

Journal of Veterinary Cardiology

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

Manuscript Number:	JVC-D-22-00039R3
Article Type:	Original article
Keywords:	Echocardiography; Equivocal; Cardiac Biomarkers; survival; Sudden cardiac death
Corresponding Author:	Hannah Stephenson UNITED KINGDOM
First Author:	Samar El Sharkawy, PhD
Order of Authors:	Samar El Sharkawy, PhD Joanna Dukes-McEwan, PhD Hisham Abdelrahman, PhD Hannah Stephenson, BVMS
Abstract:	<p>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.</p>
Response to Reviewers:	

HS Cardiology Ltd

Dalton House

9 Dalton Square

Lancaster

LA1 1WD

hannah@hscardiology.co.uk

1st March 2023

Dr Michele Borgarelli and Dr Sonja Fonfara

Editors-in-Chief

Journal of Veterinary Cardiology

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

On behalf of the authors:

Samar El Sharkawy PhD ^{a,b} , Joanna Dukes-McEwan PhD^a, Hisham Abdelrahman PhD^c ,
Hannah Stephenson BVMS^{a,d}

^a Cardiology Service, Small Animal Teaching Hospital, Department of Small Animal Clinical Sciences, School of Veterinary Science, University of Liverpool, Leahurst Campus, Chester High Road, Neston, Cheshire CH64 7TE, UK.

^b Department of Surgery, Anaesthesiology, and Radiology, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt ^c Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt

^d HS Cardiology Ltd, Dalton House, 9 Dalton Square, Lancaster LA1 1WD

Corresponding author:

Hannah Stephenson Email: hannah@hscardiology.co.uk

1
2 **Long-term Outcome and Troponin I Concentrations in Great Danes**
3
4 **Screened for Dilated Cardiomyopathy: An Observational Retrospective**
5 **Epidemiological Study**
6
7
8
9

10
11
12
13
14 **AUTHORS**
15

16 Samar El Sharkawy PhD ^{a,b}

17
18
19
20 Joanna Dukes-McEwan PhD^a

21
22
23 Hisham Abdelrahman PhD^c

24
25
26 Hannah Stephenson BVMS^{a,d}

27
28
29 ^a Cardiology Service, Small Animal Teaching Hospital, Department of Small
30 Animal Clinical Sciences, School of Veterinary Science, University of Liverpool,
31 Leahurst Campus, Chester High Road, Neston, Cheshire CH64 7TE, UK.
32
33

34
35
36
37 ^b Department of Surgery, Anaesthesiology, and Radiology, Faculty of
38 Veterinary Medicine, Cairo University, Giza 12211, Egypt ^c Department of
39 Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo
40 University, Giza 12211, Egypt
41
42
43
44
45

46
47 ^d HS Cardiology Ltd, Dalton House, 9 Dalton Square, Lancaster LA1 1WD
48
49

50
51 Corresponding author:

52
53
54 Hannah Stephenson Email: hannah@hscardiology.co.uk
55
56
57
58
59
60
61
62
63
64
65

ABSTRACT

1
2 Introduction/Objectives: Dilated cardiomyopathy (DCM) is common in Great Danes
3 (GD) but screening for this condition can be challenging. We hypothesised that cardiac
4 troponin-I (cTnI) concentration is elevated in GD with DCM and/or ventricular
5 arrhythmias (VA), and is associated with reduced survival time in GD.
6
7

8
9
10
11
12 Animals: One hundred and twenty-four client-owned GD, assigned
13 echocardiographically as normal (n= 53), equivocal (n= 37), preclinical DCM (DCM-P)
14 (n= 21) or clinical DCM (DCM-C) (n= 13).
15
16
17

18
19 Methods: Retrospective epidemiological study. Echocardiographic diagnosis, VA and
20 contemporaneous cTnI concentrations were recorded. Diagnostic accuracy and cTnI
21 cut-offs were determined with receiver operating characteristic analyses. Effects of
22 cTnI concentration and disease status on survival and cause of death were explored.
23
24
25
26

27
28 Results: Median cTnI was greater in DCM-C (0.6 ng/mL [25th-75th percentiles: 0.41-
29 1.71 ng/mL]) and GD with VA (0.5 ng/mL [0.27- 0.80 ng/mL], $P < 0.001$). Elevated cTnI
30 detected these dogs with good accuracy (Area under the curve [AUC]: 0.78 – 0.85;
31 cut-offs 0.199 - 0.34 ng/mL). Thirty-eight GD (30.6%) suffered a cardiac death (CD);
32 GD suffering CD (0.25 ng/mL [0.21- 0.53 ng/mL]) and specifically sudden cardiac
33 death (SCD) (0.51 ng/mL [0.23- 0.72 ng/mL]) had higher cTnI than GD dying of other
34 causes (0.20 ng/mL [0.14- 0.35 ng/mL]; $P < 0.001$). Elevated cTnI (>0.199 ng/mL) was
35 associated with shorter long-term survival (1.25 years) and increased risk of SCD.
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

Conclusions: Cardiac troponin-I concentration is a useful adjunctive screening tool.
Elevated cTnI is a negative prognostic indicator.

