STANDARD ARTICLE

Etiology and outcome of extreme neutrophilic leukocytosis: A multi-institutional retrospective study of 269 dogs

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Abstract

Background: The magnitude of diagnostic abnormalities can influence the perception of clinical outcome. Extreme neutrophilic leukocytosis (ENL) is an uncommon finding caused by markedly increased granulopoiesis. A lack of recent, large-scale studies limits our understanding of the importance, causation, and prognosis associated with ENL in dogs.

Hypothesis/Objectives: Describe disease categories (DC) identified in dogs with ENL and identify variables associated with survival. We hypothesized that factors including fever, segmented and band neutrophil counts, and DC would be negatively associated with survival.

Animals: Two-hundred sixty-nine dogs with ENL (segmented neutrophils $\geq 50 \times 10^3$ cells/μL) presented to the veterinary teaching hospitals at Auburn University (n = 164), the University of Missouri (n = 81), and Oklahoma State University (n = 24) between January 1, 2009 and December 31, 2019.

Methods: Retrospective study. Demographic data and outcome variables including temperature, CBC findings, DC, duration of hospitalization (DOH) and outcome were acquired from the medical record. Statistical analyses included chi-squared and Kruskal-Wallis tests, and Pearson product moment correlations with a $P < .05$ significance level.

Results: Mortality was 41%. Survival differed with DC ($P = .002$). Mortality was higher ($P < .05$) in dogs with neoplasia (56.2%) vs immune-mediated disease (20.5%) or tissue damage/necrosis (19%). Weight ($P = .001$, $r = -0.14$) and total neutrophil count ($P = .04$, $r = -0.02$) were weakly negatively associated with survival whereas DOH was weakly positively associated with survival ($P = .03$, $r = 0.14$).

Conclusions and Clinical Importance: Mortality in dogs with ENL is high but differed according to DC. Only weak correlations between clinical or clinicopathologic variables and mortality were identified. Extreme neutrophilic leukocytosis should be

interpreted in conjunction with the underlying disease process, and not broadly used to predict clinical outcome.

KEYWORDS

canine, leukemoid, prognosis, risk-factors, survival

1 | INTRODUCTION

Extreme neutrophilic leukocytosis (ENL), characterized by a segmented neutrophil count ≥50 × 10³/μL, is an uncommon clinicopathologic finding in dogs.[1-4] This magnitude of neutrophilia can be alarming to clinicians and clients, leading to subjective perceptions of a negative prognosis.[5] These biases have the potential to influence clinical decision making in the absence of objective data.[5] Furthermore, ENL represents a clinical challenge because granulopoiesis may be nonspecifically stimulated by a variety of underlying disease processes including local or systemic inflammation, infection, immune-mediated disease, neoplasia, tissue damage, or a combination of these.[1-4,6-10] The existing literature on ENL in dogs is limited, with few studies published within the last 2 decades.[3,4,6-9,11,12] These studies have come to different conclusions about the underlying etiologies and prognostic relevance of ENL in dogs.[3,4,6] The lack of recent large-scale studies has limited our current understanding of the associated diseases, potential prognostic markers, and clinical outcome in dogs with ENL.[3,4,6] This limited understanding can lead to extensive, costly evaluations or nonspecific empirical recommendations, including euthanasia.[3,4]

The objectives of our multi-institutional, retrospective study were to describe the conditions, factors influencing survival, and clinical outcomes in dogs with ENL presented to 3 veterinary teaching hospitals. We hypothesized that body weight and temperature, CBC findings including total white blood cell (WBC) count, segmented and band neutrophil count, as well as neutrophil toxicity, disease category, and duration of hospitalization would be associated with outcome.

