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Use of physical examination, electrocardiography, radiography, and biomarkers to predict echocardiographic stage B2 myxomatous mitral valve disease in preclinical Cavalier King Charles Spaniels☆

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Selected results from the study were presented in abstract form at the 2020 and 2021 virtual ECVIM Congresses as well as the 2021 virtual ACVIM Forum and the 2022 ACVIM Forum.

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KEYWORDS
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Abstract *Introduction:* Cavalier King Charles Spaniels (CKCS) are predisposed to developing myxomatous mitral valve disease (MMVD). Dogs with stage B2 MMVD benefit from medication.
Objectives: To develop (1) breed-specific cut-offs for individual screening tests and (2) predictive models utilizing physical examination (PE), ECG, radiograph, and blood-based biomarker variables in combination for identification of echocardiographic stage B2 MMVD in preclinical CKCS.
Animals: Adult, preclinical CKCS not receiving cardiac medications (N = 226).
Materials and methods: Prospective, cross-sectional study. Enrolled CKCS underwent PE, ECG, radiography, Doppler blood pressure measurement, echocardiography, and biomarker testing. Dogs were grouped by MMVD stage using echocardiography only. The discriminatory ability of individual tests to identify stage B2 was assessed, and prediction models were developed using variables derived from four 'tests' (PE, ECG, radiography, and biomarkers).
Results: N-terminal pro-B-type natriuretic peptide (NT-proBNP) and radiographic vertebral heart size (VHS) had the best discriminatory ability of individual diagnostic tests to differentiate stage A/B1 CKCS from stage B2, with an area under the curve (AUC) of 0.855 and 0.843, respectively. An NT-proBNP ≥1138 pmol/L or a VHS ≥11.5 had high specificity for predicting stage B2 (90.1% and 90.6%, respectively). Prediction models incorporating variables from multiple tests had better discriminatory ability than single tests. The four-test prediction model had an AUC of 0.971. Three and two-test models had AUCs ranging between 0.925—0.959 and 0.895—0.949, respectively.
Conclusions: Both NT-proBNP and VHS have good utility for predicting echocardiographic stage B2 MMVD in CKCS as individual tests. Prediction models incorporating multiple test variables have superior discriminatory ability.

Abbreviations

ACVIM	American College of Veterinary Internal Medicine
AUC	area under the curve
CKCS	Cavalier King Charles Spaniels
LA	left atrium
LV	left ventricle
MMVD	myxomatous mitral valve disease
MR	mitral regurgitation
NT-proBNP	N-terminal pro-B-type natriuretic peptide
PE	physical examination
ROC	receiver operator characteristic
SDMA	symmetric dimethylarginine
VHS	vertebral heart size
VLAS	vertebral left atrial size

Introduction

Cavalier King Charles Spaniels (CKCS) are predisposed to developing myxomatous mitral valve disease (MMVD) [1—4]. Use of pimobendan in dogs with preclinical MMVD meeting specific echocardiographic and radiographic criteria for cardiomegaly, significantly delays the onset of congestive heart failure and confers an overall survival benefit [5], with dogs meeting these criteria considered to be stage B2 based on the current American College of Veterinary Internal Medicine (ACVIM) MMVD staging scheme [6]. Although echocardiography is the preferred diagnostic test to confirm stage B2 MMVD, in many cases, it is not able to be pursued, with more readily available diagnostic tests often utilized as preliminary screening tests to identify high-risk dogs. The recent development of predictive models for preclinical dogs with MMVD weighing 2—25 kg has shown that a combination of history, physical examination (PE), and blood test variables including the cardiac biomarker N-terminal pro-B-type natriuretic peptide (NT-proBNP) have good predictive ability to identify stage B2 dogs, with this type of model being superior to any single diagnostic test alone [7]. Relatively, few dogs in that study had thoracic radiographs, however, limiting the ability to fully explore the potential additive benefit of this diagnostic test, and no ECG variables were explored [7].

While NT-proBNP provides diagnostic value in the clinical assessment of cardiac disease in dogs and is shown to increase with increasing severity of MMVD [7—11], significant differences in normal NT-proBNP values have been documented between different breeds of dogs [12]. This suggests that biomarker cut-offs or predictive models including NT-proBNP that are derived from non-breed-specific data may not perform as well as breed-specific models or cut-offs for predicting stage B2 MMVD. Similarly, objective radiographic measurements of cardiac size such as the vertebral heart size (VHS) [13] are also known to vary by breed, with a variety of breed-specific normal VHS reference intervals previously published. The newer vertebral left atrial size (VLAS) measurement [14], though comparatively less studied, has recent breed-specific normal data published as well [15,16].

The objectives of this study, therefore, were (1) to develop breed-specific cut-offs for individual cardiac screening tests and (2) to develop predictive models utilizing PE, ECG, radiograph, and blood-based biomarker variables in combination for the identification of stage B2 MMVD in preclinical CKCS diagnosed based on echocardiographic criteria only.

Animals, materials, and methods

Adult, apparently healthy, client-owned CKCS receiving no cardiac medications were prospectively recruited for this cross-sectional study via social media posts and by contacting local and regional CKCS breed clubs and associations. Exclusion criteria included age <1 year, history of active or chronically managed non-cardiac illness that could impact the cardiovascular system, and diagnosis of congenital or other non-MMVD cardiac disease known prior to the study or revealed during the completion of study diagnostics. Additionally, if creatinine or symmetric dimethylarginine (SDMA) results obtained as part of the study protocol were above the reported reference range, dogs were excluded from analysis. Dogs with mild elevation in blood urea nitrogen (<20.0% increase above the upper limit of normal) in the face of normal creatinine, SDMA, and blood pressure (BP) measurements were allowed to remain in the analysis.

Data were collected during three-day enrollment clinics held in December 2018, July 2019, and January 2020. Dog owners completed patient history forms for each CKCS to provide basic demographic data, confirm that the dog was not receiving prohibited cardiac medications, disclose all current medications and supplements being administered, and answer five clinical history questions. The five questions included: does your dog (1) have a cough, (2) have breathing difficulty, (3) currently have an abnormal activity level at home, (4) have a history of fainting spells, and (5) currently have an abnormal appetite. A yes answer to any of these questions prompted additional probing questions to provide further detail and clarification. In addition, each owner completed a functional evaluation of cardiac health questionnaire [17] for dogs enrolled in clinics two and three. Diagnostically, each enrolled CKCS underwent a PE, one right lateral thoracic radiograph, a 30-s six-lead ECG, Doppler BP measurement, a standardized echocardiogram including two-dimensional, M-mode, and Doppler blood flow imaging, and venous blood sampling. The order in which these tests were completed was based on operator availability at each of the testing stations.

Physical examination was performed by a single cardiologist (SG) in all dogs. Heart murmurs were graded between 0 and 6 based on the modified Levine scale [18]. Additionally, the presence or absence of a mid-systolic click or an auscultable arrhythmia was noted for each dog. Heart rate was not recorded during the physical exam.

