Usefulness of Cardiac Biomarker Screening to Detect Dilated Cardiomyopathy in UK Dobermanns

Joanna Dukes-McEwan BVMS, MVM, PhD, DVC, DipECVIM-CA(Cardiology), FRCVS

Department of Small Animal Clinical Science, School of Veterinary Science, Leahurst Campus, University of Liverpool, Chester High Road, Neston CH64 7TE.

J.Dukes-McEwan@liverpool.ac.uk 0151 795 6100 (Corresponding author)

Kathryn E Garven BVMS CertVC MRCVS

MBM Veterinary Group, 21 Hill Street, Kilmarnock, KA3 1HF.
katygarven@mbmvetgroup.co.uk

Jordi Lopez Alvarez DVM, PhD, DipECVIM-CA(Cardiology), MRCVS

Davies Veterinary Specialists. Manor Farm Business Park, Higham Gobion, Hitchin, Herts SG5 3HR.

Current affiliations:

1) Memvet - Centre de Referència Veterinària. C/Reina Esclaramunda 6, baixos, 07003 Palma, Mallorca, Illes Balears.
2) Fundació Hospital Clínic Veterinari de la Universitat Autònoma de Barcelona. Campus UAB, Carrer de l'Hospital, s/n, 08193 Cerdanyola del Vallès, Barcelona.
3) Telemedicine Consultant at Idexx.
jordilopeza@gmail.com

Pedro Oliveira DVC, DipECVIM-CA (Cardiology), MRCVS

Davies Veterinary Specialists. Manor Farm Business Park, Higham Gobion, Hitchin, Herts SG5 3HR.

Pedro.Oliveira@vetspecialists.co.uk

Paul F. Motskula DVM, MSc, MvetMed, PhD, DipECVIM-CA(Cardiology), MRCVS

Anderson-Moores Veterinary Specialists. The Granary, Bunstead Barns, Poles Ln, Hursley, Winchester SO21 2LL.

Current affiliations:

1) Vetekar – Veterinary Cardiology Consultancy. Fortuuna 35-24, Tartu 50603, Estonia
2) Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 62, 51006 Tartu, Estonia.
3) Telemedicine Consultant at Idexx

Paul.motskula@emu.ee

Ruth Willis BVM&S, DVC, MRCVS

Holter Monitoring Services. Dick White Referrals, Station Farm, London Road, Six Mile Bottom, Cambridgeshire CB8 0UH

Current address: Sarah Smith Veterinary Cardiology; Ivy Court, Willington Rd, Etwall, Derby DE65 6JG

ruth@sarahsmithcardiology.co.uk

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Conflicts of interest

JLA & PFM are telemedicine consultants for IDEXX laboratories. The authors declare no other potential conflicts of interest. Support of this project is acknowledged above.

Ethics statement

Institutional ethical approval had been awarded for this study (VREC164).

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Structured Summary

Objectives: To assess the efficacy of two cardiac biomarker (CBM) assays (N-terminal proBNP (NTproBNP) and high sensitivity cardiac Troponin I (hs cTnI) (Beckman Coulter Access) in detecting Dobermann dilated cardiomyopathy.

Methods: Dobermanns undergoing CBM testing were screened by echocardiography (Echo) and Holter monitoring, then assigned to a group: normal, equivocal, arrhythmia form of DCM (DCM-Holter), echocardiographic form of DCM (DCM-Echo) or both (DCM-Both). Some were reassessed to identify final status. Initial CBM results were compared to final status. Receiver operating characteristic (ROC) curves were used to identify area under the curve (AUC) and corresponding sensitivity (Se), specificity (Sp) for different cut-offs (CO) for each CBM.

Results: 118 Dobermanns with CBM data had Echo/Holter assessment. Repeat assessment was carried out in 47 Dobermanns after 394.5 $\pm$ 151.0 days. Seventeen dogs changed group between initial and final status. The final status of 59 was normal, 9 were equivocal and 50 had DCM (prevalence 42.4%). Of the DCM group, 25 had DCM-both, 13 DCM-Echo and 12 DCM-Holter. ROC AUC = 0.807 for NTproBNP (Se 0.69 & Sp 0.81) and 0.873 for hs cTnI (Se 0.77 & Sp 0.86). When both Se and Sp were optimised for all forms of DCM, NTproBNP cut-off was 626 pmol/L (Se & Sp 0.79) and hs cTnI cut-off was 0.056 ng/mL (Se & Sp 0.84). ROC AUC was higher for DCM-Echo (NT-proBNP 0.883; hs cTnI 0.907) than DCM-Holter.

Clinical significance: CBM screening may be useful to select Dobermanns which would benefit from further assessment by Echo and Holter.

Key Words:

Troponin I, N-terminal Pro-Brain Natriuretic Peptide, Doberman pinscher, Echocardiography, Holter monitoring

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Introduction

Dilated cardiomyopathy (DCM) has high prevalence in the Dobermann breed (Wess *et al.* 2010b). DCM is familial but inheritance is complex (Simpson *et al.* 2015) with several loci or genes reported (Mausberg *et al.* 2011, Meurs *et al.* 2012, Owczarek-Lipska *et al.* 2013, Meurs *et al.* 2019). Therefore, a simple genetic test therefore cannot reliably identify Dobermanns at risk of developing DCM. Owners, breeders and veterinary surgeons therefore still need to rely on clinical screening tools to identify individual Dobermanns with DCM.

Dobermann DCM is associated with ventricular arrhythmias, which may or may not be associated with the echocardiographic changes typical of DCM (Wess *et al.* 2017). Affected Dobermanns with DCM have a long, preclinical (occult) phase lasting years and it is important to identify these individuals to avoid breeding from affected dogs and also to benefit the individual dog (Summerfield *et al.* 2012). Current "gold standard" recommendations for screening Dobermanns for DCM are regular echocardiography and Holter monitoring (Wess *et al.* 2017). The accuracy of cardiac biomarkers for DCM has been investigated; Wess and colleagues showed that the cardiac biomarkers Troponin I[a] (Wess *et al.* 2010c) and N-terminal pro-brain natriuretic peptide[b] (NTproBNP) (Wess *et al.* 2011) each separately showed reasonable sensitivity and specificity at detecting clinical and pre-clinical Dobermann DCM. They also identified incipient cases, i.e. those which were initially normal but later developed echocardiographic or Holter abnormalities. An ultrasensitive Troponin I[c] assay provided greater sensitivity at detecting incipient cases, which developed DCM within 18 months of "last normal" screening (Klüser *et al.* 2019). The second-generation NTproBNP assay[d] has been assessed prospectively in Dobermanns, along with the ultrasensitive Troponin I[c] assay and the PDK4 genetic test (Gordon *et al.* 2015).

