Leukocyte Ratios and Prognosis in Dogs with Primary Immune-Mediated Hemolytic Anemia: A Pilot Study

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Short Report

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Abstract

Canine immune-mediated hemolytic anemia (IMHA) is a life-threatening condition that is commonly associated with neutrophilia and monocytosis. Leukocyte ratios have been found to have prognostic value in humans and animals affected by a range of inflammatory, infectious, and neoplastic disorders. We hypothesized that in primary IMHA, neutrophil to lymphocyte (NLR), neutrophil to monocyte (NMR), band neutrophil to segmented neutrophil (BNR) and monocyte to lymphocyte (MLR) ratios would be higher in dogs that did not survive to discharge. Medical records of dogs diagnosed with IMHA at two veterinary teaching hospitals were retrospectively reviewed. Twenty-three of the 72 included dogs do not survive to discharge. NLR, NMR, BNR and MLR ratios were compared between dogs that survived to discharge and dogs that died or were euthanized. None of the ratios were significantly different between survivors and non-survivors (P = 0.14-0.99). Area under the Receiver Operating Characteristic (ROC) curve for prediction of non-survival ranged from 0.5 (95% confidence interval 0.38-0.62) for MLR to 0.61 (0.49-0.72) for NMR and was not significantly different from 0.5 for any ratio (P = 0.29-0.99). After exclusion of 31 dogs that received one or both of immunosuppression and blood transfusion before presentation, the area under the ROC curve for prediction of survival was significantly different from 0.5 for MLR (0.78, P = 0.01) and NMR (0.78, P = 0.0002). This pilot study suggests MLR and NMR may be of prognostic value in untreated dogs with IMHA.

Introduction

Canine immune-mediated hemolytic anemia (IMHA) is a common and life-threatening disease, with approximately 30% mortality during initial hospitalization. The ability to identify the most severely affected dogs at initial presentation would allow stratification for clinical trials, and a more personalized approach to treatment, with more aggressive therapies targeted at the patients at highest risk of death or euthanasia. A prognostic algorithm, the canine hemolytic anemia objective score (CHAOS), has been shown to be associated with prognosis in an independent validation cohort. However, the area under the ROC curve for prediction of non-survival was relatively low (0.688, 95% confidence interval 0.618-0.758). This indicates a continuing need to develop robust prognostic markers for IMHA (Ishihara et al. 2010; Goggs et al. 2015; Swann & Skelly 2015).

IMHA is highly inflammatory, and commonly results in neutrophilia, frequently with a left shift, and monocytosis (McManus et al. 2001; Kjelgaard-Hansen et al. 2011). Higher concentrations of GM-CSF, a cytokine that promotes granulocyte and monocyte production, have been reported in dogs that die within thirty days of diagnosis (Kjelgaard-Hansen et al. 2011). Monocytosis and increases in cytokines associated with monocyte activation have also been linked to poor prognosis in IMHA (Kjelgaard-Hansen et al. 2011; Piek et al. 2011).

Studies in dogs affected by a range of inflammatory and infectious disorders suggest that calculation of leukocyte ratios may provide additional prognostic information (Becher et al. 2021; Benvenuti et al. 2020; Conway et al. 2021; Pierini et al. 2020).

We hypothesized that in primary IMHA, segmented neutrophil to lymphocyte (NLR), segmented neutrophil to monocyte (NMR), band neutrophil to segmented neutrophil (BNR) and monocyte to lymphocyte (MLR) ratios would be higher in dogs that did not survive to discharge. The aims of the study were (1) to compare the leukocyte ratios between dogs hospitalized for IMHA that survived to discharge and dogs that died or were euthanized and (2) to determine if any ratio had an area under the ROC curve for prediction of non-survival that was significantly different from 0.5.

Materials And Methods

Electronic medical records databases at the Veterinary Teaching Hospitals of the University of Pisa, Italy (VTH-UdP) and Texas A&M University, USA (VTH-TAMU), were retrospectively reviewed to identify dogs hospitalized and treated for primary IMHA between February 2010 and March 2018.