Digital thoracic radiographs were acquired by trained radiology technicians and stored for each participating CKCS. Measurement of VHS [13] and VLAS [14] was performed on a digital DICOM viewer at the completion of study enrollment by a cardiology research intern (TS) trained in the measurement of VHS and VLAS who was blinded to both identity and MMVD stage. The ventral border of the caudal vena cava was used as the starting point for the short axis measurement of the VHS on all dogs for consistency.

All ECGs were recorded in right lateral recumbency using a commercially available six-lead ECG system[e] and stored for later measurement and analysis. After study enrollment was complete, the following variables were measured on all ECGs by a trained rotating veterinary intern (BGB) at a sweep speed of 100 mm/s: P wave duration, P wave amplitude, PR interval, R wave peak time, QRS duration, R wave amplitude, and QT interval. Twenty sequential RR intervals were used to calculate the average heart rate and vasovagal tonus index to assess heart rate variability [19]. The P wave duration and QRS duration were also

[e] CardioPet ECG Device, IDEXX Laboratories, Westbrook, Maine, USA.

summed to create a P + QRS duration for each dog. This measurement was collected to investigate the utility of combining two ECG durations that have the potential to lengthen in the setting of left atrial and left ventricular enlargement, respectively.

All Doppler BP measurements were obtained by a single, registered veterinary technician with extensive training and experience in BP measurement within the Cardiology Department at Texas A&M University. Blood pressure was obtained as recommended by consensus guidelines [20]; however, given the battery of tests performed in a short period of time, some degree of stress could not entirely be eliminated from the situation and dogs were not with their owners at the time that BP was assessed. Additionally, dogs did not always have a full 5–10 min of acclimatization time prior to the BP measurement being obtained.

Echocardiographic measurements were performed in real time, and the average of three measurements was transcribed directly onto each dog's study form. Echocardiograms were completed on multiple different ultrasound machines with different, phased array cardiac ultrasound probes of appropriate frequency for CKCS body size by board-certified veterinary cardiologists (SW, ABS, RF, JV, and BB) or third-year cardiology residents (SK and JS) with dogs imaged in both right and left lateral recumbency. Mitral valve anatomy was assessed from right parasternal four-chamber views and left apical four-chamber views, and subjective evidence of mitral valve prolapse or flail leaflet(s) was recorded as yes or no in addition to noting the presence or absence of mitral regurgitation (MR) and quantifying its severity based on the percentage of the left atrium (LA) area filled by the color Doppler MR jet as assessed real time by the sonographer (none, trivial, mild = <20.0%, moderate = 20.0–40.0%, or severe = >40.0% of the LA area) [21]. The assessment of MR severity was based on the maximum color Doppler jet size appreciated throughout the systolic period. The internal diameter of the left ventricle (LV) at end diastole and end systole were measured on an LV short axis M-mode image at the level of the papillary muscles, with these measurements subsequently normalized to body weight [22]. Left atrial to aortic root ratio [23] was measured from a short axis view at the heart base immediately after aortic valve closure. All CKCS were staged based on echocardiography alone using these measurements of the LV and LA based on current MMVD consensus guidelines as stages A, B1, or B2 [6]. Of note, because CKCS were staged based only on their echocardiogram results, a CKCS without a heart murmur could be staged as B1 based on mitral valve remodeling, with or without MR that was not detectable by auscultation. Additional standard echocardiographic measurements were also performed to ensure dogs did not have pre-existing, congenital, or acquired cardiac disease other than MMVD. Dilated cardiomyopathy was diagnosed if left ventricular internal diameter at end systole was elevated based on breed-specific reference intervals adjusted for body weight, and fractional shortening was decreased <25.0% [24] in the absence of moderate to severe MR, as outlined by a recent review on screening for dilated cardiomyopathy in dogs [25].

Blood samples were immediately processed, aliquoted, and frozen at 0 °C. At the conclusion of each of the three enrollment clinics, the samples were transferred frozen on dry ice and stored at −80 °C. Ultrasensitive cardiac troponin I was measured using the commercially available assay offered by the Texas A&M Gastrointestinal Laboratory at the time of the study[f] within two days of the samples being stored at −80 °C after each of the three enrollment clinics. The remainder of the samples were stored at −80 °C until batch analysis could be completed on samples from all three enrollment clinics. Samples were then shipped on dry ice to IDEXX Laboratories (Portland, Maine) for measurement of NT-proBNP and a renal panel including blood urea nitrogen, creatinine, albumin, phosphorus, potassium, and SDMA.

Statistical analysis

Descriptive statistics are reported as median (25th–75th percentile), minimum, and maximum for continuous variables as the data were not normally distributed. Categorical variables are reported as proportions. The correlation between NT-proBNP and systolic BP was explored with Spearman correlation coefficients (r_s).

Receiver operator characteristic curves (ROC) were constructed for selected individual diagnostic tests/measurements to evaluate their ability to discriminate CKCS in echocardiographic stage B2 from those in stages A and B1 combined. A lower, middle, and upper cut-off were selected based on ROC curve analysis for each variable, with these cut-offs aimed at identifying high

[f] Advia Centaur TnI-Ultra, Siemens Medical Solutions Diagnostics, New York, NY, USA.

sensitivity (>90%), optimized sensitivity and specificity combined, and high specificity (>90%), respectively.

Prediction models were developed using exploratory binary forward stepwise regression analysis, using a method that begins with a model that contains no variables, then starts adding the most significant candidate variables one after another until a pre-specified stopping rule is reached or until all variables under consideration are included in the model. The criteria for model entry were $P<0.050$ and $P>0.050$ for removal. The stopping criteria were: 100 iterations = 100 and convergence = 0.00001. All candidate variables from each test category were evaluated as a 'cluster', with the four test categories being (1) history/PE, (2) blood tests, (3) ECG, and 4) radiographic measurements. These four initial regression models identified a total of seven significant candidate variables which were carried forward into multitest analyses [26,27]. The significant candidate variables for each test category were identified as: (1) history/PE: history of cough yes/no and murmur grade, (2) blood tests: NT-proBNP and SDMA, (3) ECG: P + QRS duration and heart rate derived from the ECG, and (4) radiographic measurements: VHS. These seven significant candidate variables were entered into all models as continuous variables with the exception of the historical question of whether cough was present or not and murmur grade, which were entered as categorical variables. All models evaluated a binary response variable of echocardiographic stage B2 CKCS vs. stage A and stage B1 CKCS combined into a single group. The best model (based on the area under the curve (AUC) for this comparison) was developed by entering all seven significant candidate variables from the four 'tests' into a forward stepwise analysis, which the statistical program then carried out. Additional model iterations were explored in which only two or three 'tests' were available to assess the additive value of additional diagnostic testing, including three iterations in which history/PE variables were not available to mimic a telemedicine scenario in which diagnostics may be available with unknown or inaccurate knowledge of murmur grade or clinical history. Overall model significance was assessed by the likelihood, score, and Wald tests. The significance of individual predictors was evaluated with Wald chi-square statistic for the retained regression coefficients and goodness-of-fit was assessed by the Hosmer—Lemeshow test. Predicted probabilities were assessed through the construction of classification tables that reported specificity, sensitivity, and percent of correct classifications using a cut-point threshold of 0.5. Models were compared using AUC. ROC curves were then constructed for the predicted probabilities for each regression model to determine the 95% confidence intervals of the model and the sensitivity, specificity, and overall accuracy for three predicted probability cut-offs. The lower cut-offs were selected for relatively high sensitivity (≥90.0%). The middle cut-off was selected based on the highest combined sensitivity and specificity. The highest cut-off was selected for a high specificity (≥90.0%). Statistical tests were considered significant if the P-value was <0.050.