2 | MATERIALS AND METHODS

2.1 | Case selection

Medical records of dogs presented to the veterinary teaching hospitals at Auburn University (AU-VTH), the University of Missouri (MU-VHC) and Oklahoma State University (OK-VMH) between January 1, 2009 and December 31, 2019 were retrospectively reviewed. Dogs were included if they had ENL (segmented neutrophils ≥50 × 10³ cells/μL) identified on a CBC reviewed by a board-certified clinical pathologist and a complete medical record.[1,2,13] Dogs diagnosed with either acute or chronic myeloid leukemia, based on the morphological appearance of blasts cells in circulation, were excluded. Dogs receiving granulocyte colony-stimulating factor also were excluded from evaluation. Reference ranges were based on healthy adult dogs and determined independently by the clinical pathology laboratories at the AU-VTH, MU-VHC, and OK-VMH. Segmented neutrophil reference ranges across all institutions were 2.27-11.4 × 10³ cells/μL.

Demographic data (age, sex, breed, body weight), total WBC count, neutrophil counts (segmented and band), neutrophil toxicity, rectal temperature concurrent with CBC, diagnosis, duration of hospitalization, and case outcome (ie, hospital discharge, natural death, or euthanasia) were acquired from the medical record. Duration of hospitalization was calculated from the date of the first CBC with ENL until death or discharge. Dogs were assigned to 1 of the following disease categories based on ante- or postmortem diagnosis: infectious/inflammatory (INF/INF), immune-mediated (IM), neoplastic (NEO), tissue damage/necrosis (TD/N), >1 diagnosis (>1D), or open. Dogs were assigned to the NEO category based on a definitive or presumptive diagnosis of neoplasia. Although dogs with myeloid leukemia were excluded, those with lymphoid leukemia and presumed paraneoplastic ENL were included in the NEO category. Dogs were assigned to the >1D category if they had diagnoses from >1 of the above categories that were thought to contribute to the ENL (eg, immune-mediated hemolytic anemia and neoplasia, large necrotic tumor). If dogs had >1 diagnosis belonging to the same category, they were only listed under that single category (eg, mammary carcinoma and a splenic mass). If no diagnosis could be determined, the dog was assigned to the open category. Dogs assigned to the open category are described herein but were not included in statistical evaluation because chronic myeloid leukemia could not be definitively excluded in this population.

2.2 | Statistical analysis

Statistical analysis was performed using a commercial software package (SAS, version 9.4, SS institute Inc, Cary, North Carolina). The normality assumption was evaluated using Proc Univariate of SAS. All variables were not normally distributed with exception of temperature. Categorical variables, such as fever (Y/N), neutrophil toxicity (none/mild/moderate/marked), and survival (Y/N) were analyzed by using a chi-squared test. For comparing among the categories of disease (INF/INF, IM, NEO, Open, TD/N, and >1 DG), a Kruskal-Wallis test was used to determine significance for quantitative variables that were not normally distributed. If significance was present, pairwise 2-sided multiple comparison analysis was performed by a Dwass, Steel, Critchlow-Fligner multiple comparison post hoc analysis. For temperature, which was normally distributed, Proc Mixed of SAS was used to determine the differences among the categories of disease. The correlation between survival and other categorical variables was

TABLE 1 Represented breeds

Dogs (n)	Breed
36	Mixed breed
27	Labrador Retriever
15	Golden Retriever
13	German Shepherd
9	Beagle, Chihuahua
8	American Cocker Spaniel, American Staffordshire Terrier, Poodle (standard)
7	Yorkshire Terrier
6	Dachshund (standard), Pomeranian, Pug, West Highland White Terrier, Shih Tzu
5	Border Collie
4	Australian Shepherd, Boxer, Great Dane, English Pointer
3	American Eskimo, Australian Cattle Dog, Cavalier King Charles Spaniel, American Bulldog, Doberman Pinscher, English Springer Spaniel, Weimaraner, Shetland Sheepdog, Shar-Pei, Maltese, Poodle (miniature), German Shorthaired Pointer
2	Bichon Frise, Boston Terrier, Dachshund (miniature), Jack Russel Terrier, Chow Chow, Papillon, Siberian Husky, Scottish Terrier, Rottweiler, Schnauzer (miniature)
1	Alaskan Malamute, Brussels Griffon, Basset Hound, Border Collie, Brittany Spaniel, English Cocker Spaniel, Wirehaired Pointing Griffon, Rat Terrier, Rhodesian Ridgeback, Pembroke Welsh Corgi, Poodle (toy), Petit Basset Griffon, French Bulldog, German Spitz, Irish Setter, Tibetan Terrier, Pekingese, Schnauzer (standard), Kerry Blue Terrier, Mastiff, Miniature Pinscher, Unknown