To the authors' knowledge, there are no published reports of the Beckman Coulter Access high sensitivity cTnI assay[e] being used prospectively in Dobermanns to identify preclinical DCM although it has been used to generate canine reference ranges including Dobermanns (Oyama & Sisson 2004).

We hypothesised that CBM screening with both the hs cTnI assay[e] and second-generation NTproBNP assay[d] would improve the sensitivity and specificity of detection and discrimination between DCM-affected and healthy Dobermanns better than either test alone.

Study aims were (i) to investigate the sensitivity and specificity of the hs cTnI[e] assay in identifying Dobermanns with DCM compared with echocardiography and Holter monitoring; (ii) to report on the sensitivity and specificity of the NTproBNP[d] assay in identifying UK Dobermanns with DCM compared with echocardiography and Holter monitoring; (iii) to investigate whether the combination of hs cTnI and second-generation NTproBNP improves identification of DCM.

Materials and Methods

This was a prospective, observational study. The cardiac biomarker (CBM) study was conducted between January 2015 and January 2017. Institutional ethical approval had been awarded (XXX redacted for review).

Dobermanns with CBM data available were eligible for inclusion. During the study period, physical examination and blood sampling was carried out at Dobermann shows by a veterinary surgeon. Blood samples were taken into EDTA for NTproBNP and either serum or EDTA tubes for hs cTnI (Klüser *et al.* 2019). Samples were centrifuged within 1 hour, and plasma / serum separated and stored at -20°C or -4°C prior to shipping to the laboratory within 24 hours, at ambient temperature.

Dobermanns who presented for evaluation by a participating cardiologist were also eligible if contemporaneous CBM results were available. Some cases had clinical signs which prompted the cardiovascular assessment and others presented for routine DCM screening by echocardiography and Holter monitoring (Echo/Holter).

Dogs with hs cTnI (ref. <0.07 ng/mL[f]) and / or NTproBNP (ref. <735 pmol/L (Gordon *et al.* 2013)) concentrations above the laboratory reference ranges were included in the abnormal CBM group. Dobermanns in the normal CBM group had both hs cTnI and NTproBNP concentrations within reference ranges. From the normal CBM group, Dobermanns were selected from show testing and invited for Echo/Holter if they were ≥ 4 years old, had an unremarkable physical examination documented by the attending veterinary surgeon and were considered to be healthy by their owners. The age of ≥ 4 years old was selected so that the screened population was likely to have a higher prevalence of DCM to minimise false negative results with the screening tests. For Dobermanns presenting to a cardiologist, any age was permitted, provided that CBM results and echo / Holter data were available and any non-cardiac condition was noted.

Echo was carried out by veterinarians with a post-graduate qualification in cardiology following two-dimensional (2D) and M-mode recommendations as previously described (Wess *et al.* 2010a, 2010b, 2017). Doppler echocardiographic studies (colour flow and spectral) were sufficiently detailed to exclude other congenital or acquired cardiac diseases.

Holter recordings were scheduled to be over approximately 24 hours, and studies of <18 hours were excluded. Analysis of the Holter recordings was by a single author (initials redacted for review). Ambulatory ECG recording data were acquired using a commercial ambulatory ECG monitor (Lifecard Compact Flash (CF); Spacelabs Healthcare) at a sampling frequency of 1024Hz and stored on a 90 megabyte removable compact flash card. Commercially available Holter software (Pathfinder version 9; Spacelabs Healthcare) was used to perform standardised semi-automatic arrhythmia analysis. From the Holter analyses, Dobermanns were considered to be normal if they had fewer than 50 ventricular premature complexes (VPCs) over 24 hours, abnormal if they had >100 VPCs/24 hours, and equivocal if they had 50 – 100 VPCs/24 hours (Wess and others 2010b). The total number of VPCs and their complexity (couplets, triplets, salvos or runs of ventricular tachycardia) were noted (Wess *et al.* 2017). If couplets, triplets or runs were closely coupled (instantaneous rate >250 bpm) but the absolute VPC/24 hour count was <50, these were classified as equivocal.

Based on Echo/Holter results, Dobermanns were classified as follows:

1) Apparently healthy (no echo or Holter abnormalities)
2) DCM-Echo: Echo abnormal; presence of congestive heart failure noted: DCM-CHF

3) DCM-Holter: Holter abnormal or atrial fibrillation (AF)
4) DCM-Both: Both Echo / Holter Abnormal (with or without DCM-CHF)
5) Equivocal (for either Echo or Holter or both).

After 12 months, a number of dogs were invited back from the apparently healthy and equivocal groups for repeat screening by cardiac biomarkers and Echo/Holter. In particular, Dobermanns with abnormal CBM test(s) but initially unremarkable echocardiography and Holter monitoring results were re-examined. Dobermanns from the DCM groups were also reassessed as clinically indicated. Owner updates were sought at the end of the study (January 2019), to provide information about the final status of their dog (alive, dead, cause of death if known). Cause of death was categorized as sudden, cardiac (death or euthanasia due to cardiac causes) and other (non-cardiac).

Data analyses and Statistical methods

Data from each dog were collated in an Excel spreadsheet (2016; Microsoft Office) and statistical analyses were carried using SigmaPlot 14 (Systat). To include data from animals with cardiac biomarker results below the detection limit of the assays, for NTproBNP, values reported as <250 pmol/L were assigned a value of 249, and for hs cTnI, values reported as <0.01 ng/mL were assigned a value of 0.009. If NT-pro-BNP was >10,000 pmol/L, it was assigned a value of 10,001. The Shapiro-Wilk test was used to assess for normal distribution of data spread and the Brown-Forsythe test was used to test for equal variance. Basic descriptive statistics for normally distributed data included mean and standard deviation or median (interquartile range) for non-normally distributed data or if data showed unequal variance. To compare continuous normally distributed data for two groups (e.g. male / female), the unpaired T-test was used. To compare three or more groups with normally distributed data, one-way analysis of variance (ANOVA) was used with the Holm-Sidak test for multiple pairwise comparisons. If data were not normally distributed, the Kruskal-Wallis ANOVA on ranks was used, with Dunn's method for multiple pairwise comparisons. Categorical data (e.g. males, females) were compared using the Chi squared test. To explore for any associations (e.g. age, cardiac biomarker data), scatter plots were constructed. As cardiac biomarker data were not normally distributed, Spearman's rank order correlation was used to investigate presence, strength and significance of any associations.

The initial CBM results were compared with the final known status of the dog (NORMAL, EQUIVOCAL, DCM-echo, DCM-Holter, DCM-Both) and noted to be concordant or discordant with the final diagnosis. Incipient results were included (i.e. normal echo and Holter at time of first CBM sampling, but later developed echo and / or Holter evidence of DCM on repeat assessment).

