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Long-term Outcome and Troponin I Concentrations in Great Danes Screened for Dilated Cardiomyopathy: An Observational Retrospective Epidemiological Study

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Abstract:	Introduction/Objectives: Dilated cardiomyopathy (DCM) is common in Great Danes (GD) but screening for this condition can be challenging. We hypothesised that cardiac troponin-I (cTnI) concentration is elevated in GD with DCM and/or ventricular arrhythmias (VA), and is associated with reduced survival time in GD.Animals: One hundred and twenty-four client-owned GD, assigned echocardiographically as normal (n= 53), equivocal (n= 37), preclinical DCM (DCM-P) (n= 21) or clinical DCM (DCM-C) (n= 13).Methods: Retrospective epidemiological study. Echocardiographic diagnosis, VA and contemporaneous cTnI concentrations were recorded. Diagnostic accuracy and cTnI cut-offs were determined with receiver operating characteristic analyses. Effects of cTnI concentration and disease status on survival and cause of death were explored.Results: Median cTnI was greater in DCM-C (0.6 ng/mL [25th-75th percentiles: 0.41-1.71 ng/mL]) and GD with VA (0.5 ng/mL [0.27- 0.80 ng/mL], P < 0.001). Elevated cTnI detected these dogs with good accuracy (Area under the curve [AUC]: 0.78 – 0.85; cut-offs 0.199 - 0.34 ng/mL). Thirty-eight GD (30.6%) suffered a cardiac death (CD); GD suffering CD (0.25 ng/mL [0.21- 0.53 ng/mL]) and specifically sudden cardiac death (SCD) (0.51 ng/mL [0.23- 0.72 ng/mL]) had higher cTnI than GD dying of other causes (0.20 ng/mL [0.14- 0.35 ng/mL]; P < 0.001). Elevated cTnI (>0.199 ng/mL) was associated with shorter long-term survival (1.25 years) and increased risk of SCD. Great Danes with VA had shorter survival times (0.97 years).Conclusions: Cardiac troponin-I concentration is a useful adjunctive screening tool. Elevated cTnI is a negative prognostic indicator.
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Editors-in-Chief

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Dear Editors,

Thank you for your conditional acceptance of our manuscript. We believe we have addressed all the points in your email, response from reviewers and annotated PDF document. Please let us know if we need to do anything further.

Please accept my apologies for the late submission of this final revision, which has been due to me being on annual leave and then contracting Covid.

Yours sincerely,

Hannah Stephenson

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Running head: Troponin I as a prognostic indicator for Great Danes.

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Key words: Echocardiography; Equivocal; Cardiac Biomarkers; Survival; Sudden cardiac death.

TABLE OF ABBREVIATIONS

AF	atrial fibrillation
AUC	area under the curve
CD	cardiac death
CI	confidence intervals
cTnI	cardiac troponin I (Immulite assay)
DCM	dilated cardiomyopathy
DCM-C	clinical dilated cardiomyopathy
DCM-P	preclinical dilated cardiomyopathy
ECHO	echocardiography
ECG	electrocardiography
EQUIV	equivocal
EU-CD	euthanasia due to cardiac causes.
EU-NC	euthanasia due to non-cardiac causes.
GD	Great Danes
NC	non-cardiac
NORM	normal
SCD	sudden cardiac death
T_{cTnI}	time-to-death after last cardiac Troponin I
VA	ventricular arrhythmias

VPC	ventricular premature complex

INTRODUCTION

Dilated cardiomyopathy (DCM) is the second most common cardiac disease in dogs [1], causing significant morbidity and mortality in large to giant breed dogs [1-5]. The reported prevalence of DCM in great Danes (GD) in prospective screening studies ranged from 11.8 to 35.6% [5, 6]. Dilated cardiomyopathy is usually an adult-onset acquired disease and often has a prolonged preclinical phase, where dogs show no clinical signs of cardiac disease. This provides a particular problem in breeding populations, where dogs may develop acquired disease after producing progeny.

Once the disease becomes apparent, GD are expected to have a lower median survival time than the other breeds [2]. In addition, unreported sudden cardiac deaths (SCD) in GD were found to occur in a large proportion of sires and dams at early ages [5]. Despite the known high prevalence in the breed, data describing longitudinal progression by serial examinations in GD is lacking [5, 6]. In addition, long-term follow-up of GD screened for DCM has not been described, or examined for any associations with echocardiographic, other clinical criteria, or clinical pathology data.

Primary DCM is suspected to be hereditary and has been found to be associated with the presence of genetic loci in a number of breeds [1]. A genome-wide study on GD and Newfoundland dogs identified the association between DCM and the presence of different genetic loci indicating different genetic risk factors [7], but currently, there is no specific genetic marker for DCM in GD which might identify dogs at risk of developing DCM later in life.

In the absence of an accurate genetic test, early monitoring of GD and other breeds with familial DCM has been advocated using echocardiographic and electrocardiographic (ECG) screening to detect the asymptomatic stage [4, 8]. Early detection of preclinical DCM by echocardiography (ECHO) may provide an opportunity to initiate medical therapy to delay the onset of congestive heart failure, in addition to

excluding the affected dogs from breeding programs [2-4, 8]. In other breeds with a high risk of SCD, Holter monitoring is also a recommended screening tool for ventricular arrhythmias (VA) [4].

Cardiac troponin I (cTnI) is a specific marker of recent or ongoing myocardial injury [9, 10]. It is an intracellular protein, so it is not usually released into the circulation in high concentrations [10]. Concentrations of cTnI have been found to increase with myocardial injury in non-cardiac diseases [11-15] as well as in canine mitral valve disease [16, 17] and DCM [18]. Shorter survival times with increased cTnI concentrations were also found in dogs with DCM and other acquired cardiac diseases [16, 19, 20]. Large studies investigating the diagnostic utility of cTnI in the GD are lacking. One small study has previously reported that cardiac biomarkers, including high sensitivity cTnI, cannot be used as a standalone test for DCM in the breed [21]. Despite the high prevalence of DCM in GD and a suggested increased risk of SCD, little is known about disease outcomes or causes of death in this breed [5, 22].

We hypothesised that cTnI would be significantly higher in GD with DCM and those with VA, than those without, and that cTnI levels would be negatively associated with survival time. The objectives of this observational retrospective epidemiological study were: (i) to investigate the ability of the cTnI to predict echocardiographic evidence of DCM or presence of VA in GD (ii) to describe causes of death in GD which had been screened for DCM at least once and (iii) to explore any association between cTnI concentration at the time of screening and time to death.

ANIMALS, MATERIALS, AND METHODS

Study population

Medical records were retrieved for all GD that had cardiac examinations at the Small Animal Teaching Hospital, University of Liverpool including ECHO performed between

July 2008 and 2018. To be eligible for inclusion, ECHO reports and cTnI [e] concentration at the time of the last scoring had to be available (subsequent examinations were performed in some GD). As well as GD presenting for DCM screening, GD presenting as clinical cases to the cardiology service were also eligible for inclusion. GD eligible for screening were ≥ four years old, but clinical cases could be of any age. The DCM screening population included GD owned by clients and breeders across the United Kingdom. Clinical cases were referred by a primary veterinarian for investigations of clinical signs such as a murmur, arrhythmia, or presenting problems such as cough, exercise intolerance, syncope, etc.

Great Danes were not eligible for inclusion if a different (e.g. high sensitivity) cTnI assay was used, or there was no cTnI result available. Great Danes with other primary acquired or congenital heart diseases and significant systemic diseases were excluded. Signalment data were retrieved including sex, neuter status, age at the time of the assessment, and body weight.