To explore the impact of including dogs with no heart murmur in the models, all models were re-run excluding all stage A CKCS and stage B1 CKCS that did not have a heart murmur.

Decision tree analysis was used to develop a single three-test decision tree algorithm. To mimic a more clinically relevant scenario, heart murmurs were collapsed into two categories including none/soft (grades 0, 1 and 2) and moderate/thrilling (grades 3, 4, 5, and 6). A simplified regression equation using only the three variables from the decision tree algorithm (murmur, VHS, and NT-proBNP) was used to derive example prediction probabilities for demonstration purposes.

A statistical software package[g] was used for analysis.

Results

A total of 235 CKCS were enrolled in the study. Five CKCS were excluded due to diagnosis of cardiac disease other than MMVD including a ventricular septal defect (n = 1), patent ductus arteriosus (n = 1), third-degree atrioventricular block (n = 1), and dilated cardiomyopathy (n = 2). Four CKCS were excluded due to creatinine or SDMA values above reference range. The remaining 226 CKCS made up the analyzed study population. One 1.75-year-old dog (stage A) with a blood urea nitrogen of 43 mg/dL (reference range: 9—31 mg/dL) was allowed to remain in the analysis despite the >20% elevation as this was considered spurious in the face of a creatinine of 0.5 mg/dL, SDMA of 7, and systemic BP of 121 mmHg. There were 14 CKCS (6.2%) in stage A, 167 (73.9%) in stage B1, and 45 (19.9%) in stage B2.

[g] Addinsoft (2022) XLSTAT statistical and data analysis solution, New York, NY, USA. https://www.xlstat.com/en.

Table 1 Demographic, historical, and physical examination variables for the entire population of 226 Cavalier King Charles Spaniels and broken down by echocardiographically determined American College of Veterinary Internal Medicine myxomatous mitral valve disease stage (A, B1, or B2). Quantitative variables are expressed as median (25th—75th percentile) [range] while proportional variables are expressed as n (%).

Variable	Number of missing observations	Entire population (n = 226)	Stage A (n = 14)	Stage B1 (n = 167)	Stage B2 (n = 45)
Age (years)	0	7.2 (5.0—9.5) [1.2—14.7]	2.6 (1.8—3.8) [1.3—8.0]	6.9 (4.9—9.5) [1.2—14.7]	9.0 (7.9—10.3) [5.3—13.0]
Sex	0	Female: 136 (60.2%)	Female: 7 (50.0%)	Female: 104 (62.3%)	Female: 25 (55.6%)
		Male: 90 (39.8%)	Male: 7 (50.0%)	Male: 63 (37.7%)	Male: 20 (44.4%)
Coat color	0	BL: 119 (52.7%)	BL: 10 (71.4%)	BL: 86 (51.5%)	BL: 23 (51.1%)
		BT: 18 (8.0%)	BT: 0 (0.0%)	BT: 14 (8.4%)	BT: 4 (8.9%)
		R: 22 (9.7%)	R: 0 (0.0%)	R: 17 (10.2%)	R: 5 (11.1%)
		TRI: 67 (29.6%)	TRI: 4 (28.6%)	TRI: 50 (29.9%)	TRI: 13 (28.9%)
History of cough (yes/no)	0	Yes: 35 (15.5%)	Yes: 2 (14.3%)	Yes: 19 (11.4%)	Yes: 14 (31.1%)
		No: 191 (84.5%)	No: 12 (85.7%)	No: 148 (88.6%)	No: 31 (68.9%)
History of breathing difficulty (yes/no)	4	Yes: 13 (5.9%)	Yes: 0 (0.0%)	Yes: 10 (6.1%)	Yes: 3 (7.0%)
		No: 209 (94.1%)	No: 14 (100.0%)	No: 155 (93.9%)	No: 40 (93.0%)
History of abnormal activity level (yes/no)	4	Yes: 4 (1.8%)	Yes: 0 (0.0%)	Yes: 4 (2.4%)	Yes: 0 (0.0%)
		No: 218 (98.2%)	No: 14 (100.0%)	No: 161 (97.6%)	No: 43 (100.0%)
History of fainting spells (yes/no)	4	Yes: 3 (1.4%)	Yes: 0 (0.0%)	Yes: 2 (1.2%)	Yes: 1 (2.3%)
		No: 219 (98.6%)	No: 14 (100.0%)	No: 163 (98.8%)	No: 42 (97.7%)
History of abnormal appetite (yes/no)	4	Yes: 2 (0.9%)	Yes: 0 (0.0%)	Yes: 1 (0.6%)	Yes: 1 (2.3%)
		No: 220 (99.1%)	No: 14 (100.0%)	No: 164 (99.4%)	No: 42 (97.7%)
FETCH score	70	0 (0—1) [0—58]	0 (0—0) [0—1]	0 (0—1) [0—58]	2 (0—10) [0—26]
Body weight (kg)	0	8.2 (7.2—9.6) [4.4—15.6]	8.2 (7.2—8.4) [5.4—12.3]	8.2 (7.2—9.7) [4.4—15.6]	8.8 (7.5—9.4) [4.7—12.8]
Body condition score	8	5.5 (5—6.5) [3.5—9]	5.3 (4.9—6.0) [4.5—6.5]	5.5 (5.0—6.5) [4.0—9.0]	6.0 (5.0—6.5) [3.5—8.5]
Murmur grade	0	Grade 0: 38 (16.8%)	Grade 0: 11 (78.6%)	Grade 0: 27 (16.2%)	Grade 0: 0 (0.0%)
		Grade 1: 20 (8.8%)	Grade 1: 0 (0.0%)	Grade 1: 20 (12.0%)	Grade 1: 0 (0.0%)
		Grade 2: 47 (20.8%)	Grade 2: 2 (14.3%)	Grade 2: 45 (26.9%)	Grade 2: 0 (0.0%)
		Grade 3: 38 (16.8%)	Grade 3: 1 (7.1%)	Grade 3: 33 (19.8%)	Grade 3: 4 (8.9%)
		Grade 4: 44 (19.5%)	Grade 4: 0 (0.0%)	Grade 4: 26 (15.6%)	Grade 4: 18 (40.0%)
		Grade 5: 20 (8.8%)	Grade 5: 0 (0.0%)	Grade 5: 8 (4.8%)	Grade 5: 12 (26.7%)
		Grade 6: 19 (8.4%)	Grade 6: (0.0%)	Grade 6: 8 (4.8%)	Grade 6: 11 (24.4%)
Auscultable premature beats		Yes: 64 (28.3%)	Yes: 3 (21.4%)	Yes: 52 (31.1%)	Yes: 12 (26.7%)
		No: 162 (71.7%)	No: 11 (78.6%)	No: 115 (68.9%)	No: 33 (73.3%)

BL: Blenheim; BT: black and tan; R: ruby; TRI: tricolor.

