ARTICLE IN PRESS

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
x (x): x-x, 2026
DOI: 10.9775/kvfd.2026.37248

RESEARCH ARTICLE

A Canine Inflammatory-Metabolic Risk Index Across ACVIM Stages of Myxomatous Mitral Valve Disease: A Composite Biomarker Approach

Tuğba VARLIK[1], Didem ALGAN[1], Zeki YILMAZ [1(*)]

[1] Bursa Uludağ University, Faculty of Veterinary Medicine, Department of Internal Medicine, TR-16059 Bursa - TÜRKİYE

(*) **Corresponding author:**
Zeki Yılmaz
Phone: +90 224 294 08 09
E-mail: zyilmaz@uludag.edu.tr

How to cite this article?

Varlık T, Algan D, Yılmaz Z: A Canine Inflammatory-Metabolic Risk Index Across ACVIM Stages of Myxomatous Mitral Valve Disease: A Composite Biomarker Approach. *Kafkas Univ Vet Fak Derg*, x (x): x-x, 2026. DOI: 10.9775/kvfd.2026.37248

Article ID: KVFD-2026-37248
Received: 07.06.2026
Accepted: 21.08.2026
Published Online: 24.08.2026

Abstract

Myxomatous mitral valve disease (MMVD) is associated with progressive structural cardiac remodeling and systemic inflammatory and metabolic alterations. This study aimed to develop and preliminarily evaluate Canine Inflammatory-Metabolic Risk Index (CIMRI) as a composite biomarker, assess its differences across ACVIM stages and associations with echocardiographic indices, and explore its ability to discriminate dogs with current or previous congestive heart failure (CHF) from those without CHF. Fifty client-owned dogs were retrospectively enrolled and equally distributed across five disease stages. CIMRI was calculated using routinely available laboratory variables reflecting immune response, metabolic balance, and electrolyte status, normalized to total protein concentration. Associations with echocardiographic variables were assessed using Spearman's correlation analysis, and discriminatory performance was evaluated by receiver operating characteristic (ROC) analysis. CIMRI differed significantly across ACVIM stages, with the highest values observed in Stage D ($P<0.001$), and correlated positively with LA/Ao ($\rho = 0.631$, $P<0.001$) and LVIDdN ($\rho = 0.561$, $P<0.01$). ROC analysis yielded an AUC of 0.929 (95% CI: 0.837-1.000) for discriminating dogs with current or previous CHF (Stages C-D) from those without current or previous CHF (Stages A-B2). At the optimal cut-off of >5.8, sensitivity and specificity were 95.0% and 90.0%, respectively. These findings support further evaluation of CIMRI as an exploratory composite biomarker associated with MMVD severity and CHF status in larger independent, prospective, and longitudinal populations.

Keywords: Canine, Composite Index, Echocardiography, Inflammatory-metabolic index, Myxomatous mitral valve disease

Introduction

Myxomatous mitral valve disease (MMVD) is the most common acquired cardiac disorder in dogs. The disease is characterized by progressive degeneration of the mitral valve apparatus, leading to mitral regurgitation, left atrial (LA) enlargement, and eventually congestive heart failure (CHF) [1,2]. Although the staging system proposed by the American College of Veterinary Internal Medicine (ACVIM) provides a useful framework, substantial heterogeneity exists within each stage regarding progression rates, clinical deterioration, and survival outcomes [3]. Consequently, improved tools are needed to more precisely characterize disease severity and biological heterogeneity among affected dogs.

Traditionally, MMVD progression has been attributed to hemodynamic overload resulting from chronic mitral regurgitation. Echocardiographic indices such as the left atrial-to-aortic root ratio (LA:Ao), left ventricular (LV) dimensions, and systolic function parameters are widely used to quantify cardiac remodeling and disease severity [1,2,4-6]. However, growing evidence suggests that MMVD is not solely a mechanical disorder but involves complex systemic processes. While individual biomarkers provide valuable information, each reflects only a single aspect of the disease pathophysiology [7]. Composite indices that integrate multiple complementary physiological domains have shown potential clinical utility in both veterinary and human medicine. For instance, the hemoglobin, albumin, lymphocyte, and platelet (HALP) score predicts short-term mortality in dogs with CHF secondary to MMVD [8], and the Inflammatory Metabolic Index (IMI) and Metabolic-Inflammatory Stress Index (MISI) are effective in human clinical conditions [9]. These approaches suggest that a multidimensional metric can provide a more comprehensive representation of systemic disease burden than isolated parameters [7,10].

Several inflammatory, metabolic, and neurohormonal biomarkers have been individually investigated in canine MMVD; however, they are typically evaluated in isolation and may provide only a partial representation of the complex systemic processes underlying disease

progression [8,11-13]. MMVD is increasingly recognized as a multisystemic disorder, wherein inflammatory activation, metabolic dysregulation, neurohormonal disturbances, and systemic physiological status interact to influence cardiac remodeling and clinical outcomes [7,9,14]. However, integrating these complementary domains into a single quantitative index may provide a more comprehensive assessment of the systemic alterations associated with MMVD. A composite metric could therefore improve disease characterization and provide a more holistic assessment of systemic involvement associated with MMVD severity.

Based on this rationale, we developed the Canine Inflammatory-Metabolic Risk Index (CIMRI). Similar to previously described composite inflammatory and metabolic indices, CIMRI was designed as a pragmatic integrative score that combines complementary biological domains using routinely available laboratory variables. Specifically, the index incorporates inflammatory (neutrophil-to-lymphocyte ratio [NLR]) [15,16], acute-phase response (C-reactive protein-to-albumin ratio [CRP/Alb]) [17,18], metabolic (total cholesterol-to-glucose ratio [Chol/Glu]) [19], neurohormonal-electrolyte (sodium-to-potassium ratio [Na/K]) [20-21] and systemic protein status (total protein [TP]) into a single composite metric. Total protein was incorporated as a normalization factor to account for inter-individual variation in circulating protein concentrations and overall serum protein milieu, thereby providing contextual scaling of the combined inflammatory-metabolic signal rather than functioning as an independent disease marker.