Note: (n) reflects the number of dogs per breed represented in this study.

performed by using a chi-squared test or Fisher Exact test. For the correlation between survived to discharge and other quantitative variables, Proc Corr of SAS was used. Data for categorical variables are presented as (n) and percentage, data for quantitative variables are presented as median and interquartile range (IQR), and data for temperature are presented as mean and SD. Level of significance was set at $P < .05$.

3 | RESULTS

Two hundred sixty-nine dogs met our inclusion criteria: AU-VTH (n = 164), MU-VHC (n = 81), OK-VMH (n = 24). Sixty-three breeds were represented (Table 1). One hundred forty-five dogs were male (38 intact; 107 castrated) and 124 were female (21 intact; 103 spayed). The median (IQR) age was 7.5 years (5-10; range, 2 months to 16 years). The median (IQR) weight was 20 kg (7.8-28; range, 0.9-94 kg). Rectal temperatures concurrent with the date of ENL diagnosis were available for 264/269 dogs. The mean ± SD rectal temperature was 101.5 °F (38.6°C) ±1.65 (0.6) (range, 96.4-107.0 °F [35.8-41.7°C]). Sixty-six dogs (25%) were considered febrile (rectal temperature > 102.5 °F [39.2°C]) whereas 9 dogs (3.4%) were hypothermic (<99.0 °F [37.2°C]).

The median (IQR) total WBC count across all groups was 73.3×10^3 cells/μL (63.0-93.0) (range, $51.8-888.0 \times 10^3$ cells/μL). The median (IQR) segmented neutrophil count was 63.8×10^3 cells/μL (54.7-74.2) (range, $50.1-197.9 \times 10^3$ cells/μL). Band neutrophil count was above reference range (ie, left shift) in 202 dogs with a median (IQR) band neutrophil count of 1.9×10^3 cells/μL (0.53-5.42; range, $0-29.6 \times 10^3$ cells/μL). Neutrophil toxicity was graded by a board-certified clinical pathologist in 244/269 dogs as none (n = 159,

TABLE 2 Individual diagnosis considered to be contributing to the development of the extreme neutrophilic leukocytosis (ENL)