The study was originally approved by the institutional Committee on Research Ethics and the LUPA consortium ethics work-package. Further ethical approval was gained from the institutional Veterinary ethics research committee prior to seeking follow-up information about each GD from primary veterinarians or owners (VREC 916 & VREC 917); this was sought between March and July 2020 with an owner questionnaire or a telephone conversation. If the dog was deceased, the date of death, veterinarian or owner-reported cause of death was recorded and whether the death was sudden, natural but following signs of illness or by euthanasia was documented.

Cardiovascular examination

The reason for presentation and physical examination findings were reviewed. All included GD had a health assessment including complete blood count and

biochemistry. Some, but not all dogs had a total thyroxine concentration measured. Concentration of cTnI was measured from heparinised plasma using an Immulite assay [23].

Echocardiographic examinations [f] were conducted in unsedated GDs lying in right and left lateral recumbency on an ECHO table with simultaneous ECG monitoring as previously described [5]. Images were stored as cine-loops of three cardiac cycles for later offline analyses [g]. M-mode derived left ventricular internal dimensions obtained from short-axis views at level of chordae tendinae, in diastole and systole were indexed to body weight (normalized left ventricular internal dimension at end diastole and normalized left ventricular internal dimension at end systole) by allometric scaling [24] and fractional shortening was calculated. From right parasternal long-axis views which optimised the left ventricular length and area, and included the left ventricular apex, Simpson's method of discs was used to estimate the left ventricular end-diastolic and end-systolic volumes. A standard formula was used to calculate body surface area from body weight automatically [25]. Left ventricular volumes indexed to body surface area (end diastolic volume index and end systolic volume index) were calculated. The ejection fraction was calculated (ventricular end-diastolic volume - end-systolic volume/ end-diastolic volume) and expressed as a percentage. The sphericity index was calculated from the maximum left ventricular length during end- diastole indexed to left ventricular internal dimension at end diastole.

Based on the ECHO results and information from clinical records, GD were assigned into four groups according to previously published ECHO parameters [5, 21]; apparently healthy at the time examination (NORM), equivocal (EQUIV), preclinical DCM (DCM-P), and clinical DCM (DCM-C). For dogs with multiple ECHO

examinations, the data and score from the most recent examination with contemporaneous Immulite cTnI concentration were used in the analysis.

In addition to ECG monitoring during ECHO, some but not all GD had a standard six-lead ECG and/or 24-hour ambulatory ECG examination (Holter), at the clinician's discretion. If the results of ECG were recorded in the notes, GD were classified as suffering from VA or not. If only an in-house ECG had been recorded, at least one ventricular premature complex (VPC) during the examination was interpreted as suffering from VA. If a Holter had been performed, then >100 VPC/24 h was also considered as suffering from VA [26]. We also included GD with >20 VPC/24 h plus evidence of complexity (presence of couplets, triplets and/or ventricular tachycardia) in the same group. Any other arrhythmia noted during these examinations were recorded.

Laboratory analysis

For comparability and accuracy, all heparinised samples submitted for cTnI analysis used the same analyser[e] [23] on the same day as the echocardiographic assessment. Concentrations of cTnI <0.15 ng/mL were considered normal [7, 27]. The limit of detection for the analyser was changed from 0.2 ng/mL to 0 to enable recording of low concentrations of cTnI, while appreciating that the precision of the assay at low concentrations is less satisfactory [23]. In animals that were re-presented to the cardiology service for follow-up, only the final cTnI concentrations obtained contemporaneously with ECHO were used for this study.

Classification of death (survival data)

Survival (follow-up outcome) data and causes of death were retrieved from the medical records or after seeking information from referring veterinarians or the owners of each GD. Classification of the death causes was based on the judgement of the attending

veterinarian and by personal communication with owners. Only a small minority of GD underwent post-mortem examination.

In deceased GD, the death was then classified as either cardiac or non-cardiac, and further subclassified into a total of five categories. Deaths were classified as SCD when deaths were unexpected and not preceded by any other clinical signs such as dyspnoea. If GD were diagnosed with DCM prior to death and had evidence of congestive heart failure signs, the death was classified as a natural cardiac death (CD), or if the patient was euthanised, as euthanasia due to cardiac disease (EU-CD). If the death was associated with signs of other systemic conditions, with no evidence of congestive heart failure signs, the death was considered non-cardiac either due to natural causes (NC) or euthanasia (EU-NC). Animals that were alive at the end of the study were noted and censored. If information could not be retrieved after attempts to contact the owner or primary veterinarian, these were lost to follow-up and also censored from the time of last contact.

Statistical analyses

One-way analysis of variance tests (ANOVA) were used to compare body weight, age at the most recent admission, cTnI concentrations, and ECHO parameters' values among ECHO groups, survival status, and causes of death. Levene's test was used to evaluate the homogeneity of variances (homoscedasticity), and the Shapiro–Wilk test was utilized for normality analysis of the variables. The data that were not normally distributed or violated homogeneity of variance assumption were analysed with the Kruskal-Wallis test. The Tukey's Studentized Range (HSD) test was used for post-hoc analysis. If two groups were compared, the Mann-Whitney U test was used for non-parametric analysis. All P values less than 0.05 were considered statistically significant [h].

Receiver operating characteristic curve analyses were performed to determine optimal cutoff values of cTnI for different disease states (ECHO group or VA) [i]. The optimal cTnI cutoff points for each variable were determined by the point of maximum Youden's index (Youden's J statistic).

Kaplan-Meier survival analyses were performed for estimation of median survival times or time-to-death or follow-up time after last cTnI (T_{cTnI}) and survival probabilities of GD after the last cTnI test on the basis of cTnI concentration and presence of VA. Log-rank tests were used to compare the survival probability among ECHO groups, causes of death, and presence of VA. To determine the sole effect of cTnI concentration on the T_{cTnI} of GD, a univariate (single outcome variable) simple (one predictor variable) Cox proportional hazard regression model was used.

Finally, binary logistic regression was used to explore the associations between the presence of VA and SCD, and the concentration of cTnI and probability of suffering SCD.

RESULTS

Data for 134 GD were retrieved, and 10 dogs were excluded from the analysis either due to significant cardiac or systemic illnesses (cardiac causes: left ventricular hypertrophy, pulmonic stenosis and mitral dysplasia; and non-cardiac causes: systemic hypertension, idiopathic chylothorax and neurological diseases). The study population therefore consisted of 124 dogs and demographic and echocardiographic data for these dogs is presented in supplementary Table A online.

There were 53 NORM dogs, 37 EQUIV dogs, 21 DCM-P dogs, and 13 DCM-C dogs. There were no differences in age or body weight between groups. As expected, left ventricular dimensions and volumes were higher in DCM-P and DCM-C dogs than

NORM and EQUIV dogs, whereas the fractional shortening and the sphericity index were significantly lower in DCM-P and DCM-C dogs ($P < 0.001$).

The results of ECG assessment were recorded in 114 GD. Of these GD, 83 had undergone Holter analysis, with the rest having only an in-house ECG recording. Thirty-six GD (31.5%) were classified as affected by VA. Eight GD had atrial fibrillation (AF), with AF cases in each echo group (these data are available in Supplementary Table B online). Other arrhythmias included supraventricular premature complexes, non-sustained supraventricular tachycardia and first degree atrioventricular block.

Eighty-six (69%) GD had cTnI above the reference interval of 0.15 ng/mL. Cardiac troponin I level was significantly higher in DCM-C dogs than those in other groups ($P < 0.001$), while there was no significant difference in cTnI between NORM, EQUIV, and DCM-P dogs (Table 1; Fig.1A). Median cTnI levels were also significantly higher in dogs with VA (0.50 ng/mL [25^{th}–75^{th} percentiles: 0.27–0.80 ng/mL]) than those who had no VA (0.19 ng/mL [0.09–0.30 ng/mL]; $P < 0.001$; Fig. 1B).