We hypothesized that CIMRI would capture systemic alterations associated with MMVD severity. Therefore, this study aimed to develop and preliminarily evaluate CIMRI as a composite biomarker integrating routinely available inflammatory, metabolic, and electrolyte-related variables in dogs with MMVD. Specifically, we assessed differences in CIMRI across ACVIM stages, examined its associations with selected echocardiographic indices, and explored its ability to discriminate dogs with current or previous CHF (Stages C-D) from those without current or previous CHF (Stages A-B2).

Material and Methods

Ethical Statement

This retrospective study was conducted using previously collected clinical data obtained during routine veterinary care. The Bursa Uludağ University Animal Experiments Local Ethics Committee (HADYEK, Bursa, Türkiye) reviewed the study protocol and confirmed that formal ethical approval was not required because the study was retrospective and based exclusively on archived clinical records (Decision No: B.30.2.ULU.0.8Z.00.00; Date: 22 June 2026). Written informed consent covering diagnostic procedures and the use of clinical data had been obtained from the owners of all dogs upon admission to the veterinary hospital.

Study Population

This retrospective study was conducted between March 2019 and March 2026 at the Veterinary Hospital. A total of 50 client-owned dogs of various breeds, ages, body weights, and both sexes were enrolled. Dogs were staged according to the ACVIM consensus classification for MMVD [3]:

- **Stage A (healthy/controls, n=10):** Dogs without clinical or echocardiographic evidence of MMVD.
- Asymptomatic dogs with an audible heart murmur were further stratified into Stage B1 and B2 according to the presence or absence of structural cardiac remodeling.
    - **Stage B1 (n=10):** Asymptomatic dogs without structural remodeling of the heart (no radiographic cardiomegaly, normal LA/Ao and LV dimensions on echocardiography).
    - **Stage B2 (n=10):** Asymptomatic dogs showing structural remodeling of the heart (echocardiographic LA and/or LV enlargement) without clinical signs of heart failure.
- **Stage C (n=10):** Dogs with current or past clinical signs of heart failure associated with MMVD, responsive to standard therapy.
- **Stage D (n=10):** Dogs with refractory heart failure, showing clinical signs despite optimized therapy.

Blood Sampling and Laboratory Analyses

Complete blood count (CBC) analysis provided 24 hematologic parameters (HM5 analyzer, Abaxis, USA). The NLR was manually calculated for this study. Routine serum biochemistry panels were measured in all dogs, and specific parameters relevant to this study included glucose, cholesterol, sodium (Na), potassium (K), C-reactive protein (CRP), albumin (Alb), and total protein (TP) (serum biochemical analyzer, Fuji Dri-Chem NX600V IC), as described previously [14,23].

Echocardiographic Assessment

Cardiac evaluation was performed in a quiet examination room without sedation, with precautions taken to minimize sound and motion artifacts. Conventional echocardiography was conducted using standard right parasternal short-axis (RPSAx) and right parasternal long-axis (RPLAx) views in accordance with ACVIM

recommendations [3]. Two-dimensional imaging together with pulsed-wave (PW), continuous-wave (CW), and color Doppler modalities were used (Vivid S60N Ultra, GE Healthcare, Norway) as reported in our previous studies [14,16,23].

The LA/Ao was measured at the aortic valve level from the RPSAx view. Left ventricular internal diameter in diastole (LVIDd) was obtained from RPLAx M-mode recordings. To account for body size differences, LVIDd was normalized using an allometric scaling method to derive the normalized left ventricular internal diameter in diastole (LVIDdN), calculated according to the formula [3]:

LVIDdN = LVIDd (cm)/BW (kg)^0.294

After entering the patient's body weight into the echocardiographic system and measuring LVIDd, the normalized value was automatically generated during offline analysis within the RPLAx LV M-mode measurements by the echocardiographic software (Vivid EchoPack, GE Healthcare, Version 204).

Left ventricular systolic function was assessed using M-mode measurements obtained from the RPLAx view, and ejection fraction (EF) and fractional shortening (FS) were calculated using the Teichholz method. Mitral annular plane systolic excursion (MAPSE) was measured from the septal insertion of the mitral annulus in the apical four-chamber view using M-mode. Mitral inflow velocities were recorded from the apical four-chamber view using ECG-guided pulsed-wave Doppler to determine the early (E) and late (A) diastolic velocities, and the mitral E/A ratio (MV E/A) was calculated [24]. All measurements were averaged over three consecutive cardiac cycles.

These parameters were selected because they can be readily obtained using standard echocardiographic equipment and are widely used in clinical practice and research to evaluate cardiac structure and function. Although several additional morphological and functional variables were recorded during routine echocardiographic examinations, only those relevant to the objectives of the present study were included in the final analysis.

Inclusion and Exclusion Criteria

Dogs diagnosed with MMVD were considered for inclusion if complete hematologic, biochemical, and echocardiographic data were available. Young (≤3 years) and senior (>10 years) dogs were excluded to minimize age-related variability. Dogs with comorbidities such as pulmonary hypertension, hypothyroidism, cancer, or any other systemic disease were also excluded. Animals that had received medications including corticosteroids, non-steroidal anti-inflammatory drugs, antibiotics, positive inotropes, diuretics, or vaccinations within 10 days before evaluation were excluded, as these treatments could affect hematologic and biochemical profiles. An exception was made for dogs in Stage D MMVD, as these animals were already refractory and exhibited CHF signs despite standard therapy, making recent medication use clinically unavoidable [7,14,23].