For the VTH-UdP cases, diagnosis of IMHA was based on evidence of hemolysis and/or autoagglutination associated with positive detection of anti-erythrocyte antibodies with flow cytometry (performed at San Marco Laboratory, in Padova, Italy) and/or marked spherocytosis on blood smear evaluation. For the VTH-TAMU cases, diagnosis of IMHA was based on evidence of hemolysis and at least one of spherocytosis or positive saline agglutination test. Cases were excluded if clinical investigations, as directed by the attending clinician, identified a non-immune-mediated cause of hemolysis or an iatrogenic, infectious, or neoplastic trigger for secondary IMHA.

Signalment, treatment before and after presentation, complete blood count (CBC) and other diagnostic test results, and case outcome were manually recorded from the medical records. CBC were obtained using ProCyte Dx® (IDEXX) at the VTH-UdP, and Cell-Dyn® 3700 (IDEXX) was used for cases from 2010-2017 and Siemens Advia 2120® analyzer for cases from 2017-2018 at VTH-TAMU. At both institutions, microscopic blood smear examination, including a 100-200 cell leukocyte differential count, was performed.

For each dog, the following ratios were calculated from the manual leukocyte differentials: NLR, NMR, BNR, and MLR ratio. If dogs had more than one CBC performed, the first result was used for leukocyte ratio calculation. Data was non-normal, so each ratio was compared between dogs that did and did not survive to discharge by Mann-Whitney-U test. For each ratio, it was determined if the area under the ROC curve for prediction of non-survival was significantly different from 0.5. These analyses were repeated after exclusion of dogs that received immunosuppression and/or blood transfusion before presentation, and after exclusion of dogs that were euthanized within 24 hours of admission. Statistical significance was defined as $P < 0.05$. All statistics were performed using MedCalc Statistical Software version 19.1.3 (MedCalc Software bv, Ostend, Belgium).

Results

Ninety-one dogs met our case definition, but 19 dogs were treated as out-patients, and were excluded from analysis. Demographic information for the remaining 72 dogs is summarized in Table 1.

Table 1
Demographic information for dogs hospitalized at the VTH-UdP and VTH-TAMU for treatment of primary IMHA.

	VTH-UdP (n=29)	VTH-TAMU (n=43)
Breed	Beagle (1), Bolognese (1), Border Collie (1), Boxer (1), Bull Terrier (2), Cocker Spaniel (3), German Shepherd (1), Lagotto Romagnolo (1), Maltese (2), Mixed breed (15), Poodle (1)	Alaskan Malamute (1), American Pit Bull Terrier (1), Australian Heeler (1), Beagle (1), Boston Terrier (1), Chihuahua (6), Cocker Spaniel (1), Dachshund (3), English Bulldog (1), English Pointer (1), English Springer Spaniel (1), German Shepherd (1), Golden Retriever (1), Havanese (1), Italian Greyhound (1), Labrador Retriever (3), Lhasa Apso (1), Maltese (1), Mastiff (1), Miniature Poodle (1), Miniature Schnauzer (2), Mixed breed (5), Pekingese (1), Shih Tzu (4), Welsh Springer Spaniel (1), Yorkshire Terrier (1)
Gender	Female intact (7), Female spayed (12), Male intact (7), Male neutered (3)	Female intact (6), Female spayed (26), Male intact (3), Male neutered (8)
Median age, range (years)	6 (2-14)	7 (1-14)
Median body weight, range (kg)	18.0 (4.3-44.7)	7.1 (1.8-76.2)

Selected clinical and laboratory results are summarized in Table 2.

Table 2
Clinical and laboratory findings for dogs hospitalized for treatment of primary IMHA.

