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Article

Evaluation of Galectin-3 in Dogs with Atrial Fibrillation

Giulia Arcuri [1], Carlotta Valente [1], Giovanni Romito [2], Federico Bonsembiante [1], Chiara Mazzoldi [2], Barbara Contiero [1], Helen Poser [1] and Carlo Guglielmini [1]*

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Simple Summary: Cardiac fibrosis is a common manifestation of heart disease that leads to the deterioration of cardiac function and the development of cardiac arrhythmias. Atrial fibrillation (AF) is particularly influenced by cardiac fibrosis, which is considered one of the primary factors in its development. Various diagnostic technique can be employed to assess myocardial fibrosis, including cardiac imaging and the evaluation of circulating biomarkers. Among these biomarkers, galectin-3 (Gal-3) is notable for its involvement in inflammation and tissue fibrosis associated with cardiac disease. In humans, several studies have reported that increased serum Gal-3 concentration is a risk factor for AF. In this study, we evaluated the serum concentration of Gal-3 in 30 dogs with AF associated with different cardiac diseases. Seventeen clinically healthy dogs and 33 dogs with cardiac disease but without AF served as controls. Our findings indicated no significant difference in Gal-3 concentration between healthy dogs and dogs with cardiac disease, regardless of the presence of AF. Gal-3 showed a significant positive correlation with age. The results of this study suggest that Gal-3 does not have predictive value for the development of AF in dogs but it is associated with advanced age.

Abstract: Galectin-3 (Gal-3) is a lectin associated with fibrosis and inflammation, and increased circulating concentrations are considered a risk factor for atrial fibrillation (AF) in humans. This retrospective study aimed to evaluate the serum concentration of Gal-3 in dogs with cardiac disease, both with and without AF. Dogs with AF associated with congenital and acquired heart diseases were selected, while clinically healthy dogs and dogs with heart diseases but without AF served as controls. We statistically compared the serum concentration of Gal-3, assessed using a commercial canine-specific ELISA kit, among healthy dogs and dogs with heart disease with and without AF. Additionally, associations between Gal-3 and clinical and echocardiographic variables were evaluated. A total of 80 dogs were included, of which 17/80 (21.2%) were clinically healthy and 63/80 (78.8%) had heart disease, with 30/63 (47.6%) having AF. No significant difference in Gal-3 concentration was found between healthy dogs (3.90 ± 0.38 ng/mL) and dogs with heart disease, either with or without AF (3.45 ± 0.28 ng/mL, P=0.226 and 4.46 ± 0.27 ng/mL, P=0.286, respectively). Gal-3 showed a significant positive correlation with age (r=0.46, P < 0.001). The results of this study suggest that Gal-3 does not have predictive value for the development of AF in dogs, but it is associated with advanced age.

Keywords: canine; biomarker; cardiac disease; echocardiography

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1. Introduction

Galectin 3 (Gal-3) is a beta-galactoside-binding protein belonging to the lectin family that is released by activated macrophages. It plays a vital role in many physiological cellular functions, including cellular growth, differentiation, proliferation, apoptosis, cellular adhesion, and tissue repair [1].

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In human medicine, increased Gal-3 has been reported to be associated with a variety of disorders, including congestive heart failure (CHF), renal failure, diabetes mellitus, and cancer [2–5]. Its involvement in the pathogenesis of cardiovascular diseases has been extensively studied, particularly with reference to its role in inflammatory processes and tissue fibrosis. An increase in Gal-3 concentration stimulates the release of several mediators that promote cardiac fibroblast proliferation, collagen synthesis and deposition, and ventricular dysfunction [5–8]. Consequently, Gal-3 is considered a biomarker predictive of cardiac remodeling and adverse cardiac events, including the risk of onset of CHF and myocardial fibrosis [5,9–12].

Inflammation and fibrosis of the myocardium are particularly important in the etiology of atrial fibrillation (AF). The pathophysiology of AF is complex and involves, among other factors, atrial pro-inflammatory responses that lead to electrical and structural remodeling associated with myocardial fibrosis. This process creates a pro-arrhythmic substrate that promotes the onset of AF [13–15]. Due to these mechanisms, several studies have investigated the possible relationship between serum concentration of Gal-3 and the risk of developing AF in humans, revealing a correlation between increased Gal-3 levels and an increased risk of AF [16–19].

In veterinary medicine, a few recent studies have evaluated the role of Gal-3 in dogs and cats with cardiac and noncardiac diseases, such as endocrine, dermatologic, or neoplastic disorders [20–23]. Specifically, some studies have demonstrated increased Gal-3 concentration in dogs with congenital or acquired heart disease, including myxomatous mitral valve disease (MMVD) and dilated cardiomyopathy (DCM) [20,24–28]. As in humans, AF is the most common supraventricular arrhythmia in dogs [29–32] and typically occurs secondary to cardiac diseases associated with left atrial enlargement [33,34]. Additionally, the development of AF is associated with a worse prognosis in dogs with MMVD and DCM [35–37]. Thus, investigating the correlation between serum Gal-3 concentration and the risk of developing AF could be useful in the clinical evaluation of dogs with heart disease. To the authors' knowledge, no studies have yet investigated the relationship between Gal-3 and AF in dogs.

Therefore, the aim of this study was to evaluate the serum concentration of Gal-3 in dogs, with cardiac disease with or without AF. We hypothesized that the presence of AF would be associated with an increased serum concentration of Gal-3.

2. Materials and Methods

2.1. Animals

In this retrospective study, clinical data of dogs visited from July 2017 to October 2023 at the Cardiologic Units of the Veterinary Teaching Hospital (VTH) of the University of Padua and Bologna were reviewed. Dogs with congenital heart diseases (CHD), MMVD or DCM associated with AF were selected.

The diagnosis and classification of heart disease were performed based on previously described clinical and echocardiographic criteria [38–43]. The presence or absence of AF was based on a 6-/12-lead surface ECG of at least 3-minute duration. Specifically, the diagnosis of AF was based on the combined presence of the following findings: lack of recognizable P waves and irregularly irregular cardiac rhythm with narrow QRS complexes [44,45].

Clinically healthy dogs and dogs with heart disease but without AF were included in the study as control groups. These control animals were selected from the database of the same VTHs during the same time period. Specifically, clinically healthy dogs had normal results of clinical and echocardiographic examination and unremarkable CBC and biochemical profile findings, whereas dogs with heart disease were chosen based on the same type and stage of cardiac disease as those with AF. The presence of concomitant noncardiac disease was not an exclusion criterion in this study, but this information was noted.

Each dog included in this study underwent blood sampling for routine CBC and biochemical profile. Serum samples were kept at room temperature for 15 minutes to allow for stable clot formation and then were centrifuged for 10 minutes. An aliquot of serum was then harvested and frozen at -80°C until batch analysis for the evaluation of Gal-3 concentration.

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2.2. Echocardiographic Examination

At each VTH, an experienced operator (G.R., H.P., and C.G.) performed the echocardiographic examination using echocardiographic units (iE33 and CX50 ultrasound systems, Philips Healthcare) equipped with dedicated multifrequency phased array transducers (S5-1 and S3-8 MHz) and continuous ECG tracing.

The measurements of left ventricular diastolic diameter (LVDD) and left ventricular systolic diameter (LVSD) were obtained from M-mode short-axis echocardiographic images at the level of the *chordae tendineae*. Left ventricular diastolic and systolic measurements were then transformed using the described allometric scaling system to obtain normalized measurements for bodyweight (LVDDn and LVSDn, respectively) [46]. Fractional shortening (FS) was then calculated using the standard formula. Left atrial diameter (LA) and aortic root diameter (Ao) were measured at early diastole from 2D echocardiographic short axis images obtained at the level of the heart base, and the LA:Ao ratio then was calculated [47]. The trans-mitral blood flow was examined using pulsed-wave Doppler from the left apical four-chamber view by positioning the sample volume at the tip of the mitral valve leaflets, and the peak velocity of the early diastolic wave (E Mitral) was obtained. All measurements were replicated on 3 or 5 consecutive beats, in dogs without or with AF, respectively, and the mean values were calculated.

