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Degenerative Left Shift as a Prognostic Tool in Cats

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Background: A degenerative left shift (DLS) is reported to be a poor prognostic indicator in dogs and cats. Limited data in dogs and no studies in cats have been published to investigate this claim.

Hypothesis/Objectives: To characterize the feline population affected by DLS and to determine if the presence and severity of DLS are associated with increased risk of euthanasia or death.

Animals: One hundred and eight cats with DLS (cases) and 322 cats without DLS (controls) presented to the University of California, Davis Veterinary Medical Teaching Hospital between April 1, 1995 and April 1, 2010.

Methods: Retrospective case–control study. All cases had a CBC performed within 24 hours of presentation in which immature granulocytic precursors exceeded mature neutrophils. Controls were matched by year of presentation and primary diagnosis. Survival analysis was used to determine risk of death or euthanasia from DLS and other potential predictors of outcome.

Results: Cases were more likely to die or be euthanized in hospital compared to controls (60/108 [56%] versus 107/322 [33%]). DLS was a significant predictor of death or euthanasia in hospitalized cats in both univariate and multivariate analysis (hazard ratio, 1.57; 95% confidence interval, 1.13–2.18). Trend analysis showed an increasing trend in the hazard of euthanasia or death with increasing severity of DLS.

Conclusions and Clinical Importance: Cats with DLS are 1.57 times more likely to die or be euthanized in hospital than cats without DLS. In addition, increasing severity of DLS is associated with increased likelihood of death or euthanasia.

Key words: CBC; Feline leukemia virus; Neutrophil; Sepsis; Survival.

Neutrophils are the most abundant circulating leukocyte in cats and play a fundamental role in the innate immune response.[1,2] Neutrophil homeostasis is maintained by a balance among neutrophil production, bone marrow storage, intravascular margination, clearance, and destruction. Neutrophil production is limited by physiologic capacity, but demand for neutrophils from an inflammatory nidus can vary markedly. To compensate for any potential discrepancy between supply and potential tissue requirements, the feline bone marrow has a large storage pool of neutrophils.[3,4] The marrow storage pool of neutrophils can be depleted in times of increased demand. When this reserve is exhausted, granulocytic precursors are released into circulation, and the leukogram is defined as left shifted.[5] Neutrophilia with a left shift generally suggests that the bone marrow is able to match supply of neutrophils with the demands of an inflammatory nidus. In contrast, leukopenia or higher numbers of granulocytic precursors than mature neutrophil suggests an inability of the bone marrow to meet tissue demands.[5–7]

A degenerative left shift (DLS) occurs when immature granulocytic precursors outnumber mature neutrophils.[6–10] This is the current, and most widely accepted definition, but one possible limitation in the interpretation of DLS using such terms is lack of consideration of the total leukocyte number. It is unknown if a DLS in the face of neutrophilia would have the same implications as a DLS with concurrent neutropenia. Studies have shown that leukopenia and marked neutrophilia can be associated with increased mortality in cats.[4,11,12]

Abbreviations:

CBC	complete blood count
DLS	degenerative left shift
N/I	mature neutrophil to immature neutrophil ratio

In species that have a large storage pool of neutrophils, a DLS is commonly reported to confer a poor prognosis.[6–10] Currently, only a single study in dogs has been published to investigate this hypothesis.[13] In that study, it was found that hospitalized dogs with a DLS were nearly twice as likely to die or be euthanized as control dogs with the same disease diagnosis. A retrospective study on neutrophil counts in cats found that the presence of a left shift was significantly associated with mortality, but regenerative (n = 51) and degenerative (n = 3) left shifts were analyzed collectively.[4] Similar to dogs, cats have a large storage pool of neutrophils, and intravascular compartmentalization of neutrophils also is similar when comparing these species.[3] The intravascular distribution of neutrophils in cats, however, is unique in that approximately 70% of total blood neutrophils reside in the marginated neutrophil pool, compared to 50% in both dogs and humans.[3,14,15]

The objectives of this study were to characterize the feline population affected by DLS presenting to a veterinary teaching hospital, and to determine whether or not the presence or severity of DLS increased the risk of in-hospital death or euthanasia. Given previous

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findings in dogs, we hypothesized that an increased risk of death or euthanasia would be associated with DLS, but the severity of the DLS would not affect outcome.

