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Retrospective evaluation of systemic hypertension in dogs with non-associative (primary) immune mediated hemolytic anemia

ABBREVIATIONS

AKI, acute kidney injury

BP, blood pressure

CHAOS, canine hemolytic anemia objective score

ICU, intensive care unit

IMHA, immune mediated hemolytic anemia

PCV, packed cell volume

SBP, systolic blood pressure

ABSTRACT

Objective:

Primary aim: To report the prevalence of arterial hypertension in a population of dogs with non-associative immune mediated hemolytic anemia (IMHA) on presentation and during hospitalization. Secondary aim: To determine the relationships of systolic blood pressure (SBP) with mortality and a prognostic indicator, the canine hemolytic anemia objective score.

Design:

Retrospective observational study (December 2016-April 2019).

Setting:

University teaching hospital.

Animals:

26 clinical dogs presenting to the ICU (intensive care unit) with non-associative (primary) IMHA and a control group of 23 clinical dogs with idiopathic epilepsy hospitalized in the ICU for seizure treatment or monitoring.

Interventions:

None

Measurements and Main Results:

Hypertension was defined as SBP ≥160mmHg and severe hypertension ≥180mmHg. . Mean SBP was significantly elevated in IMHA dogs (161mmHg SD 21) compared to ICU control dogs (138mmHg SD 14; P<0.005). Hypertension was present in 13/26 (50.0%) dogs across the period of hospitalization and was severe in 3/26 (11.5%). During at least one day of hospitalization 18/26 (69.2%) dogs were hypertensive and 8/26 (34.6%) were severely hypertensive). Hypertension was not associated with short-term mortality or canine hemolytic anemia objective score.

Conclusions: In this retrospective study, hypertension was more prevalent in dogs with non-associative IMHA than a control population of ICU-hospitalized dogs. An association between auto-immune conditions and hypertension has been previously reported in people, but not within a canine population. Hypertension in dogs may have an inflammatory or auto-immune etiology. SBP should be monitored closely in canine IMHA, in case anti-hypertensive treatment is required.

1. INTRODUCTION

Canine immune mediated hemolytic anemia (IMHA) is one of the most common autoimmune disorders in dogs. Most cases are non-associative (previously called primary or idiopathic). [1–3] Despite research into immunosuppressive treatments and prognostic indicators, there is still a significant mortality and morbidity associated with the disease.[1,4,5] Therefore, there is a need to further understand the systemic consequences of IMHA and identify novel therapeutic targets.

Hypertension is a negative prognostic indicator in people with autoimmune disease. [6] It is a major risk factor for human cardiovascular and renal disease, and, if uncontrolled, is associated with poorer outcomes in both people and dogs. [7–9] Recent studies suggest an inflammatory etiology to human hypertension,[7] raising the possibility that treatment of hypertension as a consequence of IMHA could improve clinical outcome in affected dogs. To date, the prevalence of hypertension in dogs with IMHA, and its association with clinical outcomes, remains unknown.

We hypothesized that the incidence of hypertension is increased in dogs with IMHA. The primary aim of this study was to compare the prevalence of hypertension in dogs hospitalized due to IMHA, with a control population of hospitalized dogs. Secondary aims were to explore the relationships of systolic blood pressure (SBP) with mortality and the canine hemolytic anemia objective score (CHAOS) [c], a clinical prognostic indicator.

2. MATERIALS AND METHODS

2.1 Case selection

The study population for this retrospective study consisted of client-owned dogs, presented to a university teaching hospital between December 2016 and April 2019, and hospitalized in the intensive care unit (ICU). Hospital electronic records were searched to identify patients that met the inclusion criteria for the test and control populations.