2.3. Measurement of Serum Galectin-3 Concentration

Galectin-3 concentration was measured in canine serum samples using a commercially available ELISA kit (RayBio Canine Galectin-3 ELISA kit, RayBiotech, Norcross, GA, USA) with a detection range of 2-500 pg/mL. According to a previous study [28], serum samples were diluted 1:30 with the diluent included in the assay prior to analysis. This dilution ensured that all samples fell within the detectable range of the assay, and no additional dilutions were necessary. Measurements were performed following the manufacturer's assay procedure, with all samples analyzed in duplicate. Data analysis utilized the mean of the two measurements. Optical density was measured using a multimode microplate reader (Victor X4, PerkinElmer, Waltham, MA, USA), at a wavelength of 450 nm.

2.4. Statistical Analysis

Data were analyzed using commercial software (SAS 9.4, SAS Institute Inc., Cary, NC, USA). A sample size calculation was performed to determine the number of dogs to be included in the study. Based on results from previous studies that measured Gal-3 concentrations, we calculated the sample size to achieve a power of 0.8 and a significance level (α) of 0.05 [24,27]. These calculations indicated that groups ranging from 8 to 17 dogs would be sufficient to detect a difference in Gal-3 concentrations.

Demographic and clinical characteristics included breed, sex, age, bodyweight, presence of heart disease and corresponding severity evaluated according to the American College of Veterinary Internal Medicine (ACVIM) classification [39], ongoing treatment at the time of examination, presence of other concurrent diseases, presence or absence of AF. The following continuous echocardiographic variables were considered: LA, Ao, LA:Ao, LVDDn, LVSDn, FS, and E Mitral. Normality of data was assessed using the Shapiro-Wilk test. Comparisons were made between healthy dogs and dogs with heart disease with and without AF, and between healthy dogs and dogs with CHD, DCM, and MVD. Additionally, in dogs with heart disease, comparisons were made between those with compensated and decompensated heart disease (ACVIM classes B1+B2 and C+D, respectively [39,41]).

Normally distributed data were reported as means and standard deviation and were compared using the Student t-test for comparisons between two groups or one-way ANOVA for comparisons among more than two groups. Non-normally distributed data were reported as median and range (minimum-maximum) and were compared using nonparametric tests (Mann-Whitney test for comparisons between two groups or Kruskal-Wallis for comparisons among more than two groups).

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Post-hoc pairwise comparisons were performed using Bonferroni's correction. Associations between variables were evaluated using Spearman's rank correlation coefficient (r). For all analyses, a P value <0.05 was considered significant, except for Bonferroni's correction where P<0.017 and P<0.008 and were considered significant for three and six comparisons, respectively.

3. Results

3.1. Study Population and Echocardiographic Parameters

A total of 80 dogs were included in this study, comprising 34 (42.5%) females (three spayed and 31 intact) and 46 (57.5%) males (two castrated and 44 intact). The mean age was 9.3 ± 3.5 years, and the median bodyweight was 22.1 kg (range 2.2-120 kg). Most dogs were purebred (63 dogs, 78.7%). The most frequently represented breed was Doberman Pinscher (six dogs, 7.5%) followed by Miniature Pinscher, Jack Russel Terrier, and American Staffordshire Terrier (four dogs each, 5%), and by Cavalier King Charles Spaniel, Dogue de Bordeaux, and Border Collie (three dogs each, 3.7%). Other breeds were represented by one or two dogs each.

Seventeen (21.2%) dogs were clinically healthy, while 63 (78.8%) had cardiac disease, including seven (11.1%), 16 (25.4%), and 40 (63.5%) dogs with CHD, DCM, and MMVD, respectively. Congenital heart disease included patent ductus arteriosus (six cases) and mitral dysplasia (one case). Among dogs with cardiac disease, 30 (47.6%) had AF, while 33 (52.4%) maintained a sinus rhythm. Of those with AF, four dogs (13.3%) had CHD, eight dogs (26.7%) had DCM, and 18 dogs (60%) had MMVD.

Twenty-three dogs (28.7%) had concurrent noncardiac diseases, including neoplastic (five dogs, 6.25%), dermatological (five dogs, 6.25%), neurological (four dogs, 3.2%), gastrointestinal (four dogs, 3.2%), endocrine (two dogs, 1.6%), orthopedic (two dogs, 1.6%), and other various (three dogs, 2.4%) diseases. Specifically, neoplastic diseases included chronic leukemia, intracranial mass, lung, intestinal and hepatoid gland neoplasia (one dog each). Among dermatological diseases, two dogs each had otitis and food allergies, and one dog had atopic dermatitis. Neurological disorders included herniated intervertebral discs (two dogs), and Wobbler syndrome and Chiari-like syndrome (one dog each). In the gastrointestinal group, two dogs had food-responsive enteropathy and one dog each had an esophageal foreign body and immune-mediated enteropathy. Endocrine disease included hypoadrenocorticism, hyperadrenocorticism, and hypothyroidism (one dog each), while orthopedic disorders included joint pain and hip-elbow dysplasia (one dog each). Finally, three dogs had chronic kidney disease, pyorrhea, and peritoneal hernia (one dog each). At the time of study enrolment, 28 (35%) dogs were receiving cardiac treatment (CT), noncardiac treatment (OT) or both. Among those receiving CT, 22 (27.5%) dogs were receiving diuretics (furosemide or torasemide), 19 (23.7%) pimobendan, 17 (21.2%) ACE inhibitors (benazepril or enalapril), 12 (15%) spironolactone, and two (2.5%) digoxin.

Table 1. presents a comparison of clinical and echocardiographic variables between healthy dogs and dogs with cardiac disease with or without AF. Dogs with AF were heavier (P=0.001), predominantly in ACVIM stage C or D (P<0.001) and had higher LA and E mitral compared to healthy dogs or dogs with cardiac disease but without AF (P<0.001 for both comparisons). There were no significant differences found regarding breed (P=0.754), sex (P=0.176), and mean age (P=0.123) among these groups. Among dogs with cardiac disease, there was no significant difference regarding the presence of concurrent disease (P=0.279) and type of treatment at the time of admission (P = 0.99 for CT and P = 0.603 for CT + OT) between those with or without AF.

Table 1. Clinical and echocardiographic data obtained from 80 dogs divided into three groups: healthy dogs and dogs with heart disease with or without atrial fibrillation (AF).

Variable	Category	Healthy group (N=17)	AF group (N=30)	No AF group (N=33)	Overall P-value
Breed	Purebred, N (%)	14/18 (78%)	25/30 (83%)	25/33 (76%)	0.754
Sex	Male/Female	7/11	19/11	21/12	0.176
Age (years)	Mean ± SD	10 ± 3	8±3	10±4	0.123