Descriptive statistics outlining the demographics, history, and functional evaluation of cardiac health questionnaire results and PE findings of the population are presented in Table 1. From a history perspective, in this population of preclinical CKCS, there were 35 dogs (15.5% of the total population) that had a history of a cough, 13 dogs (5.9%) had a history of breathing difficulty, four dogs (1.8%) had a history of abnormal activity level, three dogs (1.4%) had a history of having had fainting spells, and two dogs (0.9%) had a history of an abnormal appetite. Additional history provided by the owners in dogs noted to having breathing difficulty included comments such as a previous diagnosis of laryngeal paralysis, hyperventilation when excited, snoring (with or without reverse sneezing), increased panting or heavy breathing when walking or excited, and different breathing patterns at night (one since puppyhood, the other noted to have a history of abnormal tonsillar swelling). In the three dogs in which fainting was reported, one had fainted four times in association with exercise but routinely ran three miles per day at the time of evaluation, one fainted in association with breeding on a single occasion several years prior to the study evaluation, and the third was noted to have the back legs 'give out' for 30–60 s after heavy exercise. These dogs were subsequently classified as stages B2, B1, and B1, respectively. None of the three dogs were noted to have evidence of pulmonary hypertension on their echocardiogram, and all three had a tricuspid regurgitation velocity <2.6 m/s.

The percentage of CKCS with each category of murmur grade is also broken down by echocardiographic ACVIM stage as shown in Table 1. There were 27 CKCS categorized as stage B1 that did not have a heart murmur detected on auscultation. Of these 27 dogs, two had moderate MR on their echocardiogram, 12 had mild MR, and 13 had none or trivial MR but had mitral valve remodeling consistent with MMVD such as mitral valve prolapse and/or thickening. In CKCS that were stage B2, 91.1% had a heart murmur that was a grade 4/6 or louder. Twelve stage B1 dogs were noted to have mid-systolic clicks. Additionally, auscultable premature beats were detectable on auscultation in 21.4%, 31.1%, and 26.7% of stages A, B1 and B2 dogs, respectively.

Descriptive statistics for all individual diagnostic test results or measurements are presented in Table 2. Fourteen dogs had a BP between 160 and 169 mmHg and five had a BP between 170 and 180 mmHg, all 19 of which had normal blood urea nitrogen, creatinine, and SDMA. No correlation between NT-proBNP and systolic BP was appreciated ($P = 0.448$ and $r_s = 0.003$). Diagnostic results for cardiac biomarkers showed increasing NT-proBNP and ultrasensitive cardiac troponin I values with increasing stage of MMVD, while renal panel markers were similar across all groups.

Electrocardiogram variables are also displayed in Table 2. Of note, 26.5% of the total population of CKCS were noted to have single supraventricular premature contractions during their 30-s ECG, with that percentage relatively unchanged across MMVD stages A, B1, and B2. There were also seven CKCS that had second-degree atrioventricular block identified during their ECG and one dog with single ventricular premature contractions was identified.

Descriptive statistics for the VHS and VLAS measurements as well as the combined VHS + VLAS measurement are also presented in Table 2. As expected, each of the objective radiographic measurements of cardiac size increased with increasing stage of MMVD. This parallels the increasing echocardiographic LV and LA size demonstrated by the median left ventricular internal diameter at end diastole normalized to body weight and left atrial to aortic root ratio values across groups that is also presented in Table 2.

Receiver operator characteristic curve analyses for selected sole diagnostic variables are presented in Table 3, including NT-proBNP, VHS, VLAS, VHS + VLAS, and P + QRS duration. All tests showed good discriminatory ability to differentiate between echocardiographic stage B2 vs. stage A + B1 combined. The AUC was highest for NT-proBNP at 0.855. Of the radiographic measurements, VHS slightly outperformed VLAS. The combined VHS + VLAS measurement also marginally outperformed VHS alone based on AUC; however, the confidence intervals for all variables overlapped suggesting they were not statistically different. Even the sole ECG measurement combining the P and QRS durations had good discriminatory ability with an AUC of 0.813.

A summary of the best regression analysis and all explored two and three-test iterations are presented in Table 4. The model equations generate an output result using a predicted probability. To understand the clinical implication of these predicted probabilities, they were used to construct ROC curves. The results of the ROC curve analysis of the predicted probabilities derived from each of the regression models are summarized in Supplemental Table 1, with the best four-test model ROC curve presented in Figure 1. The ROC curves allow models to be compared using AUC, and predicted

Table 2 Diagnostic test variables for the entire population of 226 Cavalier King Charles Spaniels and broken down by echocardiographically determined American College of Veterinary Internal Medicine myxomatous mitral valve disease stage (A, B1, or B2). Quantitative variables are expressed as median (25th—75th percentile) [range] while proportional variables are expressed as n (%).

