (18.0%)	54 (100.0%)	<0.0001
No Left Shift[3]	20 (76.9%)	297 (94.9%)	146 (82.0%)	463 (100.0%)	
All cats	26 (100.0%)	313 (100.0%)	178 (100.0%)	517 (100.0%)	

1) within reference interval; 2) neutrophil cytoplasmic toxic changes; 3) neutrophil band count >1,000/μL when total neutrophil count was within or above reference interval, or above 10% of the total neutrophil count in cases with neutropenia.

Table 4: Survival, duration of hospitalization and treatment cost in 517 cats grouped by their absolute neutrophil count

Parameter		Neutropenia	Neutrophils WRI[1]	Neutrophilia	All cats	*P* value
Hospitalization (days)*	n	26	307	174	507	0.002
	Mean±SD[2]	2.2±2.4	1.9±2.6[a]	2.8±2.9[a]	2.2±2.7	
Cost* (NIS)	n	26	307	176	509	0.001
	Mean±SD[2]	2002 ±1214[a]	1451±1011[b]	1764±1053[c]	1587±1049	
Survivors	n (%)	18 (69.3)	257 (84.3)	119 (68.0)	394 (77.8)	<0.0001
Non survivors	n (%)	8 (30.7)	48 (15.7)	56 (32.0)	112 (22.2)	

1) within reference interval; 2) standard deviation; *) normal distribution; a b c) groups designated by different letters differed significantly (P<0.017) in *post hoc* analysis.

When the neutrophilia group was divided into quartiles, there was no significant association between quartile and mortality rate. The mortality rate was examined in cats presenting none, one, two, or three of the following: left shift, toxicity and neutrophil count abnormalities (i.e. neutropenia or neutrophilia). The mortality rates were 16%, 17%, 33% and 42% when none, one, two or three of these three phenomena occurred concurrently, and these differences were significant (P <0.01) among all subgroups.

DISCUSSION

Physiologic and pathologic neutrophil count abnormalities are common in cats, reflecting hematopoietic and immune system response to an array of molecular signals and cytokines (1-4). Neutrophil count alterations have been extensively studied in humans; however, relatively little has been published regarding this subject in cats, with most studies focusing on changes in neutrophil counts in specific diseases. Only one study of 29 cats has investigated the associations between neutropenia, its possible etiologies and mortality (18), while another has characterized cats presenting with extreme neutrophilic leukocytosis (8). The present study characterized the neutrophil counts and occurrence of cytoplasmic toxicity and left shift in a large ill-cat population in a hospital setting. It is the largest one of its kind, and the first to evaluate the neutrophil count in general, rather than focusing on particular diseases.

Excluding the neutrophil and WBC counts, there were no other significant hematological differences between the three groups of cats. The latter correlated with the neutrophil count, which was to be expected, because neutrophils comprise the largest blood leukocyte subpopulation in cats (5).

The higher frequency of hyperglobulinemia concurrently with hypoalbuminemia in the neutrophilia group most likely was due to a higher frequency of inflammatory conditions in this group. Inflammation is characterized by an acute phase response, where serum positive acute phase proteins (i.e., α- and β-globulins) and immunoglobulins (mostly γ-globulins) concentrations increase, while the hepatic production of albumin (a negative acute phase protein) is decreased, although the latter is not as well established in cats compared to dogs (19, 20). Additionally, gastrointestinal or urinary albumin loss, capillary leakage or hepatic insufficiency may have also contributed to the observed higher frequency of hypoalbuminemia in this group.

Serum CK activity was significantly higher in cats with neutrophilia or neutropenia. Albeit highly specific to muscle (striated or myocardial) injury, CK activity is also very sensitive to any insult, and mild to moderate increases have been demonstrated in a plethora of conditions in ill cats (21, 22). Increased CK activity may result from intramuscular injections, trauma, restraint, as well as spurious increases in cases of *in vitro* hemolysis (21-23). There were no significant differences in the prevalence of etiologies that induce muscle injury among groups, with exception of HRS, which was more frequent in the neutropenia group. Therefore, increased CK activity may potentially serve as a marker of a more severe disease, despite its low specificity (21).