Materials and Methods

Medical records from an electronic database were retrospectively reviewed for cats having an initial CBC performed within 24 hours of presentation to the William R. Pritchard Veterinary Medical Teaching Hospital at the University of California, Davis between April 1, 1995 and April 1, 2010 that showed the sum of immature granulocytic precursors and unclassifiable cells to be greater than mature neutrophils. Criteria for inclusion into the study included a CBC performed within 24 hours of presentation to the hospital that showed an immature granulocyte count higher than that of mature neutrophils, defined as a DLS. Only results from the first CBC were used. Cases were excluded if the mature neutrophil count exceeded the immature neutrophil count, the disease diagnosis was unknown, and if any chemotherapeutic agents were used within 1 month of presentation. Cats with neoplastic blast cells or precursors in the granulocytic line were not included as cases, because the pathogenesis of the DLS in these cats was considered different from the other cases.

Controls subsequently were chosen based on the final diagnosis of cases and matched to within 1 year of the corresponding case. For inclusion into the study, controls were required to have had a CBC performed within 24 hours of presentation. Controls were excluded from the study if DLS was seen at any stage of hospitalization. Three controls were randomly chosen using a random number generator[16] for each case, as available, to increase study power and efficiency.[17]

Data retrieved from the medical records included signalment (age, sex, neuter status, and breed), presence or absence of prior treatment, clinical diagnoses, CBC results, hospitalization duration, and discharge status (alive, dead, or euthanized). Hematologic parameters from the automated CBC included total white blood cell and mature neutrophil count. From April 1, 1995 to September 1, 2001, hematologic parameters were analyzed with a Baker Systems 9110 Plus Hematology Analyzer[a] and from September 1, 2001 to April 1 2010, hematologic parameters were analyzed with an ADVIA 120 Hematology System[b] using the species-specific setting in the MultiSpecies System Software.[c] Manual leukocyte differential counts of 200 cells were performed on all cases to obtain numbers of band neutrophils, metamyelocytes, myelocytes, and promyeloyctes as well as any unclassifiable cells. Toxicity also was assessed according to previously reported guidelines.[18] Differential counts were performed by various diplomates of the American College of Veterinary Pathologists (Clinical Pathology) or technicians and clinical laboratory scientists licensed by the State of California. Classification of granulocytic precursor cells in the laboratory was based on previously published recommendations.[19,20] All unclassifiable cells were reviewed by board-certified veterinary clinical pathologists at the time of submission.

Statistical Analyses

DLS was the main predictor variable for this study, although predictor variables related to neutrophil kinetics and toxicity also were evaluated, including total neutrophil count (categorical: neutrophilia, normal neutrophil count, or neutropenia), an indicator for shift severity (presence or absence of earlier neutrophil precursors [metamyelocytes, myelocytes, promyelocytes]), and neutrophil toxicity (categorical: marked, moderate, slight, or no toxicity). Additional potential predictor variables included age category (≤3 years, >3 to ≤6 years, >6 years to ≤9 years, >9 years to ≤12 years, and >12 years), sex, neuter status, previous treatment status (yes or no), breed category (domestic shorthair, domestic medium or longhair, and purebred or purebred mix), and disease category (septic peritonitis, FeLV, pyothorax, wounds, and other).