To ensure reliable echocardiographic and laboratory measurements, dogs exhibiting excessive excitement, stress, or respiratory distress were excluded, as these factors could induce stress leukogram patterns, transient hyperglycemia, and interfere with accurate cardiac assessment. Only dogs meeting all inclusion criteria and with high quality, artifact free echocardiographic images were enrolled in the study.

CIMRI Calculation and Integration

CIMRI was calculated as follows:

CIMRI = (NLR + CRP/Alb + Chol/Glu + Na/K)/TP

This composite index integrates four complementary pathophysiological dimensions: systemic inflammation (NLR), acute-phase protein balance (serum CRP/Alb), metabolic homeostasis (serum Chol/Glu), and neurohormonal-electrolyte regulation (Na/K). Total protein was incorporated as a scaling factor to account for inter-individual variation in circulating protein concentrations and overall serum protein milieu. Similar normalization approaches are commonly employed in biomarker research to reduce variability related to differences in protein content and biological matrix composition. Rather than representing an independent disease marker, TP was included to contextualize the composite inflammatory-metabolic signal within the individual's systemic protein status. By combining these parameters into a single metric, CIMRI was designed to provide an integrative representation of biological processes associated with MMVD progression. The additive structure of CIMRI was intentionally chosen to maintain clinical simplicity and facilitate bedside applicability using routinely available biomarkers. Equal weighting was applied to minimize model overfitting given the relatively limited sample size. Weighted approaches, such as regression-derived or machine-learning-based coefficients, typically require large training datasets to yield stable and generalizable weight estimates; applying such methods to a modest, single-center cohort would risk overfitting and reduce the reproducibility of the index in independent populations. Furthermore, equal weighting avoids assumptions about the relative pathophysiological importance of each component, which remains incompletely defined in canine MMVD, and preserves a transparent, easily interpretable formula that can be readily calculated in clinical practice without specialized statistical software. This pragmatic approach is consistent with previously described composite inflammatory

and metabolic indices in both human and veterinary medicine [9,27], which have similarly prioritized simplicity and clinical applicability over statistically optimized weighting in their initial development stages. Consistent with previously proposed composite inflammatory and metabolic indices, the present formulation was intended as a pragmatic integrative score rather than a mechanistic mathematical model. The CIMRI conceptual framework was illustrated in *Fig. 1*.

Statistical Analysis

The study included five groups: one healthy control group and four groups of dogs with varying severity of MMVD. Distributional assumptions for all continuous variables were assessed using the Shapiro-Wilk test. As CIMRI scores were not normally distributed across the groups, the non-parametric Kruskal-Wallis test was used to compare CIMRI values between the groups, followed by Dunn's multiple comparisons test for post hoc pairwise comparisons. Sex distribution among groups was compared using the Pearson chi-square test. The relationships between CIMRI scores and echocardiographic parameters were evaluated using Spearman's rank correlation analysis. Spearman's rank correlation coefficients (ρ) were reported with their 95% confidence intervals (CIs) and exact P values. As these analyses were exploratory, no adjustment for multiple testing was applied; therefore, the corresponding P values should be interpreted accordingly. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the ability of CIMRI to discriminate between dogs with current or previous CHF (Stages C-D, n=20) and those without CHF (Stages A-B2, n=30). Statistical significance was set at P<0.05. All analyses were performed using SigmaPlot Statistical and Graphing Software (California, USA) and MedCalc (version 20.218, Ostend, Belgium). Data are presented as mean ± standard deviation (SD).

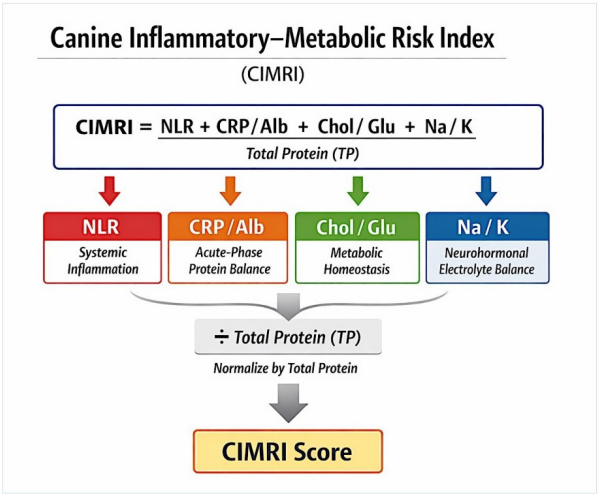

Fig 1. Schematic representation of the CIMRI - Integrated Risk Assessment in MMVD. The index integrates four complementary pathophysiological dimensions-systemic inflammation (neutrophil-to-lymphocyte ratio, NLR), acute-phase protein balance (C-reactive protein to albumin ratio, CRP/Alb), metabolic homeostasis (cholesterol to glucose ratio, Chol/Glu), and neurohormonal-electrolyte regulation (sodium to potassium ratio, Na/K)-and normalizes them by total protein (TP) to generate a single composite score. CIMRI reflects multisystemic disease activity in dogs with MMVD and correlates with echocardiographic measures and clinical disease stage (ACVIM classification)

Results

Study Population

A total of 50 small to medium breed dogs were included, distributed evenly across five groups: one healthy control group and four MMVD severity groups. The overall population had a mean age of 7.3±2.4 years, with 26 males and 24 females. Breed distribution included Cavalier King Charles Spaniel (n=18), Terrier (n=8), Toy Poodle (n=8), Pug (n=6), Pomeranian (n=4), Yorkshire Terrier (n=2), and mixed breeds (n=4). Clinical characteristics and echocardiographic measurements according to ACVIM stages are presented in *Table 1*. Age, body weight, sex distribution, EF, FS, MAPSE, and MV E/A ratio did not differ significantly among groups (P>0.05). LA/Ao and LVDdN differed significantly among ACVIM stages, with higher values observed in the dogs with CHF (Stages C and D; *Table 1*).