Receiver operating characteristic (ROC) curves were constructed for each of the initial hs cTnI and NTproBNP results and including the final diagnosis for each dog as DCM (all forms) or Normal; Dobermanns with equivocal echo or Holter data were excluded from this analysis. Area under the curve (AUC) was calculated. To optimise both sensitivity and specificity for all forms of DCM, graphs of sensitivity and specificity for different cut-offs for each biomarker were constructed, and the point at which the curves crossed was selected as the cut-off which optimised both. In addition, similar analyses were applied to DCM-Echo (with or without arrhythmias) and DCM-Holter (with or without echo changes) groups. The cut-offs optimising both sensitivity and specificity for both DCM-echo and DCM-Holter were determined.

Using the prevalence identified in this population (42.4%; see results), from the sensitivity and specificity data for different cut-offs of both cardiac biomarkers, the positive and negative predictive values and positive and negative likelihood ratios were determined for each biomarker test being above or below each cut-off. The statistical software determined an optimal operating point from the sensitivity and specificity data, which was calculated as *Sensitivity – m(1-Specificity)*, where *m* is the slope of the tangent to the ROC curve determined by pre-test probability and false positive / false negative test cost ratio (arbitrarily defined as 1).

Results

A total of 118 Dobermanns were included in the study (Figure 1). Descriptive statistics of their signalment, initial cardiac biomarker results and final status are shown in Table 1. A total of 50 Dobermanns were documented to have DCM (all forms; DCM-all) implying a prevalence of 42.4%. The dogs from the DCM-both group were older than in the Normal group (P=0.022) and the DCM-both group contained significantly more males (P=0.032).

The data for NTproBNP and hs cTnI concentrations in Table 1 are from the initial assessment. There is a significant difference between the groups for NTproBNP and hs cTnI (both P<0.001) (Figures 2A; 2B). There was a modest correlation between NTproBNP and cTnI (Rs=0.456; P<0.001). For the normal group of 59 dogs, there was no association between NTproBNP and age, but a modest positive association of hs cTnI concentration and age was identified (Rs=0.364; P=0.005).

A total of 17 dogs changed group between initial and final status (Figure 1). The CBM results were separated as being concordant or discordant with the final cardiac status (Table 2). There were 4 dogs with abnormal NTproBNP (two also with abnormal hs cTnI) who were initially echo / Holter normal, who subsequently developed DCM (2 DCM-Echo; 2 DCM-Holter). An additional dog with abnormal hs cTnI and normal NTproBNP was initially normal but later developed DCM-echo. The numbers in each group at final diagnosis with concordant or discordant CBM results are shown (Table 2).

For the ROC curve analysis, when the laboratory cut-offs for the cardiac biomarker data were used, the areas under the curve (AUC) and sensitivity (Se) and specificity (Sp) for all forms of DCM and both cardiac biomarkers are shown (Table 3; Figure 3A). The AUCs were 0.870 for cTnI and 0.807 for NTproBNP (see Table 3 for the confidence intervals). For the laboratory cut-off of <0.07 ng/mL for cTnI, the Se and Sp were 0.77 and 0.86 respectively. For the cut-off recommended by the laboratory for screening Dobermanns for DCM of <735 pmol/L, Se and Sp were 0.69 and 0.81 respectively (Table 3). When both Se and Sp were optimised, for all forms of DCM, a cut-off for hs cTnI of 0.056 ng/mL and NTproBNP of 626 pmol/L gave both Se & Sp of 0.838 for hs cTnI and 0.787 for NTproBNP respectively (Table 3). Identification of DCM-echo had greater Se & Sp (cTnI 0.85; NTproBNP 0.81) for slightly higher cut-offs of hs cTnI and NTproBNP with AUCs of 0.907 and 0.883 respectively (Table 3; Figure 3B). Identification of DCM-Holter had slightly lower Se & Sp (hs cTnI 0.846; NTproBNP 0.779) and lower AUCs (0.892 and 0.804) respectively for their cut-offs (Table 3; Figure 3C).

For the prevalence of DCM (all forms) at 42.4%, the positive likelihood ratio (i.e. positive test indicates likelihood of some form of DCM), the negative likelihood ratios and positive and negative predictive values of the cardiac biomarker tests at different cut-offs are shown (Table 4). The optimal cut-off points were determined (Table 4; Figure 4A; 4B).

At the time of data analysis, 42 out of 118 dogs were known to be dead (35.6% of the population). Age of death for all Dobermanns was 8.95 ± 2.5 years. Twenty-six deaths were believed to be cardiac in origin. Of these, 15 deaths were sudden (mainly dying during sleep rather than on exercise), 11 died or were euthanised because of cardiac disease (ten because of congestive heart failure and one because of recurrent syncope affecting quality of life). For non-cardiac causes of death (n=16), there were 8 dogs with neoplasia, 2 had gastric dilatation / volvulus, 3 dogs were euthanised due to old age or mobility issues (including 1 cervical spondylopathy), 1 due to signs associated with a portosystemic shunt, 1 bitch died during whelping and 2 had unknown causes of death. There was no significant difference between the ages of death of normal and the DCM groups (P=0.091).

Discussion

In this study, the Beckman Coulter Access hs cTnI assay[e] performed better than the 2nd generation NTproBNP assay[d], based on comparisons of ROC AUCs for all forms of DCM in this population of Dobermanns. We confirmed the findings of other studies that NTproBNP had good AUCs (Wess *et al.* 2011, Singletary *et al.* 2012, Gordon *et al.* 2015) especially for the echo form of DCM. The data presented here show that the AUC, sensitivity and specificity for the Beckman Coulter Access hs cTnI assay[e] was superior to the Immulite[a] assay (Wess *et al.* 2010c) and similar to the Advia Centaur ultra-sensitive cTnI assay[c] (Klüser *et al.* 2019). Our results with hs cTnI[e] showed better detection of DCM-echo than DCM-

Holter based on AUC results. This was also reported by Wess and colleagues for the Immulite assay[a] (Wess *et al.* 2010c) but not for the ultra-sensitive assay[c] (Klüser *et al.* 2019), which had similar ROC AUCs for both DCM-Echo and DCM-Holter. Troponin I is likely to be increased due to cardiomyocyte injury in all forms of DCM, and those with DCM-Echo may have more advanced disease (Wess *et al.* 2010c). In our study, when hs cTnI was compared in Dobermanns with DCM and their cause of death (sudden, cardiac or non-cardiac), there was a significant difference between groups (P=0.019) with higher hs cTnI values in sudden death or cardiac death than in dogs with DCM who died of non-cardiac causes. Klüser and colleagues (2016) noted that increased ultra-sensitive cTnI[c] was significantly higher in Dobermanns suffering a sudden cardiac death (SCD), additional to the influence of severity of left ventricular dilatation. In this study, we did not separate SCD from other forms of cardiac death, due to low numbers.