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

10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

AUTHORS

6 Samar El Sharkawy PhD ^{a,b}

7 Joanna Dukes-McEwan PhD^a

8 Hisham Abdelrahman PhD^c

9 Hannah Stephenson BVMS^{a,d}

10 ^a Cardiology Service, Small Animal Teaching Hospital, Department of Small
11 Animal Clinical Sciences, School of Veterinary Science, University of Liverpool,
12 Leahurst Campus, Chester High Road, Neston, Cheshire CH64 7TE, UK.

13 ^b Department of Surgery, Anaesthesiology, and Radiology, Faculty of
14 Veterinary Medicine, Cairo University, Giza 12211, Egypt ^c Department of
15 Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo
16 University, Giza 12211, Egypt

17 ^d HS Cardiology Ltd, Dalton House, 9 Dalton Square, Lancaster LA1 1WD

18 Corresponding author:

19 Hannah Stephenson Email: hannah@hscardiology.co.uk

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

22 **Acknowledgements**

1
2 23 This study was generously supported by grants from the European Commission
3
4 24 (LUPA-GA 201270; the LUPA project), the Great Dane Breed Council (UK), and the
5
6 25 Kennel Club Charitable Trust (UK). In addition, it was supported by donations from
7
8
9 26 Great Dane owning private benefactors. The work would not have been possible
10
11 27 without the support and participation of Great Dane owners and their dogs. The authors
12
13
14 28 are especially grateful to Joan Toohey for her administration and coordination of the
15
16 29 project. Members of the cardiology service, past and present all participated in
17
18
19 30 generating the data and screening some of the Great Danes in this study, especially
20
21 31 Jordi Lopez-Alvarez, Chris Linney, and Brigitte Pedro.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

32 ABSTRACT

1 33 Introduction/Objectives: Dilated cardiomyopathy (DCM) is common in Great Danes
2
3 34 (GD) but screening for this condition can be challenging. We hypothesised that cardiac
4
5 35 troponin-I (cTnI) concentration is elevated in GD with DCM and/or ventricular
6
7
8 36 arrhythmias (VA), and is associated with reduced survival time in GD.

10 37 Animals: One hundred and twenty-four client-owned GD, assigned
11
12 38 echocardiographically as normal (n= 53), equivocal (n= 37), preclinical DCM (DCM-P)
13
14 39 (n= 21) or clinical DCM (DCM-C) (n= 13).

17 40 Methods: Retrospective epidemiological study. Echocardiographic diagnosis, VA and
18
19
20 41 contemporaneous cTnI concentrations were recorded. Diagnostic accuracy and cTnI
21
22 42 cut-offs were determined with receiver operating characteristic analyses. Effects of
23
24 43 cTnI concentration and disease status on survival and cause of death were explored.

27 44 Results: Median cTnI was greater in DCM-C (0.6 ng/mL [25th-75th percentiles: 0.41-
28
29 45 1.71 ng/mL]) and GD with VA (0.5 ng/mL [0.27- 0.80 ng/mL], P < 0.001). Elevated cTnI
30
31 46 detected these dogs with good accuracy (Area under the curve [AUC]: 0.78 – 0.85;
32
33 47 cut-offs 0.199 - 0.34 ng/mL). Thirty-eight GD (30.6%) suffered a cardiac death (CD);
34
35 48 GD suffering CD (0.25 ng/mL [0.21- 0.53 ng/mL]) and specifically sudden cardiac death
36
37 49 (SCD) (0.51 ng/mL [0.23- 0.72 ng/mL]) had higher cTnI than GD dying of other causes
38
39 50 (0.20 ng/mL [0.14- 0.35 ng/mL]; P < 0.001). Elevated cTnI (>0.199 ng/mL) was
40
41 51 associated with shorter long-term survival (1.25 years) and increased risk of SCD.
42
43 52 Great Danes with VA had shorter survival times (0.97 years).

46 53 Conclusions: Cardiac troponin-I concentration is a useful adjunctive screening tool.
47
48 54 Elevated cTnI is a negative prognostic indicator.

54 55
55
56 56 Key words: Echocardiography; Equivocal; Cardiac Biomarkers; Survival; Sudden
57
58 57 cardiac death.