Descriptive analyses for all variables were performed. In order to assess potential predictor variables for independence, Pearson's Chi-squared test was used. Both univariate and multivariate survival analyses were conducted using days of hospitalization as the time-to-event variable and death or euthanasia, versus alive at the time of discharge, as the event of interest. Univariate survival analysis was conducted by the Kaplan-Meier method, and significant differences between groups were assessed by the log-rank test. For multivariate analysis, a stratified Cox proportional hazards model was constructed, including those variables statistically significant in univariate analysis and those of biologic importance, yielding HRs and 95% CIs. Chi-square testing of the scaled Schoenfeld residuals was used to assess the validity of the proportional hazards assumption, whereas chi-square testing of likelihood ratios was used to assess the no-interaction assumption of the stratified model.[21] Variables with P-values ≤.05 were considered statistically significant.

In addition, trends for increasing hazard of death or euthanasia with increasing severity of DLS were analyzed by categorizing the mature neutrophil to immature neutrophil (N/I) ratio into quartiles (cut points at 0.37, 0.58, 0.77) for all DLS cases. All analyses were conducted by R version 3.0.1.[22]

Results

One hundred and eighteen cats were identified as potential cases based on the initial database search for patients with the sum of immature granulocytes and unclassifiable cells exceeding mature neutrophils. Ten cases were excluded, the majority being FeLV-positive cats with neoplastic blast cells contributing to the granulocytic line (n = 6), with others including chemotherapy within 1 month of presentation (n = 2), unknown disease diagnosis (n = 1), and mature neutrophils exceeding immature neutrophils after review of unclassifiable cells, which were lymphoid in origin (n = 1). A total of 108 cats were included as cases, along with 322 cats selected as controls.

Descriptive data for all animals, also broken down by cases versus controls, are summarized in Table 1. Sixteen disease diagnoses were identified for cases. However, 12 of those categories had sample sizes of only 1–8 cases and therefore were grouped together in the "Other" disease category; diseases represented in this category with the total number of cases and controls in parentheses include pancreatitis (n = 32), pyelonephritis (n = 32), pneumonia (n = 28), gastroenteritis (n = 16), hepatopathy (n = 12), feline immunodeficiency virus (n = 8), retroperitoneal abscess (n = 4), arterial thromboembolus (n = 4), feline infectious peritonitis (n = 4), pyometra (n = 4), lymphoma (n = 4), and methimazole toxicity (n = 4).

For both cases and controls, the average age was 7 years, and the average number of days hospitalized was similar: 4.2 (range, 1–21) for cases and 3.6 (range, 1–22) for controls. Breed and neuter status categories were similarly distributed between cases and controls. Cases were more likely to have marked neutrophil toxicity (51% for cases versus 10% for controls) and less

Table 1. Descriptive statistics for study variables for both cats with (cases) and without (controls) DLS.

Variable	Overall Count (%)	Cases (N = 108)	Controls (N = 322)
Sex			
Male	237 (55)	66 (61)	171 (53)
Female	191 (45)	42 (39)	149 (47)
Neuter status			
Yes	379 (89)	96 (89)	283 (88)
No	49 (11)	12 (11)	37 (12)
Discharge status			
Dead/euthanized	167 (39)	60 (56)	107 (33)
Alive	263 (61)	48 (44)	215 (67)
DLS			
Present	108 (25)	108 (100)	0
Absent	322 (75)	0	322 (100)
Neutrophil count category			
Neutropenia	78 (18)	54 (50)	24 (7)
Normal neutrophil count	144 (34)	43 (40)	101 (32)
Neutrophilia	208 (48)	11 (10)	197 (61)
Shift severity			
Precursor absence	331 (77)	51 (47)	280 (87)
Precursor presence	99 (23)	57 (53)	42 (13)
Neutrophil toxicity			
None	135 (32)	8 (7)	127 (40)
Slight	99 (23)	8 (7)	91 (28)
Moderate	108 (25)	37 (35)	71 (22)
Marked	87 (20)	54 (51)	33 (10)
Disease category			
Septic Peritonitis	90 (21)	32 (21)	67 (21)
Pyothorax	72 (17)	18 (17)	54 (17)
FeLV	60 (14)	15 (14)	45 (14)
Wounds	56 (13)	14 (13)	42 (13)
Other	152 (35)	38 (35)	114 (35)
Previous treatment			
Yes	142 (33)	21 (19)	121 (38)
No	288 (67)	87 (81)	201 (62)
Breed			
Domestic Shorthair	274 (64)	75 (69)	199 (62)
DMH/DLH	88 (20)	17 (16)	71 (22)
Other	68 (16)	16 (15)	52 (16)
Hospital days			
Range (average)	1–22 (3.8)	1–21 (4.2)	1–22 (3.6)
Age in years, continuous			
Range (average)	0.4–21.2 (7)	0.4–21.2 (7)	1–18 (7)
Age in years, categorical			
<3	98 (23)	26 (24)	72 (22)
3–6	98 (23)	28 (26)	70 (22)
6–9	74 (17)	18 (17)	56 (18)
9–12	75 (18)	19 (18)	56 (18)
≥12	81 (19)	16 (15)	65 (20)