Cardiac Troponin I may be elevated due to non-cardiac disease (Wess *et al.* 2017), so a value above the cut-off does not necessarily indicate presence of DCM. However, an abnormal result does indicate a Dobermann who would benefit from further cardiac and other veterinary examinations.

NTproBNP has been said to be not clinically useful to identify DCM-Holter (Wess *et al.* 2017). Our results using the second-generation assay appear to be slightly more discriminatory for DCM-Holter at a higher cut-off of NT-proBNP. However, for the ROC curve analysis, the DCM-Holter group included all Dobermanns meeting Holter criteria for DCM, including dogs which were abnormal on echo, which will have had increased myocardial wall stress. This group also included dogs with atrial fibrillation (n=5) which all also had significant ventricular arrhythmia and DCM-Echo and were in congestive heart failure.

It is interesting that this study had higher cut-offs for NTproBNP than those published previously. The results of the first-generation assay and second-generation assay are not interchangeable, as previously reported (Cahill *et al.* 2015). However, the second-generation assay was designed to give similar results to the first generation NTproBNP assay (Wess *et al.* 2017). Another study using the second-generation assay in 449 Dobermanns also gave a lower cut-off of 548 pmol/L and better AUC and sensitivity (AUC 0.91, Se 100%, Sp 80%) for echo-DCM (Gordon *et al.* 2015) than presented here. The reason for the difference is unclear, but may reflect much lower numbers in our study, or that elevated NTproBNP may reflect non-cardiac disease (e.g. renal or respiratory conditions), or the considerable biological day to day variability of this assay (Wess *et al.* 2017, Winter *et al.* 2017).

The second-generation assay using an EDTA plasma sample is stable at ambient temperature for 48 hours (Cahill *et al.* 2015). In our study, some dogs underwent cardiac biomarker testing at dog shows at weekends, with plasma samples refrigerated or ideally frozen prior to shipping. It is possible that NTproBNP degraded due to variable or uncertain sample handling and delays in processing. This is therefore a limitation of this study. Sample degradation could potentially explain the inferior performance of the NTproBNP assay in this population of dogs, compared with previous publications. If this had been a factor, however, one would expect lower cut-offs rather than higher as sample degradation would have affected all samples.

It is important for a diagnostic test to have high specificity and positive predictive value (PPV) so that only dogs which may benefit from diagnostic and therapeutic interventions are identified. However, for DCM screening, high sensitivity and negative predictive value (NPV) are preferable. This permits identification of any affected individual and enables diagnostic interventions and treatments which can influence outcome (O'Grady *et al.* 2008, 2009, Summerfield *et al.* 2012). Based on the results from this study, the NPV of the hs cTnI test was up to 0.86 (specificity 0.8), which means that up to 14% of negative tests (<0.055 ng/mL) might be false negatives (i.e. have DCM). For NTproBNP, the NPV was up to 0.85 (specificity 0.78), so 15% of negative tests (<603pmol/L) are false negatives (affected cases). However, the authors propose that Dobermanns are screened by cardiac biomarker screening on an annual basis, so a Dobermann with DCM should eventually be detected by the cardiac biomarker screening. It must be emphasised that the CBM results do not replace the gold-standard screening of echo / Holter but they might help triage Dobermanns which benefit from full screening. The authors recommend serial screening (e.g. annually) in all Dobermanns, since this DCM is an acquired disease which may only be manifest in later life. Normal results from CBM analysis (and Echo or Holter screening) in a young Dobermann do not preclude the possibility that DCM may manifest in the future. Although in this study, only initial CBM results were compared with the last known phenotype, CBM data cannot be expected to predict future development of DCM in the long-term, even though this study and those by Wess and colleagues (2010c; 2011) indicated possible detection of incipient cases in the short-term.

In the 2014 UK Kennel Club survey, the Dobermanns breed had the shortest average survival time, with mean age of death 7.67 years, and the most common cause of death was cardiomyopathy, accounting for 19% of deaths (Lewis *et al.* 2018). Whilst our study shows an older mean age of death (8.95 $\pm$ 2.5 years; including all causes of death), the increasing impact of DCM on the breed's longevity has considerable welfare importance. Identifying more Dobermanns in the early stages of the disease in a cost-effective way may reduce prevalence of disease if these dogs are not bred, as well as benefiting individual affected Dobermanns by allowing treatment which prolongs the asymptomatic phase of DCM (Summerfield *et al.* 2012). Future prospective studies are required to see if CBM screening and widening access to pre-DCM testing will reduce mortality or prevalence of DCM in Dobermanns.

Limitations

Not many Dobermanns, especially from the normal group, underwent a repeat assessment. Therefore, the recorded cardiac status may not be accurate and some dogs may have eventually developed DCM. This study included relatively low numbers and it is possible that selecting which Dobermanns had a repeat assessment may have introduced a bias to these findings.

Troponin I and NTproBNP can be significantly elevated in various systemic diseases as noted by Wess and colleagues (2017). Although the dogs included were considered healthy by their owners, and no significant abnormalities suggesting systemic disease were noted on physical examination by the participating cardiologist, no biochemistry, haematology or thyroid function testing was undertaken to confirm health status of most Dobermanns in this study (other than clinical cases).

Multiple cardiologists, using different echocardiography machines and software, participated in the study and there was no consideration of the repeatability between cardiologists assessed as part of this study. A strength of the study was the same cardiologist carried out all the Holter analyses on the same system. However, we considered abnormal Holter recordings as those with >100 VPCs/24 hours (Wess *et al.* 2010b), in contrast to more recent recommendations, where >300 VPCs /24 hours are considered abnormal on a single recording (Wess *et al.* 2017). However, this would have not altered the classification of most dogs in this study.

Conclusions

UK Dobermanns have a high prevalence of DCM and this is of major welfare importance in the breed. CBM testing, with both high sensitivity cTnI and second generation NTproBNP assays, can be used to: screen Dobermanns for DCM in a cost-effective manner in general practice; identify individuals for further diagnostic echocardiographic and Holter assessment; and thereby permit early therapeutic intervention in the preclinical phase and removal of affected individuals from breeding programmes. The authors recommend annual cardiac biomarker screening for Dobermanns, to allow detection of initially false negative cases. The authors recommend serial testing of both hs cTnI and NTproBNP since an affected Dobermann may have a single CBM above the cut-off. In a Dobermann with an abnormal CBM result, even if Echo and Holter are initially unremarkable, repeat screening (e.g. in 12 months) is important in order to detect incipient cases.