Receiver operator characteristic curve analysis showed that in general, cTnI performed with good accuracy to differentiate different ECHO groups, performing best to differentiate NORM from DCM-P (Area under the curve [AUC] 0.78; 95% confidence interval [CI]: 0.68–0.89; $P < 0.001$) and NORM from DCM-C dogs (AUC 0.85; 95% CI: 0.73–0.97; $P < 0.001$). When the groups were combined, cTnI was able to differentiate between all GD with DCM (DCM-C + DCM-P) from all GD without DCM (NORM + EQUIV) with good accuracy (AUC 0.80; 95% CI: 0.72–0.88; $P < 0.001$). A cTnI concentration of 0.199 ng/mL yielded the highest Youden index (146.1) at a sensitivity of 88.2% and a specificity of 58.9% (Fig. 2A).

Cardiac troponin I also differentiated dogs with VA on ECG from those without documented VA with good accuracy (AUC 0.82; 95% CI: 0.74–0.91; $P < 0.001$). A cTnI

concentration of 0.34 ng/mL yielded the highest Youden index (151.78) at a sensitivity of 69% and a specificity of 83% (Fig. 2B).

At the time of writing, eight GD were lost to follow up, seven GD were alive and 109 GD had died (Table 1). Thirty-eight GD (30.6%) were determined to have died due to cardiac disease (SCD, CD, and EU-CD). No GD with DCM were alive at the time of analysis. Sudden cardiac death was common, with 53% of cardiac deaths being SCD. Nineteen GD that had been classified as NORM or EQUIV on exam ultimately suffered a cardiac death, with 12 (63%) of these being SCD.

Great Danes which died or were euthanised as a consequence of cardiac disease had significantly higher cTnI concentration (0.44 ng/mL [25^{th}–75^{th} percentiles: 0.19–0.69 ng/mL]; *n* = 38) than those dogs that died from or were euthanised due to non-cardiac causes (0.20 ng/mL [25^{th}–75^{th} percentiles: 0.14–0.35 ng/mL]; *n* = 71; P < 0.001; Table 1). In addition, cTnI was also significantly higher in all GD suffering SCD (0.51 ng/mL [25^{th}–75^{th} percentiles: 0.23–0.72 ng/mL]; *n* = 20) than those dying from or euthanised for non-cardiac causes (P < 0.001; Table 1; Fig.3A). Considering only NORM/EQUIV dogs, median cTnI concentration of GD suffering SCD (0.33 ng/mL [25^{th}–75^{th} percentiles: 0.20–0.52 ng/mL]; *n* = 12) was significantly higher (P = 0.026) than that of GDs dying of non-cardiac causes (0.20 ng/mL [25^{th}–75^{th} percentiles: 0.10–0.31 ng/mL]; *n* = 57; Fig. 3B).

Median survival times for dogs in this study are summarised in table 2. Median T_{cTnI} was significantly different among ECHO groups (P < 0.001). Kaplan-Meier survival analysis showed that GD with cTnI level lower than 0.199 ng/mL had significantly longer T_{cTnI} (3.15 years [95% CI: 2.26–3.29 years]; *n* = 57; P = 0.001) than GD with cTnI level higher than this cutoff (1.25 years [95% CI: 1.37–2.23 years]; *n* = 67; Fig. 4A). In addition, GD without VA had significantly longer T_{cTnI} (2.44 years; 25^{th}–75^{th}

percentiles: 0.76–3.94 years; *n* = 78; P = 0.002) than those GD exhibiting VA (0.97 years [25th–75th percentiles: 0.36–3.06 years]; *n* = 36; Fig. 4B).

Cox proportional hazards analysis showed a significant effect of cTnI concentration on survival time, with each 0.1 ng/mL increase in cTnI, the hazard rate increased by 14% (95% CI: 9.7–18.6%; Wald $\chi^2_{(1)} = 43.07$, P < 0.001). No association was identified between presence of VA and SCD (Logistic regression: $\chi^2_{(1)} = 2.85$, P = 0.091); 95% CI for the odds ratio = 0.16–1.15. There was a significant positive relationship between cTnI level and probability of suffering SCD (Logistic regression: $\chi^2_{(1)} = 0.93$; P = 0.032; Fig. 5). The following equation developed from binary logistic regression analysis can be used to estimate probability of SCD at any cTnI level (within the studied range [0 to 3.27 ng/mL]):

$$Probability\ of\ SCD = \frac{exp^{[-1.98 + (0.9315*cTnI)]}}{1 + exp^{[-1.98 + (0.9315*cTnI)]}}$$

DISCUSSION

In this population of GD, cTnI was a useful tool to identify GD with DCM, and also to detect GD with VA. Higher concentrations of cTnI were also associated with shorter survival times, and dogs suffering SCD had significantly higher concentrations of cTnI.

Owners and breeders of GD may wish to screen their dogs for DCM to try and reduce the risk of transmission to progeny, and/or to initiate treatment in the preclinical stages of the disease. Screening for DCM in GD usually includes regular ECHO and potentially Holter monitoring, which can be expensive for the owners of these dogs. In addition, accurate echocardiographic diagnosis of DCM is dependent on operator experience, which limits the availability and usefulness of this test.

This study shows that cTnI is moderately accurate in determining if a GD has echocardiographic evidence of DCM. A cTnI concentration of 0.199 ng/mL detected dogs with DCM with a sensitivity of 88.2% and a specificity of 58.9%. At the currently cited reference range of <0.15 ng/mL for this Immulite assay, it showed 97% sensitivity and 42% specificity. Great Danes with VA in our study also had higher levels of cTnI. A cTnI concentration of 0.34 ng/mL detected dogs with VA with a sensitivity of 69% and a specificity of 83%. At the currently cited reference range of <0.15 ng/mL for this Immulite assay, it showed 97% sensitivity and 36% specificity. Similar findings were reported in a study of cTnI in Dobermanns [18].

Sixty-nine percent of GD in this study had elevated cTnI based on the reference interval for the assay used (0.15 ng/mL). Elevated cTnI has been reported in dogs with systemic disease [15, 28], azotaemia [15], gastric dilatation-volvulus [13], snake-bite envenomation [14], Ehrlichiosis [12] and pyometra [11]. We excluded any dog identified as having systemic disease at the time of examination, but we cannot completely exclude that some patients were suffering from other conditions that might affect their cTnI concentration. It is also possible that there is some breed variation in circulating cTnI levels, as suggested in other breeds [29, 30] and indeed a higher normal cTnI level has been reported for this breed in a previous study albeit using a different assay [21]. Alternatively, some of these GD may have had early myocardial disease that was not detectable on ECHO; this has been reported in Dobermanns, where incipient DCM was identified by cTnI assays [18, 28].

Despite the low specificity of cTnI for detection of DCM and/or VA, an increased value identifies a GD which benefits from a full veterinary health assessment. We propose that cTnI has the potential for use as a first-line screening test in apparently healthy GD, allowing owners to prioritise those GD for full screening.

Sudden cardiac death is common in dogs with DCM, particularly in Dobermanns, where SCD is associated with increased heart size but also the presence of malignant VA [31]. In GD, sudden death is also reported in populations affected by DCM [5]. Early identification of affected dogs might allow anti-arrhythmic treatment to be initiated and prevent SCD in some cases [32].

Sudden death was common in our population of GD, with 16% of all GD suffering SCD, accounting for 53% of all cardiac deaths. Interestingly, some dogs determined as NORM or EQUIV by ECHO also suffered cardiac deaths, with 63% of these being SCD. This is in keeping with the natural history of DCM in other breeds such as Dobermanns, where SCD can occur prior to any echocardiographic changes [31].

Importantly, GD suffering SCD had higher concentrations of cTnI in this study. Even GD without DCM on ECHO (NORM+EQUIV) that suffered later SCD had higher cTnI levels than other GD in these ECHO groups. Probability of SCD was shown to increase with increasing cTnI concentration. These findings are similar to reported increases in cTnI in Dobermanns suffering SCD [31]. Great Danes with VA identified on ECG also had shorter survival times.