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Body weight (Kg)	Median (min-max)	13.7 (2.2-48.5)^{b}	30 (4-120)^{a}	12 (2.4-48.2)^{b}	0.001
Concurrent disease	Yes, N (%)	-	4/30 (13%)	9/33 (27%)	0.279
ACVIM stages	C+D, N (%)	-	28/30 (93%)	12/33 (36%)	<0.001
Treatment ad admission	CT, N (%)	-	19 (63%)	22 (67%)	0.99
	CT + OT, N (%)	-	5 (17%)	3 (9%)	0.603
	NT, N (%)	-	6 (20%)	8 (24%)	0.919
LA (cm)	Median (min-max)	2.3 (1.4-3.6)^{c}	5.6 (3.3-8.5)^{a}	3.7 (2.6-6.7)^{b}	<0.001
Ao (cm)	Median (min-max)	1.7 (1.0-2.7)^{b}	2.5 (1.1-3.1)^{a}	1.97 (1.2-3.2)^{ab}	<0.001
LA:Ao	Median (min-max)	1.4 (1.2-1.9)^{b}	2.2 (1.7-4.2)^{a}	2.0 (1.5-3.2)^{a}	<0.001
LVDDn	Mean ± SD	1.38 ± 0.07^{b}	2.09 ± 0.05^{a}	2.08 ± 0.05^{a}	<0.001
LVSDn	Mean ± SD	0.86 ± 0.08^{b}	1.34 ± 0.06^{a}	1.25 ± 0.05^{a}	<0.001
FS (%)	Mean ± SD	34.9 ± 3.3	29.6 ± 2.5	36 ± 2.4	0.159
E Mitral (m/s)	Mean ± SD	0.67 ± 0.08^{c}	1.48 ± 0.06^{a}	1.15 ± 0.06^{b}	<0.001

Data are expressed as mean ± standard deviation (SD) or median (range). Different superscript letters along rows mean significant different values (P<0.017); Abbreviations: N, number of dogs; ACVIM, American College of Veterinary Internal Medicine; CT, cardiac treatment; OT, other treatment, NT, no treatment; LA, left atrial diameter; Ao, aortic diameter; LA:Ao, left atrial diameter to aortic diameter ratio; LVDDn, left ventricular diastolic diameter normalized to body weight; LVSDn, left ventricular systolic diameter normalized to body weight; FS, Fractional shortening; E mitral, mitral valve maximal E wave velocity.

Table 2 presents a comparison of clinical and echocardiographic variables between healthy dogs and dogs with different types of cardiac diseases. Dogs with CHD and DCM were predominantly purebred compared to those with MMVD and clinically healthy dogs (P=0.025). Females were more prevalent in dogs with CHD, while males were more prevalent in dogs with DCM (P=0.001). Dogs with MMVD were older, whereas dogs with DCM were heavier (P<0.001 for both comparisons). A significant difference was found regarding ACVIM stages between dogs with MMVD and those with DCM (P=0.029). Not surprisingly, dogs with cardiac disease had higher LA and LA:Ao ratio, normalized left ventricular diameters, and E mitral compared to healthy dogs (P<0.001 for all comparisons). Additionally, dogs with DCM had reduced FS, whereas dogs with MMVD had increased FS (P<0.001). No significant differences were found regarding concurrent disease (P=0.351) and treatment administered at the time of admission (P=0.981 for CT and P=0.546 for CT + OT).

Table 2. Clinical and echocardiographic data obtained from 80 dogs divided according to presence and type of heart disease.

Variable	**Category**	**Healthy group (N=17)**	**CHD group (N=7)**	**DCM group (N=16)**	**MMVD group (N=40)**	**Overall P-value**
Breed	Purebred, N (%)	14/18 (78%)^{ab}	7/7 (100%)^{a}	16/16 (100%)^{a}	27/40 (68%)^{b}	0.025
Sex	Male/Female	7/11^{b}	0/7^{c}	13/3^{a}	27/13^{ab}	0.001
Age (years)	Mean ± SD	10±3^{a}	3±3^{c}	7±2^{b}	11±3^{a}	<0.001
Body weight (kg)	Median (min-max)	13.7 (2.2-48.5)^{b}	23 (2.4-37.1)^{b}	42 (32.6-120)^{a}	11 (4-72)^{b}	<0.001
Concurrent disease	Yes, N (%)	-	0	4/16 (25%)	9/40 (23%)	0.351
ACVIM stages	C+D, N (%)	-	4/7 (57%)^{ab}	6/16 (38%)^{b}	30/40 (75%)^{a}	0.029
Treatment ad admission	CT	-	5/7 (71%)	10/16 (63%)	26/40 (65%)	0.918
	CT + OT	-	0	2/16 (13%)	6/40 (15%)	0.546
	NT	-	2/7 (29%)	4/16 (25%)	8/40 (20%)	0.84
LA (cm)	Median (min-max)	2.3 (1.4-3.6)^{b}	5.8 (2.6-6.8)^{a}	4.9 (3.7-6.6)^{a}	4.4 (2.82-8.5)^{a}	<0.001
Ao (cm)	Median (min-max)	1.7 (1.0-2.7)^{b}	2.4 (1.4-3.0)^{a}	2.6 (2.2-3.1)^{a}	1.7 (1.1-3.2)^{b}	<0.001
LA:Ao	Median (min-max)	1.4 (1.2-1.9)^{c}	2.2 (1.6-2.4)^{ab}	1.8 (1.5-3.0)^{b}	2.3 (1.63-4.2)^{a}	<0.001
LVDDn	Mean ± SD	1.38 ± 0.07^{c}	2.38 ± 0.11^{a}	1.93 ± 0.08^{b}	2.09 ± 0.05^{ab}	<0.001
LVSDn	Mean ± SD	0.86 ± 0.08^{c}	1.59 ± 0.11^{a}	1.55 ± 0.07^{a}	1.13 ± 0.04^{b}	<0.001
FS (%)	Mean ± SD	34.9 ± 3.3^{b}	28.9 ± 3.5^{b}	13.4 ± 2.3^{c}	41.5 ± 1.5^{a}	<0.001

E Mitral (m/s)	Mean ± SD	0.67 ± 0.08[c]	1.57 ± 0.13[a]	1.05 ± 0.09[b]	1.36 ± 0.06[a]	<0.001

Data are expressed as mean ± standard deviation (SD) or median (range). Different superscript letters along rows mean significant different values (P<0.008); Abbreviations: N, number of dogs, CHD, congenital heart disease, DCM, dilated cardiomyopathy, MMVD, myxomatous mitral valve disease ; ACVIM, American College of Veterinary Internal Medicine; CT, cardiac treatment; OT, other treatment, NT, no treatment; LA, left atrial diameter; Ao, aortic diameter; LA:Ao, left atrial diameter to aortic diameter ratio; LVDDn, left ventricular diastolic diameter normalized to body weight; LVSDn, left ventricular systolic diameter normalized to body weight; FS, Fractional shortening; E mitral, mitral valve maximal E wave velocity.

3.2. Serum Galectin-3 Concentration

The median serum Gal-3 concentration in dogs with cardiac disease without AF was 4.46 ± 0.27 ng/mL, which was significantly higher compared to dogs with cardiac disease with AF (3.45 ± 0.28 ng/mL, P=0.009) (Figure 1). There was no significant difference in Gal-3 concentration between healthy dogs (3.90 ± 0.38 ng/mL) and dogs with cardiac disease with AF or without AF (P=0.226 and P=0.286, respectively).

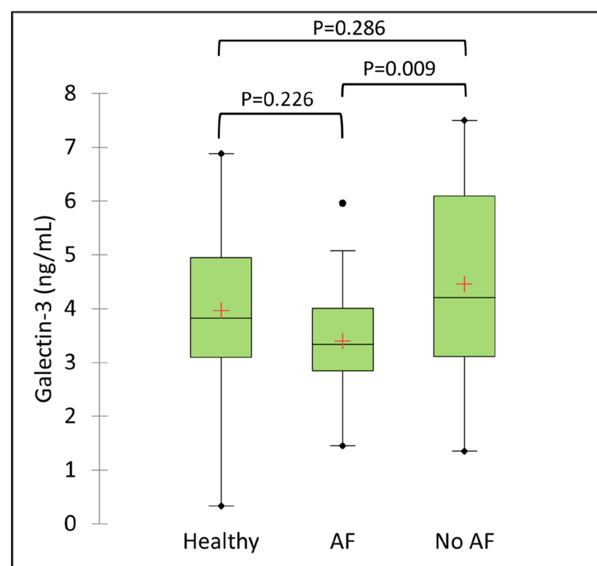

Figure 1. Boxplot showing serum Gal-3 concentration in clinically healthy dogs and dogs with heart disease with or without atrial fibrillation (AF).