Disease category	Individual diagnosis
INF/INF (total n = 79)	Abscess (19), pyometra (12), pancreatitis (11), pneumonia—bacterial (10), septic peritonitis (9), septicemia (8), hepatozoonosis (8), endocarditis (5), pneumonia—aspiration (5), necrotic lymphanditis (3), vasculitis (2), thromboembolism (2), inflammatory bowel disease (2), discospondylitis (2), disseminated intravascular coagulation (2), heartworm disease (2), sterile peritonitis (2), acute respiratory distress syndrome (2), cholecystitis/cholangiohepatitis (2), pyogranulomatous myositis (1), pyoderma (1), suppurative pleuritis (1), parvovirus (1), incisional infection (1), necrosuppurative cellulitis/myositis (1), foreign body (1), pyothorax (1), fungal pneumonia (1), gallbladder mucocoele (1), epidural empyema (1), pyogranulomatous enteritis and colitis (1), meningitis (1), sterile nodular panniculitis (1), babesia (1), suppurative epididymitis (1), steatitis (1), hepatitis (1), bartonella (1), clostridial enterotyphlocolitis (1), pneumonitis (1), sialoadenitis (1), fungal dermatitis/panniculitis (1), anaplasmosis (1), colorectal polyps (1), gangrenous mastitis (1), diabetic ketoacidosis (1) suppurative gastritis (1)
IM (total n = 39)	Immune-mediated hemolytic anemia (18), immune-mediated thrombocytopenia (14), immune-mediated polyarthritis (7), Evan's syndrome (5), steroid responsive meningitis-arteritis (2), pemphigus foliaceous (1), masticatory muscle myositis (1)
NEO (total n = 74)	Lymphoma (15), mass—splenic (8), hemangiosarcoma (7), histiocytic sarcoma (5), chronic lymphocytic leukemia (4), mass—liver (4), mass—lungs (3), mast cell tumor (3), nasal carcinoma (2), osteosarcoma (2), hepatic carcinoma (2), renal carcinoma (2), mammary adenocarcinoma (1), mass—small intestine (1), spindle cell neoplasia—liver (1), hepatic sarcoma (1), carcinomatosis (1), round cell tumor—skin (1), anaplastic sarcoma (1), cranial mediastinal sarcoma (1), splenic stromal sarcoma (1), pulmonary adenocarcinoma (1), leiomyosarcoma (1), acute lymphoblastic leukemia (1), mass—bladder (1), melanoma (1), peripheral nerve sheath tumor (1), pancreatic adenocarcinoma (1), renal adenocarcinoma (1), intestinal adenocarcinoma (1), pulmonary carcinoma (1), adrenal mass (1), mass—prostate (1), liver sarcoma (1), omental sarcoma (1), intestinal sarcoma (1), mass—kidney (1)
TD/N (total n = 21)	Gastric ulceration (5), gastric necrosis (3), splenic torsion (3), lung lobe torsion (2), trauma (1), lingual necrosis (1), oronasal fistula (1), splenic infarction (1), bite wounds (1), other wound (1), drug-induced liver failure/necrosis (1)

Note: A single dog may have more than 1 diagnosis. Frequency (n) in parenthesis.
Abbreviations: IM, immune-mediated; INF/INF, infectious/inflammatory; NEO, neoplasia; TD/N, tissue damage/necrosis.

TABLE 3 Association between survival to discharge and quantitative variables

| Variable | Survived | | P-value[*] | r |
	Yes	No		
Age (y)	7 (5-10)	8 (5.7-10)	.71	
BW (kg)	14.0 (7.2-25.3)	22.3 (10-33.5)	.001*	−.14
Temperature (°F)	101.4 ± 1.61	101.7 ± 1.70	.06	
Total WBC ($\times 10^3$/μL)	72.0 (62.0-87.3)	74.6 (65.3-95.1)	.1	
Segmented neutrophils ($\times 10^3$/μL)	62.0 (53.8-72.3)	65.3 (56.8-78.5)	.04*	−.02
Band neutrophils ($\times 10^3$/μL)	1.8 (0-5.29)	2.4 (0.7-5.8)	.37	
Duration of hospitalization (d)	3 (1-5)	2 (1-3)	.03*	.14

Note: Statistical significance denoted by (*). Data are presented as median (IQR) except for temperature (normally distributed) which is presented as a mean ± SD. For parameters reaching statistical significance, Pearson correlations coefficients were calculated (r). Dogs in the OPEN were excluded from statistical analysis.
Abbreviations: BW, body weight; WBC, white blood cell.