	VTH-UdP (n=29)		VTH-TAMU (n=43)	
	Survivors (n=19)	Non-survivors (n=10)	Survivors (n=30)	Non-survivors (n=13)
PCV at admission (%)	14 (5-27)	13 (9-22)	16 (3-34)	20 (8-29)
Reticulocyte count ($x10^3/\mu L$)[a]	218 (27-705)	150 (12-227)	153 (47.2-424)	260 (241-269)
Positive saline agglutination test	5/19	4/10	23/30	11/13
Positive anti-RBC antibodies detection	11/12	7/7	Not performed	Not performed
Spherocytes occurrence ≥ 1+	14/19	9/10	27/30	11/13
Platelets count ($x\ 10^3/\mu L$)[b]	200 (15-440)	93 (51-314)	157 (73-528)	264 (112-491)
Leukocyte count ($x10^3/\mu L$)	33 (8-65)	27 (13-96)	24 (7-64)	28 (12-58)
Segmented neutrophils count ($x10^3/\mu L$)	20 (5-52)	19 (11-54)	18 (4-56)	20 (10-47)
Presence of left shift[c]	16/19	8/10	22/43	8/13
Total Bilirubin (mg/dL)	0.9 (0.2-4.0)	2.5 (0.2-9.2)	1.8 (0.3-25.2)	5.6 (0.9-53.9)
Hemolysed plasma	0/19	8/10	14/43	11/13
Icteric plasma	11/19	6/10	14/43	5/13
CHAOS score	2 (0-6)	3 (0-5)	3 (0-5)	4 (2-7)

[a] Automated reticulocyte counts were excluded if agglutination was present. [b] Automated platelet counts were excluded if clumping was present on blood smear examination. [c] A left shift was considered present if, based on a manual differential, there were >300 band neutrophils/μL. The continuous data are reported as median and range, the categorical data are reported as number of positive cases over the total cases examined.

Testing for vector borne disease was performed in 29/43 VTH-TAMU cases and 10/27 VTH-UdP cases. For VTH-TAMU cases, thoracic radiographs were performed in 41/43; abdominal radiographs in 30/43 and abdominal ultrasound in 33/43. For VTH-UdP, thoracic radiographs were performed in 12/29, abdominal radiographs in 3/29 and abdominal ultrasound in 23/29.

Twenty-three dogs died or were euthanized before discharge, including 10/29 (34%) of the dogs presenting to the VTH-UdP and 13/43 (30%) of the dogs presenting to VTH-TAMU. Of the non-survivors,

one dog treated at VTH-TAMU died spontaneously, while the remaining non-survivors at VTH-TAMU, and all non-survivors at VTH-UdP were euthanized. For non-survivors, median time to death or euthanasia was 3.5 days (range 2-8 days) at the VTH-UdP, and 3 days (range 0-7 days) at VTH-TAMU. For survivors, median length of hospitalization was 5 days (range 1-11 days) at the VTH-UdP and 4 days (range 1-19 days) at VTH-TAMU.

Of the VTH-UdP cases, 4/10 non-survivors and 12/19 survivors were receiving at least one immunosuppressive medication at initial presentation (prednisone n=7, prednisolone n=5, cortisone n=3, azathioprine n=2, dexamethasone n=2, and mycophenolate n=1). At initial presentation, 1/19 survivors was receiving clopidogrel and 1/19 survivors was receiving unfractionated heparin. Before presentation, 3/10 non-survivors and 3/30 survivors had received at least one transfusion with Packed Red Blood Cells (PRBC) or Stored Whole Blood (SWB).

Of the VTH-TAMU cases, 4/13 non-survivors and 7/30 survivors were receiving at least one immunosuppressive medication at initial presentation (prednisone n =11, azathioprine n=4, dexamethasone n=5, and cyclosporine n=2). At initial presentation, 2/30 survivors were receiving aspirin. Before presentation, 3/13 non-survivors and 5/30 survivors had received at least one transfusion with PRBC or SWB.