Among dogs with heart disease, animals with MMVD had higher median serum concentration of Gal-3 (4.61 ± 0.22 ng/mL) compared to dogs with DCM (2.75 ± 0.34 ng/mL, P<0.001) or CHD (3.17 ± 0.52 ng/mL, P=0.007) (Figure 2). There was no significant difference in median Gal-3 concentration between dogs with CHD and dogs with DCM (P=0.846). Additionally, there was no significant difference in median Gal-3 concentration between healthy dogs and dogs with heart disease (P=0.112 for CHD, P=0.016 for DCM, and P=0.119 for MMVD).

In dogs with MMVD, those without AF had higher median serum concentration of Gal-3 compared to those with AF (5.35 ± 0.27 ng/mL and 3.69 ± 0.30 ng/mL, respectively, P<0.01). Conversely, median serum concentration of Gal 3 in dogs with DCM was not significantly different between those with or without AF (2.65 ± 0.37 ng/mL and 2.84 ± 0.37 ng/mL, respectively, P=0.716). Additionally, no significant difference in median serum concentration of Gal-3 was found between dogs with compensated heart disease (ACVIM stage B1-B2) and those with decompensated heart disease (ACVIM stages C-D) (3.69 ± 0.33 ng/mL and 4.14 ± 0.25 ng/mL, respectively, P=0.29).

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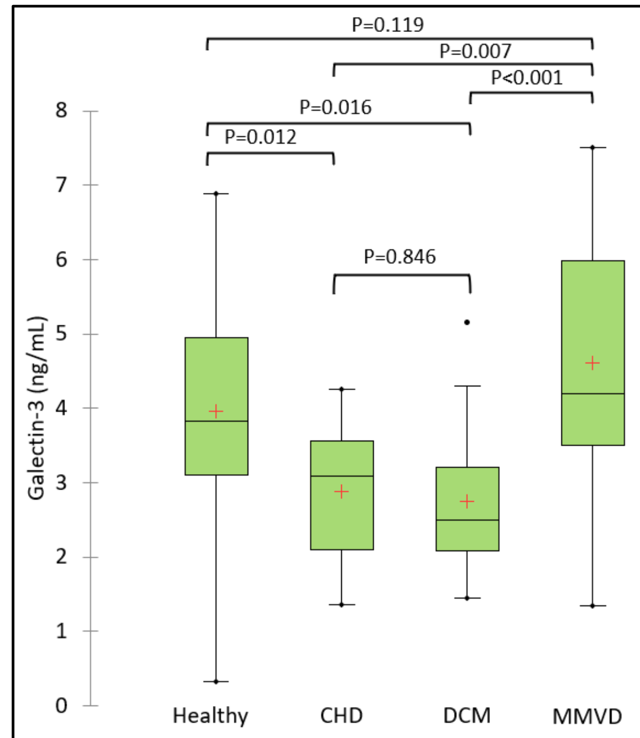

Figure 2. Boxplot showing serum Gal-3 concentration in clinically healthy dogs and dogs with congenital (CHD) or acquired heart disease (DCM, dilated cardiomyopathy; MMVD, myxomatous mitral valve disease).

Table 3 shows the correlations between serum concentration of Gal-3 and clinical and echocardiographic variables. Specifically, a significant positive correlation was found between Gal-3 and age (r=0.46, P<0.001) as well as fractional shortening (r=0.41, P<0.001). Additionally, there was a significant negative correlation between Gal-3 and body weight (r=-0.40, P<0.001) and aortic root diameter (r=-0.35, P=0.002).

Table 3. Results of Spearman's rank correlation test showing the correlation between serum concentration of Gal-3 and clinical and echocardiographic variables.

Variable	r	P-value
Age (years)	0.46	<0.001
BW (Kg)	-0.40	<0.001
LA (cm)	-0.19	0.087
Ao (cm)	-0.35	0.002
LA:Ao	0.18	0.118
LVDDn	0.19	0.092
LVSDn	-0.18	0.101
FS (%)	0.41	<0.001
E Mitral (m/s)	0.10	0.357

Abbreviations: BW, bodyweight; LA, left atrial diameter; Ao, aortic diameter; LA:Ao, left atrial diameter to aortic diameter ratio; LVDDn, left ventricular diastolic diameter normalized to body weight; LVSDn, left ventricular systolic diameter normalized to body weight; FS, Fractional shortening; E mitral, mitral valve maximal E wave velocity.

4. Discussion

The main findings of this study were that serum concentration of Gal-3 does not increase in dogs with secondary AF compared to those with cardiac disease maintaining a sinus rhythm. Among

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canine cardiac diseases, MMVD is associated with higher Gal-3 concentration, and advanced age is additionally correlated with an increased level of this biomarker in the dog.

The characteristics of dogs with AF secondary to cardiac disease in this study are consistent with those reported in previous studies [33,48–50]. Animals with AF were heavier and had higher LA and E mitral measurements than their counterparts maintaining a sinus rhythm. Atrial structural, electrical, ionic, and functional remodelling are the main cardiac modifications underlying the development of AF, both in humans and dogs [51–54]. Left atrial structural remodelling refers to adaptive or maladaptive changes in cardiac architecture that occur at both macro- and microscopic levels [52]. Specifically, atrial enlargement and fibrosis are the most important macroscopic and microscopic changes occurring during atrial remodelling in people with AF [52].

Few studies have investigated the atrial microscopic changes that occur in dogs with MMVD or DCM, the main ones being interstitial fibrosis, myocardial fat replacement, and immune cell infiltration [55,56]. Even fewer studies have evaluated microscopic changes at the atrial level in dogs with AF [55,57]. Beyond pathological studies, the clinical evaluation of atrial changes leading to or associated with AF is challenging. Echocardiography, including speckle-tracking echocardiography (STE), can be useful for identifying left atrial remodeling and dysfunction [58–60], but is not suitable to unveil atrial microscopic changes. Advanced imaging techniques, such as cardiac magnetic resonance imaging and computed tomography, allow accurate assessment of myocardial fibrosis [61–63], but are not routinely employed in the canine clinical setting.

Therefore, increasing attention has been paid to circulating molecules, such as Gal-3, as potential biomarkers of cardiac remodelling and fibrosis in recent years. Despite these premises, Gal-3 was not found to be a risk factor for the development of AF in this study. Indeed, dogs with heart disease and maintaining a sinus rhythm had higher median serum concentration compared to those with AF. In humans, AF often occurs in elderly patients without recognizable cardiac disease (i.e., primary or lone AF) [13,64]. In these patients, cardiac fibrosis in the absence of any discernible heart disease is likely the major inciting mechanism for the development of the arrhythmia [65,66] leading to increased serum concentration of Gal-3. Furthermore, cardiac fibrosis is proportional to the amount of myocardial tissue involved, intrinsically higher in the ventricles compared to the atria and, consequently, the concentration of fibrosis-related serum biomarkers, such as Gal-3, also follows this proportionality. Therefore, we hypothesize that atrial fibrosis was likely not sufficient to result in increased serum concentration of Gal-3 in dogs of the present study with secondary AF.

Regarding the evaluation of Gal-3 according to different cardiac diseases, dogs with MMVD had significantly increased Gal-3 concentration compared to dogs with CHD and DCM, regardless of the presence of AF, but not compared to clinically healthy dogs. Cardiac fibrosis is a pathological feature of MMVD, especially at the level of papillary muscles and *chordae tendineae* [67,68]. Furthermore, one study reported evidence of fibrosis in the left atrium of dogs with MMVD, although a histopathological evaluation of the ventricles was not performed in the same animals [56]. Conversely, the main histologic features of canine DCM, the second most represented cardiac disease in dogs of this study, include the “fatty infiltration-degenerative” type, characterized by myofibril degeneration, vacuolization, and adipocyte clusters, and the “attenuated wavy fiber” type, characterized by atrophic myocardiocytes with a wavy appearance [69]. These different pathological features of the two most common canine acquired cardiac disease explain the observed increase of Gal-3 concentration in dogs with MMVD.