Sudden cardiac death in people is commonly associated with VA resulting in cardiac arrest [33]. We speculated that there might be an association between presence of VA and SCD in our population of GD, but no statistically significant association was identified. Significant VA have been documented in dogs with high levels of cTnI associated with other diseases such as envenomation [14] illustrating the importance of excluding other potential causes. We may have incorrectly classified some GD as not all dogs had Holter monitors fitted, and there is known to be day-to-day variation in VA on Holter.

Although our study does not conclusively demonstrate that VA are the cause of SCD in GD, this study confirms the importance of Holter screening of GD, regardless of ECHO findings, to identify GD which may require antiarrhythmic medication and to identify adverse prognostic indicators.

Atrial fibrillation is reported to be one of the most common arrhythmias in the GD population [5], however, it was detected in only eight GD in this study. Interestingly, NORM dogs with AF had normal cTnI levels, whereas the EQUIV dog with AF had elevated cTnI. The influence of the presence of AF on cTnI values could not be established due to the small numbers affected. Future studies would be useful to investigate any correlation.

This study has demonstrated the prognostic significance of cTnI in this population of GD, with higher levels of cTnI increasing the likelihood of death of any cause, and also increasing risk of SCD. This is in keeping with other studies investigating levels of cTnI in cardiac [16, 33] and non-cardiac disease [34]. Higher cTnI also showed higher probability for fatal outcomes in our GD population with VA.

The retrospective nature of this study results in some limitations. Many of the GD in this study were not examined on multiple occasions, which may have resulted in incorrect classification of dogs with early myocardial disease. Due to low numbers of repeat examinations, and a change at our center to the use of a high sensitivity cTnI assay, only one time point was used for analysis of cTnI in these dogs, and therefore the significance of changes in cTnI could not be evaluated. Further studies would be useful in assessing the prognostic significance of changes in cTnI over time.

This study did not utilise high sensitivity troponin assays, as this was not widely available early in the study period. High sensitivity assays have been shown to be more accurate in detection of DCM with or without VA in dogs, with improved sensitivity

particularly in the early stages of the disease [28]. Use of a high sensitivity assay may therefore have improved early detection of DCM-P dogs or dogs with VA. Further studies into the utility of high sensitivity assays and Holter monitoring in GD are warranted.

Classification of GD with VA was challenging. Although >100 VPC/24 h is generally considered to be abnormal in dogs [26], the complexity of the arrhythmia is also considered important [4, 35]. Our data suggests that most normal GD have <20 VPC/24 hours (unpublished data) and therefore we also considered GD with >20 VPC/24 hour, plus evidence of complexity, as affected by VA.

Holter monitoring was not performed in all animals, and therefore this may have led to incorrect classification of dogs as either normal or affected by VA. For example, a single VPC detected during ECHO might be associated with increased circulating catecholamines. Nevertheless, detection of at least one VPC on a five-minute ECG has been shown to have a high positive predictive value for Holter abnormalities in Dobermanns [36].

Conclusions

The current data suggest that cTnI could be a useful adjunctive screening test for DCM and VA in GD. Great Danes with DCM and/or VA have higher concentrations of cTnI. Circulating levels of cTnI have prognostic significance in this population, with higher levels seen in patients later suffering SCD.

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Table 1. Overall number (*N*), number (*n*) and percentage (of N) of GD for each survival status within each ECHO group. Median and 25th–75th percentiles of cTnI concentrations (ng/mL) are reported for each group.

Status	Cause		Median (25th–75th percentiles) of cTnI concentration (ng/mL)				
			ALL GD	ECHO group			
				Normal	EQUIV	DCM-P	DCM-C
Overall		Total *N*	124	53	37	21	13
		cTnI	0.21 (0.13–0.44)	0.18[B] (0.08–0.29)	0.19[B] (0.12–0.34)	0.39[B] (0.25–0.96)	0.60[A] (0.41–1.71)
LTFU		*n* (%)	8 (6.5)	6 (11.3)	1 (2.7)	0 (0.0)	1 (7.7)
		cTnI	0.11[b] (0.03–0.18)	0.06 (0.02–0.15)	0.15	-	0.22
Alive		*n* (%)	7 (5.6)	6 (11.3)	1 (2.7)	0 (0.0)	0 (0.0)
		cTnI	0.10[b] (0.05–0.12)	0.07 (0.05–0.10)	0.12	-	-
Dead		*n* (%)	109 (87.9)	41 (77.4)	35 (94.6)	21 (100.0)	12 (92.3)
		cTnI	0.25 (0.15–0.45)	0.20 (0.09–0.32)	0.19 (0.10–0.34)	0.39 (0.25–0.69)	0.63 (0.47–2.06)
Card-iac	All	*n* (%)	38 (30.6)	8 (19.5)	11 (31.4)	10 (47.6)	9 (75.0)
		cTnI	0.44 (0.19–0.69)	0.28 (0.14–0.44)	0.21 (0.17–0.58)	0.69 (0.39–0.80)	0.60 (0.53–2.41)
	SCD	*n* (%)	20 (16.1)	5 (12.2)	7 (20.0)	6 (28.6)	2 (16.7)
		cTnI	0.51[a] (0.23–0.72)	0.32 (0.25–0.43)	0.34 (0.18–0.68)	0.77 (0.67–0.90)	1.91 (0.56–3.27)
	CD	*n* (%)	5 (4.0)	0 (0.0)	1 (2.9)	1 (4.8)	3 (25.0)
		cTnI	0.25[ab] (0.21–0.53)	-	0.21	0.25	0.53 (0.18–2.86)
	EU-CD	*n* (%)	13 (10.5)	3 (7.3)	3 (8.6)	3 (14.3)	4 (33.3)
		cTnI	0.39[ab] (0.13–0.66)	0.09 (0.09–0.45)	0.17 (0.07–0.19)	0.69 (0.39–0.69)	0.63 (0.37–1.54)

	All	*n* (%)	71 (57.3)	33 (80.5)	24 (68.6)	11 (52.4)	3 (25.0)
		cTnI	0.20 (0.14–0.35)	0.20 (0.09–0.26)	0.18 (0.10–0.33)	0.25 (0.20–0.41)	0.81 (0.41–1.71)
Non-cardiac	NC	*n* (%)	21 (16.9)	9 (22.0)	6 (17.1)	4 (19.0)	2 (16.7)
		cTnI	0.20[b] (0.14–0.29)	0.20 (0.14–0.20)	0.12 (0.07–0.19)	0.25 (0.20–0.28)	0.61 (0.41–0.81)
	EU-NC	*n* (%)	50 (40.3)	24 (58.5)	18 (51.4)	7 (33.3)	1 (8.3)
		cTnI	0.22[b] (0.14–0.40)	0.20 (0.09–0.35)	0.25 (0.15–0.35)	0.28 (0.20–0.44)	1.71

For any cell where *n* = 1, actual values were reported instead of descriptive statistics. In the first row, representing all dogs in the study, different uppercase superscript letters indicate significant differences in median cTnI between ECHO groups ($\chi^2_{(3)} = 28.21$, $P < 0.001$). In the first column, representing the total dogs in each survival status, different lowercase superscript letters indicate significant differences in median cTnI between groups ($\chi^2_{(6)} = 14.12$, $P = 0.029$).
CD: cardiac death; cTnI: cardiac troponin I; DCM-C: clinical DCM; DCM-P: preclinical DCM; ECHO: echocardiography; EQUIV: equivocal; EU-CD: euthanasia due to cardiac causes; EU-NC: euthanasia due to non-cardiac causes; GD: Great Dane; NC: non-cardiac death; NORM: normal; LTFU: lost to follow up; SCD: sudden cardiac death.