FeLV, feline leukemia virus; DMH/DLH, domestic medium hair/domestic longhair.

likely to have been previously treated (19% for cases versus 38% for controls). In addition, cases were more likely to die or be euthanized in hospital than controls: 56% (n = 60/108) of cases versus 33% (n = 107/322) of controls.

Potential nonindependence of the following variables was indicated by Pearson's Chi-square tests: neuter status and age category ($P < .001$), disease category and age category ($P < .001$), disease category and toxic neutrophil category ($P < .001$), as well as DLS, neutrophil count, and shift severity categories (each $P < .001$). By univariate survival analysis, 8 variables were identified as significant outcome predictors, including DLS status ($P < .01$), breed category ($P < .01$), shift severity ($P = .01$), neutrophil count category ($P < .001$), and disease category ($P < .001$). Disease category also was evaluated by individual disease groups, and 3 of the 5 groups subsequently were significant, including septic peritonitis ($P = .04$), FeLV ($P < .001$), and pyothorax ($P = .005$). All other variables were nonsignificant. Log-rank test results are summarized in Table 2. Time-to-event analysis indicated that median time in hospital was 5 days for cats with DLS and 9 days for cats without DLS ($P < .01$). The Kaplan-Meier curve, shown in Figure 1, displays the difference in survival probabilities over time between cases and controls.

Trend analysis was performed using the N/I category corresponding to the most severe ratio (0–0.37) as the reference group; these results are summarized in Table 3. Although the comparison of the reference group to the second most severe group (0.37–0.58) was not significant, the trend was significant for the remaining 2 groups, and significant overall ($P < .01$).

A test of the proportional hazards (PH) assumption for the multivariate Cox PH model for all potential predictor variables indicated violation of the PH assumption for both treatment ($P < .01$) and disease category ($P = .02$) as well as a resulting violation for the overall test ($P = .04$). Testing of the PH assumption by individual disease categories indicated that only the septic peritonitis category was in violation ($P = .01$). Upon stratification on the treatment and septic peritonitis variables, the global test was no longer significant ($P = .51$), and no additional variables violated the assumption. Therefore, a stratified Cox PH model was used. Additional testing of the no-interaction assumption of the stratified Cox PH model resulted in no

Table 2. Results for univariate analyses, including the log-rank chi-square test statistics and *P*-values for significant predictor variables, and including specific disease variables, within the feline study population.

Variable	Subcategory	χ^2 Value	*P*-value
DLS		7.5	<.01
Breed category		11.2	<.01
Shift severity		6.3	.01
Neutrophil count		18.4	<.001
Disease group		31.7	<.001
	FeLV	21.6	<.001
	Pyothorax	7.9	<.01
	Septic peritonitis	4.2	.04

χ^2 value, chi-squared value for log-rank test; FeLV, feline leukemia virus.