Dogs were included in the test population if they were diagnosed with IMHA for the first time according to American College of Veterinary Internal Medicine (ACVIM) consensus criteria: PCV<37% with two or more signs of immune-mediated destruction (spherocytosis, positive saline agglutination test without washing, positive direct antiglobulin test or positive saline agglutination test that persisted with washing) and one or more signs of hemolysis (hyperbilirubinemia in the absence of hepatic disease, post hepatic cholestasis or sepsis), hemoglobinemia, hemoglobinuria or erythrocyte ghosts).[3] A further inclusion criterion was documentation of ≥2 SBP measurements in the medical record.

Dogs were excluded if they had a previous diagnosis of IMHA, precursor-targeted immune mediated anemia,[3] hyperadrenocorticism, hypertension, renal disease, diabetes mellitus, hyperaldosteronism or adrenal tumors. Previous treatment with antihypertensives, adrenoreceptor agonists, diuretics, glucocorticoids or blood products prior to the diagnosis of IMHA, incomplete hospital or blood work records were also reasons for exclusion.

The control population were dogs with a diagnosis of idiopathic epilepsy. This diagnosis was made by Diplomates of the European College of Veterinary Neurology. Dogs with compatible

clinical signs, signalment and history, and absence of systemic causes were included. The use of magnetic resonance imaging was recorded. The main inclusion criterion was that they were hospitalized in the ICU. Exclusion criteria were the same as for the test population but also included evidence of structural intracranial disease on magnetic resonance imaging, altered mentation or vomiting that increased the clinical suspicion of raised intracranial pressure, and any systemic anomaly that might manifest clinically as seizures, such as increased serum bile acids. Blood pressure (BP) measurements were excluded from analysis if dexmedetomidine or acepromazine had been administered to the patient within 24 hours preceding acquisition.

2.2 Data collection

Data collected included age, gender, neutering status, weight, heart rates at presentation, the time after admission of BP measurement, temperature at presentation, previous medical history (including medication), date(s) of administration of blood products (whole blood, packed red blood cells and intravenous immunoglobulin) if applicable, date of admission, date of euthanasia, death or discharge. Results of diagnostic testing were recorded including: blood type, complete blood count including smear evaluation, serum biochemistry, in-saline agglutination (macroscopic or microscopic), direct antiglobulin test, point-of-care vector borne diseases ELISA [a], Angiostrongylus vasorum rapid ELISA test [b], urinalysis and urine culture, and diagnostic imaging. The date and timing of medication administration including glucocorticoids, second line immunosuppressants, and amlodipine were collected. Finally, BP values, the modality used, timings, site of measurement and details of any retinal examination

were collected. Canine hemolytic anemia objective score (CHAOS) was calculated for every case [c].

2.3 Blood Pressure Measurement

BP was measured indirectly according to a hospital-wide standard operating procedure. An average value was obtained from 5-7 readings according to ACVIM guidelines. This was recorded in the hospital records alongside the site measured and modality used. [9]

Hypertension was defined as SBP of ≥160mmHg, with severe hypertension ≥180mmHg. [9] When more than one BP measurement was performed on a day, the median SBP for the day was used for analysis. [10]

2.4 Statistical analysis

Statistical analysis was performed using commercially available statistics software [d]. Data were assessed for normality by a Shapiro Wilk W test and are expressed as mean ± standard deviation (normal) or median with range (non-normal). Independent *t*-tests were used to compare normal or log transformed data between two groups, whereas Mann Whitney *U* was used for non-normal data. Fisher's exact tests compared the incidence of hypertension and severe hypertension between IMHA and control groups, and CHAOS with hypertension and severe hypertension in IMHA dogs. The correlation between SBP and CHAOS was determined by Spearman rank. Significance was set at $P<0.05$.

3 RESULTS

3.1 Group demographics

The test and control populations were comparable with respect to age (P=0.125) and weight (P=0.311). There was a wide spread of breeds.

3.1.1 IMHA (test) population:

Twenty-six dogs met the inclusion and exclusion criteria (median age 6.7 years, range 0.3-9.7 years), of which 13 were male (8 neutered, 5 entire) and 13 female (9 neutered, 4 entire). Their median weight was 15.7kg (4.5-60.8kg). Cocker Spaniels were over-represented (6/26).