Variable	Number of missing observations	Entire population (n = 226)	Stage A (n = 14)	Stage B1 (n = 167)	Stage B2 (n = 45)
Doppler blood pressure					
Heart rate during BP (beats/minute)	0	124 (108—140) [76—232]	128 (117—143) [100—172]	124 (112—140) [76—232]	116 (100—128) [84—156]
Systolic blood pressure (mmHg)	0	129 (118—143) [93—180]	119 (107—126) [103—143]	129 (119—143) [93—180]	129 (119—146) [102—170]
Biomarkers					
NT-proBNP (pmol/L)	0	738.1 (525.6—1047.0) [250.0—3656.8]	510.6 (400.6—588.7) [285.4—1044.5]	671.3 (515.2—917.3) [250.0—2208.4]	1374.3 (960.4—1972.8) [478.8—3656.8]
cTnI (ng/mL)	0	0.045 (0.022—0.079) [0.006—0.350]	0.011 (0.006—0.024) [0.006—0.068]	0.035 (0.021—0.069) [0.006—0.350]	0.074 (0.050—0.105) [0.013—0.347]
SDMA (ug/dL)	0	8.0 (7.0—10.0) [4.0—14.0]	8.0 (7.3—10.0) [6.0—14.0]	8.0 (7.0—10.0) [4.0—13.0]	8.0 (7.0—10.0) [5.0—13.0]
BUN (mg/dL)	0	16.5 (13.3—19.0) [7.0—43.0]	20.5 (17.0—21.75) [13.0—43.0]	16.0 (13.0—19.0) [7.0—35.0]	16.0 (13.0—18.0) [9.0—30.0]
Creatinine (mg/dL)	0	0.6 (0.6—0.7) [0.3—1.3]	0.7 (0.6—0.7) [0.5—1.3]	0.6 (0.6—0.7) [0.3—1.2]	0.6 (0.6—0.7) [0.3—1.2]
Albumin (g/dL)	0	3.0 (2.8—3.2) [1.5—3.7]	3.0 (2.9—3.1) [2.6—3.4]	3.0 (2.8—3.2) [1.5—3.7]	3.0 (2.8—3.2) [2.0—3.6]
Phosphorus (mg/dL)	0	4.3 (3.9—4.7) [2.2—7.4]	4.5 (4.2—5.0) [3.7—5.2]	4.3 (3.9—4.6) [2.2—7.4]	4.4 (4.1—4.8) [2.5—6.2]
Potassium mmol/L)	0	4.4 (4.2—4.6) [3.6—5.4]	4.3 (4.2—4.4) [4.0—4.7]	4.4 (4.2—4.6) [3.6—5.4]	4.5 (4.2—4.8) [3.8—5.2]
Electrocardiogram					
Heart rate during ECG (beats/minute)	3	121 (107—135) [77—250]	136 (121—162) [80—179]	122 (108—136) [77—250]	115 (102—127) [79—148]
P + QRS duration (ms)	3	73.0 (68.0—78.0) [56.0—112.0]	67.0 (64.0—72.8) [58.0—80.0]	72.0 (67.0—77.0) [56.0—112]	80.5 (76.8—86.0) [62.0—103.0]
P duration (ms)	3	37.0 (34.0—40.0) [23.0—52.0]	35.5 (34.0—36.8) [27.0—43.0]	36.0 (33.0—39.0) [25.0—49.0]	41.0 (36.8—46.0) [23.0—52.0]
QRS duration (ms)	3	36.0 (34.0—39.0) [24.0—74.0]	34.0 (31.5—35.8) [28.0—37.0]	36.0 (33.0—38.0) [24.0—74.0]	39.5 (37.0—42.3) [33.0—54.0]
PR interval (ms)	3	95.0 (86.0—107.0) [69.0—156.0]	87.0 (83.8—91.0) [78.0—108.0]	96.0 (86.0—106.0) [69.0—156.0]	98.0 (89.8—109.3) [71.0—151.0]
QT interval (ms)	3	181.0 (173.0—191.0) [142.0—227.0]	174.0 (165.5—182.5) [155.0—203.0]	181.0 (172.0—190.0) [142.0—223.0]	183.0 (176.0—192.3) [164.0—227.0]
P amplitude (mV)	3	0.20 (0.15—0.27) [0.04—0.46]	0.23 (0.17—0.27) [0.13—0.46]	0.20 (0.15—0.26) [0.04—0.41]	0.21 (0.15—0.30) [0.06—0.40]
R amplitude (mV)	3	2.6 (2.1—3.0) [1.0—5.4]	2.3 (1.9—2.5) [1.3—2.9]	2.6 (2.1—3.0) [1.1—4.6]	2.7 (2.2—3.5) [1.3—5.4]

R wave peak time (ms)	3	23.0 (22.0—25.0) [15.0—30.0]	24.5 (22.3—25.0) [20.0—28.0]	23.0 (22.0—25.0) [15.0—30.0]	24.0 (22.0—26.0) [18.0—30.0]
MEA (degrees)	3	60.0 (60.0—60.0) [0.0—120.0]	60.0 (60.0—90.0) [30.0—90.0]	60.0 (60.0—60.0) [0.0—120.0]	60.0 (60.0—90.0) [30.0—120.0]
RR-SD	3	53.1 (31.0—88.8) [3.9—195.9]	42.5 (24.7—56.5) [3.9—136.6]	54.8 (31.1—80.3) [7.1—185.1]	52.8 (32.3—103.2) [6.0—195.9]
VVTI	3	7.9 (6.9—9.0) [2.7—10.6]	7.5 (6.4—8.1) [2.7—9.8]	8.0 (6.9—8.8) [3.9—10.4]	7.9 (6.9—9.3) [3.6—10.6]
Supraventricular premature contractions (Yes/No)	3	Yes: 59 (26.5%) No: 164 (73.5%)	Yes: 4 (28.6%) No: 10 (71.4%)	Yes: 43 (26.1%) No: 122 (73.9%)	Yes: 12 (27.3%) No: 32 (72.7%)
Thoracic radiograph					
VHS	0	10.7 (10.2—11.2) [8.5—13.1]	10.1 (9.9—10.6) [9.5—11.5]	10.5 (10.2—11.0) [8.5—12.5]	11.5 (11.0—12.0) [10.0—13.1]
VLAS	0	1.9 (1.7—2.2) [1.2—3.4]	1.7 (1.6—2.0) [1.4—2.3]	1.8 (1.7—2.1) [1.2—3.2]	2.3 (1.9—2.6) [1.6—3.4]
VHS + VLAS	0	12.6 (12.0—13.3) [10.1—15.9]	12.1 (11.6—12.4) [11.1—13.5]	12.4 (11.9—13.0) [10.1—15.2]	13.7 (12.9—14.5) [11.7—15.9]
Echocardiogram					
LVIDdN	0	1.55 (1.41—1.73) [1.11—2.53]	1.29 (1.20—1.43) 1.11—1.51]	1.51 (1.39—1.62) [1.15—2.03]	1.87 (1.77—2.00) [1.71—2.53]
LA:Ao	0	1.32 (1.22—1.49) [0.76—2.77]	1.19 (1.13—1.23) [1.00—1.28]	1.30 (1.20—1.38) [0.76—1.97]	1.75 (1.68—1.86) [1.61—2.77]

BP: blood pressure; BUN: blood urea nitrogen; cTnI: ultrasensitive cardiac troponin I; ECG: electrocardiogram; MEA: mean electrical axis; NT-proBNP: N-terminal B-type natriuretic peptide; RR-SD: standard deviation of average R wave to R wave interval over 20 heart beats; SDMA: symmetric dimethylarginine; VHS: vertebral heart score; VLAS: vertebral left atrial size; VVTI: vasovagal tonus index.

Table 3 Receiver operator characteristic curve analysis to discriminate between Cavalier King Charles Spaniels in echocardiographic stage B2 vs. stages A and B1 combined using six individual diagnostic test or physical examination variables.