The neutropenia group showed significantly higher frequencies of sepsis, HRS and AKI. Neutropenia was recorded in 4/7 septic cats, and the remaining three had neutrophil counts within the reference interval**.** The definition of sepsis varies, and requires the presence of clinical signs compatible with a 'systemic inflammatory response syndrome' (SIRS), in conjunction with histopathological or culture confirmation of infection (11, 24). The classical hemodynamic changes observed in other species during SIRS, however, may not apply

to cats (11). In one retrospective study of neutropenic dogs and cats, sepsis was the presumptive cause of neutropenia in 17% of the cats (18). In another study of 29 septic cats, none had leukopenia, but differential leukocyte counts were not performed, thereby limiting interpretation of the leukogram (11). Contrary to the previous study, leukocytosis and neutrophilia were not documented in septic cats in this study, probably because the criteria used to define sepsis in this retrospective study were variable. Septic, neutropenic cats in this study had a 75% mortality rate, in contrast with 100% survival rate of the septic cats in the group with neutrophil count WRI. It would therefore seem that neutropenia is a risk factor for mortality in cats with sepsis. The mortality rate of cats with sepsis in previous studies ranged between 22% and 79% (12, 17, 25, 26). Although there was no association between neutrophil counts and mortality in one report (25), in another, neutrophil counts were lower, albeit insignificantly (P=0.058) in non-survivor cats with pyothorax (17).

Most cats with HRS herein had neutrophil counts WRI. The frequency of HRS, however, was significantly higher in the neutropenia group (4 cats) compared to the other two groups. Neutropenia was not associated with mortality in HRS, in agreement with a previous report (27). Multiple organs may be affected in cats with HRS; thereby, tissue demand for neutrophils is often increased, resulting in temporary depletion of blood reserves, and neutropenia (1, 2).

The frequency of neutrophilia was unexpectedly high in FIV-infected cats in this study (17/29 cats, 58.6%), while neutropenia was detected only in a single FIV-positive cat (3.4%), in contrast to previous reports, in which neutropenia and leukopenia were consistent findings in such cats (13, 14, 18, 28). With exception of cats in the asymptomatic stage, all other stages of FIV infection, (i.e., acute, lymphadenopathy and acquired immunodeficiency syndrome-related complex stages) are associated with neutropenia and leukopenia (29, 30). Neutrophilia occurs in 23% to 35% of FIV-positive cats, often in association with concurrent purulent bacterial diseases (30). Herein, 6/17 FIV-positive cats with neutrophilia (35%) had concurrent inflammatory diseases, while the rest had concurrent non-inflammatory disorders (e.g. non-septic pleural effusion, hepatic lipidosis and CKD). This finding suggests that the immune response of these cats to inflammatory stimuli or stress was appropriate, as manifested by an appropriate neutrophil response.

While cytoplasmic toxicity was observed in 57% of the cats in this study, left shift was detected in only 10% of our cats, and only 16% of the cats with cytoplasmic toxicity had concurrent left shift. The discrepancy between these two morphologic phenomena probably resulted from several factors. Cytoplasmic toxicity may occur during earlier disease stages in cats, even in milder disorders, compared to dogs, when changes in mature or band neutrophil counts are absent (10). Cytoplasmic toxicity is also associated with drug administration and certain metabolic derangements (e.g. hyperbilirubinemia and hypoxia), which are less likely to be associated with left shift (10, 31). Left shift, on the other hand, mostly results from increased tissue neutrophil demand, with inappropriate bone marrow granulopoiesis, leading to immature neutrophil release (2, 4, 5). Appearance of cytoplasmic toxicity may therefore serve as a harbinger of disease, preceding the occurrence of quantitative leukogram abnormalities or the occurrence of left shift. In corroboration with the assumption that cytoplasmic toxicity in cats occurs earlier in the course of disease and in milder conditions, left shift, but not cytoplasmic toxicity, was positively associated with mortality.

The frequency of left shift was significantly higher in the neutropenia and neutrophilia groups, and in only 5% of our cats with left shift the neutrophil counts were WRI. Diffuse infectious etiologies are often involved in processes which lead to increased tissue neutrophil demand, and therefore to left shift. In severe cases, band neutrophils predominate (i.e., degenerative left shift), a negative prognostic indicator (5), and in support of that, 2/3 such cats in our study did not survive.

Treatment cost was highest in the neutropenia group, followed by the neutrophilia group and the group with neutrophil count WRI, which probably reflected the severity of the disease in this cat population. A similar significant trend was observed in the duration of hospitalization, as previously described in cats (10). Neutrophil count abnormalities therefore can serve as a surrogate for the severity of disease. Duration of hospitalization and treatment cost were also significantly higher among cats with neutrophilic cytoplasmic toxicity or left shift compared to cats in which these were absent.