Investigations performed to reach a diagnosis of non-associative IMHA are summarized in Table 1. Blood pressure was measured using Doppler sphygmomanometry [e] in 25/26 cases and an oscillometric machine [f] in 1/26. No dogs showed evidence of acute kidney injury based on serial creatinine assessment. Proteinuria was found in 3/16 dogs with urine dipstick evaluation (2 trace, 1 +) Fundic exams, performed in 5/26 cases, were unremarkable.

3.1.2 Idiopathic epilepsy (control) population:

Twenty-three dogs met the inclusion criteria. Magnetic resonance imaging of the brain was performed in 20 dogs, and no structural abnormality was identified. Of the three remaining dogs, two were young Border collies and one a young Springer spaniel, whose owners declined advanced imaging. The median age was 5 years (range 1-7 years). Eleven were male (6 neutered) and 12 were female (11 neutered). Median weight was 19.9 kg (5.25-40.65 kg). Border Collies were over-represented (5/23) All BP measurements were obtained by Doppler sphygmomanometry[e].

3.2 Group comparisons of SBP and hypertension

Within the IMHA group, SBP was measured within 6 hours of admission in 24/26 dogs. Of these, 11/24 were hypertensive, including 6/11 with severe hypertension. The remaining two dogs did not have a SBP recorded until day two of hospitalization. Mean daily SBP of the population, prior to antihypertensive medication, was 161mmHg (SD 22), of which 13/26 dogs were hypertensive, and 3/26 had severe hypertension. Hypertension was present on at least one day of hospitalization in 18/26 dogs and 8/26 were severely hypertensive on at least one day. Five hypertensive dogs were treated with amlodipine. 12/26 dogs had received systemic glucocorticoids prior to referral. There was no significant difference in admission SBP (P=0.732) between dogs that had recently received systemic glucocorticoids to treat IMHA before admission (mean SBP 159, SD 11), and those that had not (mean SBP 162, SD 28). Six of the dogs that had not received glucocorticoids prior to admission were hypertensive on arrival.

Three dogs received whole blood transfusions at their referring veterinarian. In all cases this was greater than 24 hours prior to referral.There was no difference in admission SBP (P=0.357) between dogs that had received blood products prior to referral (SBP 167, SD 18) and those that had not (SBP 156, SD 23). Five dogs did not receive a transfusion during their disease course. Receiving a transfusion was not associated with SBP (P=0.231) or hypertension (P=0.163). The number of transfusions received was not associated with SBP (P=0.825) or hypertension (P=0.174).

Within the control group, SBP was measured in 18/23 dogs within six hours of admission. 5/18 were hypertensive and 2/5 were severely hypertensive. Mean daily SBP was 138mmHg (SD 14). Only 1/23 dogs was hypertensive during the period of hospitalization (170mmHg).

IMHA dogs had a significantly higher SBP than control dogs during hospitalization ($P<0.01$) (Figure 1). Dogs with IMHA had a greater prevalence of hypertension than control dogs ($P<0.01$).

3.3 SBP, CHAOS and mortality within the IMHA group

Within hospital mortality was 6/26 (23.1%), of which 5/6 were euthanized. Three out of these 6 dogs were hypertensive, with one severely hypertensive prior to amlodipine administration. Hypertension was not associated with mortality ($P=1$). CHAOS was predictive of mortality (Figure 2, $U=20$, $P=0.013$), but was not associated with hypertension or severe hypertension ($P=1$). There was no difference in SBP between dogs with a CHAOS ≥3 or CHAOS <3 (Figure 3). CHAOS was not correlated with SBP ($r=0.119$, $P=0.56$).

4. DISCUSSION

This is the first study to compare BP in dogs with IMHA with a control population. BP was increased in dogs with IMHA, and the incidence of hypertension was increased. However, hypertensive dogs were not more likely to die in the short-term. We conclude that IMHA may be a risk factor for hypertension. The presence of hypertension in IMHA in this retrospective study did not predict short-term mortality.