Stage B2 CKCS (n = 45) vs. Stage A + Stage B1 CKCS combined (n = 181)				
AUC	95% CI AUC	Cut-off (≥)	SE%	SP%
NT-proBNP (pmol/L)				
0.855	0.792–0.917	670	91.1	50.8
		1138	68.9	90.1
		1522	44.4	95.6
VHS				
0.843	0.777–0.908	10.6	91.1	55.8
		11.0	80.0	74.0
		11.5	51.1	90.6
VLAS				
0.783	0.707–0.859	1.7	95.6	27.1
		2.3	51.1	92.3
		2.6	26.7	98.3
VHS + VLAS				
0.848	0.785–0.911	12.5	91.1	50.8
		12.9	82.2	72.9
		13.6	53.3	90.6
P + QRS duration				
0.813	0.740–0.885	70	95.5	42.5
		75	81.8	68.2
		81	50.0	91.6

AUC: area under the curve; CI: confidence interval; NT-proBNP: N-terminal pro-B-type natriuretic peptide; SE: sensitivity; SP: specificity; VHS: vertebral heart size; VLAS: vertebral left atrial size.

probability to be assessed considering a variety of cut-offs related to sensitivity and specificity for diagnosis of echocardiographic stage B2 vs. stage B1/A in the present study population. The best regression model included candidate variables from each of the four 'test' categories and resulted in an AUC of 0.971. Three-test models performed similarly among themselves, regardless of which of the diagnostic tests was removed from the model with the AUC ranging between 0.943 and 0.959 with the exception of the model in which history/PE candidate variables were removed which lowered the AUC to 0.925. Two-test models also performed similarly among themselves with an AUC ranging between 0.922 and 0.949 when history/PE candidate variables were included and dropping slightly to 0.895–0.901 if they were not. The most common retained predictors of significance were NT-proBNP, VHS, murmur grade, and P + QRS duration. Of note, ultrasensitive cardiac troponin I and VLAS were not significant in the initial blood test cluster and radiograph cluster regression models, respectively, and were therefore not included in any subsequent exploratory regression models.

Exclusion of all stage A CKCS and the stage B1 CKCS that did not have heart murmurs from all models resulted in an AUC that was different by 0.016 on average (range: 0.009–0.02), with none of the new AUCs falling outside of the 95% confidence intervals for the original models.

A single, three-test decision tree algorithm was developed using heart murmur, VHS, and NT-proBNP (Fig. 2). Heart murmur was chosen as the first decision step, as this variable should be known for any examined CKCS. The VHS measurement was utilized as the second step in a three-bin fashion, followed by NT-proBNP. The decision tree demonstrates a trend of increasing confidence in an echocardiographic stage B2 diagnosis for dogs that progressively meet multiple higher specificity cut-offs, while also demonstrating increased confidence in ruling out an echocardiographic stage B2 diagnosis in dogs with no murmur or a soft murmur as well as dogs falling beneath lower thresholds for VHS and NT-proBNP. The decision tree also demonstrates that a clear gray zone exists in dogs with a VHS between 10.6 and 11.4; however, the addition of NT-proBNP does help improve discriminatory ability in CKCS that fall within this VHS range. While this provides useful general information, the degree of discriminatory ability for an individual dog can be significantly improved if specific measurements can be accounted for using the regression equations, rather than being forced to bin an individual dog according to the provided decision tree thresholds.

Table 4 Summary of prediction models using four, three, and two test models. The four test clusters included (1) history/PE, (2) blood tests, (3) ECG, and (4) radiographic measurements. The best candidate variables for each cluster were identified as: (1) history/PE: history of cough yes/no and murmur grade, (2) blood tests: NT-proBNP and SDMA, (3) ECG: P + QRS duration and heart rate derived from the ECG, and (4) radiographic measurements: VHS. All models except those that entered predictors derived from the ECG included all 226 dogs (A + B1 = 181, B2 = 45). Models that entered predictors derived from the ECG included 223 dogs (A + B1 = 179, B2 = 44). Sensitivities and specificities were calculated based on a threshold probability of 0.5.

Tests included	Four test regression models	Three test regression models				Two test regression models				
	PE NT-proBNP ECG Rad	PE NT-proBNP Rad	PE ECG Rad	PE NT-proBNP ECG	NT-proBNP ECG Rad	PE NT-proBNP	PE ECG	PE Rad	ECG Rad	NT-proBNP Rad
Number of entered predictors	7	4	5	5	4	3	4	3	3	2
Entered predictors	Cough Murmur NT-proBNP SDMA P + QRS ECG-HR VHS	Cough Murmur NT-proBNP VHS	Cough Murmur P + QRS ECG-HR VHS	Cough Murmur NT-proBNP P + QRS ECG-HR	NT-proBNP P + QRS ECG-HR VHS	Cough Murmur NT-proBNP	Cough Murmur P + QRS ECG-HR	Cough, Murmur VHS	P + QRS ECG-HR VHS	NT-proBNP VHS
AUC (95% CI)	0.971 (0.952—0.989)	0.943 (0.914—0.972)	0.957 (0.935—0.980)	0.959 (0.936—0.982)	0.925 (0.882—0.967)	0.932 (0.901—0.964)	0.949 (0.923—0.976)	0.922 (0.889—0.956)	0.895 (0.847—0.943)	0.901 (0.845—0.958)
Number of retained predictors	6	3	4	4	4	2	3	2	3	2
Retained predictors (listed in order from most to least significant)	NT-proBNP VHS P + QRS Murmur ECG-HR SDMA	NT-proBNP VHS Murmur	Murmur P + QRS ECG-HR VHS	NT-proBNP Murmur P + QRS ECG-HR	NT-proBNP VHS P + QRS ECG-HR	NT-proBNP Murmur	Murmur P + QRS ECG-HR	Murmur VHS	VHS P + QRS ECG-HR	NT-proBNP VHS
Classification table: specificity%	95.0%	94.5%	93.9%	96.1%	94.4%	94.5%	93.3%	91.7%	94.4%	95.6%
Classification table: sensitivity%	75.0%	62.2%	70.5%	72.7%	59.1%	62.2%	68.2%	57.8%	52.3%	53.3%
Classification table: % correct	91.0%	88.1%	89.2%	91.5%	87.4%	88.1%	88.3%	85.0%	86.1%	87.2%

AUC: area under the curve; CI: confidence interval; ECG: electrocardiogram; HR: heart rate; NT-proBNP: N-terminal pro-B-type natriuretic peptide; PE: physical examination; Rad: radiograph; VHS: vertebral heart size.

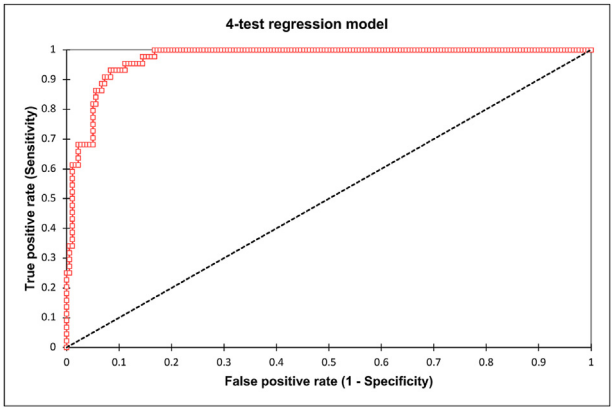

Figure 1 Receiver operator characteristic curve based on the best regression model including variables from each of the four 'test' categories (N-terminal pro-B-type natriuretic peptide, vertebral heart size, P + QRS duration, heart murmur grade, heart rate derived from the electrocardiogram, and symmetric dimethylarginine) which resulted in an AUC of 0.971 (confidence intervals: 0.952—0.989).