Footnotes:

[a] Immulite 2000 troponin I test; Siemens Healthcare Diagnostics

[b] Cardiopet proBNP test, IDEXX Laboratories (first generation assay)

[c] Advia Centaur TnI-Ultra assay; Siemens Healthcare Diagnostics

[d] Cardiopet proBNP test, IDEXX Laboratories (second generation assay); IDEXX Laboratories,

[e] Beckman Coulter Access hsTnI assay; IDEXX Laboratories, Wetherby, West Yorkshire, UK

Wetherby, West Yorkshire, UK

[f] Personal communication: Anne-Marie Porritt; IDEXX Laboratories, Wetherby, UK; (hs cTnI reference range generated from a collaboration with the Royal Veterinary College).

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List of Tables

List of Figures

Figure 1. Dobermanns in the Study.

Flow chart to show the results of Echocardiography and Holter screening following initial cardiac biomarker testing. Some Dobermanns had repeat assessments (n=47 at least 2 assessments). If they changed group, their interim status is noted (coloured boxes), with numbers which changed groups. Some Dobermanns with DCM changed category of DCM on their repeat assessment (purple arrows); numbers in the black boxes are the final known diagnosis.

Figure 2. Box and Whisker plots cardiac biomarker results for Dobermann groups.

The line representing the laboratory reference range for each biomarker is indicated

2A (left): N-terminal pro-BNP concentration (log_{10} scale). The line is the current laboratory 735 pmol/L cut-off.

2B (right): high sensitivity Troponin I concentration (log_{10} scale). The boxes define the 25^{th} – 75^{th} percentile, with median line shown. Whiskers define the 10^{th} – 90^{th} percentiles, with outlying data points indicated. The line is the current laboratory cut-off of <0.07 ng/mL.

Groups: Normal: no abnormalities identified by echocardiography or Holter monitoring at the time of (last) examination. Equivocal: equivocal based on either echocardiography or Holter monitoring results or both; not meeting criteria for normal or DCM groups. Echo-DCM: meets only echocardiographic criteria for diagnosis of DCM; Holter-DCM: meets only arrhythmia criteria for diagnosis of DCM; Both-DCM: meets both echo and Holter criteria for the diagnosis of DCM.

Figure 3. ROC curves for Dobermanns based on cardiac biomarker screening.

hs cTnI: high sensitivity Troponin I (in red); NTproBNP: N-terminal proBNP (in blue) (A: area under the curve for each ROC curve).

Figure 3A: ROC curve for all forms of DCM (DCM-all) and normal Dobermanns (equivocal Dobermanns excluded from analysis).

Figure 3B: ROC curve for Echo form of DCM (DCM-echo) (with or without significant arrhythmias) (equivocal Dobermanns excluded).

Figure 3C: ROC curve for Holter form of DCM (DCM-Holter) (with or without echocardiographic abnormalities) (equivocal Dobermanns excluded).

Figure 4. Dot Histograms for Dobermann status and cardiac biomarker concentrations

Graphs show Dobermanns with DCM (all forms) (left columns) and normal Dobermanns (right columns).

Figure 4A (left): N-terminal pro-BNP (NTproBNP) concentrations. Red line shows the optimal cut off of 603 pmol/L to detect all forms of DCM.

Figure 4B (right): High sensitivity cardiac Troponin I (hs cTnI) concentrations (log_{10} scale). Red line shows the optimal cut off of 0.065 ng/mL to detect all forms of DCM.

Table 1: Final recorded status of Dobermanns based on echocardiography and Holter examination and initial cardiac biomarker results

	Normal	DCM-all	DCM-Echo	DCM-Holter	DCM-both	DCM-CHF	Equivocal	P value
N=	59	50	13	12	25	10 (9 from DCM-both, 1 from DCM-echo group)	9	n/a
Male / Female (n=)	21 / 38	29/21	6/7	5/7	18 / 7	6/4	6/3	Comparisons: 1: Normal, all DCM **P=0.032** 2. Normal, Echo DCM, Holter DCM or Both-DCM: **P=0.024**
Age (years) (mean and standard deviation (SD) (inclusion) (min.-max.)	**6.07 ± 2.37**[1] (2.13 – 11.02)	7.13 ± 2.48 (2.3 – 12.23)	6.18 ± 1.93 (3.84 – 10.08)	7.24 ± 2.49 (4.04 – 11.42)	**7.86 ± 2.44**[1] (2.4 – 12.23)	7.59 ± 3.18 (2.4 – 12.23)	6.48 ± 1.66 (3.32 – 8.6)	Comparison: Normal, Echo DCM, Holter-DCM, Both DCM & Equivocal; **P=0.022**
Number dead (type of death)	7 (Other or unknown)	34 (14 Sudden, 12 Cardiac, 9 Other)	8 (3 sudden, 2 Cardiac (CHF), 3 Other)	7 (1 sudden, 2 cardiac, 4 other)	19 (10 sudden, 8 cardiac, 1 other)	9 (4 sudden, 8 total cardiac, 1 LTFU)	2 (both Other)	Not analysed

Deceased dogs: Timing of death after inclusion (days) (min. – max.)	494.67 ± 518.07 (12 – 1149)	439.8 ± 337.9 (15 – 1126)	582 ± 378.8 (15-1126)	702 ± 234.4 (386 – 950)	264.79 ± 254.16 (1-874)	163.89 ± 231.25 (1 – 751)	529 & 926 days (n=2)	Not analysed
Initial NTproBNP result (all in group included) Median (range: min.-max.)	**Median: 249 (range 249 - 3140)[1,4]**	Median: 1193 (range 249 – 10001)	**Median: 765.5 (range 286 – 3209)[4]**	**Median 297 (range 249 – 5180)[3]**	**Median 2399 (range 292 – 10001)[1,2,3]**	Median: 4515 (range 642 – 10001)	**Median 334 (range 249 – 1024)[2]**	**P<0.001**
Initial hs cTnI result (all in group) Median (range: min. - max.)	**Median 0.02 (Range 0.009 – 0.27)[1,2,3]**	Median 0.10 (Range 0.009 – 13.47)	**Median 0.075 (range 0.02 – 0.27)[2]**	**Median 0.09 (range 0.009 – 13.47)[3]**	**Median 0.16 (range 0.06 – 13.47)[1]**	Median 0.27 (0.09 – 1.44)	Median 0.05 (range 0.02 – 0.1)	**P<0.001**

Cells in bold which share a superscript number are significantly different from each other with post-hoc pairwise comparisons.

Abbreviations for GROUPS:
All DCM: includes data from Dobermanns with any and all forms of DCM, DCM-echo: DCM evident on echocardiography, DCM-Holter: arrhythmias noted only, DCM-both: meet both the echocardiographic and Holter criteria for diagnosis of DCM. CHF: presence of congestive heart failure (will be in Echo-DCM or Both-DCM groups; not analysed separately).