Inflammatory and metabolic parameters across different stages of MMVD are summarized in *Table 2*. The NLR and CRP/Alb differed significantly among ACVIM stages, with the highest values observed in Stage D (P<0.05). Chol/Glu, Na/K ratio, and serum TP concentration did not differ significantly among groups (P>0.05).

CIMRI Scores Across Groups

CIMRI values differed among ACVIM stages (*Fig. 2*). Mean scores were 5.3±0.1 in stage A, 4.9±0.3 in B1, 5.6±0.3 in B2, 6.9±2.0 in C, and 10.4±1.7 in D. Kruskal-Wallis analysis demonstrated significant differences among groups (P<0.01), and Dunn's post-hoc testing revealed significant pairwise differences among the groups, with the highest CIMRI values observed in Stage D (*Fig. 2*).

ROC analysis performed in all 50 dogs yielded an AUC of 0.929 (95% CI: 0.837-1.000, *P*<0.0001) for discriminating dogs with current or previous CHF (Stages C-D, n=20) from dogs without current or previous CHF (Stages A-B2, n=30). The optimal CIMRI cut-off determined by the Youden index was >5.8, yielding a sensitivity of 95.0% (95% CI: 75.1-99.9%) and specificity of 90.0% (95% CI: 73.5-97.9%). The corresponding PPV and NPV were 86.4% (95% CI: 65.1-97.1%) and 96.4% (95% CI: 81.7-99.9%), respectively (*Table 3* and *Fig. 3*).

Table 1. Clinical characteristics and echocardiographic measurements (Mean ± SD) in dogs grouped according to ACVIM MMVD stages

Parameters	Dogs with MMVD Staged to ACVIM Guideline					P-value
	A n=10	B1 n=10	B2 n=10	C n=10	D n=10	
Ages (years)	5.2±2.3	6.1±2.1	5.9±1.2	7.3±2.2	7.1±2.9	= 0.206
BW (kg)	12.1±3.4	12.3±3.3	13.4±4.5	14.2±3.5	13.2±3.9	= 0.194
Sexes (M/F)	5/5	3/7	6/4	5/5	7/3	= 0.474
La/Ao	1.2±0.1	1.3±0.1	1.8±0.3	2.0±0.3	2.1±0.4	C *vs* A, and B1; P<0.001 C *vs* B2; P=0.039 D *vs* A, and B1; P<0.001 D *vs* B2; P=0.014
LVDdN	1.3±0.1	1.3±0.2	1.5±0.2	1.5±0.2	1.6±0.3	D *vs* A; P=0.010 D *vs* B1; P=0.041 C *vs* A; P=0.093
EF %	61.1±10.8	59.7±6.0	65.7±7.8	54.5±6.9	54.6±10.3	= 0.755
FS %	34.2±6.9	32.2±5.7	35.2±6.6	28.8±9.6	29.0±9.3	= 0.705
MAPSE cm	1.4±0.1	1.0±0.1	0.9±0.2	1.0±0.3	1.1±0.4	= 0.147
MV E/A	1.5±0.2	1.7±0.3	2.1±0.5	2.2±0.5	2.2±0.6	= 0.081

ACVIM, American College of Veterinary Internal Medicine; BW, body weight; LA/Ao, left atrial-to-aortic root ratio; LVIDdN, normalized left ventricular internal diameter in diastole; EF, ejection fraction; FS, fractional shortening; MAPSE, mitral annular plane systolic excursion; MV E/A, ratio of peak early (E) to late (A) diastolic transmitral flow velocities; M, male; F, female; vs, versus

Relationships Between CIMRI and Echocardiographic Parameters

Spearman's rank correlation analysis (n=50) revealed significant positive correlations between CIMRI and LA/Ao ($\rho = 0.631$, 95% CI: 0.371-0.792, P<0.001), LVDdN ($\rho = 0.561$, 95% CI: 0.315-0.719, P<0.01), and MV E/A ($\rho = 0.456$, 95% CI: 0.193-0.676, P<0.001). CIMRI was also significantly negatively correlated with MAPSE ($\rho = -0.336$, 95% CI: -0.569 to -0.066, P=0.017). In contrast, CIMRI was not significantly correlated with EF ($\rho = -0.189$, 95% CI: −0.428 to 0.083, *P*=0.189) or FS ($\rho = -0.234$, 95% CI: −0.480 to 0.047, *P*=0.102).

Discussion

In canine MMVD, numerous clinical, hematologic, biochemical, and echocardiographic variables have been proposed as indicators of disease severity and progression. However, these parameters are typically evaluated in isolation and may therefore provide only a partial representation of the complex multisystemic processes associated with MMVD. In this context, the present study introduces and preliminarily evaluates CIMRI, an exploratory composite biomarker approach integrating systemic inflammation, acute-phase protein balance, metabolic homeostasis, electrolyte regulation, and systemic protein status into a single metric. By combining complementary physiological domains, CIMRI may capture multiple systemic alterations associated with MMVD severity. The principal findings were that CIMRI differed across ACVIM stages, with the highest values observed in Stage D; was associated with selected echocardiographic indices; and showed a high ability to

Table 2. Inflammatory, metabolic, electrolyte, and protein-related parameters (mean ± SD) across ACVIM MMVD stages in dogs