Discussion

Given the known predilection for CKCS to develop MMVD, breed-specific data to better inform risk prediction in these dogs are particularly important. The data produced by the present study have shown that single diagnostic tests do provide good population-level identification of CKCS at high risk of being echocardiographic stage B2, with multiple breed-specific cut-offs now proposed to optimize sensitivity, specificity, or a combination of both, depending upon the intended goal for a particular client and dog. The individual tests with the greatest discriminatory ability in CKCS in this study were NT-proBNP and VHS. Notably, the CKCS-specific data presented here had better discriminatory ability for predicting echocardiographic stage B2 MMVD than general, all breed data for dogs with MMVD [7], with an NT-proBNP AUC of 0.855 for CKCS compared to 0.77 for non-breed-specific data [7], and a VHS AUC of 0.843 for CKCS compared to 0.76 for non-breed-specific data [7]. The newer VLAS measurement did not perform as well as VHS in CKCS, though the summation of both VHS and VLAS performed similarly to VHS alone. Given the decreased familiarity with the VLAS measurement by general veterinary practitioners, decreased repeatability and reproducibility of VLAS compared to VHS [28], and the increased potential for errors if multiple measurements are combined, these data suggest that focusing solely on the more familiar VHS measurement may be the most clinically useful for CKCS.

On an individual dog level, the data ranges shown in Table 2 highlight the fact that there can

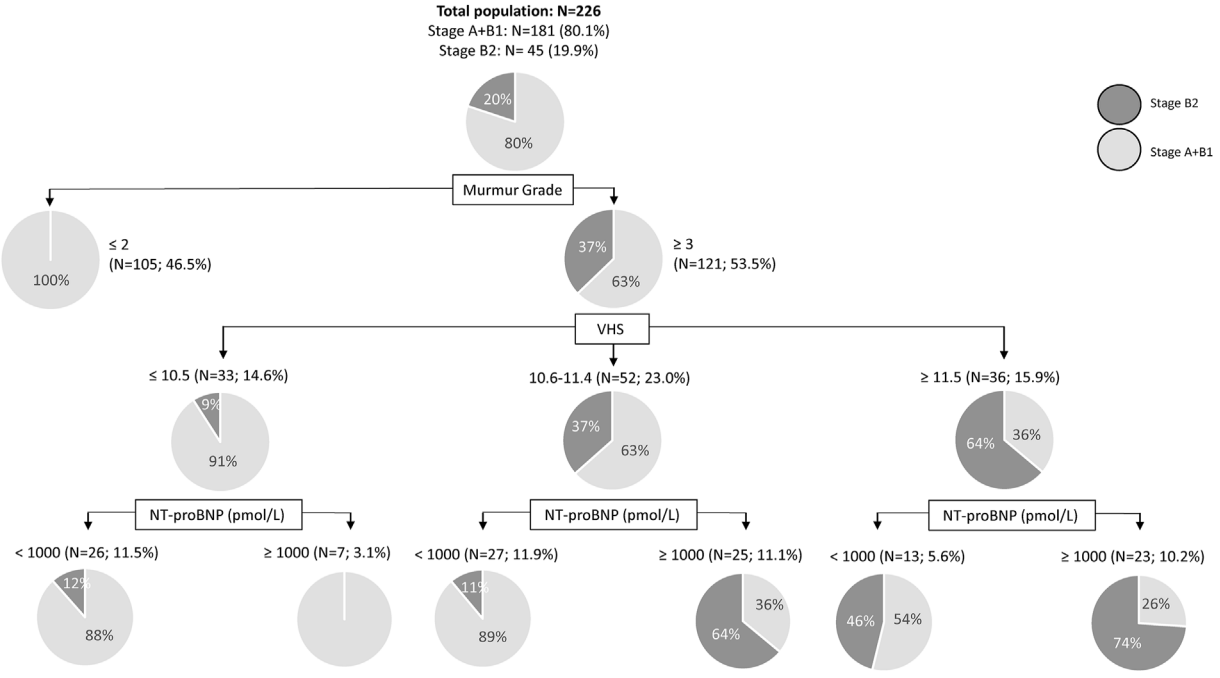

Figure 2 Decision tree algorithm using murmur grade, vertebral heart size, and NT-proBNP to discriminate asymptomatic Cavalier King Charles Spaniels in stages A and B1 combined from those in echocardiographic stage B2. N = number in cell and % = percent of total population in cell. NT-proBNP: N-terminal B-type natriuretic peptide; VHS: vertebral heart size.

be considerable variability amongst CKCS in the same MMVD stage across the investigated diagnostic tests. For example, many CKCS fell below the high specificity stage B2 VHS cut-off of 11.5 but were confirmed to be stage B2 by echocardiography. In addition, there were CKCS that were echocardiographically classified as being in stage A or B1 that met or exceeded this same VHS cut-off of 11.5. These individual cases would have reflected false negative or false positive cases, respectively, if VHS was the sole diagnostic test relied upon, and a cut-off of 11.5 was used for the prediction of stage B2 status. Reliance on any single diagnostic test inevitably requires compromise on the side of sensitivity or specificity when population-based cut-offs are utilized even in a single breed population. Given the known benefits that pimobendan provides for stage B2 dogs [5], correctly identifying these dogs in a timely fashion carries the greatest clinical significance. If single diagnostic tests are used to screen for stage B2 status in CKCS, reliance on the high sensitivity cut-offs provided by these data may be most prudent for identifying the largest number of dogs that could benefit from therapy after confirmatory echocardiography. On the other hand, if echocardiography is not pursued and cut-offs are being relied upon to determine whether to initiate pharmacologic therapy, high specificity cut-offs should be utilized to minimize false positives and erroneous treatment of stage B1 dogs.