Previous studies have found significantly increased Gal-3 concentration in dogs with MMVD compared to healthy animals [24–26], but in all these studies, the control group was composed of dogs significantly younger than those with MMVD. Conversely, in the present study, healthy dogs were cross-matched by age with those with MMVD, and both groups had a mean age greater than 10 years, whereas dogs with CHD or DCM were younger (mean age 3 and 7 years, respectively). Furthermore, we found a significant positive correlation between Gal-3 concentration and advanced age. These findings provide evidence of the correlation between Gal-3 and cardiac aging in the dog, as already reported in humans [70,71].

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An hallmark of cardiac aging is progressive ventricular remodeling characterized by myocardial hypertrophy, interstitial fibrosis, and ultimately ventricular dysfunction [72], although the underlying pathophysiological mechanisms are complex and not completely understood [73,74]. Regarding the role of Gal-3 in dogs with congenital cardiac disease, a recent study reported an increased level of circulating Gal-3 in dogs with pulmonic stenosis compared to clinically healthy dogs, with a median age of three years and one and a half year, respectively [27]. Increased right ventricular myocardial stiffness and fibrosis have been reported in dogs with pulmonic stenosis [27,75], but no dog with pulmonic stenosis was included in the present study.

Another finding in this study was the negative correlation between Gal-3 and both body weight and aortic root diameter. These results contrast to those reported in humans, where Gal-3 has been found to be positively correlated with body mass index in patients with heart disease [76]. Aged small-sized dogs, with or without MMVD, had higher levels of Gal-3 in this study, likely explaining the observed negative correlation between this molecule and body weight.

Because of its retrospective design, this study has some limitations. First, Gal-3 is not a specific cardiac biomarker, and increased circulating concentrations have been reported in other noncardiac diseases, such as diabetes mellitus, kidney disease, and cancer in humans [2–4]. Similarly, some studies have shown a role for this biomarker in dogs with chronic dermatological, endocrine, and neoplastic disorders [20,22]. In this study, some dogs presented with concurrent noncardiac diseases, including neoplastic, orthopedic, neurological, endocrine, and dermatological disorders. These comorbidities may have affected our results, but the influence of some of them on circulating Gal-3 have not been previously reported. Moreover, no difference was found in the prevalence of these comorbidities either between dogs with or without AF or between dogs with DCM or MMVD. Second, the circulating levels of Gal-3 were not analyzed in comparison with histologically proven myocardial fibrosis or imaging techniques other than standard echocardiography, which is poorly sensitive for myocardial fibrosis. In humans, an accurate assessment of myocardial fibrosis can be obtained using cardiac magnetic resonance imaging, cardiac computer tomography, or STE [61–63,77]. However, these diagnostic tools are not routinely available and performed in dogs with cardiac disease. Finally, different analytical ELISA kits have been used for the evaluation of circulating Gal-3 in dogs, and values obtained using different kits are not equivalent [24,25,28]. Therefore, it is important to note that a comparison of absolute or reference values of circulating Gal-3 measured with different kits are not interchangeable.

5. Conclusions

In conclusion, Gal-3 was not found to be a risk factor for the development of AF secondary to different cardiac diseases in dogs. Advanced age and MMVD are associated with increased circulating Gal-3 in this species, likely reflecting cardiac remodelling secondary to these conditions. Future prospective studies are warranted to elucidate the potential prognostic role of Gal-3 in aged dogs with cardiac disease.

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References

1. Blanda, V.; Bracale, U.M.; Di Taranto, M.D.; Fortunato, G. Galectin-3 in Cardiovascular Diseases. *International Journal of Molecular Sciences* **2020**, *21*, 9232, doi:10.3390/ijms21239232.
2. Chen, S.-C.; Kuo, P.-L. The Role of Galectin-3 in the Kidneys. *International Journal of Molecular Sciences* **2016**, *17*, 565, doi:10.3390/ijms17040565.
3. Vora, A.; de Lemos, J.A.; Ayers, C.; Grodin, J.L.; Lingvay, I. Association of Galectin-3 With Diabetes Mellitus in the Dallas Heart Study. *The Journal of Clinical Endocrinology & Metabolism* **2019**, *104*, 4449–4458, doi:10.1210/jc.2019-00398.
4. Cheng, D.; Liang, B.; Li, Y. Serum Galectin-3 as a Potential Marker for Gastric Cancer. *Med Sci Monit* **2015**, *21*, 755–760, doi:10.12659/MSM.892386.
5. Ho, J.E.; Liu, C.; Lyass, A.; Courchesne, P.; Pencina, M.J.; Vasan, R.S.; Larson, M.G.; Levy, D. Galectin-3, a Marker of Cardiac Fibrosis, Predicts Incident Heart Failure in the Community. *Journal of the American College of Cardiology* **2012**, *60*, 1249–1256, doi:10.1016/j.jacc.2012.04.053.
6. de Boer, R.A.; Voors, A.A.; Muntendam, P.; van Gilst, W.H.; van Veldhuisen, D.J. Galectin-3: A Novel Mediator of Heart Failure Development and Progression. *European Journal of Heart Failure* **2009**, *11*, 811–817, doi:10.1093/eurjhf/hfp097.
7. Lok, D.J.A.; Van Der Meer, P.; de la Porte, P.W.B.-A.; Lipsic, E.; Van Wijngaarden, J.; Hillege, H.L.; van Veldhuisen, D.J. Prognostic Value of Galectin-3, a Novel Marker of Fibrosis, in Patients with Chronic Heart Failure: Data from the DEAL-HF Study. *Clin Res Cardiol* **2010**, *99*, 323–328, doi:10.1007/s00392-010-0125-y.
8. de Boer, R.A.; Yu, L.; van Veldhuisen, D.J. Galectin-3 in Cardiac Remodeling and Heart Failure. *Curr Heart Fail Rep* **2010**, *7*, 1–8, doi:10.1007/s11897-010-0004-x.
9. Gehlken, C.; Suthahar, N.; Meijers, W.C.; de Boer, R.A. Galectin-3 in Heart Failure: An Update of the Last 3 Years. *Heart Failure Clinics* **2018**, *14*, 75–92, doi:10.1016/j.hfc.2017.08.009.
10. Yancy, C.W.; Jessup, M.; Bozkurt, B.; Butler, J.; Casey, D.E.; Colvin, M.M.; Drazner, M.H.; Filippatos, G.S.; Fonarow, G.C.; Givertz, M.M.; et al. 2017 ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America. *Circulation* **2017**, *136*, e137–e161, doi:10.1161/CIR.0000000000000509.
11. Lok, D.J.; Lok, S.I.; Bruggink-André de la Porte, P.W.; Badings, E.; Lipsic, E.; van Wijngaarden, J.; de Boer, R.A.; van Veldhuisen, D.J.; van der Meer, P. Galectin-3 Is an Independent Marker for Ventricular Remodeling and Mortality in Patients with Chronic Heart Failure. *Clin Res Cardiol* **2013**, *102*, 103–110, doi:10.1007/s00392-012-0500-y.
12. de Boer, R.A.; Lok, D.J.A.; Jaarsma, T.; van der Meer, P.; Voors, A.A.; Hillege, H.L.; van Veldhuisen, D.J. Predictive Value of Plasma Galectin-3 Levels in Heart Failure with Reduced and Preserved Ejection Fraction. *Annals of Medicine* **2011**, *43*, 60–68, doi:10.3109/07853890.2010.538080.
13. Joglar, J.A.; Chung, M.K.; Armbruster, A.L.; Benjamin, E.J.; Chyou, J.Y.; Cronin, E.M.; Deswal, A.; Eckhardt, L.L.; Goldberger, Z.D.; Gopinathannair, R.; et al. 2023 ACC/AHA/ACCP/HRS Guideline for the Diagnosis and Management of Atrial Fibrillation: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. *Circulation* **2024**, *149*, doi:10.1161/CIR.0000000000001193.
14. Pauklin, P.; Zilmer, M.; Eha, J.; Tootsi, K.; Kals, M.; Kampus, P. Markers of Inflammation, Oxidative Stress, and Fibrosis in Patients with Atrial Fibrillation. *Oxidative Medicine and Cellular Longevity* **2022**, *2022*, e4556671, doi:10.1155/2022/4556671.
15. Burstein, B.; Nattel, S. Atrial Fibrosis: Mechanisms and Clinical Relevance in Atrial Fibrillation. *Journal of the American College of Cardiology* **2008**, *51*, 802–809, doi:10.1016/j.jacc.2007.09.064.
16. Chen, D.; Procter, N.; Goh, V.; Liu, S.; Chua, S.J.; Assadi-Khansari, B.; Stewart, S.; Horowitz, J.D.; Sverdlov, A.L.; Ngo, D.T. New Onset Atrial Fibrillation Is Associated with Elevated Galectin-3 Levels. *International Journal of Cardiology* **2016**, *223*, 48–49, doi:10.1016/j.ijcard.2016.08.172.
17. Fashanu, O.E.; Norby, F.L.; Aguilar, D.; Ballantyne, C.M.; Hoogeveen, R.C.; Chen, L.Y.; Soliman, E.Z.; Alonso, A.; Folsom, A.R. Galectin-3 and Incidence of Atrial Fibrillation: The Atherosclerosis Risk in Communities (ARIC) Study. *American Heart Journal* **2017**, *192*, 19–25, doi:10.1016/j.ahj.2017.07.001.
18. Gong, M.; Cheung, A.; Wang, Q.; Li, G.; Goudis, C.A.; Bazoukis, G.; Lip, G.Y.H.; Baranchuk, A.; Korantzopoulos, P.; Letsas, K.P.; et al. Galectin-3 and Risk of Atrial Fibrillation: A Systematic Review and Meta-analysis. *Clinical Laboratory Analysis* **2020**, *34*, e23104, doi:10.1002/jcla.23104.
19. Procyk, G.; Czapla, A.; Jałocha, K.; Tymińska, A.; Grabowski, M.; Gąsecka, A. The Role of Galectin-3 in Atrial Fibrillation. *J Mol Med* **2023**, *101*, 1481–1492, doi:10.1007/s00109-023-02378-5.
20. Lee, G.-W.; Kang, M.-H.; Ro, W.-B.; Song, D.-W.; Park, H.-M. Circulating Galectin-3 Evaluation in Dogs With Cardiac and Non-Cardiac Diseases. *Front. Vet. Sci.* **2021**, *8*, 741210, doi:10.3389/fvets.2021.741210.
21. Vichit, P.; Rungsipipat, A.; Surachetpong, S.D. Changes of Cardiac Function in Diabetic Dogs. *Journal of Veterinary Cardiology* **2018**, *20*, 438–450, doi:10.1016/j.jvc.2018.08.001.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 July 2024 doi:10.20944/preprints202407.1611.v1