Hypertension is the single biggest contributor to the human global disease burden, and idiopathic, or essential, hypertension is the most common chronic disease in people.[7,11] As such, a great deal of research has focused on identifying its etiopathogeneses. There is increasing evidence that hypertension is an inflammatory disorder, and that oxidative stress and inflammatory infiltration of both vascular adventitia and the renal interstitium play key roles in its development. [7] Hypertension is modulated by immune cell lines, including macrophages and T cells, which mediate the hypertensive effects of angiotensin II. [7,12] Therefore, we predicted the incidence of hypertension would be increased in inflammatory or immune-mediated disease in dogs. We selected canine IMHA as a test disease because its immune-mediated etiology is well described. There is an exaggerated Th2 immune response, and an upregulated mononuclear-phagocyte system, leading to the generation of erythrocyte autoantibodies, and extravascular hemolysis. [5,13] There are also tight relationships between IMHA and inflammatory markers. C-reactive protein (CRP) is significantly increased in canine IMHA, and subsequently decreases on disease resolution. [13–15] This is relevant to our study, since in people, CRP, along with TNF-α are correlated with hypertension and can predict its future development. [7]

Improving understanding of the role of inflammation in hypertension is particularly important in autoimmune disorders, such as systemic lupus erythematosus and the human form of IMHA (termed autoimmune hemolytic anemia), in which hypertension contributes to morbidity and mortality associated with the disease. [6,16,17] Of additional therapeutic relevance is the reduction in BP that can be achieved with immunosuppressive therapy in patients with autoimmune disease, and its increase on cessation of treatment. [18] No studies, thus far, have examined the predictive value of cardiovascular risk scores and autoimmune disease, and

prospective clinical studies examining the role of immunosuppressant treatment and hypertension in people have been proposed. [19] Our data clearly demonstrate that the incidence of hypertension is also increased in a well-defined autoimmune disorder in dogs that is phenotypically similar to autoimmune hemolytic anemia. We cannot completely rule out the presence of separate, undiagnosed diseases causing associative IMHA and, by an alternative mechanism, hypertension in our patients. However, the majority of dogs in this retrospective study had extensive diagnostic testing and we feel that this is unlikely. Therefore, our data at least raise the possibility of a causal link between canine IMHA and the development of hypertension.

To address the secondary aims of the study, we investigated the relationship between hypertension with morbidity, short term mortality and CHAOS, a prognostic indicator. CHAOS was chosen because it has been shown in a follow-up study to be associated with in-hospital and 30-day mortality. [2] As expected, CHAOS was correlated with mortality, but we did not demonstrate a relationship between hypertension and mortality. This may represent a Type 2 error, since our retrospective study was small, and our in-hospital mortality was towards the lower end of reported ranges. [5]

Due to the retrospective nature of this study, reasons for euthanasia and death were not available. Owner, patient, financial, ethical and other factors may all have contributed to the decision to euthanize an animal and this has the potential to compromise the utility of mortality data. In this cohort, there was no relationship between CHAOS and either BP or hypertension suggesting that BP may not have prognostic value in the short term. However, our low mortality rate and small case numbers may mean the study was underpowered to detect this.