Overall, 517 cats were included in this study, of which 112 (21.7%) either died or were euthanized during hospitalization or within 30 days of discharge. Neutrophil count abnormalities were significantly associated with mortality (with approximately 30% mortality rate in cats with abnormal neutrophils counts) compared to cats in which the neutrophil count was WRI (16%). Nonetheless, when cats with

neutrophilia were divided into quartiles based on their neutrophil count, there was no association between the degree of deviation from upper range interval and the mortality rate. This finding is in disagreement with a previous study, in which extreme neutrophilic leukocytosis was associated with increased mortality (8). Salient differences in the frequencies of several etiologies, notably neoplasia (23% in that study, compared to 8.9% herein) and immune mediated diseases (accounting for 22% of cases in that study, while not observed herein) may account for this discrepancy.

Marked leukocytosis and neutrophilia have been associated with increased mortality in cats, dogs and humans (8, 33, 34). Interestingly, in both humans and cats, leukocytosis and neutrophilia due to non-infectious etiologies carried worse prognoses (8, 34). In a case-controlled retrospective study of 182 dogs with a WBC≥35×10^3/μL, there was a significant negative association between the total neutrophil count (i.e., mature and immature neutrophils combined) and survival (35). Our findings are in agreement with the aforementioned studies, namely that abnormalities in neutrophils counts (i.e., neutropenia and or neutrophilia) are associated with survival. However, based on the present study, the magnitude of deviation in the neutrophil count may be of lesser clinical importance as it was not associated with decreased survival. The relatively low number of neutropenic cats in our study precluded a similar analysis, and therefore this warrants further investigation.

Neutropenia is most commonly associated with increased tissue neutrophil demand or decreased production in small animals; however, although presence of neutropenia often indicates a clinically significant underlying disease, it has not been consistently associated with poor prognosis (4, 5, 6, 18, 32). In humans, its severity and duration are proportionately associated with risk of infection, morbidity and mortality, especially when patients are also febrile (6). Our findings are in agreement with those of a retrospective study of neutropenic dogs and cats, in which 16% of the animals were euthanized and 16% died.

Interestingly, this study demonstrated that a combination of neutrophil count abnormalities (i.e., neutropenia or neutrophilia), presence of neutrophilic cytoplasmic toxicity and left shift (degenerative or regenerative) in cats was associated with increased mortality. Therefore, a combination of changes in both neutrophil count and morphology (i.e., cytoplasmic toxicity and left shift) appears to be a serious negative prognostic indicator. Thus, combining the total neutrophil count along with assessment of neutrophil morphologic abnormalities is a clinically useful prognostic tool.

Despite the association between neutrophil count abnormalities and mortality, ROC analysis showed that the overall performance of the total neutrophil count as a predictor of the outcome (i.e. survival or death) in cats was moderate at best, likely because other factors affect survival, notably the underlying etiology. Therefore, although the neutrophil count is significantly associated with survival, as a sole predictor, it has only moderate sensitivity and specificity for outcome prediction.

Our study has several limitations. First, due to its retrospective nature, missing data were common, thereby limiting the statistical analyses, potentially contributing to type-II errors. Second, although this study included a rather large population, our results should be applied with caution to other populations, as the present study population may not necessarily reflect other populations in different clinical settings (e.g. in this study population immune-mediated diseases were not recorded). Third, due to the large number of different primary diagnoses, the number of cases for each was limited, thereby rendering some statistical comparison underpowered. Fourth, in certain conditions (e.g. sepsis) diagnostic criteria were not pre-set, which may have introduced variability and misdiagnoses. Lastly, in such a large scale study, over 100 statistical analyses were made. Possibly, some significant results may have occurred due to pure chance (type-I error). Therefore, our results should be interpreted with caution, and ideally should be validated by future prospective large-scale studies of different cat populations.

In conclusion, total neutrophil count abnormalities are associated with different outcomes in cats. The magnitude of deviation in the neutrophil count may however be of lesser clinical importance. Left shift is positively associated with decreased survival. Combining the total neutrophil count with neutrophil morphologic abnormalities (i.e., cytoplasmic toxicity and left shift) appears to be a clinically useful tool for prognostication in cats.

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