Parameters	Dogs with MMVD Staged to ACVIM Guideline					P-value
	A n=10	B1 n=10	B2 n=10	C n=10	D n=10	
NLR	3.6±1.3	3.8±1.0	3.8±1.2	6.5±3.4	10.5±6.4	D *vs* A, B1, and B2; P<0.05
CRP/Alb	1.3±0.9	1.0±0.3	1.5±1.6	7.2±10.4	14.5±9.1	D *vs* A, B1, and B2; P<0.05
Chol/Glu	3.1±1.3	2.8±0.7	2.9±0.6	2.1±0.9	1.8±1.1	= 0.092
Na/K	26.8±1.2	25.5±1.2	25.9±1.5	26.3±5.0	30.1±6.6	= 0.224
TP g/dL	6.4±0.2	6.5±0.4	6.3±0.3	6.4±0.8	5.8±0.9	= 0.331

ACVIM, American College of Veterinary Internal Medicine; NLR, neutrophil-to-lymphocyte ratio; CRP/Alb, serum C-reactive protein-to-albumin ratio; Chol/Glu, serum total cholesterol-to-glucose ratio; Na/K, serum sodium-to-potassium ratio; TP, serum total protein concentration; vs, versus

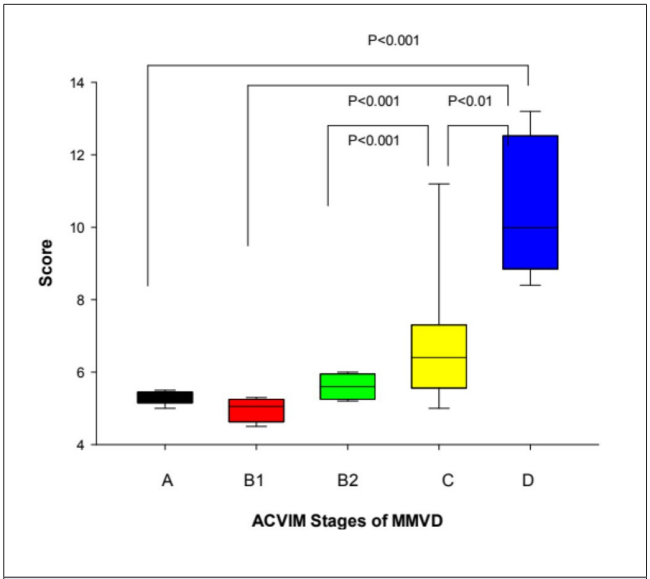

Fig 2. Box-and-whisker plots showing the distribution of Canine Inflammatory-Metabolic Risk Index (CIMRI) scores across ACVIM stages of myxomatous mitral valve disease (MMVD). The boxes represent the interquartile range, the central line indicates the median, and whiskers denote the minimum and maximum values. CIMRI scores differed significantly across ACVIM stages, with the highest values observed in Stage D. Significant pairwise comparisons are indicated (P<0.01 and P<0.001)

discriminate dogs with current or previous CHF from those without CHF in this exploratory cohort. However, these findings should not be interpreted as evidence that CIMRI reflects longitudinal disease progression, independently reflects cardiac remodeling, or has established prognostic validity.

The concept of composite indices has gained increasing recognition in both human and veterinary medicine as a means of integrating multidimensional biological information. In human medicine, indices such as the MISI and IMI have demonstrated associations with

Table 3. ROC analysis of CIMRI for discriminating dogs with current or previous congestive heart failure (CHF; ACVIM Stages C-D) from dogs without current or previous CHF (Stages A-B2)

Parameter	Estimate	95% CI
AUC	0.929	0.837-1.000
P value (AUC = 0.5)	< 0.0001	-
Optimal cut-off	>5.8	-
Sensitivity (%)	95.0	75.1-99.9
Specificity (%)	90.0	73.5-97.9
PPV (%)	86.4	65.1-97.1
NPV (%)	96.4	81.7-99.9
Youden index	0.85	-

ROC analysis included 20 dogs with current or previous CHF (Stages C-D) and 30 dogs without current or previous CHF (Stages A-B2). The optimal cut-off was determined using the Youden index. AUC, area under the receiver operating characteristic curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value

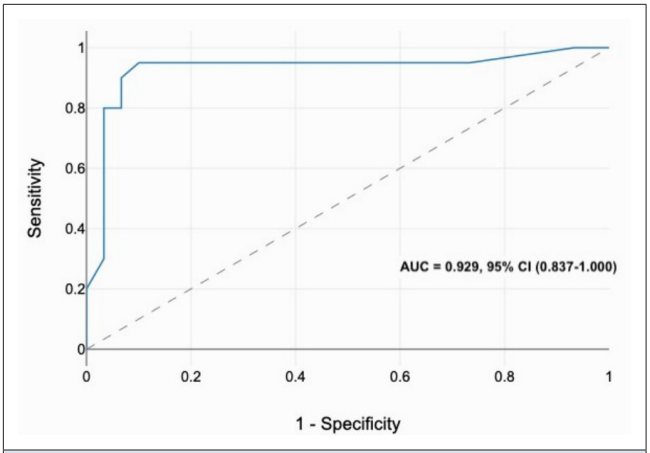

Fig 3. Receiver operating characteristic (ROC) curve analysis of CIMRI for discriminating dogs with current or previous congestive heart failure (CHF; ACVIM Stages C-D) from dogs without current or previous CHF (ACVIM Stages A-B2)

adverse outcomes in inflammatory and cardiometabolic conditions [9]. Similarly, in veterinary medicine, the HALP score has been associated with survival in dogs with CHF secondary to MMVD [8]. In addition, CBC-derived indices, including NLR, monocyte-to-lymphocyte ratio, and platelet-to-lymphocyte ratio, increase with disease severity and reflect underlying inflammatory activation [10,13,16]. Collectively, these studies support the potential value of multidimensional biomarkers in characterizing the systemic nature of cardiovascular disease.