Abbreviations:
CHF: congestive heart failure, echo: echocardiography, hs cTnI: high sensitivity Troponin I, LTFU: lost to follow-up, max: maximum, min: minimum, n/a: not applicable, NTproBNP: N-terminal pro-BNP, SD standard deviation.

Table 3: ROC curve analysis for all forms of Dobermann DCM

	Variable	Area under curve (AUC)	Cut-offs	Sensitivity (Se)	Specificity (Sp)
All DCM	hs cTnI	0.873 (95% CI: 0.804 - 0.942)	Lab: ≥0.07 ng/mL	0.77	0.86
	NTproBNP	0.807 (95% CI: 0.725 - 0.890)	Lab: ≥735 pmol/L	0.69	0.81
All DCM: optimised Se & Sp	hs cTnI	0.873 (95% CI: 0.804 - 0.942)	0.056 ng/mL	0.84	0.84
	NTproBNP	0.807 (95% CI: 0.725 - 0.890)	626 pmol/L	0.79	0.79
Echo form of DCM (echo or both, +/- CHF) (n=39) **(optimised Se & Sp)**	hs cTnI	0.907 (95% CI: 0.849 - 0.966)	0.062 ng/mL	0.85	0.85
	NTproBNP	0.883 (95% CI: 0.818 - 0.947)	678 pmol/L	0.81	0.81
Holter form of DCM (alone or with Echo) (n=38) **(optimised Se & Sp)**	hs cTnI	0.892 (95% CI: 0.818 - 0.966)	0.0615 ng/mL	0.85	0.85
	NTproBNP	0.804 (95% CI: 0.712 - 0.896)	609 pmol/L	0.78	0.78

Cut-offs for revised scoring based on optimization of both sensitivity and specificity.

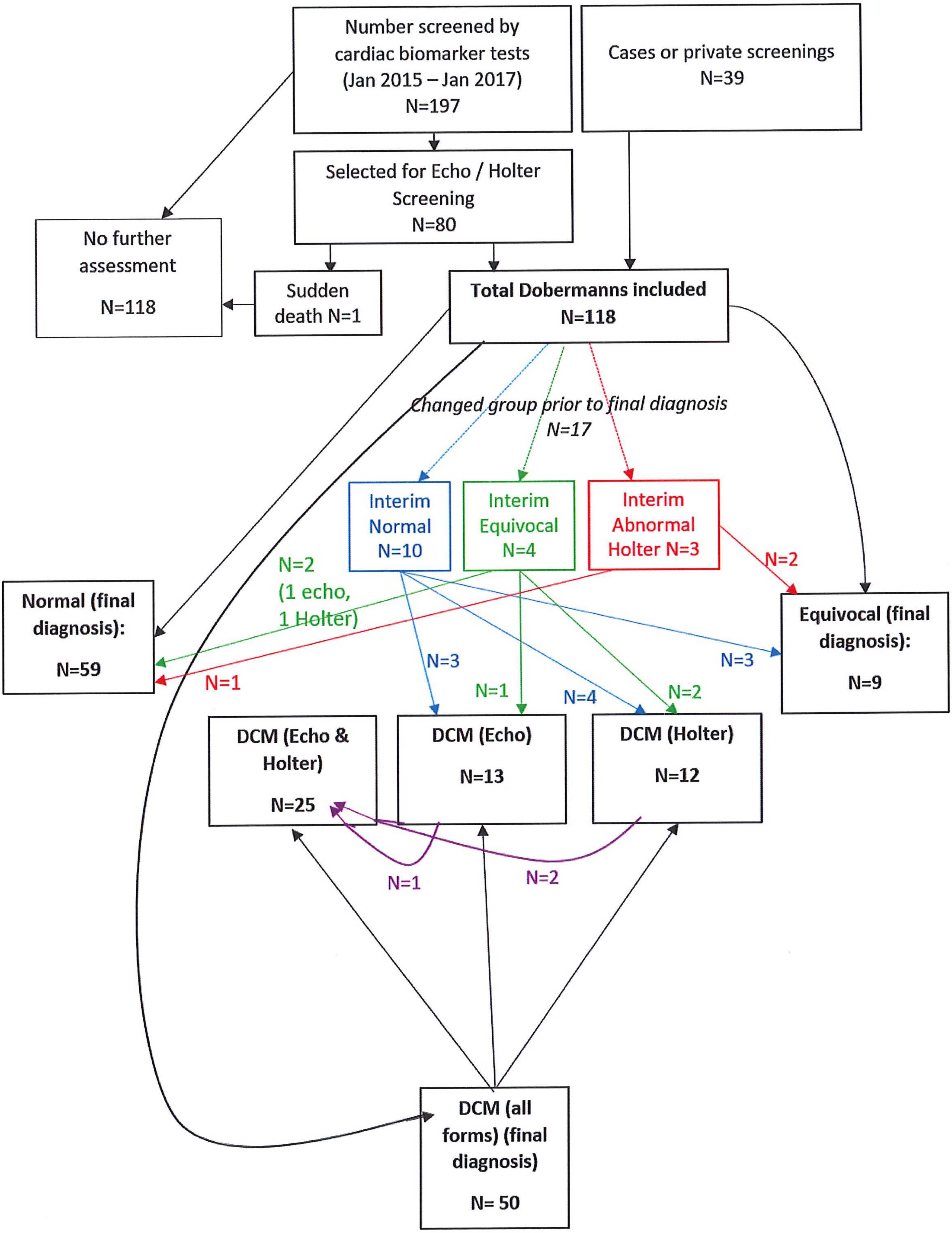
Abbreviations: CI: confidence intervals, hs cTnI: high sensitivity Troponin I, NTproBNP: N-terminal pro-BNP, Se: sensitivity, Sp: specificity

Table 4. Positive and Negative Predictive Values and Likelihood Ratios of Cardiac Biomarker Tests (various cut-offs)