Table 2. Overall number (*N*), number (*n*) and percentage (of N) of GD for each survival status within each ECHO group. Median (interquartile range; minimum – maximum) of time-to-death (or follow-up) after last cTnI analysis (years) depending on survival status, for all dogs and each ECHO group.

Status	Cause		Time-to-death (or follow-up) after last cTnI analysis (years)				
			All GDs	ECHO group			
				Normal	EQUIV	DCM-P	DCM-C
Overall		Total *N*	124	53	37	21	13
			1.97 (3.26)	2.65 (3.27)	3.01 (2.65)	1.45 (2.10)	0.22 (0.41)
			(0.00–7.27)	(0.00–7.27)	(0.00–6.73)	(0.00–4.81)	(0.00–3.91)
LTFU[a]		*n* (%)	8 (6.5)	6 (11.3)	1 (2.7)	0 (0.0)	1 (7.7)
			0.00 (1.88)	0.00 (0.00)	4.75[b]	-	0.00[b]
			(0.00–4.75)	(0.00–3.75)	-	-	-
Alive		*n* (%)	7 (5.6)	6 (11.3)	1 (2.7)	0 (0.0)	0 (0.0)
			3.48 (2.28)	3.46 (2.28)	3.70[b]	-	-
			(2.18–5.73)	(2.18–5.73)	-	-	-
Dead		*n* (%)	109 (87.9)	41 (77.4)	35 (94.6)	21 (100.0)	12 (92.3)
			1.84 (3.18)	2.77 (2.96)	2.32 (2.69)	1.45 (2.10)	0.23 (0.53)
			(0.00–7.27)	(0.00–7.27)	(0.00–6.73)	(0.00–4.81)	(0.00–3.91)
Cardiac	SCD	*n* (%)	20 (16.1)	5 (12.2)	7 (20.0)	6 (28.6)	2 (16.7)
			1.11 (2.71)	2.77 (2.63)	1.32 (3.04)	0.61 (1.67)	0.59 (0.33)
			(0.00–6.73)	(0.00–5.29)	(0.44–6.73)	(0.05–2.18)	(0.42–0.75)
	CD	*n* (%)	5 (4.0)	0 (0.0)	1 (2.9)	1 (4.8)	3 (25.0)
			0.10 (0.38)	-	3.46[b]	0.00[b]	0.10 (0.38)
			(0.00–3.46)	-	-	-	(0.01–0.39)
	EU-CD	*n* (%)	13 (10.5)	3 (7.3)	3 (8.6)	3 (14.3)	4 (33.3)

			1.75 (2.40)	1.99 (3.27)	3.16 (2.69)	1.75 (1.48)	0.20 (1.99)
			(0.00–4.03)	(0.76–4.03)	(0.94–3.63)	(0.78–2.26)	(0.00–3.91)
Non-cardiac	NC	*n* (%)	21 (16.9)	9 (22.0)	6 (17.1)	4 (19.0)	2 (16.7)
			2.99 (2.58)	4.00 (2.90)	2.28 (2.39)	2.77 (1.52)	0.73 (1.02)
			(0.05–6.95)	(0.05–6.95)	(0.30–3.71)	(0.50–3.10)	(0.22–1.24)
	EU-NC	*n* (%)	50 (40.3)	24 (58.5)	18 (51.4)	7 (33.3)	1 (8.3)
			1.98 (3.09)	2.18 (2.95)	2.16 (2.87)	1.45 (3.67)	0.00[b]
			(0.00–7.27)	(0.00–7.27)	(0.00–5.29)	(0.15–4.81)	-

[a] Follow-up times of zero in LTFU status indicate that we were not able to follow-up the survival status of those dogs since the date of last cTnI analysis. [b]Actual values were reported instead of descriptive statistics (*n* = 1). CD: cardiac death; cTnI: cardiac troponin I; DCM-C: clinical DCM; DCM-P: preclinical DCM; ECHO: echocardiography; EQUIV: equivocal; EU-CD: euthanasia due to cardiac causes; EU-NC: euthanasia due to non-cardiac causes; GD: Great Dane; NC: non-cardiac death; NORM: normal; LTFU: lost to follow up; SCD: sudden cardiac death.

Figure captions

Figure 1. Box-and-whisker plot showing the concentration of cTnI in [A] GD in each of the four echocardiographic groups and [B] in GD with/without VA (all ECHO groups). Within each box plot, horizontal line indicates the median, black circle indicates the mean, and error bars around the circle represent standard error of the mean. Different superscripts indicate statistically significant differences between groups.

cTnI: cardiac troponin I; DCM-C: clinical DCM; DCM-P: preclinical DCM; ECHO: echocardiorgaphy; EQUIV: equivocal; GD: Great Danes; NORM: normal; VA: Ventricular arrhythmias.

Figure 2. Receiver operator characteristic curves demonstrating diagnostic performance of cTnI to differentiate between GD both (A) with echocardiographic changes associated with DCM (DCM-P+ DCM-C) and those without DCM (NORM+ EQUIV) and (B) with VA noted on ECG and those showing no VA.

AUC: area under the curve; cTnI: cardiac troponin I; DCM-C: clinical DCM; DCM-P: preclinical DCM; ECG: electrocardiogram; ECHO: echocardiography; EQUIV: equivocal; GD: Great Danes; NORM: normal; VA: Ventricular arrhythmias.

Figure 3. Box and whisker plot showing the concentration of cTnI in [A] all GD suffering sudden cardiac death (SCD) versus non-cardiac deaths (NC/EU-NC) and [B] only GD without DCM [NORM + EQUIV] suffering SCD versus all non-cardiac deaths (NC/EU-NC). Within each box plot, horizontal line indicates the median, black circle indicates the mean, and error bars around the circle represent standard error of the mean. Within each figure, different superscripts indicate statistically significant differences between groups.

cTnI: cardiac troponin I; ECHO: echocardiography; EQUIV: equivocal; EU-NC: euthanasia due to non-cardiac causes; GD: Great Danes; NC: non-cardiac death; NORM: normal; SCD: sudden cardiac death.

Figure 4. Kaplan Meier curve comparing the time-to-death after last cTnI analysis (in years) [A] according to the receiver operating characteristic calculated cTnI cut-off value of 0.199 ng/mL in all GD and [B] in GD with VA recorded on ECG versus GD without detected VA.

cTnI: cardiac troponin I; ECG: electrocardiogram; GD: Great Danes; VA: Ventricular arrhythmias.

Figure 5. Predicted probability and 95% CI from a statistically significant (P = 0.032) binary logistic regression model investigating the effect of cTnI concentration on the probability of GD suffering SCD.

CI: confidence intervals; cTnI: cardiac troponin I; GD: Great Danes; SCD: sudden cardiac death.

Footnotes

[e] Immulite 2000 cTnI assay (Siemens; Surrey).

[f] Vivid 7 Echocardiography machine; GE, Buckinghamshire, UK with an M4S multifrequency phased array sector transducer.

[g] Echopac; GE, Buckinghamshire, UK.

[h] SAS. (2013). The SAS system for Windows, release 9.4. In Cary, NC, USA: SAS Institute Inc.

[i] IBM SPSS (2017). IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp

Figure 1A and 1B.