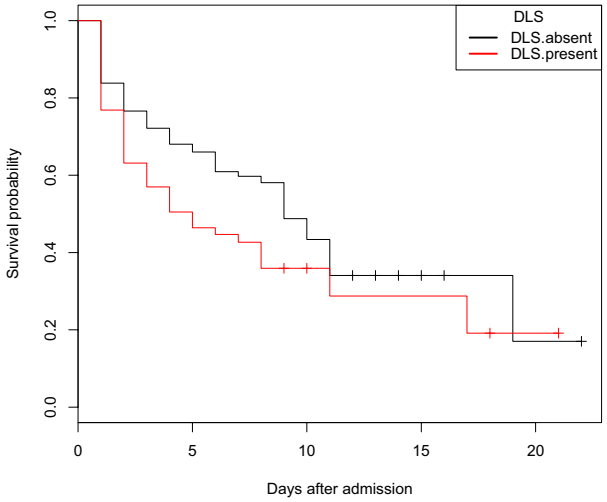

Fig 1. Kaplan-Meier curve demonstrating survival time after admission to the hospital for cats based on DLS status. The DLS.present line represents cases (bottom curve) and the DLS.absent line represents controls (top curve). Median survival time is 5 days for cases and 9 days for controls (P-value <.01).

Table 3. Summary of trend analysis results with decreasing DLS severity, as reflected by the increasing mature/immature neutrophil ratio (N/I) index in the feline case population*.

N/I level	n	HR	SE (coef.)	P-value
0–0.37	27			
0.37–0.58	27	0.64	0.34	.2
0.58–0.77	27	0.43	0.36	.02
0.77–0.98	27	0.24	0.39	<.001
Overall: Likelihood ratio test = 14.8				<.01

*The 0–0.37 N/I level is the referent level; HR, hazard ratio; SE, standard error; n, sample size for each quartile.

improvement in fit by the addition of interaction terms between stratified and nonstratified variables.

The stratified Cox PH model for multivariate survival analysis identified 3 significant predictor variables, including DLS (HR, 1.57; 95% CI, 1.13–2.18; $P < .01$), FeLV (HR, 3.37; 95% CI, 2.15–5.28; $P < .001$), and breed category (HR, 0.75; 95% CI, 0.60–0.94; $P = .01$), as shown in Table 4. Age category was nonsignificant ($P = .10$) but was left in the model because of its general importance in epidemiologic models and influence on health outcomes. All other variables that previously were significant on univariate analysis were nonsignificant in the multivariate model. Interaction terms also were evaluated. None of these terms improved model fit nor were they significant, thus they were not included in the final model.

Discussion

Hospitalized cats with DLS have 1.57-fold increase in the hazard of death or euthanasia compared to hospitalized cats without DLS after adjusting for the effect of age category, sex, neuter status, treatment, breed, left shift severity, neutrophil count, disease category, and degree of neutrophil toxicity. This supports visual evidence in the Kaplan-Meier curve of lower survival probabilities over most of the hospitalization period for those cats with DLS. Although age category was not a significant predictor of outcome, age often is strongly related to disease and mortality, thus it was retained in the final model. Among the 5 disease categories, only FeLV remained a significant predictor in the multivariate model. FeLV also was the most significant univariate predictor ($P < .001$), compared to pyothorax ($P = .05$) and septic peritonitis ($P = .04$), whereas the wounds and 'other' disease categories were nonsignificant in all analyses. Interestingly, a seemingly stronger impact of disease on outcome, as compared to the impact of DLS status, reflects findings from a similar study in dogs with septic peritonitis.[13] Given the long-term impact of FeLV on the immune system, it is perhaps not surprising that FeLV-infected cats presented for hospitalization would have a >3-fold higher hazard of death or euthanasia compared to noninfected cats.

Table 4. Summary of multivariate survival analysis results from the optimized Cox proportional hazards model, stratified by previous treatment status (yes/no) and septic peritonitis (presence/absence), for the feline study population.