58 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
-----	-------------------------------

1
2 59
3
4 60
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

61 INTRODUCTION

1 62 Dilated cardiomyopathy (DCM) is the second most common cardiac disease in dogs
2
3 63 [1], causing significant morbidity and mortality in large to giant breed dogs [1-5]. The
4
5 64 reported prevalence of DCM in great Danes (GD) in prospective screening studies
6
7
8 65 ranged from 11.8 to 35.6% [5, 6]. Dilated cardiomyopathy is usually an adult-onset
9
10 66 acquired disease and often has a prolonged preclinical phase, where dogs show no
11
12 67 clinical signs of cardiac disease. This provides a particular problem in breeding
13
14 68 populations, where dogs may develop acquired disease after producing progeny.

15
16
17
18 69 Once the disease becomes apparent, GD are expected to have a lower median
19
20 70 survival time than the other breeds [2]. In addition, unreported sudden cardiac deaths
21
22 71 (SCD) in GD were found to occur in a large proportion of sires and dams at early ages
23
24
25 72 [5]. Despite the known high prevalence in the breed, data describing longitudinal
26
27 73 progression by serial examinations in GD is lacking [5, 6]. In addition, long-term follow-
28
29 74 up of GD screened for DCM has not been described, or examined for any associations
30
31 75 with echocardiographic, other clinical criteria, or clinical pathology data.

32
33
34
35 76 Primary DCM is suspected to be hereditary and has been found to be associated with
36
37 77 the presence of genetic loci in a number of breeds [1]. A genome-wide study on GD
38
39 78 and Newfoundland dogs identified the association between DCM and the presence of
40
41 79 different genetic loci indicating different genetic risk factors [7], but currently, there is
42
43 80 no specific genetic marker for DCM in GD which might identify dogs at risk of
44
45 81 developing DCM later in life.

46
47
48
49
50
51 82 In the absence of an accurate genetic test, early monitoring of GD and other breeds
52
53 83 with familial DCM has been advocated using echocardiographic and
54
55 84 electrocardiographic (ECG) screening to detect the asymptomatic stage [4, 8]. Early
56
57 85 detection of preclinical DCM by echocardiography (ECHO) may provide an opportunity
58
59 86 to initiate medical therapy to delay the onset of congestive heart failure, in addition to
60
61
62
63
64
65

87 excluding the affected dogs from breeding programs [2-4, 8]. In other breeds with a
1 88 high risk of SCD, Holter monitoring is also a recommended screening tool for
2
3 89 ventricular arrhythmias (VA) [4].
4
5

6 90 Cardiac troponin I (cTnI) is a specific marker of recent or ongoing myocardial injury [9,
7
8 91 10]. It is an intracellular protein, so it is not usually released into the circulation in high
9
10 92 concentrations [10]. Concentrations of cTnI have been found to increase with
11
12 93 myocardial injury in non-cardiac diseases [11-15] as well as in canine mitral valve
13
14 94 disease [16, 17] and DCM [18]. Shorter survival times with increased cTnI
15
16 95 concentrations were also found in dogs with DCM and other acquired cardiac diseases
17
18 96 [16, 19, 20]. Large studies investigating the diagnostic utility of cTnI in the GD are
19
20 97 lacking. One small study has previously reported that cardiac biomarkers, including
21
22 98 high sensitivity cTnI, cannot be used as a standalone test for DCM in the breed [21].
23
24 99 Despite the high prevalence of DCM in GD and a suggested increased risk of SCD,
25
26 100 little is known about disease outcomes or causes of death in this breed [5, 22].
27
28
29
30
31
32

33
34 101 We hypothesised that cTnI would be significantly higher in GD with DCM and those
35
36 102 with VA, than those without, and that cTnI levels would be negatively associated with
37
38 103 survival time. The objectives of this observational retrospective epidemiological study
39
40 104 were: (i) to investigate the ability of the cTnI to predict echocardiographic evidence of
41
42 105 DCM or presence of VA in GD (ii) to describe causes of death in GD which had been
43
44 106 screened for DCM at least once and (iii) to explore any association between cTnI
45
46 107 concentration at the time of screening and time to death.
47
48
49
50

51 108

52 109 ANIMALS, MATERIALS, AND METHODS

53 110 **Study population**

54
55
56 111 Medical records were retrieved for all GD that had cardiac examinations at the Small
57
58 112 Animal Teaching Hospital, University of Liverpool including ECHO performed between
59
60
61
62
63
64
65

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

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

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

135 **Cardiovascular examination**

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

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

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

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

162 examinations, the data and score from the most recent examination with
1 163 contemporaneous Immulite cTnI concentration were used in the analysis.
2
3