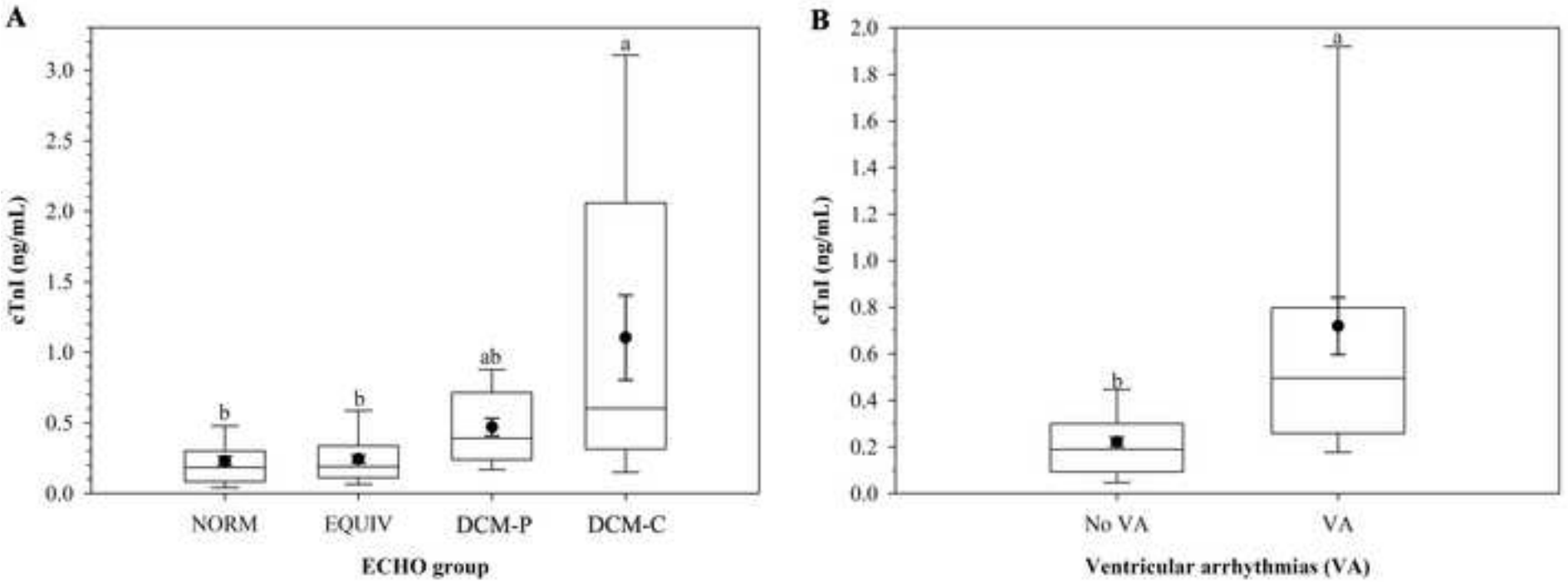

Figure 2A and 2B

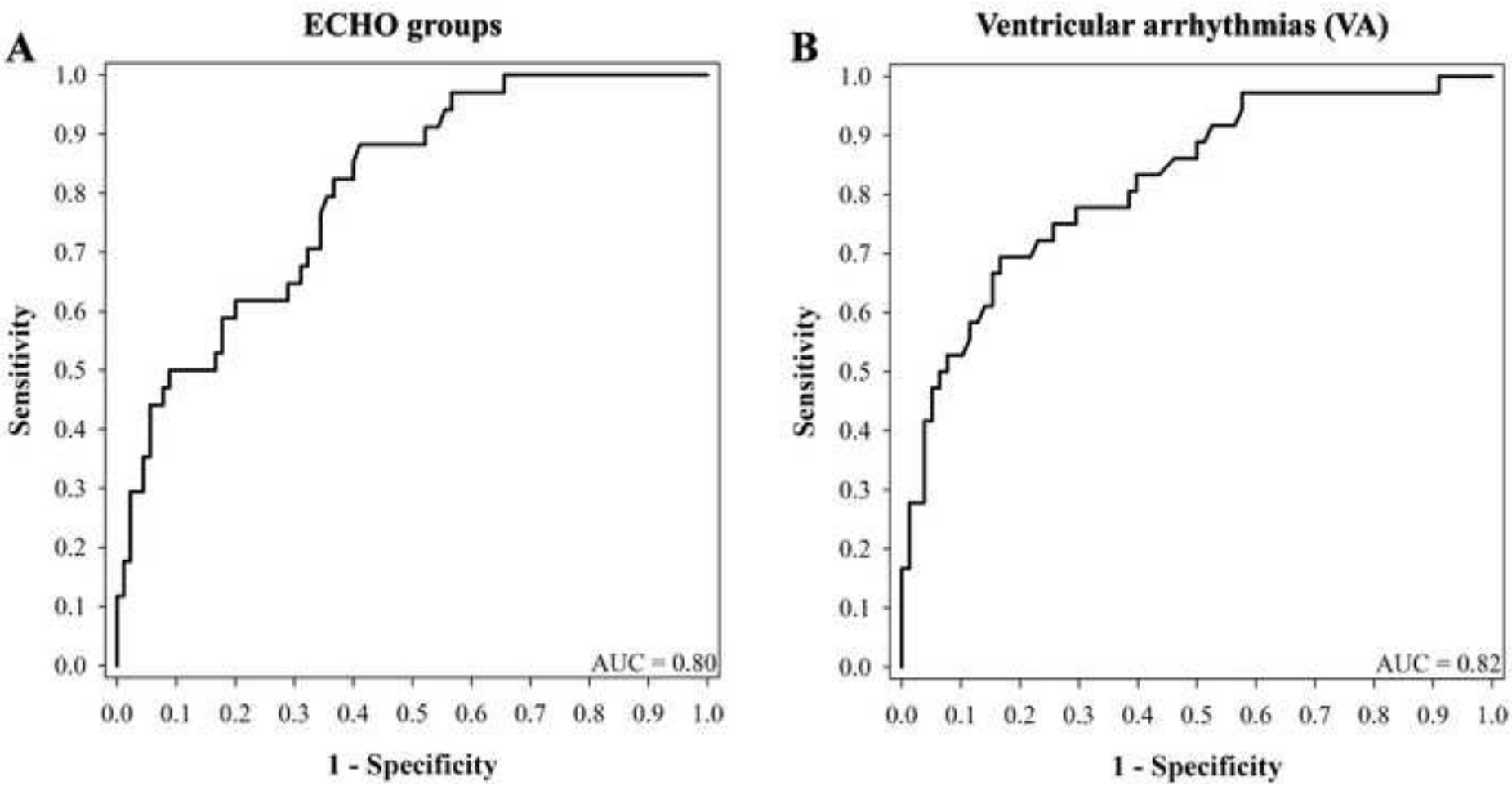

Figure 3A and 3B

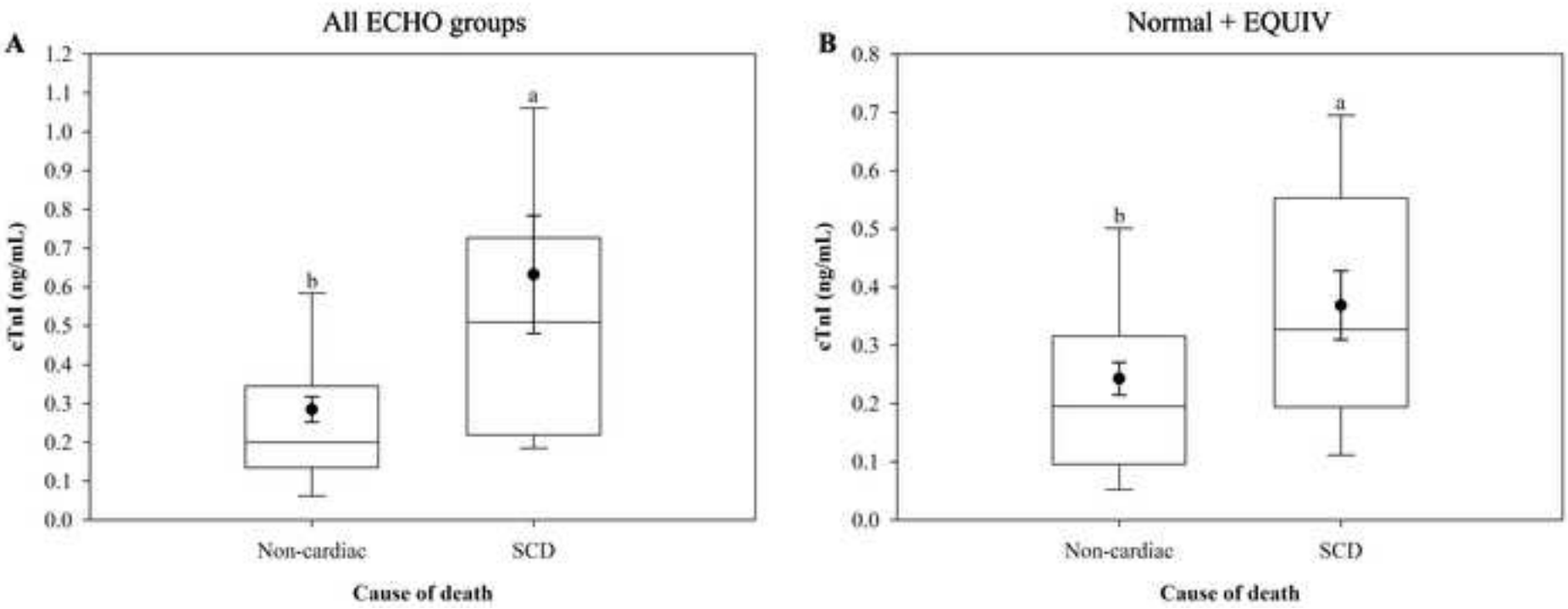

Figure 4A and 4B