When multiple diagnostic tests are combined, as they were for the prediction models in this study, improved discriminatory ability for detecting echocardiographic stage B2 MMVD in CKCS was achieved over any individual diagnostic test alone. The optimal prediction model used information derived from four different 'tests' including PE, biomarkers (NT-proBNP being the only biomarker retained), ECG, and radiographs and produced excellent discriminatory ability with an AUC of 0.971 and the highest sensitivity (76.2%), specificity (95.5%), and % correct classification (91.8%) based on classification table construction. Relatively little discriminatory ability was lost when prediction models were limited to three diagnostic tests, and four out of five models limited to two diagnostic tests still had an AUC exceeding 0.90. Regarding specificity and sensitivity, models with two or three diagnostic tests saw relatively unchanged specificity; however, some models (particularly those lacking PE variables) saw worsened sensitivity. Overall, these findings support the theory that veterinarians can have good confidence in predicting echocardiographic stage B2 status in CKCS using whichever combination of these tests is most readily available and affordable for a particular client. To most confidently rule out echocardiographic stage B2 status, however, the use of all four diagnostic tests is most ideal based on the four-test model having the highest sensitivity. Furthermore, the use of multiple diagnostic tests may help guard against erroneous conclusions from any single test due to inherent limitations or variability that may be associated with that individual test. Lastly, similar to the individual diagnostic tests, the discriminatory ability of these breed-specific prediction models (whether they were two, three, or four test models) outperformed comparable, non-breed-specific models where the AUC was 0.84 [7].

Notably, the prediction model data also highlights the clinical importance of a thorough PE in the setting of MMVD. Murmur grade was one of the most commonly retained predictors in the various prediction models, and AUC was slightly lower in models in which PE findings were not available compared to those in which they were. This makes sense when considering that 91.1% of echocardiographic stage B2 CKCS had a heart murmur intensity of grade 4/6 or louder compared to 25.2% of stage B1 CKCS and 0% of stage A CKCS. It also emphasizes the reality that the most clinically relevant PE distinction is likely between a dog having no heart murmur or a soft heart murmur (i.e. grade 0—3/6) vs. a dog having a loud murmur (grade 4—6/6).

From an ECG standpoint, the novel measurement in which P wave duration and QRS duration were summed provided surprisingly good discriminatory ability as a sole diagnostic test (AUC 0.813) and was frequently retained in prediction models that included ECG variables. The simplicity of this measurement is appealing, particularly in a telemedicine setting in which digital ECG data can easily be measured with a high degree of precision. Additionally, both P wave duration and QRS duration have been shown to be consistent across different recording devices, body positions, and with and without butorphanol sedation which makes these measurements versatile in a clinical setting [29]. The identification of supraventricular premature contractions in CKCS, on the other hand, was consistent across dogs in all stages of MMVD in this study at 26—29%. This suggests that CKCS are predisposed to developing these abnormal beats independent of MMVD stage and the presence or absence of atrial dilation. Lastly, the significance of the average HR obtained from the ECG trace remains unknown, despite its retention in several prediction models. Interestingly, the average HR obtained from the ECG trended down

as disease stage worsened. It is possible that this finding related more to the dog's age and improved tolerance for lateral restraint than to the HR having a specific bearing on MMVD stage itself. Unfortunately, HR was not obtained during the PE and thus could not be compared to the HR obtained during the ECG or investigated in the prediction models.

Retention of SDMA in the best prediction model is also an interesting finding, though its clinical significance is questionable. SDMA is primarily considered to be a renal biomarker and has been validated in dogs as such [30]. Along with creatinine, SDMA is a core component of the current International Renal Interest Society (IRIS) staging system for chronic kidney disease in dogs. While SDMA has been associated with worsened outcomes in humans with acute myocardial infarction and coronary artery disease [31,32], this type of cardiovascular disease is rare in dogs and does not apply to the population at hand. With the exception of one small study that used a non-ACVIM cardiac staging scheme [33], concentrations of SDMA have not been shown to vary across dogs in different MMVD stages [34,35], including one study specifically evaluating preclinical CKCS [36]. This seems to be in alignment with the SDMA concentrations derived in the present study, which appeared essentially identical across stages A, B1, and B2. Prospective validation of these prediction models should help to clarify the significance, or lack thereof, of SDMA in the ability to predict stage B2 status in preclinical CKCS with MMVD.

A final factor to consider when interpreting this data is the relatively recent change in what constitutes stage B1 according to the current ACVIM consensus guidelines for MMVD [6]. According to the 2019 consensus, stage B1 now includes dogs with LA enlargement or LV enlargement as long as both left-sided chambers are not sufficiently enlarged to cross the combined threshold for initiation of treatment, compared to the previous stage B1 in which both LA and LV chamber sizes were required to be normal. This distinction may make the identification of diagnostic test cut-offs more challenging by increasing the heterogeneity of the B1 population. Additionally, it currently remains unclear whether dogs in stage B1 that do have enlargement of one of their left-sided cardiac chambers require increased scrutiny or an earlier reassessment period than stage B1 dogs that lack any evidence of left-sided enlargement at all.

Ultimately, the chief benefit of prediction models such as those presented here is the ability to predict risk on an individual dog level. To utilize the models in this fashion, however, requires a user-friendly interface rather than manually inputting a given dog's data into a complicated regression equation. This work is on-going, with prospective validation of the presented models in progress and an internet-based interface under development. In the meantime, population-based cut-offs can immediately be put to use to improve screening and identification of CKCS with echocardiographic stage B2 MMVD, as can the decision tree algorithm.

There are several limitations of the present study including the known tendency for regression analysis to overfit a dataset. Additionally, there is a less-than-ideal number of events/explanatory variables, and automated selection of explanatory variables increases the chance of type I error, whereby some variables may appear to be significant only by chance. Prospective validation of these data is needed to confirm model performance. Additionally, auscultation, radiographic, and ECG measurements were performed in this study by operators with advanced training in cardiology. It is possible that model performance could be diminished by input from less experienced operators. Because of this possibility, we felt that all CKCS (both with and without a murmur) should be included in the main models, as dogs can be mistakenly identified as having a heart murmur when they do not, and vice versa. Additionally, NT-proBNP is known to have high individual variability in dogs, with the potential for spurious, false positive results to occur on occasion [37,38]. This should be taken into account if NT-proBNP is relied upon as a sole diagnostic tool. Finally, a digital ECG system is needed to obtain the level of precision required to utilize the P + QRS duration cut-offs presented in this dataset. Taken together, these limitations highlight the benefit of relying on multitest models rather than single diagnostic tests, if possible, to diminish the likelihood that one erroneous test result leads to misclassification of an individual dog's disease probability.

Conclusions

In conclusion, NT-proBNP and VHS are the best individual diagnostic tests to discriminate preclinical CKCS in stages A and B1 from those in echocardiographic stage B2 with various breed-specific cut-offs having now been proposed. Prediction models incorporating variables from multiple tests have improved discriminatory ability compared to any individual test, with NT-proBNP,

VHS, murmur grade, and P + QRS duration being the most useful predictors in each test category.

Conflicts of Interest Statement

Drs. Gordon, Saunders, Fries, Vitt, Boutet, and Kadotani have received funding from Boehringer Ingelheim Animal Health GmbH within the last five years for some or all of the following activities: research, travel, speaking fees, consultancy fees, and preparation of educational materials. Drs. Gordon, Wesselowski and Saunders have received funding from IDEXX laboratories within the last five years for some or all of the following activities: research, travel, speaking fees, consultancy fees, and preparation of educational materials.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi https://doi.org/10.1016/j.jvc.2023.10.001.

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