22. Ribeiro, C.; Santos, M.S.; DE Matos, A.J.; Barros, R.; Gärtner, F.; Rutteman, G.R.; DE Oliveira, J.T. Serum Galectin-3 Levels in Dogs with Metastatic and Non-Metastatic Mammary Tumors. In Vivo **2016**, *30*, 13–16.
23. Stack, J.P.; Fries, R.C.; Kruckman, L.; Kadotani, S.; Wallace, G. Galectin-3 as a Novel Biomarker in Cats with Hypertrophic Cardiomyopathy. *J Vet Cardiol* **2023**, *48*, 54–62, doi:10.1016/j.jvc.2023.06.003.
24. Sakarin, S.; Rungsipipat, A.; Surachetpong, S.D. Galectin-3 in Cardiac Muscle and Circulation of Dogs with Degenerative Mitral Valve Disease. *Journal of Veterinary Cardiology* **2016**, *18*, 34–46, doi:10.1016/j.jvc.2015.10.007.
25. Rešetar Maslov, D.; Farkaš, V.; Rubić, I.; Kuleš, J.; Beletić, A.; Beer Ljubić, B.; Šmit, I.; Mrljak, V.; Torti, M. Serum Proteomic Profiles Reflect the Stages of Myxomatous Mitral Valve Disease in Dogs. *International Journal of Molecular Sciences* **2023**, *24*, 7142, doi:10.3390/ijms24087142.
26. Kim, Y.-M.; Kim, S.-W.; Kim, J.-H. Galectin-3 Is Able to Differentiate Dogs with Myxomatous Mitral Valve Disease from Healthy Control Dogs. *Am J Vet Res* **2023**, *84*, ajvr.23.03.0063, doi:10.2460/ajvr.23.03.0063.
27. Winter, R.L.; Maneval, K.L.; Ferrel, C.S.; Clark, W.A.; Herrold, E.J.; Rhinehart, J.D. Evaluation of Right Ventricular Diastolic Function, Systolic Function, and Circulating Galectin-3 Concentrations in Dogs with Pulmonary Stenosis. *Veterinary Internal Medicne* **2023**, *37*, 2030–2038, doi:10.1111/jvim.16890.
28. Klein, S.; Nolte, I.; Granados-Soler, J.L.; Lietz, P.; Sehn, M.; Raue, J.F.; Rohn, K.; Packeiser, E.-M.; Bach, J.-P. Evaluation of New and Old Biomarkers in Dogs with Degenerative Mitral Valve Disease. *BMC Vet Res* **2022**, *18*, 256, doi:10.1186/s12917-022-03343-z.
29. Buchanan, J.W. Spontaneous Arrhythmias and Conduction Disturbances in Domestic Animals. *Ann N Y Acad Sci* **1965**, *127*, 224–238, doi:10.1111/j.1749-6632.1965.tb49405.x.
30. Noszczyk-Nowak, A.; Michałek, M.; Kałuża, E.; Cepiel, A.; Pasławska, U. Prevalence of Arrhythmias in Dogs Examined between 2008 and 2014. *J Vet Res* **2017**, *61*, 103–110, doi:10.1515/jvetres-2017-0013.
31. Hellemans, A.; Schittekatte, M.; Covents, M.; Smets, P. Diagnosis and Management of Arrhythmias in Dogs: A Cross-Sectional Online Survey among Flemish Veterinary Practitioners. *Vet Rec Open* **2022**, *9*, e35, doi:10.1002/vro2.35.
32. Romito, G.; Guglielmini, C.; Poser, H.; Baron Toaldo, M. Lorenz Plot Analysis in Dogs with Sinus Rhythm and Tachyarrhythmias. *Animals* **2021**, *11*, 1645, doi:10.3390/ani11061645.
33. Guglielmini, C.; Chetboul, V.; Pietra, M.; Pouchelon, J.L.; Capucci, A.; Cipone, M. Influence of Left Atrial Enlargement and Body Weight on the Development of Atrial Fibrillation: Retrospective Study on 205 Dogs. *The Veterinary Journal* **2000**, *160*, 235–241, doi:10.1053/tvjl.2000.0506.
34. Arcuri, G.; Valente, C.; Perini, C.; Guglielmini, C. Risk Factors for Atrial Fibrillation in the Dog: A Systematic Review. *Vet Sci* **2024**, *11*, 47, doi:10.3390/vetsci11010047.
35. Friederich, J.; Seuß, A.C.; Wess, G. The Role of Atrial Fibrillation as a Prognostic Factor in Doberman Pinschers with Dilated Cardiomyopathy and Congestive Heart Failure. *The Veterinary Journal* **2020**, *264*, 105535, doi:10.1016/j.tvjl.2020.105535.
36. Jung, S.W.; Sun, W.; Griffiths, L.G.; Kittleson, M.D. Atrial Fibrillation as a Prognostic Indicator in Medium to Large-Sized Dogs with Myxomatous Mitral Valvular Degeneration and Congestive Heart Failure. *J Vet Intern Med* **2016**, *30*, 51–57, doi:10.1111/jvim.13800.
37. Borgeat, K.; Pack, M.; Harris, J.; Laver, A.; Seo, J.; Belachsen, O.; Hannabuss, J.; Todd, J.; Ferasin, L.; Payne, J.R. Prevalence of Sudden Cardiac Death in Dogs with Atrial Fibrillation. *J Vet Intern Med* **2021**, *35*, 2588–2595, doi:10.1111/jvim.16297.
38. Chetboul, V.; Tissier, R. Echocardiographic Assessment of Canine Degenerative Mitral Valve Disease. *J Vet Cardiol* **2012**, *14*, 127–148, doi:10.1016/j.jvc.2011.11.005.
39. Keene, B.W.; Atkins, C.E.; Bonagura, J.D.; Fox, P.R.; Häggström, J.; Fuentes, V.L.; Oyama, M.A.; Rush, J.E.; Stepien, R.; Uechi, M. ACVIM Consensus Guidelines for the Diagnosis and Treatment of Myxomatous Mitral Valve Disease in Dogs. *Journal of Veterinary Internal Medicine* **2019**, *33*, 1127–1140, doi:10.1111/jvim.15488.
40. Bonagura, J.D.; Visser, L.C. Echocardiographic Assessment of Dilated Cardiomyopathy in Dogs. *J Vet Cardiol* **2022**, *40*, 15–50, doi:10.1016/j.jvc.2021.08.004.
41. Wess, G. Screening for Dilated Cardiomyopathy in Dogs. *J Vet Cardiol* **2022**, *40*, 51–68, doi:10.1016/j.jvc.2021.09.004.
42. Wess, G.; Domenech, O.; Dukes-McEwan, J.; Häggström, J.; Gordon, S. European Society of Veterinary Cardiology Screening Guidelines for Dilated Cardiomyopathy in Doberman Pinschers. *J Vet Cardiol* **2017**, *19*, 405–415, doi:10.1016/j.jvc.2017.08.006.
43. Meurs, K.M.; Stern, J.A.; Sisson, D.D.; Kittleson, M.D.; Cunningham, S.M.; Ames, M.K.; Atkins, C.E.; DeFrancesco, T.; Hodge, T.E.; Keene, B.W.; et al. Association of Dilated Cardiomyopathy with the Striatin Mutation Genotype in Boxer Dogs. *J Vet Intern Med* **2013**, *27*, 1437–1440, doi:10.1111/jvim.12163.
44. Hindricks, G.; Potpara, T.; Dagres, N.; Arbelo, E.; Bax, J.J.; Blomström-Lundqvist, C.; Boriani, G.; Castella, M.; Dan, G.-A.; Dilaveris, P.E.; et al. 2020 ESC Guidelines for the Diagnosis and Management of Atrial Fibrillation Developed in Collaboration with the European Association for Cardio-Thoracic Surgery (EACTS). *European Heart Journal* **2021**, *42*, 373–498, doi:10.1093/eurheartj/ehaa612.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 July 2024 doi:10.20944/preprints202407.1611.v1