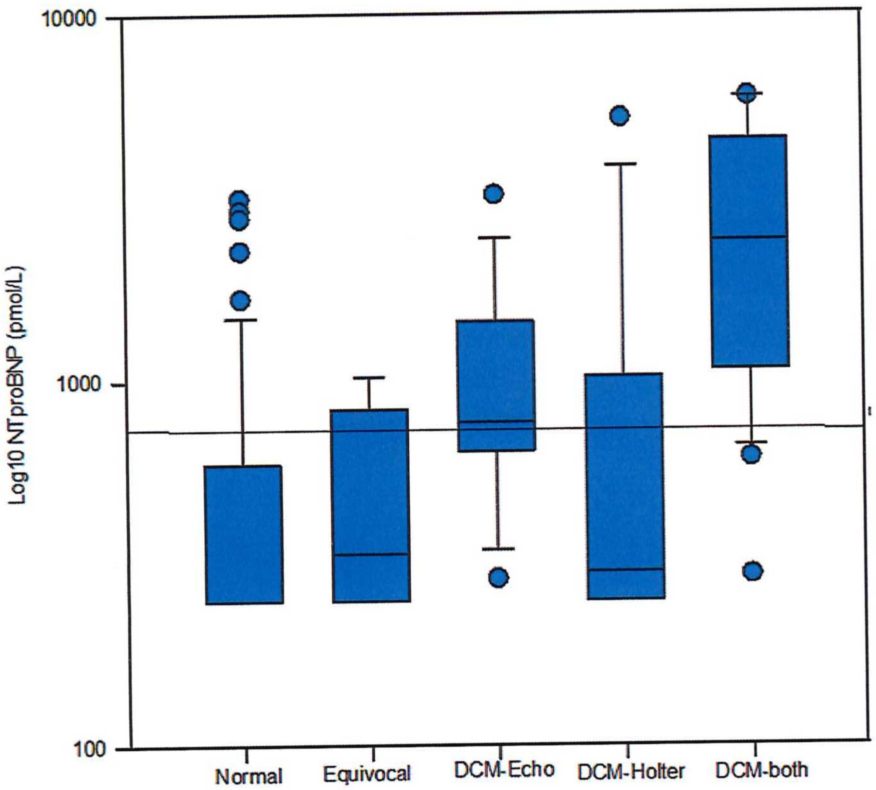
	Cut-off	Se	Sp	PPV	NPV	LR+	LR-
hs cTnI (ng/mL)	0.055	0.83	0.80	0.75	*0.86*	4.07	0.22
	0.065	0.77	0.86	0.81	0.84	5.67	0.27
	0.265	0.17	<u>0.98</u>	<u>0.88</u>	0.62	10.24	0.84
NTproBNP (pmol/L)	**603**	0.81	0.78	0.73	*0.85*	3.67	0.25
	623	0.79	0.79	0.72	0.83	3.58	0.27
	638	0.77	0.78	0.72	0.82	3.49	0.30
	664	0.75	0.78	0.71	0.81	3.40	0.32
	688	0.75	0.80	0.73	0.81	3.69	0.31
	2920	0.25	<u>0.98</u>	<u>0.92</u>	0.64	14.79	0.76

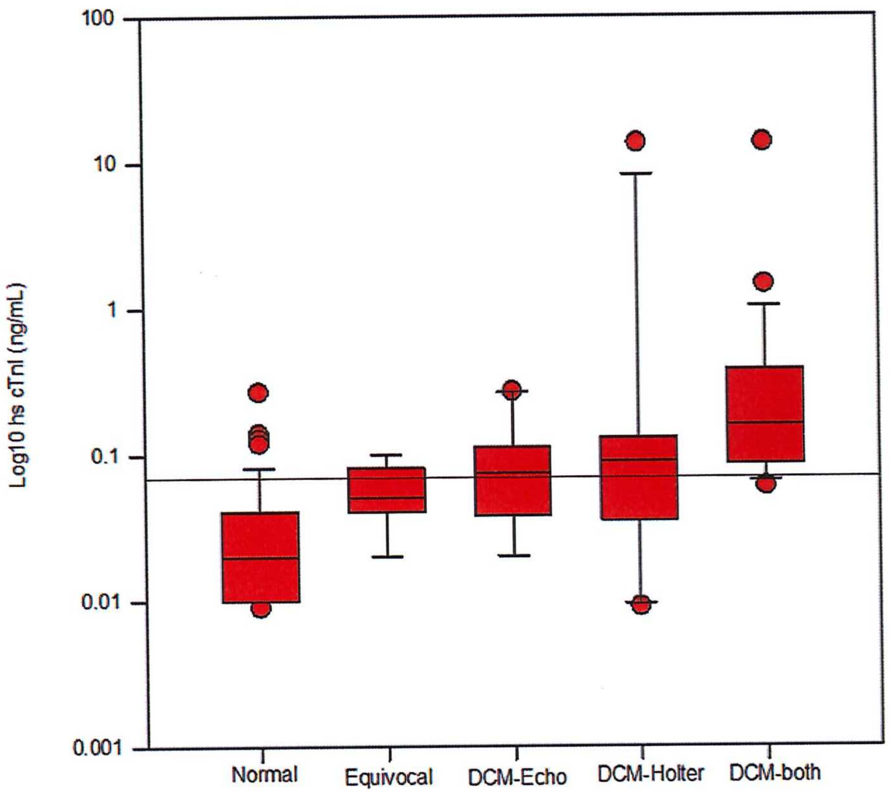
Abbreviations: hs cTnI: high sensitivity cardiac Troponin I, NTproBNP: N-terminal proBNP, Se: sensitivity, Sp: specificity, PPV: Positive predictive value, NPV: negative predictive value, LR+: positive likelihood ratio, LR-: negative likelihood ratio.

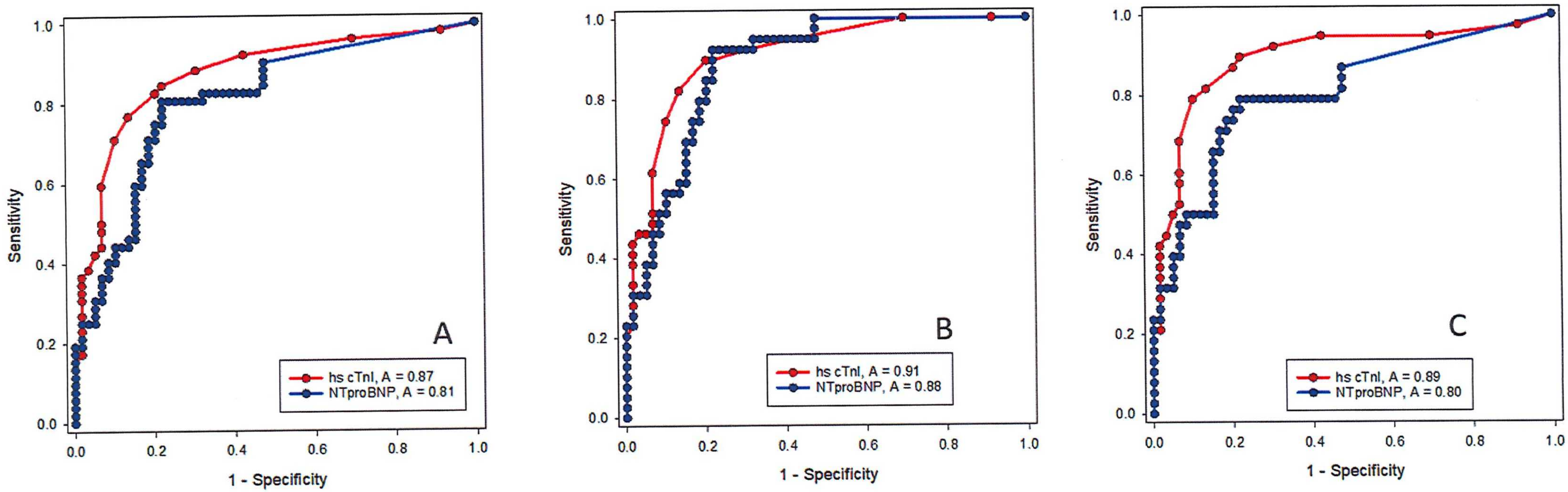
Data for all forms of DCM included. Prevalence in this population was 42.4%, used to calculate these variables. In bold, optimal operating point for cut-offs. Underlined: maximum specificity and positive predictive value where data were available for all columns of this table. Italics: maximum negative predictive value.