TABLE 5 Evaluation of categorical variables across disease category

| Variable | | Disease category | | | | | | P-value* |
		INF/INF (n = 79)	IM (n = 39)	NEO (n = 74)	Open (n = 21)	TD/N (n-21)	>1DG (n = 35)	
Fever	Yes	20/79 (25.3%)	5/38 (13.2%)	21/71 (29.6%)	5/20 (25.0%)	8/21 (38.1%)	7/35 (20.0%)	.3
	No	59/79 (74.7%)	33/38 (86.8%)	50/71 (70.4%)	15/20 (75.0%)	13/21 (61.9%)	28/35 (80.0%)	
Neutrophil toxicity	None	46/73 (63.0%)	24/37 (64.9%)	41/62 (66.1%)	14/20 (70.0%)	16/19 (84.2%)	18/33 (54.6%)	.29
	Mild	24/73 (32.9%)	10/37 (27.0%)	15/62 (24.2%)	5/20 (25.0%)	3/19 (15.8%)	12/33 (36.4%)	
	Moderate	2/73 (2.7%)	3/37 (8.1%)	6/62 (9.7%)	1/20 (5.0%)	0/19 (0.0%)	1/33 (3.0%)	
	Marked	1/73 (1.4%)	0/37 (0.0%)	0/62 (0.0%)	0/20 (0.0%)	0/19 (0.0%)	2/33 (6.1%)	
Survival to DC	Yes	45/79 (57.0%)	31/39 (79.5%)	33/74 (44.6%)	14/21 (66.7%)	17/21 (81.0%)	18/35 (51.4%)	.002*
	No	34/79 (43.0%)	8/39 (20.5%)	41/74 (55.4%)	7/21 (33.3%)	4/21 (19.1%)	17/35 (48.6%)	
Outcome	DC	44/79 (55.7%)	31/39 (79.5%)	32/73 (43.8%)	14/21 (66.7%)	17/21 (81.0%)	18/35 (51.4%)	
	Died	11/79 (13.9%)	4/39 (10.3%)	4/73 (5.48%)	0/21 (0.0%)	3/21 (14.3%)	4/35 (11.4%)	.0003*
	Euthanized	24/79 (30.4%)	4/39 (10.3%)	37/73* (50.7%)	7/21 (33.3%)	1/21 (4.8%)	13/35 (37.1%)	

Note: Statistical significance denoted by (*). Dogs in the OPEN category are reported but were excluded from statistical analysis.
Abbreviations: >1DG, greater than 1 diagnosis category; DC, discharge; IM, immune mediated; INF/INF, infectious/inflammatory; TD/N, tissue damage/necrosis; NEO, neoplasia.

65%), slight (n = 69, 28%), moderate (n = 13, 5%), or marked (n = 3, 1%) based on previously published criteria.[14]

One hundred thirteen diagnoses were derived from the medical records (Table 2). Disease category distribution was as follows: INF/INF (n = 79/269, 29%), NEO (n = 74/269, 28%), IM (n = 39/269, 14%), >1D (n = 35/269, 13%), TD/N (n = 21/269, 8%), and Open (n = 21/269, 8%). Sixty-one of 79 dogs in the INF/INF group were diagnosed with a suspected infectious disease, and 58/61 were suspicious of, or confirmed to be, bacterial in origin. A cytological or histopathologic diagnosis of neoplasia was achieved in 54/74 dogs. The remaining dogs had a presumptive diagnosis of neoplasia based on diagnostic imaging and the totality of clinical opinion considering factors such as negative infectious disease testing (eg, fungal antigen testing). The average duration of hospitalization was 3.2 days (range, 0-30 days). One hundred and fifty-eight of 269 dogs (59%)

survived to discharge. One hundred and eleven dogs of 269 (41%) did not survive to discharge and either were euthanized (n = 86/111, 77%) or died (n = 25/111, 23%).

Comparison of demographic and physical examination variables across disease categories yielded significant differences for age. Dogs in the disease category NEO were significantly older than those in the INF/INF, IM, and TD/N categories (P < .001). Age did not, however, correlate with survival (Table 3; P = .71). Across all disease categories, increased body weight was weakly associated with decreased survival (Table 3; P = .001, r = −0.14). Temperature (P = .14) and presence of fever (Table 5; P = .3) did not differ between disease categories. Fever was not significantly more common in dogs with an infectious disease process (P = .31).

Comparison of clinicopathologic variables across disease categories indicated a significantly higher total WBC count for patients in

TABLE 4 Median (IQR) for quantitative variables (nonnormally distributed) evaluated by Kruskal-Wallis test with $P < 0.05$ significant level (denoted by *)