45. Pedro, B.; Fontes-Sousa, A.P.; Gelzer, A.R. Diagnosis and Management of Canine Atrial Fibrillation. *Vet J* **2020**, *265*, 105549, doi:10.1016/j.tvjl.2020.105549.
46. Cornell, C.C.; Kittleson, M.D.; Della Torre, P.; Häggström, J.; Lombard, C.W.; Pedersen, H.D.; Vollmar, A.; Wey, A. Allometric Scaling of M-Mode Cardiac Measurements in Normal Adult Dogs. *J Vet Intern Med* **2004**, *18*, 311–321, doi:10.1892/0891-6640(2004)18<311:asomcm>2.0.co;2.
47. Rishniw, M.; Erb, H.N. Evaluation of Four 2-Dimensional Echocardiographic Methods of Assessing Left Atrial Size in Dogs. *J Vet Intern Med* **2000**, *14*, 429–435, doi:10.1892/0891-6640(2000)014<0429:eofemo>2.3.co;2.
48. Guglielmini, C.; Goncalves Sousa, M.; Baron Toaldo, M.; Valente, C.; Bentivoglio, V.; Mazzoldi, C.; Bergamin, I.; Drigo, M.; Poser, H. Prevalence and Risk Factors for Atrial Fibrillation in Dogs with Myxomatous Mitral Valve Disease. *J Vet Intern Med* **2020**, *34*, 2223–2231, doi:10.1111/jvim.15927.
49. Guglielmini, C.; Valente, C.; Romito, G.; Mazzoldi, C.; Baron Toaldo, M.; Goncalves Sousa, M.; Wolf, M.; Beluque, T.; Domenech, O.; Patata, V.; et al. Risk Factors for Atrial Fibrillation in Dogs with Dilated Cardiomyopathy. *Front Vet Sci* **2023**, *10*, 1183689, doi:10.3389/fvets.2023.1183689.
50. Romito, G.; Darida, S.; Valente, C.; Poser, H.; Contiero, B.; Cipone, M.; Guglielmini, C. Prevalence and Prognostic Role of L Wave and Selected Clinical and Echocardiographic Variables in Dogs with Atrial Fibrillation. *J Vet Intern Med* **2023**, *37*, 47–57, doi:10.1111/jvim.16584.
51. Pedro, B.; Fontes-Sousa, A.P.; Gelzer, A.R. Canine Atrial Fibrillation: Pathophysiology, Epidemiology and Classification. *The Veterinary Journal* **2020**, *265*, 105548, doi:10.1016/j.tvjl.2020.105548.
52. Pathak, R.; Lau, D.H.; Mahajan, R.; Sanders, P. Structural and Functional Remodeling of the Left Atrium: Clinical and Therapeutic Implications for Atrial Fibrillation. *J Atr Fibrillation* **2013**, *6*, 986, doi:10.4022/jafib.986.
53. Brundel, B.J.J.M.; Melnyk, P.; Rivard, L.; Nattel, S. The Pathology of Atrial Fibrillation in Dogs. *J Vet Cardiol* **2005**, *7*, 121–129, doi:10.1016/j.jvc.2005.07.001.
54. Goette, A.; Kalman, J.M.; Aguinaga, L.; Akar, J.; Cabrera, J.A.; Chen, S.A.; Chugh, S.S.; Corradi, D.; D'Avila, A.; Dobrev, D.; et al. EHRA/HRS/APHRS/SOLAECE Expert Consensus on Atrial Cardiomyopathies: Definition, Characterisation, and Clinical Implication. *Journal of Arrhythmia* **2016**, *32*, 247–278, doi:10.1016/j.joa.2016.05.002.
55. Vollmar, A.C.; Aupperle, H. Cardiac Pathology in Irish Wolfhounds with Heart Disease. *Journal of Veterinary Cardiology* **2016**, *18*, 57–70, doi:10.1016/j.jvc.2015.10.001.
56. Janus, I.; Noszczyk-Nowak, A.; Nowak, M.; Ciaputa, R.; Kandefer-Gola, M.; Pasławska, U. A Comparison of the Histopathologic Pattern of the Left Atrium in Canine Dilated Cardiomyopathy and Chronic Mitral Valve Disease. *BMC Veterinary Research* **2016**, *12*, 3, doi:10.1186/s12917-015-0626-z.
57. Tursi, M.; Mazzotta, E.; Biasato, I.; Poser, H.; Guglielmini, C. Pathology in Practice. *J Am Vet Med Assoc* **2016**, *248*, 1359–1361, doi:10.2460/javma.248.12.1359.
58. Baron Toaldo, M.; Romito, G.; Guglielmini, C.; Diana, A.; Pelle, N.G.; Contiero, B.; Cipone, M. Assessment of Left Atrial Deformation and Function by 2-Dimensional Speckle Tracking Echocardiography in Healthy Dogs and Dogs With Myxomatous Mitral Valve Disease. *J Vet Intern Med* **2017**, *31*, 641–649, doi:10.1111/jvim.14722.
59. Caivano, D.; Rishniw, M.; Birettoni, F.; Patata, V.; Giorgi, M.E.; Porciello, F. Left Atrial Deformation and Phasic Function Determined by Two-Dimensional Speckle-Tracking Echocardiography in Dogs with Myxomatous Mitral Valve Disease. *J Vet Cardiol* **2018**, *20*, 102–114, doi:10.1016/j.jvc.2018.01.002.
60. Baron Toaldo, M.; Mazzoldi, C.; Romito, G.; Poser, H.; Contiero, B.; Cipone, M.; Guglielmini, C. Echocardiographic Predictors of First Onset of Atrial Fibrillation in Dogs with Myxomatous Mitral Valve Disease. *J Vet Intern Med* **2020**, *34*, 1787–1793, doi:10.1111/jvim.15860.
61. Guglielmo, M.; Pontone, G. Clinical Implications of Cardiac Magnetic Resonance Imaging Fibrosis. *European Heart Journal Supplements* **2022**, *24*, I123–I126, doi:10.1093/eurheartjsupp/suac085.
62. Mewton, N.; Liu, C.Y.; Croisille, P.; Bluemke, D.; Lima, J.A.C. Assessment of Myocardial Fibrosis With Cardiovascular Magnetic Resonance. *Journal of the American College of Cardiology* **2011**, *57*, 891–903, doi:10.1016/j.jacc.2010.11.013.
63. Ravassa, S.; López, B.; Treibel, T.A.; San José, G.; Losada-Fuentenebro, B.; Tapia, L.; Bayés-Genís, A.; Díez, J.; González, A. Cardiac Fibrosis in Heart Failure: Focus on Non-Invasive Diagnosis and Emerging Therapeutic Strategies. *Molecular Aspects of Medicine* **2023**, *93*, 101194, doi:10.1016/j.mam.2023.101194.
64. Pison, L.; Hocini, M.; Potpara, T.S.; Todd, D.; Chen, J.; Blomstrom-Lundqvist, C.; Blomstrom-Lundqvist, C.; Bongiorni, M.G.; Pison, L.; Proclemer, A.; et al. Work-up and Management of Lone Atrial Fibrillation: Results of the European Heart Rhythm Association Survey. *Europace* **2014**, *16*, 1521–1523, doi:10.1093/europace/euu277.
65. Frustaci, A.; Caldarulo, M.; Buffon, A.; Bellocci, F.; Fenici, R.; Melina, D. Cardiac Biopsy in Patients with "Primary" Atrial Fibrillation. Histologic Evidence of Occult Myocardial Diseases. *Chest* **1991**, *100*, 303–306, doi:10.1378/chest.100.2.303.
66. Mahnkopf, C.; Badger, T.J.; Burgon, N.S.; Daccarett, M.; Haslam, T.S.; Badger, C.T.; McGann, C.J.; Akoum, N.; Kholmovski, E.; Macleod, R.S.; et al. Evaluation of the Left Atrial Substrate in Patients with Lone Atrial