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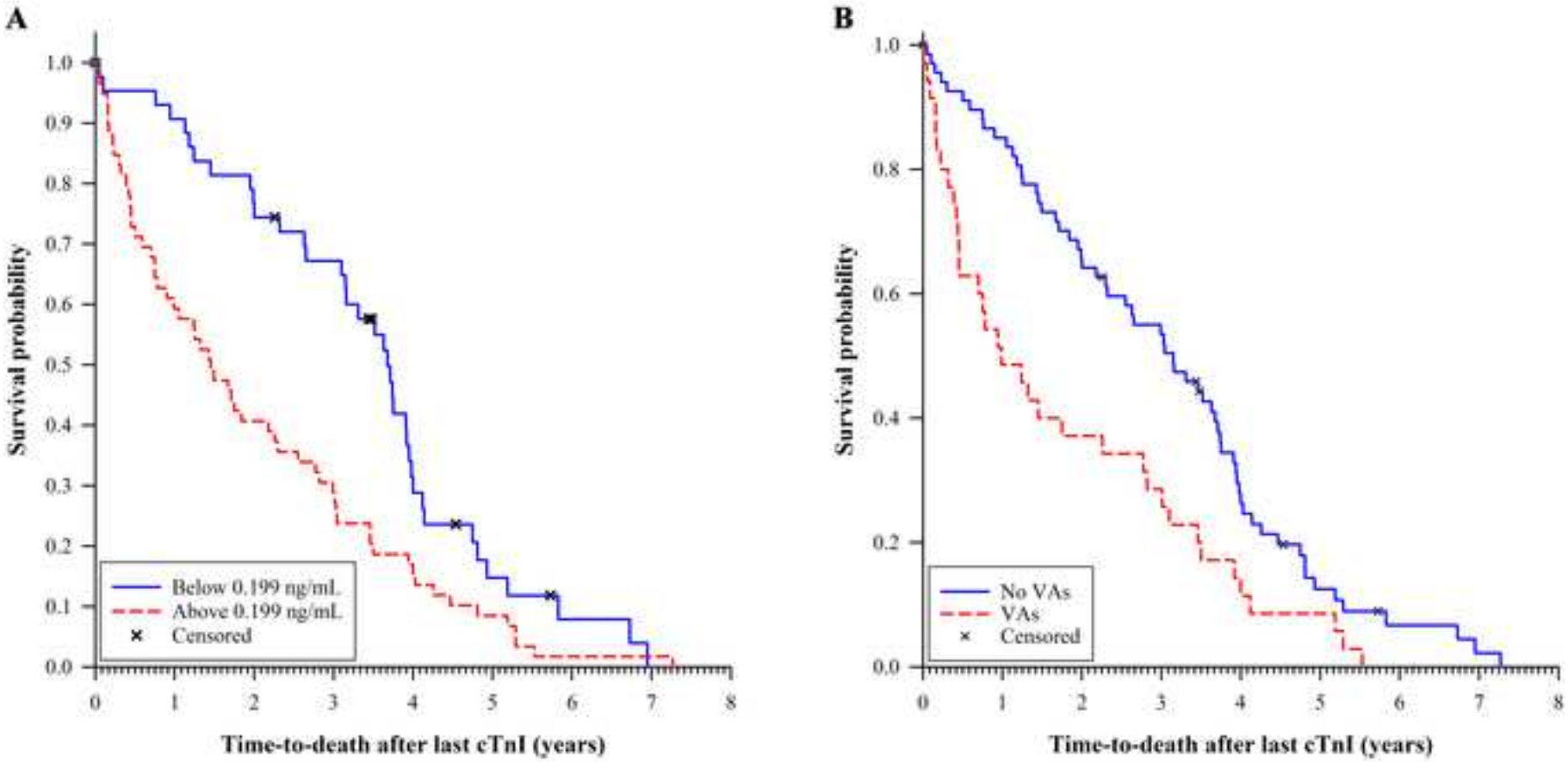

Figure 5

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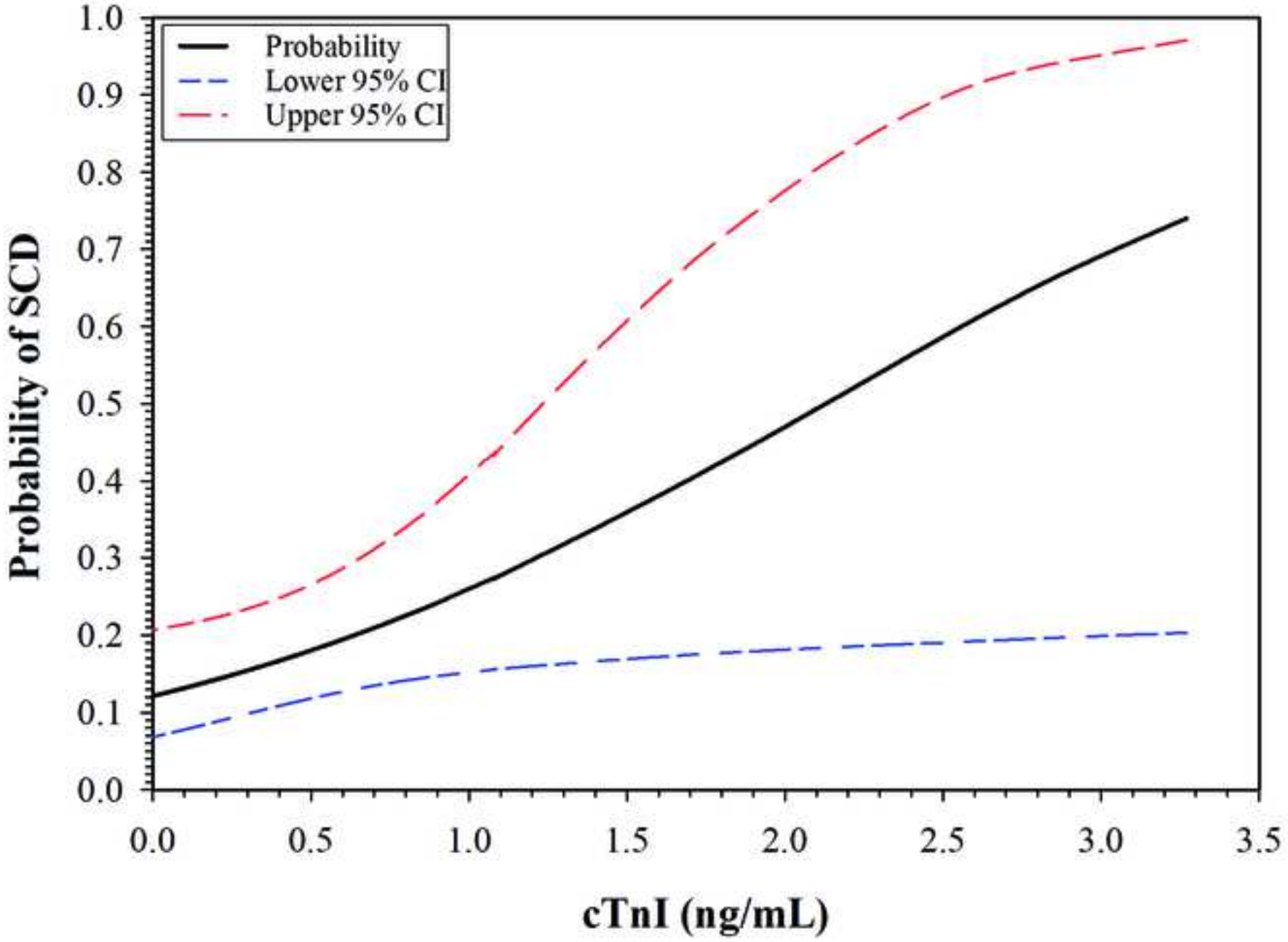