241 Blood pressure is affected by many physiological variables such as hemodynamic status,
242 pyrexia and cardiovascular integrity all of which can be altered due to the reduced tissue
243 oxygenation and changed rheology in IMHA. As an individual's BP is a result of a multitude of
244 interacting factors this could well reduce the utility of BP as a short-term prognostic indicator.
245 However, the mid to long-term consequences of hypertension for IMHA, in which day-to-day
246 variations are less likely to have a confounding effect, have yet to be determined. Risk factors
247 for mortality in canine IMHA include hypercoaguability and kidney injury. [2,20,21] Creatinine, a
248 marker of glomerular filtration rate, is predictive of 30-day mortality, and since glomerular injury
249 results from hypertension, it raises the possibility of hypertension as contributing to the
250 mortality in these patients.[9] Hypercoagulability, another risk factor for mortality, was not
251 assessed in this study. However, the activation of macrophages and subsequent tissue factor
252 expression could be linked to the activation of immune cells and increased expression of
253 endothelin and angiotensin II receptors. [7,22] Further, large, prospective studies would be
254 required to investigate these hypotheses.
255 There are multiple limitations to this study, in part due to its retrospective nature. Non-
256 invasive BP monitoring was performed in all cases, and two different methods were used
257 (Doppler and oscillometric). Invasive blood pressure monitoring is the gold standard and
258 discrepancies have been noted between it, oscillometric and Doppler measurements. [9] Arterial
259 catheter placement is invasive and could be contraindicated in a group of anemic patients due to
260 the risk of hemorrhage. In addition, the placement of an intra-arterial catheter in a group of
261 hyper-coagulable patients could further increase the risk of a vascular event.[23] The retrospective
262 nature means that we cannot exclude operator variability when obtaining BP measurements,

263 despite a hospital-wide policy on how these are obtained. Similarly, a full diagnostic evaluation
264 was not performed in all cases and it is possible that some of the dogs had an unidentified cause
265 of IMHA. Our exclusion criteria screened for animals with other clinical conditions associated
266 with hypertension. Three dogs had mild proteinuria present, one was hypertensive (164mmHg)
267 with an albumin of 23.4g/L, a urine protein:creatinine ratio was not tested. Although we cannot
268 definitively rule out protein losing nephropathy in this case, we suspect this mild
269 hypoalbuminemia is secondary to albumin being a negative acute phase protein associated with
270 the systemic inflammation present in canine IMHA. Proteinuria has been demonstrated
271 secondary to systemic inflammation and the use of steroids. [24,25]

272 It is difficult to control for behavioral factors such as patient stress, which could
273 contribute to situational hypertension, or the white coat phenomenon. However, we tried to
274 ameliorate for these effects with the use of a control group of dogs hospitalized
275 contemporaneously within the same ICU setting. Due to the heterogenous nature of an ICU
276 population a good comparable control group was difficult to establish. In our institution all dogs
277 at risk of seizure activity are hospitalized in the ICU. The 23 cases used in the control group
278 included 7 hospitalized for treatment of status epilepticus, and 16 with idiopathic epilepsy that
279 were boarding whilst awaiting other procedures. All these dogs were selected because of their
280 similar contemporaneous numbers, consistency of phenotype and absence of confounding co-
281 morbidities. The control group had an increased number of hypertensive dogs on arrival (5/18)
282 compared to the single hypertensive animal across the period of hospitalization. This likely
283 reflects the situational hypertension associated with hospital admission, or recent seizure, which
284 reduces as patients acclimatize to the hospital environment. In comparison the incidence of

hypertension in the canine IMHA population remained high, suggesting a genuine effect of the condition.

Blood pressure measurements were excluded if sedatives had been administered within 24 hours or if there was a known or clinical suspicion of intracranial pressure to avoid confounding effects on BP. Anti-epileptic medications have not been shown to alter BP in dogs, however they may sedate animals and blunt any situational hypertension. [26,27] However in the 16 dogs that were stable on long-term medication, we believe this effect would have been reduced. Clinically, our experience is that these dogs are often more stressed in the ICU than the critical patients.

Future prospective studies would benefit from an anemic control group. However a substantial sized group may be difficult to ascertain having excluded hemorrhagic patients, who are likely hypovolaemic and patients with anemias secondary to chronic inflammatory disease.