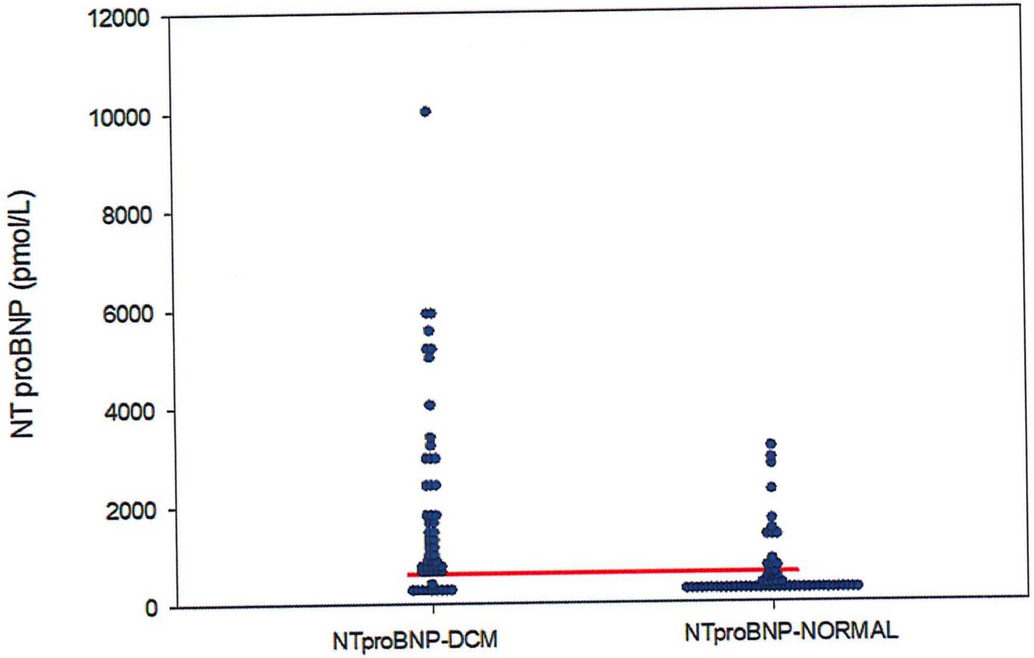


Number screened by cardiac biomarker tests (Jan 2015 – Jan 2017) N=197
Cases or private screenings N=39
Selected for Echo / Holter Screening N=80
No further assessment N=118
Sudden death N=1
Total Dobermanns included N=118
Changed group prior to final diagnosis N=17
Interim Normal N=10
Interim Equivocal N=4
Interim Abnormal Holter N=3
N=2 (1 echo, 1 Holter)
N=2
Normal (final diagnosis): N=59
Equivocal (final diagnosis): N=9
N=1
N=3
N=1
N=4
N=2
N=3
DCM (Echo & Holter) N=25
DCM (Echo) N=13
DCM (Holter) N=12
N=1
N=2
DCM (all forms) (final diagnosis) N= 50

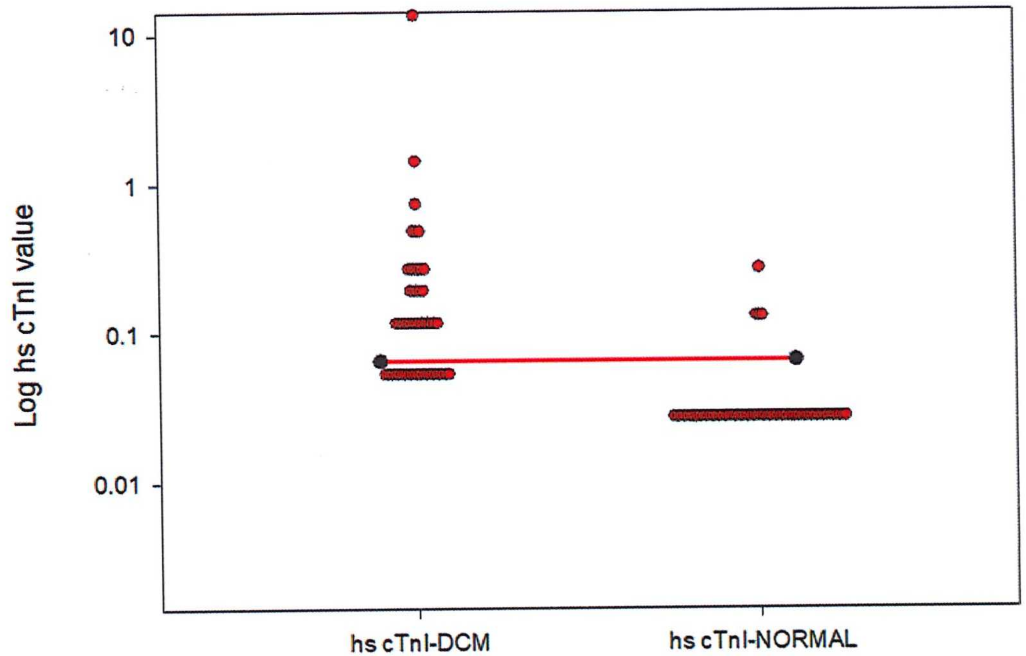

Log10 NTproBNP (pmol/L)
10000
1000
100
Normal
Equivocal
DCM-Echo
DCM-Holter
DCM-both


Log10 hs cTnI (ng/mL)
100
10
1
0.1
0.01
0.001
Normal
Equivocal
DCM-Echo
DCM-Holter
DCM-both


Sensitivity
1 - Specificity
A
hs cTnI, A = 0.87
NTproBNP, A = 0.81
B
hs cTnI, A = 0.91
NTproBNP, A = 0.88
C
hs cTnI, A = 0.89
NTproBNP, A = 0.80



Cutoff > 603 pmol/L.
Sens = 0.81
Spec = 0.78



Cut-off > 0.065 ng/mL
Sens = 0.77
Spec = 0.86