Variable	Diagnosis category						
	INF/INF (n = 79) Median (IQR)	IM (n = 39) Median (IQR)	NEO (n = 74) Median (IQR)	Open (n = 21) Median (IQR)	TD/N (n = 21) Median (IQR)	>1D (n = 35) Median (IQR)	P-value*
Age (y)	6.00 (4.00-9.00)	7.00 (6.00-9.00)	9.00 (7.00-11.00)	8.00 (3.00-11.00)	5.00 (3.00-10.00)	8.00 (6.00-10.00)	<.001*
BW (kg)	13.30 (6.50-27.60)	14.80 (7.70-24.40)	24.65 (11.91-31.45)	10.50 (5.46-17.75)	12.30 (6.40-22.70)	15.90 (12.40-30.10)	.06
Total WBC ($\times 10^3/\mu$L)	69.81 (62.23-84.80)	70.93 (59.54-87.85)	77.40 (65.90-106.30)	70.40 (61.32-84.31)	72.70 (67.70-77.30)	73.89 (60.25-85.97)	.1
Seg neutrophil ($\times 10^3/\mu$L)	63.75 (53.87-72.21)	58.87 (52.60-71.02)	65.11 (57.88-38.97)	64.06 (52.82-79.25)	62.89 (60.25-71.24)	61.11 (54.46-69.38)	.07
Band neutrophils ($\times 10^3/\mu$L)	1.71 (0.00-5.48)	4.34 (0.82-6.67)	2.24 (0.66-5.58)	1.67 (0.00-2.45)	1.44 (0.77-3.41)	1.72 (0.00-5.27)	.31
DOH (d)	3.00 (1.00-6.00)	3.00 (1.00-5.00)	1.50 (0.00-2.00)	2.00 (0.00-4.00)	3.00 (2.00-6.00)	2.00 (1.00-5.00)	<.001*

Note: Dogs in the OPEN category are reported but were excluded from statistical analysis.

Abbreviations: >1D, greater than 1 diagnosis category; BW, body weight; DOH, duration of hospitalization; IM, immune-mediated; INF/INF, infectious/inflammatory; IQR, interquartile range; NEO, neoplasia; TD/N, tissue damage/necrosis; WBC, white blood cell.

the NEO disease category compared to those in the INF/INF disease category (Table 4; $P = .04$). This difference was found to reflect the presence of 5 dogs with nonmyeloid leukemia included in our patient population. When these dogs were excluded, the median (IQR) total leukocyte count for dogs in the NEO category was $77.4 \times 10^3/\mu$L (65.9-$106.3 \times 10^3/\mu$L) and was no longer significantly increased compared to other groups ($P = .06$). These dogs therefore were excluded from statistical evaluation where total white blood cell count was an outcome variable. No differences in the number of segmented neutrophils, band neutrophils, or neutrophil toxicity were identified among disease categories (Tables 4 and 5). Segmented neutrophil count had a weak negative correlation with survival (Table 3; $P = .04$, $r = -0.02$). Total white blood cell count, band neutrophil numbers, and neutrophil toxicity were not correlated with survival (Tables 5 and 6).

Duration of hospitalization was significantly different among disease categories (Table 4). Dogs in the NEO disease category had shorter duration of hospitalization compared to those in the INF/INF, IM, and TD/N disease categories ($P < .001$). Increased duration of hospitalization was weakly associated with a higher chance of survival (Table 3; $P = .008$, $r = 0.16$).

Survival ($P = .002$) and outcome (survival to discharge, euthanasia, natural death; $P < .001$) were associated with disease category. Dogs in the IM (79.5%) and TD/N (81%) disease categories had higher likelihood of survival to discharge as compared to dogs in the NEO category ($P < .05$; Table 5). Dogs in the NEO disease category had the lowest percentage discharged (43.8%) and the highest percentage euthanized (50.7%; Table 5).

4 | DISCUSSION

An ENL might influence clinical decision making by its presence alone.[5] The absence of recent objective clinical data regarding causation, potential prognostic indicators, and outcome in dogs with ENL represents a limitation to our understanding and management of such cases. The information currently available is from individual case reports or specific patient groups with no large retrospective studies published within the last decade.[3,4,6-9,11,12] Improved diagnostic and therapeutic capabilities warrant reexamination of this patient population to improve clinical decision making. This study is the largest and first multi-institutional study evaluating dogs with ENL of any cause.