All dogs received systemic corticosteroids as part of their treatment protocol for IMHA. The BP response to corticosteroids is highly variable. For example, dogs with hyperadrenocorticism have an increased risk of hypertension, [9] and dexamethasone at a high dose of 0.5mg/kg has been shown to increase BP in dogs. [28] However, this statistically significant increase is below the threshold for clinical hypertension, and only occurs after 28 days. [9,28,29] Furthermore, neither methylprednisolone administered at 10mg/kg/day for 10 days nor hydrocortisone, at 3.3mg/kg three times a day for 42 days, induces significant increases in BP in dogs, [30,31] and oral prednisolone at anti-inflammatory doses does not increase plasma volume in healthy dogs. [32] The administration of glucocorticoids did not affect our dogs' SBP at

306 presentation. Therefore, we believe that steroid administration is not contributing to our
307 findings in this study.

308 The administration of blood products could lead to volume overload in cases of
309 euvolemic anemia. However, neither the administration of a transfusion prior to referral nor the
310 number of transfusions received was associated with SBP or hypertension. SBP decreased,
311 remained static and increased following transfusions in individual cases. We therefore believe
312 that fluid overload is not contributing to our findings in this study.

313 ## 5. CONCLUSION

314 In this retrospective study, BP was increased and the prevalence of hypertension was
315 increased in dogs with non-associative IMHA compared to a control population of ICU dogs.
316 Clinicians should be aware of an increased risk of hypertension in IMHA dogs, allowing them to
317 instigate appropriate BP monitoring and timely anti-hypertensive therapy. A relationship
318 between increased BP and IMHA has not been demonstrated before in dogs. Further prospective
319 studies are warranted to determine the etiological role of inflammation in canine hypertension
320 and whether anti-hypertensive therapy contributes to a more favorable long term clinical
321 outcome.

322

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Table 1: Summary of all tests performed to exclude associated causes of IMHA.

Investigation performed	Result supportive of diagnosis	Numbers of cases tested	Numbers with consistent result
Blood Smear evaluation	Spherocytes >3/x100 oil immersion field, Absence of Heinz bodies or intracellular organisms in RBCs	26/26	25/26
Packed Cell	Anemia	26/26	26/26

Volume	PCV<37%		
Biochemistry	Bilirubin (total) >6,8umol/L	26/26	21/26
Insaline agglutination test	Micro or macroscopic agglutination	26/26	26/26
4DX Snap test*	Negative	26/26	26/26
Thoracic imaging (radiography or computed tomography)	No underlying trigger for hemolysis identified	26/26	26/26
Abdominal imaging (ultrasonography or computed tomography)	No underlying trigger for hemolysis identified	26/26	26/26
Angiodetect	Negative	18/26	18/18
Urinalysis	Inactive sediment	24/26	24/24
	Hemoglobinuria	16/26	13/16
	Bilirubinuria	16/26	16/16
Urine culture at presentation	Negative	9/26	9/9
Direct antiglobulin test	Positive	5/26	3/5

***** SNAP4Dx, (screening for antigens of *A. phagocytophilum, A. platys, B. burgdorferi, E. canis, E. ewingii, D. immitis*) Idexx Laboratories, Westbrook, Maine

Figure legends:

Figure 1. Box and whisker plot representing median systolic blood pressure (mmHg) and interquartile range in dogs with IMHA or control population.

Figure 2. Individual value plot of CHAOS in survivors and non-survivors. CHAOS was predictive of mortality

Figure 3.Box and whisker plot representing median systolic blood pressure (mmHg) and interquartile range in dogs with CHAOS <3 and CHAOX ≥3

Figure 4. Graph to show CHAOS does not correlate with SBP (*r=0.119,*P=0.56)

Footnotes:

[a] SNAP4Dx, (screening for antigens of *A. phagocytophilum, A. platys, B. burgdorferi, E. canis, E. ewingii, D. immitis*) Idexx Laboratories, Westbrook, Maine

[b]AngioDetect rapid assay [Idexx Laboratories, Westbrook, Maine

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[e] Ultrasonic Doppler Flow Detector, Parks Medical Electronics, Inc. Aloha, OR

[f] Cardell Model 9401, Sharn Veterinary Inc., Orchard Park, NY