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 July 2024 doi:10.20944/preprints202407.1611.v1

Fibrillation Using Delayed-Enhanced MRI: Implications for Disease Progression and Response to Catheter Ablation. *Heart Rhythm* **2010**, *7*, 1475–1481, doi:10.1016/j.hrthm.2010.06.030.

67. Falk, T.; Jönsson, L.; Olsen, L.H.; Pedersen, H.D. Arteriosclerotic Changes in the Myocardium, Lung, and Kidney in Dogs with Chronic Congestive Heart Failure and Myxomatous Mitral Valve Disease. *Cardiovasc Pathol* **2006**, *15*, 185–193, doi:10.1016/j.carpath.2006.04.003.
68. Falk, T.; Jönsson, L. Ischaemic Heart Disease in the Dog: A Review of 65 Cases. *J Small Anim Pract* **2000**, *41*, 97–103, doi:10.1111/j.1748-5827.2000.tb03173.x.
69. Tidholm, A.; Jönsson, L. Histologic Characterization of Canine Dilated Cardiomyopathy. *Vet Pathol* **2005**, *42*, 1–8, doi:10.1354/vp.42-1-1.
70. de Boer, R.A.; van Veldhuisen, D.J.; Gansevoort, R.T.; Muller Kobold, A.C.; van Gilst, W.H.; Hillege, H.L.; Bakker, S.J.L.; van der Harst, P. The Fibrosis Marker Galectin-3 and Outcome in the General Population. *Journal of Internal Medicine* **2012**, *272*, 55–64, doi:10.1111/j.1365-2796.2011.02476.x.
71. Seropian, I.M.; Cassaglia, P.; Miksztowicz, V.; González, G.E. Unraveling the Role of Galectin-3 in Cardiac Pathology and Physiology. *Front Physiol* **2023**, *14*, 1304735, doi:10.3389/fphys.2023.1304735.
72. Loffredo, F.S.; Nikolova, A.P.; Pancoast, J.R.; Lee, R.T. Heart Failure with Preserved Ejection Fraction: Molecular Pathways of the Aging Myocardium. *Circ Res* **2014**, *115*, 97–107, doi:10.1161/CIRCRESAHA.115.302929.
73. Frangogiannis, N.G. Cardiac Fibrosis: Cell Biological Mechanisms, Molecular Pathways and Therapeutic Opportunities. *Mol Aspects Med* **2019**, *65*, 70–99, doi:10.1016/j.mam.2018.07.001.
74. Biernacka, A.; Frangogiannis, N.G. Aging and Cardiac Fibrosis. *Aging and Disease* **2011**, *2*, 158–173.
75. da Silveira, J.S.; Scansen, B.A.; Wassenaar, P.A.; Raterman, B.; Eleswarpu, C.; Jin, N.; Mo, X.; White, R.D.; Bonagura, J.D.; Kolipaka, A. Quantification of Myocardial Stiffness Using Magnetic Resonance Elastography in Right Ventricular Hypertrophy: Initial Feasibility in Dogs. *Magn Reson Imaging* **2016**, *34*, 26–34, doi:10.1016/j.mri.2015.10.001.
76. Horiuchi, Y.U.; Wettersten, N.; Vanveldhuisen, D.J.; Mueller, C.; Nowak, R.; Hogan, C.; Kontos, M.C.; Cannon, C.M.; Birkhahn, R.; Vilke, G.M.; et al. The Influence of Body Mass Index on Clinical Interpretation of Established and Novel Biomarkers in Acute Heart Failure. *J Card Fail* **2023**, *29*, 1121–1131, doi:10.1016/j.cardfail.2023.03.029.
77. Mariana Barros Melo Da Silveira, M.; Victor Batista Cabral, J.; Tavares Xavier, A.; Palmeira Do Ó, K.; Francisco De Moura Junior, J.; Tavares De Carvalho, O.; Bezerra Mendes Filho, E.; Furtado De Mendonça Belmont, T.; Maria Del Castillo, J.; Jesus Barreto De Melo Rêgo, M.; et al. The Role of Galectin-3 in Patients with Permanent and Paroxysmal Atrial Fibrillation and Echocardiographic Parameters of Left Atrial Fibrosis. *Mol Biol Rep* **2023**, *50*, 9019–9027, doi:10.1007/s11033-023-08774-x.

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