Of the dogs admitted to the VTH-UdP, 3/10 non-survivors were euthanized without further treatment. The remaining 7 non-survivors all received at least one immunosuppressive medication during hospitalization (dexamethasone n=4, methylprednisolone n=2, and betamethasone n=1). Five non-survivors received at least one anti-thrombotic agent, (unfractionated heparin n=5, and aspirin n=1). Three non-survivors received at least one transfusion of PRBC, and one received a transfusion of SWB. Of the dogs admitted to the VTH-UdP that survived to discharge, therapy information was not available for 4/19, and the remaining 15/19 received at least one immunosuppressive medication (prednisone n=6, prednisolone n=6, betamethasone n=4, azathioprine n=3, cyclosporine n=3, and dexamethasone n=2). At least one anti-thrombotic was administered to 9/19 VTH-UdP survivors during hospitalization (unfractionated heparin n=7, clopidogrel n=3, and aspirin n=1). Of the VTH-UdP survivors, 4/19 received at least one transfusion of PRBC during hospitalization.

Of the dogs admitted to VTH-TAMU, 3/13 non-survivors were euthanized without further treatment. The remaining 10 non-survivors all received at least one immunosuppressive medication during hospitalization (cyclosporine n=9, dexamethasone n=6, prednisone n=5, prednisolone n=2, and mycophenolate n=1). Five non-survivors received at least one anti-thrombotic agent (aspirin n=4, clopidogrel n=3, and dalteparin sodium n=2). Eight non-survivors received at least one transfusion of packed red blood cells. Of the dogs admitted to VTH-TAMU that survived to discharge, 29/30 dogs received at least one immunosuppressive medication (prednisone n=27, cyclosporine n=21, dexamethasone n=17, mycophenolate n=9, prednisolone n=7, intravenous human immunoglobulin n=6, and azathioprine n=2). At least one anti-thrombotic was administered to 29/30 VTH-TAMU survivors

during hospitalization (clopidogrel n=20, aspirin n=12, dalteparin sodium n=4, and rivaroxaban n=3). Of the VTH-TAMU survivors, 21/30 received at least one transfusion of PRBC during hospitalization.

None of the leukocyte ratios were significantly different between survivors and non-survivors or had an area under the ROC curve for prediction of survival significantly different from 0.5 (Supplementary, Table 1). After exclusion of 31 dogs that received one or both of immunosuppression and blood transfusion before presentation, area under the ROC curve for prediction of survival was significantly different from 0.5 for MLR and NMR (Table 3). To reduce the impact of possible financially driven euthanasia, analysis was repeated after exclusion of 3 dogs euthanized on the first day of hospitalization. None of the leukocyte ratios were significantly different between survivors and non-survivors or had an area under the ROC curve for prediction of survival significantly different from 0.5 (Supplementary, Table 2).

Table 3
Associations between leukocyte ratios and death or euthanasia for dogs hospitalized for treatment of primary IMHA, after exclusion of dogs that received immunosuppression and/or blood transfusion before presentation.

Leukocyte ratio	Median (25th -75th percentile)		P-value	Area under the ROC curve (95% CI)	P-value
	Survivors (n=28)	Non-survivors (n=13)			
BNR	0.04 (0.02-0.09)	0.02 (0.00-0.05)	0.11	0.66 (0.49-0.80)	0.09
MLR	1.61 (0.98-3.00)	0.71 (0.43-1.08)	0.005	0.78 (0.62-0.89)	0.01
NLR	11.80 (7.23-20.87)	14.50 (4.47-23.21)	0.83	0.52 (0.36-0.68)	0.84
NMR	6.95 (4.35-13.53)	17.80 (12.55-23.72)	0.004	0.78 (0.63-0.90)	0.0002

BNR: Band neutrophil to segmented neutrophil ratio; CI: confidence interval; MLR: Monocyte to lymphocyte ratio; NER: Segmented neutrophil to eosinophil ratio; NLR: Segmented neutrophil to lymphocyte ratio; NMR: Segmented neutrophil to monocyte ratio; ROC: Receiver operator characteristic.

Discussion

In this population of dogs hospitalized for primary IMHA, none of the tested leukocyte ratios were different between patients that survived to discharge and those that died or were euthanized. Based on ROC curve analysis, none of the tested ratios performed significantly better than chance to predict survival. However, a subgroup analysis including only dogs that had not received immunosuppression or blood transfusion before presentation identified lower MLR and higher NMR in non-survivors compared to survivors. In this group, the area under the ROC curve was significantly different from 0.5 for MLR and NMR, suggesting these ratios may have prognostic value in untreated dogs.