Supplementary Table.

Table A. Demographic and echocardiographic values for 124 GD.

Parameter		ECHO Groups				Comparison	
		Normal	EQUIV	DCM-P	DCM-C	$\chi^2_{(3)}$	P-value
Number		53	37	21	13		
A) Demographic characteristics							
Sex	Males (neutered)	25 (8)	13 (0)	8 (2)	9 (3)		
	Females (neutered)	28 (9)	24 (9)	13 (2)	4 (2)		
Weight (kg)		63.73 ± 1.22 (49.20–90.30)	64.70 ± 1.39 (50.00–90.50)	65.72 ± 2.48 (52.30–92.60)	67.14 ± 2.63 (52.70–90.00)	1.69	**0.683**
Age (years)		6.22 ± 0.28 (3.25–10.75)	6.39 ± 0.32 (1.67–11.42)	7.02 ± 0.40 (4.00–10.25)	6.96 ± 0.50 (2.83–9.00)	4.50	**0.213**
B) Echocardiographic values							
LVIDd (mm)		50.4[b] (47.20–52.50)	53.40[b] (48.10–55.8)	63.50[a] (59.82–70.70)	73.30[a] (60.80–80.10)	59.85	**<0.001**
LVIDd-N		1.49[c] (1.38–1.55)	1.56[c] (1.45–1.67)	1.87[b] ± 0.26 (1.74–2.00)	2.15[a] (1.77–2.28)	58.91	**<0.001**
LVIDs (mm)		37.90[d] (35.00–39.80)	42.5[c] (39.20–44.20)	55.30[b] (45.90–56.70)	60.20[a] (51.40–73.40)	74.45	**<0.001**
LVIDs-N		1.01[c] (0.95–1.09)	1.11[c] (1.07–1.19)	1.41[b] (1.27–1.56)	1.53[a] (1.37–1.88)	63.45	**<0.001**
FS (%)		25.00[a] ± 7.50 (10–40)	22.00[a] ± 5.25 (13–33)	15.00[b] ± 8.00 (7–32)	14.00[c] ± 12.50 (4–23)	$F_{3,124}$ =601.38	**<0.001**
EDV (mL)		129.00[c] (112.0–149.0)	138[c] (121.0–151.0)	180.00[b] (156.0–223.0)	213.00[a] (181.0–325.0)	51.06	**<0.001**
ESV (mL)		56.00[c] (49.00–66.00)	73.50[c] (64.00–83.00)	117.00[b] (79.00–139.0)	148.00[a] (112.0–208.0)	66.95	**<0.001**
EDVI (ml/m^2)		80.91[c] (69.92–91.44)	84.49[c] (76.11–88.28)	108.55[b] (96.67–132.8)	126.53[a] (105.7–198.8)	59.52	**<0.001**
ESVI (mL/m^2)		36.30[c] (31.90–41.50)	45.40[c] (41.60–52.60)	67.70[b] (53.60–83.50)	85.00[a] (67.80–117.2)	72.77	**<0.001**
EF (%)		54.2[a] (50.40- 59.25)	43.95[b] (40.93- 47.73)	41.00[b] (33.70- 47.60)	27.70[b] (24.65- 46.95)	50.96	**<0.001**
SI		1.80[a] (1.70–1.90)	1.70[a] (1.60–1.90)	1.40[b] (1.30–1.60)	1.30[b] (1.20–1.50)	49.15	**<0.001**

Normally distributed data (age, weight, and FS%) are reported as mean ± *SE* (minimum– maximum) while non-normally distributed data are presented as median (25^{th} -75^{th} percentiles). Within one row, different superscripts represent a significant difference between the groups ($P < 0.05$).

CD: cardiac death; cTnI: cardiac troponin I; DCM-C: clinical DCM; DCM-P: preclinical DCM; ECHO: echocardiography; EDV: end-diastolic volume; EDVI: end-diastolic volume indexed for body surface area; EQUIV: equivocal; ESV: end-systolic volume; ESVI: end-systolic volume indexed for body surface area; EU-CD: euthanasia due to cardiac causes; EU-NC: euthanasia due to non-cardiac causes; FS: fractional shortening; EF: ejection fraction; GD: Great Dane; LVIDd: left ventricular internal dimension in diastole; LVIDd-N: normalised left ventricular internal dimension in diastole; LVIDs: left ventricular internal dimension in systole; LVIDd-N: normalised left ventricular internal dimension in systole; NC: non-cardiac death; NORM: normal; LTFU: lost to follow up; SCD: sudden cardiac death; SI: sphericity index.

Table B. Atrial fibrillation and ventricular arrhythmias detected during ECG and/ Holter monitoring

		ECHO group				All GD
		Normal	**EQUIV**	**DCM-P**	**DCM-C**	
Number		53	37	21	13	
VA	n	7	11	10	8	36
AF	n	1	1	3	3	8
VA and AF	n	-	-	2	2	4

AF: atrial fibrillation; ECHO: echocardiography; GD: Great Danes; NORM: normal; DCM-C: clinical DCM; DCM-P: preclinical DCM; EQUIV: equivocal; VA: ventricular arrhythmias.

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The following information is required for submission:

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The ICMJE recommends that authorship be based on the following 4 criteria:

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
2. Drafting the work or revising it critically for important intellectual content; AND
3. Final approval of the version to be published; AND
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Please specify the contribution of **each author** to the paper, e.g. study concept or design, data collection, data analysis or interpretation, writing the paper, others, who have contributed in other ways, should be listed as contributors.

Samar El Sharkawy: conception of the research, acquisition, analysis and interpretation of data, drafting and revising content, final approval of version to be published
Joanna Dukes McEwan: conception of the research, data acquisition and interpretation, drafting and revising content, final approval of version to be published
Hisham Abdelrahman: analysis and interpretation of data, revising content, final approval of version to be published
Hannah Stephenson: conception of the research, data acquisition and interpretation, drafting and revising content, final approval of version to be published

As **Corresponding Author** I hereby confirm that all listed authors in the submission meet these Criteria.

Corresponding author:……Hannah Stephenson.....

Please add signature here: 

Date: …28th March 2022….