Consistent with this framework, CIMRI values tended to be higher in more advanced ACVIM stages, with the highest values observed in Stage D, and were positively correlated with echocardiographic indicators of cardiac remodeling, particularly LA/Ao and LVIDdN. These findings agree with previous reports linking increasing MMVD severity to systemic inflammatory and biochemical alterations [23,25]. A positive association was also observed between CIMRI and MV E/A. CIMRI was negatively correlated with MAPSE, although this association was weaker than those observed for LA/Ao and LVIDdN. In contrast, correlations between CIMRI and EF and FS were weak and not statistically significant. Collectively, these findings indicate that CIMRI was associated with indices of cardiac chamber enlargement and selected functional echocardiographic variables, although the strength of these associations varied among parameters. Given the cross-sectional design, these associations should not be interpreted as evidence that CIMRI independently reflects or predicts cardiac remodeling.

A notable finding of the present study was that only NLR and CRP/Alb demonstrated stage-dependent variation among the individual CIMRI components, whereas metabolic (Chol/Glu and TP) and electrolyte (Na/K) parameters remained relatively stable. This pattern is

biologically plausible because progressive inflammatory activation and acute-phase responses are recognized features of advancing MMVD, particularly in dogs with CHF [6,17,18,23,26]. In contrast, metabolic and electrolyte parameters are often subject to tighter physiological regulation and compensatory mechanisms, which may limit their ability to discriminate disease stages when evaluated individually.

Despite the relatively limited discriminatory capacity of some individual components, CIMRI tended to show higher values in the more advanced ACVIM stages, particularly Stage D. This observation suggests a potential advantage of composite indices, namely the integration of multiple biological signals into a single metric that may capture complementary systemic alterations. As a composite biomarker approach, CIMRI integrates laboratory variables representing several systemic domains potentially associated with MMVD severity; however, its incremental value over individual biomarkers was not evaluated in the present study. Similar observations have been reported in studies evaluating composite inflammatory-metabolic indices in cardiovascular disease [27]. From a clinical perspective, CIMRI may provide complementary biological information alongside conventional echocardiographic assessment, particularly in intermediate disease stages where progression patterns may be variable.

An additional observation was that CIMRI values were numerically lower in stage B1 than in stage A. However, the difference was small and likely reflects biological variability within early disease stages rather than a meaningful pathological distinction. Given the limited number of dogs included in each group, this finding should be interpreted cautiously and confirmed in larger populations.

ROC analysis showed a high discriminatory ability of CIMRI within the present cohort for discriminating dogs with current or previous CHF (Stages C-D) from those without current or previous CHF (Stages A-B2). It is important to note that the ROC endpoint represents CHF status rather than structural cardiac remodeling, since Stage B2 dogs already exhibit cardiac enlargement as defined by the ACVIM staging criteria. Therefore, the ROC findings and the correlations observed between CIMRI and echocardiographic indices of cardiac remodeling address related but distinct aspects of MMVD and should be interpreted separately. The AUC of 0.929, together with a sensitivity of 95.0% and a specificity of 90.0% at the Youden-derived cut-off point of >5.8, indicates a high level of discrimination within the present cohort.

An important consideration is that CIMRI was intentionally designed as a simple and clinically applicable composite score based on routinely available laboratory variables. Equal weighting of the individual components was preferred to maintain ease of calculation and facilitate potential use in daily clinical practice. The inclusion of total protein in the denominator was intended to account for variations in protein status that may influence inflammatory and metabolic biomarkers. Because the primary objective of this study was to explore the potential clinical utility of a composite inflammatory-metabolic index rather than to develop a predictive algorithm, advanced weighting approaches such as multivariable regression-based coefficients or machine-learning optimization were not applied. Future studies involving larger populations should evaluate alternative weighting strategies and validate the mathematical structure of CIMRI.

Several limitations should be acknowledged. The study population consisted primarily of small- to medium-sized breeds, which may limit the generalizability of the findings. The relatively small number of dogs within each disease stage should also be considered when interpreting the results, as individual outlier values may have disproportionately influenced some biomarker components, particularly inflammatory variables such as NLR and CRP/Alb. Although total protein was incorporated as a scaling factor, the mathematical implications of this normalization strategy require further investigation. Alternative weighting and normalization approaches may improve the performance and interpretability of the index in future studies. Although CIMRI showed high discriminatory ability for current or previous CHF status within the present cohort, this finding requires validation in larger, independent populations before its clinical applicability can be established. Moreover, the incremental value of CIMRI over its individual components, particularly NLR and CRP/Alb, was not formally evaluated. Therefore, the present findings should not be interpreted as demonstrating superiority of CIMRI over these individual markers. In addition, the equal weighting strategy used in CIMRI was selected pragmatically and may not fully reflect the relative biological contribution of each component. The correlation analyses were exploratory, and no adjustment for multiple comparisons was applied; therefore, particularly the weaker associations should be interpreted cautiously. The cross-sectional design precludes assessment of prognostic value over time, and longitudinal studies are required to determine whether CIMRI is associated with clinical outcomes such as progression to heart failure or survival. Finally, although inclusion of Stage D dogs reflects real-world clinical conditions, ongoing cardiovascular therapy may have influenced some inflammatory, metabolic, and electrolyte variables included in the index.