Variable	HR	95% CI	P-value
DLS	1.57	(1.13, 2.18)	<.01
FeLV	3.37	(2.15, 5.28)	<.001
Breed category	0.75	(0.60, 0.94)	.01
Age	1.1	(0.98, 1.23)	.1

HR, hazard ratio; CI, confidence interval; DLS, degenerative left shift; FeLV, feline leukemia virus.

In addition to DLS and FeLV, breed category was a significant predictor. Additional investigation of this variable indicated that this significance was found only in the purebred group. Purebred (including purebred cross) cats had a 1.3-fold lower risk of death or euthanasia than other groups (domestic short-, medium-, and long-hair cats). The purebred group only represented 16 of the 108 cases, and the CI was very close to 1 (95% CI, 0.60–0.94). This finding could be an artifact of small sample size and might differ with analysis of a larger data set. Decision to treat by owners of purebred cats also may have affected this result.

The nonindependence suggested by Pearson's Chi-square testing for DLS, neutrophil count, and shift severity is not surprising. Each of these variables is a measure of the degree of change in neutrophils and their precursors. Thus, they represent similar information from each animal. In evaluating all 3 variables, DLS and neutrophil count category were the strongest predictors in separate multivariate models, but DLS provided the better model fit and thus was used in the final multivariate model. In addition, age and neuter status were correlated, presumably because younger animals are less likely to have been spayed or neutered

compared to older animals. The correlation between disease category and neutrophil toxicity also might be expected, given that prevalence of toxicity has been reported to be significantly higher in certain diseases, many of which were seen in this study.[18]

In an interesting contrast to a similar study in dogs,[13] trend analysis showed an increasing trend in the hazard of death or euthanasia with an increasing severity of DLS both overall ($P < .01$) and between 2 of the 3 N/I ratio quartile comparisons. For cats, it seems that not only is the presence of DLS important for predicting outcome, but so is the magnitude of DLS. The N/I ratio is simply a ratio of immature to mature neutrophils, and does not take into account total leukocyte numbers. Although no studies have been performed in cats to validate the N/I ratio, several studies in dogs and humans have shown this ratio to be a more accurate measure of the severity of infection than blood granulocyte count.[23,24] Furthermore, the total neutrophil count and other measures of left shift severity analyzed in this study (presence of granulocytic precursors earlier than bands) did not have a significant effect on outcome.

Because of the retrospective nature of the study, there were limitations in the analyses and interpretations. One concern is observer bias, because this study spanned 15 years during which several technicians and clinical pathologists reviewed the blood smears used for DLS categorization. Band and segmented neutrophils cannot always be distinguished clearly, which might have led to misclassification of patients, especially those with similar band and mature counts. In addition, disease diagnoses often were based on clinical judgment, which makes standardizing diagnoses across time and clinician difficult.

The generalizability of this study is limited to cats presenting to a tertiary referral hospital, and even then, not all DLS cases might have been captured, because not every animal has a CBC performed. Also, as with many retrospective epidemiologic studies, information on additional potential confounding variables was not available or was difficult to measure, such as the impact of preconceived clinician attitudes toward DLS, and the ability of owners to afford treatment. As an additional statistical limitation, it was not possible to estimate the HR for either the treatment category or the septic peritonitis group, because the multivariate analysis was stratified by these variables because of their violation of the PH assumption of the Cox PH model. In univariate analysis, however, treatment was not significant ($P = .78$) and septic peritonitis was only minimally significant ($P = .04$).

Conclusions

Even after controlling for possible confounding factors, cats with DLS are 1.57 times more likely to die or be euthanized in hospital relative to control cats with the same disease. In addition, increased severity of DLS as measured by the N/I ratio results in an increased trend in death or euthanasia in hospitalized cats.

Footnotes

[a] BioChem ImmunoSystems Inc, Allentown, PA
[b] Siemens Healthcare Diagnostics Inc, Tarrytown, NY
[c] Siemens Medical Solutions Diagnostics Inc, Tarrytown, NY

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Conflict of Interest: Authors disclose no conflict of interest.

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