Not surprisingly, ENL was associated with a wide variety of conditions.[3,4] Interestingly, and in contrast to the human medical literature, the degree of neutrophilia, band neutrophils, or toxic change in neutrophils failed to reflect a particular underlying etiology.[13,15] Infectious/inflammatory conditions were the most frequently encountered disease category in our study, and most of the cases of infectious disease were bacterial. Although bacterial infection was common in this population of dogs with ENL, it represented only 58/269 (22%) of the total cases evaluated. As such, antibiotic use may be best reserved for dogs with additional evidence of bacterial infection rather than used

TABLE 6 Evaluation of the association between survival to discharge and categorical variables

Variable		Survived		P-value (chi-square)	P-value (Fisher Exact)
		Yes	No		
Fever	Yes	32/158 (20.25%)	34/106 (32.08%)	.03*	NA
	No	126/158 (79.75%)	72/106 (67.92%)		
Neutrophil toxicity	None	101/150 (67.33%)	58/94 (61.70%)	.63	.59
	Mild	41/150 (27.33%)	28/94 (29.79%)		
	Moderate	7/150 (4.67%)	6/94 (6.38%)		
	Marked	1/150 (0.67%)	2/94 (2.13%)		
Diagnosis category	IM	31/158 (19.62%)	8/111 (7.21%)	.002*	.002*
	INF/INF	45/158 (28.48%)	43/111 (38.74%)		
	TD/N	17/158 (10.76%)	4/111 (3.60%)		
	NEO	33/158 (20.89%)	41/111 (36.94%)		
	Open	14/158 (8.86%)	7/111 (6.31%)		
	>1D	18/158 (11.39%)	17/111 (15.32%)		

Note: Parameters meeting statistical significance are denoted by (*), Results were corroborated by a Fisher's exact where cells having counts of <5 were expected. Fever defined as temperature >102.5 °F. Dogs in the OPEN category are reported but were excluded from statistical analysis.
Abbreviations: >1DG, greater than 1 diagnosis category; IM, immune mediated; INF/INF, infectious/inflammatory; NA, not applicable; NEO, neoplasia; TD/N, tissue damage/necrosis.

as primary empirical treatment in dogs with ENL. This finding contrasts with the human medical literature, in which bacterial infection has been reported as the most common cause of ENL.[13,15] Similar to other ENL studies in companion animals, we found that fever was present in only 25% of cases, was not associated with any particular disease category, and was no more likely in dogs with infection than in those with other causes of ENL.[4] This finding is in contrast to the human medical literature in which fever was most often associated with infectious disease.[13,15] As such, fever should not be the only additional piece of evidence used to justify administration of antibiotics. Previous publications examining specific diseases associated with ENL determined that dogs with fever were less likely to survive.[4,16,17] However, in our study fever was not found to be a predictor of survival.

The second most common disease category was NEO. This finding is consistent with the veterinary and human medical literature in which tumors, even in the absence of infection, are associated with increased neutrophil counts.[1,11,12,18-22] Dogs with acute or chronic myeloid leukemia were excluded from our study, but dogs with lymphocytic leukemia were not excluded. This difference reflects our objective of evaluating causes of neutrophil counts ≥50 × 10³ cells/µL in dogs. Total WBC count was found to be different among disease categories, with dogs in the NEO category having a statistically higher number of total circulating leukocytes. However, when dogs with lymphocytic leukemia were excluded, total WBC was no longer significant. It should be noted that a minority of dogs included in the NEO category did not have a confirmed cytological or histopathological diagnosis of neoplasia. In these cases, the working presumptive diagnosis was used, usually based on identification of mass(es) and the use of ancillary testing to rule out applicable infectious differential diagnoses (eg, fungal antigen testing to rule out mycotic causes of

pulmonary masses). This approach allows for potential overlap between the NEO and INF/INF disease categories. We consider this a limitation of the study but believe that inclusion of these presumed cases of neoplasia is important because this assessment likely informed clinical decision making.