In conclusion, the findings of this study provide preliminary support for CIMRI as a composite biomarker approach integrating routinely available inflammatory, metabolic, and electrolyte-related variables in dogs with MMVD. CIMRI differed across ACVIM stages, was associated with selected echocardiographic indices, and showed potential for discriminating dogs with current or previous CHF from those without CHF. Although these findings are encouraging, the cross-sectional design does not establish longitudinal disease progression, prognostic value, or an independent relationship with cardiac remodeling. Larger prospective and longitudinal studies in the independent canine populations are required to validate its clinical applicability and prognostic value.

Declarations

Availability of Data and Materials: The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgements: The authors declare that there are no acknowledgements.

Financial Support: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical Statement: This study was reviewed by the Bursa Uludağ University Local Ethics Committee for Animal Experiments (Decision No: B.30.2.ULU.0.8Z.00.00, Decision Date: 22 June 2026).

Conflict of Interest: The authors declared that there is no conflict of interest.

Declaration of Generative Artificial Intelligence (AI): The authors used generative artificial intelligence (AI) solely for language editing. All scientific content, study design, data analysis, interpretation of the results were performed by the authors.

Ethical Compliance Declaration: The authors declare that the study was conducted in accordance with all applicable animal welfare and research ethics principles, institutional and national/international regulations, and the requirements of the relevant ethics committees and funding/supporting institutions, where applicable. The authors accept full responsibility for the ethical integrity of the study.

Author Contributions: Z.Y.: Conceptualization, Methodology, Supervision, Data analysis, Development of the Canine Inflammatory-Metabolic Risk Index (CIMRI), Writing-original draft, Final approval. T.V.: Investigation, Data curation, Formal analysis, Writing-review & editing, Final approval. D.A.: Data curation, Validation, Writing-review & editing, Final approval.

References

1. **Borgarelli M, Buchanan JW:** Historical review, epidemiology and natural history of degenerative mitral valve disease. *J Vet Cardiol,* 14 (1): 93-101, 2012. DOI: 10.1016/j.jvc.2012.01.011
2. **Boswood A, Häggström J, Gordon SG, Wess G, Stepien RL, Oyama MA, Keene BW, Bonagura J, MacDonald KA, Patteson M, Smith S, Fox PR, Sanderson K, Woolley R, Szatmári V, Menaut P, Church WM, O'Sullivan ML, Jaudon JP, Kresken JG, Watson P:** Effect of pimobendan in dogs with preclinical myxomatous mitral valve disease and cardiomegaly: the EPIC study - A randomized clinical trial. *J Vet Intern Med,* 30 (6): 1765-1779, 2016. DOI: 10.1111/jvim.14586
3. **Keene BW, Atkins CE, Bonagura JD, Fox PR, Häggström J, Fuentes VL, Oyama MA, Rush JE, Stepien R, Uechi M:** ACVIM consensus guidelines for the diagnosis and treatment of myxomatous mitral valve disease in dogs. *J Vet Intern Med,* 33 (3): 1127-1140, 2019. DOI: 10.1111/jvim.15488
4. **Baisan RA, Turcu CA, Bilboc LM, Maftei MC, Vulpe V, Ohad DG:** Quantification of respiratory sinus arrhythmia in control dogs and in dogs with various severity classes of myxomatous mitral valve disease. *J Vet Cardiol,* 55, 48-56, 2024. DOI: 10.1016/j.jvc.2024.08.001
5. **Vezzosi T, Grosso G, Vatne L, Porciello F, Dall'Aglio E, Guglielmini C, Broch H, Dickson D, Croce M, Patata V, Marchesotti F, Tognetti R, Rishniw M, Domenech O:** Risk stratification using mitral insufficiency echocardiographic score 2 in dogs with preclinical mitral valve disease. *J Vet Intern Med,* 39 (5):e70215, 2025. DOI: 10.1111/jvim.70215
6. **Zhang F, Li K, Yongchao Z:** Inhibitory effect of doxycycline on MMP2/9 and its improvement in cardiac function and left ventricular remodeling after myocardial infarction in rats. *Kafkas Univ Vet Fak Derg,* 31 (3): 361-366, 2025. DOI: 10.9775/kvfd.2024.33390
7. **Turgut K, Şen İ, İnce ME, Naseri A, Süleymanoğlu H, Ertan M, Sağmanligi V:** The end-systolic wall stress/end-systolic volume index ratio for systolic function in Anatolian shepherd dogs with stage B2 degenerative mitral valve disease. *Kafkas Univ Vet Fak Derg,* 26 (2): 299-303, 2020. DOI: 10.9775/kvfd.2019.22661
8. **Park J, Chae Y, Lee S, Koo Y, Kim H, Kang BT, Yun T:** The hemoglobin, albumin, lymphocyte, and platelet score as a prognostic indicator for dogs with congestive heart failure secondary to myxomatous mitral valve disease. *Vet Sci,* 12 (9):908, 2025. DOI: 10.3390/vetsci12090908
9. **Zorrilla-Alfaro SM, Lechuga-Garcia NA, Araujo-Conejo A, Ramirez-Hernandez LA, Garza-Veloz I, Mauricio-Gonzalez A, Delgado-Enciso I, Rodriguez-Sanchez IP, Martinez-Fierro ML:** Inflammatory metabolic index and metabolic-inflammatory stress index as new biomarkers for complicated and perforated acute appendicitis. *J Clin Med,* 14 (15):5281, 2025. DOI: 10.3390/jcm14155281
10. **Bi W, Liu Y, Wu W, Lian S, Pei Z, Zhu F, Meng Q:** Association of nine composite inflammatory indices with cardiovascular diseases in US adults: National health and nutrition examination survey (NHANES, 2005-2018). *J Health Popul Nutr,* 45:55, 2026. DOI: 10.1186/s41043-025-01222-5
11. **Ljungvall I, Höglund K, Lilliehöök I, Oyama MA, Tidholm A, Tvedten H, Häggström J:** Serum serotonin concentration is associated with severity of myxomatous mitral valve disease in dogs. *J Vet Intern Med*, 27 (5): 1105-1112, 2013. DOI: 10.1111/jvim.12137
12. **Galizzi A, Bagardi M, Stranieri A, Zanaboni AM, Malchiodi D, Borromeo V, Brambilla PG, Locatelli C:** Factors affecting the urinary aldosterone-to-creatinine ratio in healthy dogs and dogs with naturally occurring myxomatous mitral valve disease. *BMC Vet Res,* 17:15, 2021. DOI: 10.1186/s12917-020-02716-6
13. **Jung MJ, Kim JH:** Prognostic efficacy of complete blood count indices for assessing the presence and the progression of myxomatous mitral valve disease in dogs. *Animals,* 13 (18):2821, 2023. DOI: 10.3390/ani13182821
14. **Saril A, Kocaturk M, Shimada K, Uemura A, Akgün E, Levent P, Baykal AT, Prieto AM, Agudelo CF, Tanaka R, Ceron JJ, Koch J, Yilmaz Z:** Serum proteomic changes in dogs with different stages of chronic heart failure. *Animals,* 12 (4):490, 2022. DOI: 10.3390/ani12040490
15. **Ku D, Chae Y, Kim C, Koo Y, Lee D, Yun T, Chang D, Kang BT, Yang MP, Kim H:** Severity of myxomatous mitral valve disease in dogs may be predicted using neutrophil-to-lymphocyte and monocyte-to-lymphocyte ratio. *Am J Vet Res,* 84 (6):ajvr.23.01.0012, 2023. DOI: 10.2460/ajvr.23.01.0012
16. **Kocaturk M, Saril A, Oz AD, Rubio CP, Ceron JJ, Yilmaz Z:** Neutrophil-to-lymphocyte ratio and red blood cell distribution width to platelet ratio and their relationships with inflammatory and antioxidant status in dogs with different stages of heart failure due to