Previous studies examining leukocyte ratios in dogs showed mixed results. Higher NLR was associated with markers of more severe inflammatory bowel disease (Becher et al. 2021; Benvenuti et al. 2020). Few studies have investigated the ability of NLR to distinguish sepsis from systemic inflammatory response syndrome in dogs, finding no discriminatory ability for septic peritonitis (Hodgson et al. 2018) and pneumonia (Conway et al 2021), and rather good chance to be useful for acute pancreatitis (Neumann S. 2021). A weak association between lower NLR and sepsis and between higher MLR and non-survival in dogs with sepsis was found (Pierini et al. 2020).

In the current study, the association between survival and NMR and MLR only in dogs that had not received immunosuppression or blood transfusion at the time of presentation suggests that effects of therapy may have obscured the relationship between leukocyte ratios and survival in our study population. Prior immunosuppression may have dampened inflammation, reducing the sensitivity of our study for detecting a relationship between leukocyte ratios and prognosis. Additionally, exogenous corticosteroids may have distorted the leukocyte ratios, by inducing neutrophilia, monocytosis and lymphopenia. However, it is likely that corticosteroid doses were higher in our population of dogs with autoimmune disease. Blood transfusion has also been shown to have immunomodulatory effects, which may have influenced our findings (Remy et al. 2018).

Alternatively, limitations in study design may have obscured a relationship between leukocyte ratios and prognosis. Notably, we combined dogs from two geographical locations. A higher proportion of the American cases were neutered than in Italy, and median body weight was lower in the American population, reflecting a higher number of toy breed dogs. It is also possible that breed and age may be of greater relevance to leukocyte ratios than other prognostic markers, as these factors are known to influence leukocyte differentials (Miglio et al. 2020; Mongillo et al. 2015). By combining dogs from the two locations, we may have introduced other sources of heterogeneity that have limited the power of our study to identify an association between leukocyte ratios and prognosis.

The two laboratories contributing to this study also used different instrumentation, and no attempt was made to harmonize instrument performance between the two locations. However, it should be noted that leukocyte ratios were calculated from the manual differential percentages. It is possible that the heterogeneity of our population may have obscured relationships between leukocyte ratios and outcome, it should also be considered that ratios associated with prognosis only in a very strictly defined study population would likely have limited external validity. As leukocyte differentials are likely to change over time, future studies examining the effect of duration of clinical signs on the prognostic value of leukocyte ratios would be valuable.

The retrospective nature of the study may have introduced other limitations. The retrospective data set prevented use of a standardized follow up time, and survival to discharge is likely a less reliable indicator of survival than mortality at a set time, such as thirty days.

Conclusion

In conclusion, this retrospective study did not identify a relationship between leukocyte ratios and survival to discharge in the general population of dogs hospitalized for treatment of primary IMHA. However, our findings suggest that MLR and NMR may have prognostic value in untreated dogs presenting with IMHA. Further studies are needed to confirm this finding is generalizable, and at this time, these ratios should not be used to direct clinical decisions.

Declarations

Author Contributions: Conceptualization, G.L and A.G.; methodology, G.L, A.G., and C.A.; software, C.A., G.L.; validation, G.L. and A.G.; formal analysis, G.L, A.G., and C.A; investigation, G.L, A.G., and C.A.; resources, G.L.; data curation, C.A, G.DF.; writing—original draft preparation, G.L, A.G., and C.A.; writing—review and editing, G.L, A.G., C.A. and G.D.F.; visualization, G.L, A.G., C.A. and G.DF; supervision, G.L., and A.G; project administration, GL; funding acquisition, GL. All authors have read and agreed to the published version of the manuscript.

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Ethics approval: This research study was conducted retrospectively from data obtained for clinical purposes. As no additional testing was performed other than those required for diagnosis and monitoring and confidentiality is maintained, ethical approval was not required at either institution (please refer also to Materials and Methods).

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