Duration of hospitalization was significantly different among dogs in various disease categories. Dogs with neoplastic diseases had a shorter hospitalization period compared to those in the INF/INF category. The shortened duration of hospitalization may be attributed to owner-elected euthanasia upon diagnosis.[4] Increased duration of hospitalization was weakly associated with a higher chance of survival, and a diagnosis of IM or TD/N disease was a positive prognostic indicator. Dogs with IM and TD/N disease were hospitalized longer and had a higher survival percentage (79.5% and 81%, respectively) compared to dogs in the NEO category (43.8%; Table 4). This difference may be a consequence of diseases within this category with lower mortality rates but that require hospitalization, such as immune-mediated hemolytic anemia or severe wounds.[17,23] A client's choice of early euthanasia based on diagnosis or other factors likely influenced these survival statistics.

The overall mortality rate of dogs in our study was 41%, which is lower than the 62% mortality rate previously reported in dogs with ENL.[4] Interestingly, the cutoff for ENL in the prior study was lower, requiring that total leukocyte counts, not neutrophil counts, be > 50 × 10³/µL.[4] The difference in mortality may be attributed to new diagnostic and treatment options developed over the 20 years between these 2 studies. Despite this improvement, 41% mortality is substantial, and frank discussion with owners is warranted when an ENL is present. Although the total segmented neutrophil count was found to be statistically weakly negatively correlated with survival, the median (IQR) segmented neutrophil counts for survivors and

nonsurvivors were 62 (53.8-72.3) and 65.3 (56.8-78.5) respectively, negating the clinical relevance of this finding.

Our study had several limitations. Although 269 dogs met our inclusion criteria, these were not equally distributed across institutions. Data retrieval for the entire collection period was possible for AU-VTH and MU-VHC, but complete data retrieval could not be accomplished at OK-VMH because of a change in the medical records system. As such, the number of cases retrieved from the OK-VMH does not necessarily reflect the actual number of ENL cases at that institution. For this reason, institutional comparisons were not attempted. Although not evaluated, geographical variation in conditions contributing to ENL may be expected. For example, certain infections (eg, hepatozoonosis) are known to frequently cause ENL, but are not found in all locations.[1]

Furthermore, the retrospective nature of our study limited the ability to standardize specific analyses. The different hematology analyzers used across institutions may have caused variations in CBC results. However, these variations were likely small and may more accurately represent the variation among hematology analyzers in practice. Blood smears were read by multiple clinical pathologists, possibly leading to subjective variation in band neutrophil count and assessment of neutrophil toxicity. Diagnostic testing may have been limited in some cases, resulting in missed diagnoses (eg, failure to obtain blood culture might have resulted in a missed diagnosis of sepsis). The open category was included to describe the dogs with an ENL of otherwise unclassified cause or for which the cause(s) could not be determined. We felt it important to document these cases, but because chronic myeloid leukemia could not be effectively excluded from this population, they were excluded from statistical analysis. Chronic myeloid leukemia should be considered for dogs with ENL, particularly in absence of other identifiable causes. Finally, diagnoses could not be standardized by a single clinician and were based on multiple clinicians' clinical judgment across the 3 institutions over 10 years.

5 | CONCLUSION

Extreme neutrophilic leukocytosis is an uncommon clinical finding, but dogs with this finding, have a high mortality rate (41%). Although INF/INF diseases were most the most common cause of ENL, bacterial infection accounted for only a minority of cases (22%). Overall, NEO (n = 74) causes were more common than bacterial infection (n = 58). Perhaps surprisingly, band neutrophil count and toxic changes were not associated with higher mortality. Although segmented neutrophil counts were very weakly associated with survival, the clinical relevance of this finding is questionable. A diagnosis of neoplasia and higher body weight were weak negative prognostic indicators for survival. Diagnosis of immune-mediated or traumatic disease and longer duration of hospitalization were positive prognostic indicators. Category of disease associated with ENL had the largest impact on survival. Clinicians should consider a range of factors that can affect the overall prognosis, rather than assuming that the magnitude of neutrophilia is associated with a worse prognosis.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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