myxomatous mitral valve disease. *Vet Res Commun,* 48 (4): 2477-2487, 2024. DOI: 10.1007/s11259-024-10431-y

17. **Reimann MJ, Ljungvall I, Hillström A, Møller JE, Hagman R, Falk T, Höglund K, Häggström J, Olsen LH:** Increased serum C-reactive protein concentrations in dogs with congestive heart failure due to myxomatous mitral valve disease. *Vet J,* 209, 113-118, 2016. DOI: 10.1016/j.tvjl.2015.12.006
18. **Pah AM, Serban S, Mateescu DM, Cotet IG, Muresan CO, Ilie AC, Buleu F, Craciun ML, Crisan S, Avram A:** Systemic inflammatory biomarkers (interleukin-6, high-sensitivity C-reactive protein, and neutrophil-to-lymphocyte ratio) and prognosis in heart failure: A meta-analysis of prospective cohort studies. *J Clin Med,* 14 (23):8610, 2025. DOI: 10.3390/jcm14238610
19. **Chen J, Yan L, Chen J:** Association between fasting glucose/high-density lipoprotein cholesterol ratio and cardiovascular disease risk in Chinese middle-aged and older adults: A longitudinal study. *Front Cardiovasc Med,* 12:1609891, 2025. DOI: 10.3389/fcvm.2025.1609891
20. **Wang K, Chu C, Hu J, Wang Y, Zheng W, Lv Y, Yan Y, Ma Q, Mu J:** Effect of salt intake on the serum cardiotrophin-1 levels in Chinese adults. *Ann Nutr Metab,* 73 (4): 302-309, 2018. DOI: 10.1159/000494436
21. **Okafor CR, Wariboko M, Abdullahi O, Olurunkemi O, Sawyer D, Okafor HE:** Serum sodium-potassium ratio as a predictor of long-term heart failure morbidity. *Circ Cardiovasc Qual Outcomes,* 6 (1):295, 2013. DOI: 10.1161/CIRCOUTCOMES.6.suppl_1.A295
22. **Valentini A, Heilmann RM, Kühne A, Biagini L, De Bellis D, Rossi G:** The renin-angiotensin-aldosterone system (RAAS): Beyond cardiovascular regulation. *Vet Sci,* 12 (8):777, 2025. DOI: 10.3390/vetsci12080777
23. **Rubio CP, Saril A, Kocaturk M, Tanaka R, Koch J, Ceron JJ, Yilmaz Z:** Changes of inflammatory and oxidative stress biomarkers in dogs with different stages of heart failure. *BMC Vet Res,* 16:433, 2020. DOI: 10.1186/s12917-020-02650-7
24. **Boon JA:** Veterinary Echocardiography. 2nd ed., 3-552, Wiley-Blackwell, Ames, IA, USA, 2010.
25. **Zois NE, Moesgaard SG, Kjelgaard-Hansen M, Rasmussen CE, Falk T, Fossing C, Häggström J, Pedersen HD, Olsen LH:** Circulating cytokine concentrations in dogs with different degrees of myxomatous mitral valve disease. *Vet J,* 192 (1): 106-111, 2012. DOI: 10.1016/j.tvjl.2011.05.009
26. **Domanjko Petrič A, Lukman T, Verk B, Nemec Svete A:** Systemic inflammation in dogs with advanced-stage heart failure. *Acta Vet Scand,* 60:20, 2018. DOI: 10.1186/s13028-018-0372-x
27. **Sun Y, Guo Y, Ma S, Mao Z, Meng D, Xuan K, Lu R, Pan X, Zhu X:** Association of C-reactive protein-triglyceride glucose index with the incidence and mortality of cardiovascular disease: A retrospective cohort study. *Cardiovasc Diabetol,* 24:313, 2025. DOI: 10.1186/s12933-025-02835-0