4 164 In addition to ECG monitoring during ECHO, some but not all GD had a standard six-
5
6
7 165 lead ECG and/or 24-hour ambulatory ECG examination (Holter), at the clinician's
8
9 166 discretion. If the results of ECG were recorded in the notes, GD were classified as
10
11
12 167 suffering from VA or not. If only an in-house ECG had been recorded, at least one
13
14 168 ventricular premature complex (VPC) during the examination was interpreted as
15
16 169 suffering from VA. If a Holter had been performed, then >100 VPC/24 h was also
17
18
19 170 considered as suffering from VA [26]. We also included GD with >20 VPC/24 h plus
20
21 171 evidence of complexity (presence of couplets, triplets and/or ventricular tachycardia)
22
23
24 172 in the same group. Any other arrhythmia noted during these examinations were
25
26 173 recorded.
27
28

30 174 **Laboratory analysis**

31
32

33 175 For comparability and accuracy, all heparinised samples submitted for cTnI analysis
34
35 176 used the same analyser^e [23] on the same day as the echocardiographic assessment.
36
37
38 177 Concentrations of cTnI <0.15 ng/mL were considered normal [7, 27]. The limit of
39
40
41 178 detection for the analyser was changed from 0.2 ng/mL to 0 to enable recording of low
42
43 179 concentrations of cTnI, while appreciating that the precision of the assay at low
44
45 180 concentrations is less satisfactory [23]. In animals that were re-presented to the
46
47
48 181 cardiology service for follow-up, only the final cTnI concentrations obtained
49
50 182 contemporaneously with ECHO were used for this study.
51
52
53

54 183 **Classification of death (survival data)**

55
56

57 184 Survival (follow-up outcome) data and causes of death were retrieved from the medical
58
59
60 185 records or after seeking information from referring veterinarians or the owners of each
61
62 186 GD. Classification of the death causes was based on the judgement of the attending
63
64
65

187 veterinarian and by personal communication with owners. Only a small minority of GD
1 188 underwent post-mortem examination.

2
3
4 189 In deceased GD, the death was then classified as either cardiac or non-cardiac, and
5
6 190 further subclassified into a total of five categories. Deaths were classified as SCD when
7
8
9 191 deaths were unexpected and not preceded by any other clinical signs such as
10
11 192 dyspnoea. If GD were diagnosed with DCM prior to death and had evidence of
12
13 193 congestive heart failure signs, the death was classified as a natural cardiac death (CD),
14
15 194 or if the patient was euthanised, as euthanasia due to cardiac disease (EU-CD). If the
16
17 195 death was associated with signs of other systemic conditions, with no evidence of
18
19 196 congestive heart failure signs, the death was considered non-cardiac either due to
20
21 197 natural causes (NC) or euthanasia (EU-NC). Animals that were alive at the end of the
22
23 198 study were noted and censored. If information could not be retrieved after attempts to
24
25 199 contact the owner or primary veterinarian, these were lost to follow-up and also
26
27 200 censored from the time of last contact.
28
29
30
31
32
33

34 201 **Statistical analyses**

35
36
37
38 202 One-way analysis of variance tests (ANOVA) were used to compare body weight, age
39
40 203 at the most recent admission, cTnI concentrations, and ECHO parameters' values
41
42 204 among ECHO groups, survival status, and causes of death. Levene's test was used to
43
44 205 evaluate the homogeneity of variances (homoscedasticity), and the Shapiro–Wilk test
45
46 206 was utilized for normality analysis of the variables. The data that were not normally
47
48 207 distributed or violated homogeneity of variance assumption were analysed with the
49
50 208 Kruskal-Wallis test. The Tukey's Studentized Range (HSD) test was used for post-hoc
51
52 209 analysis. If two groups were compared, the Mann-Whitney U test was used for non-
53
54 210 parametric analysis. All P values less than 0.05 were considered statistically significant
55
56
57
58 211 h.
59
60
61
62
63
64
65

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

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

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

226 RESULTS

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

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

236 NORM and EQUIV dogs, whereas the fractional shortening and the sphericity index
1 237 were significantly lower in DCM-P and DCM-C dogs ($P < 0.001$).
2
3

4 238 The results of ECG assessment were recorded in 114 GD. Of these GD, 83 had
5
6
7 239 undergone Holter analysis, with the rest having only an in-house ECG recording.
8
9 240 Thirty-six GD (31.5%) were classified as affected by VA. Eight GD had atrial fibrillation
10
11 241 (AF), with AF cases in each echo group (these data are available in Supplementary
12
13
14 242 Table B online). Other arrhythmias included supraventricular premature complexes,
15
16 243 non-sustained supraventricular tachycardia and first degree atrioventricular block.
17
18
19

20 244 Eighty-six (69%) GD had cTnI above the reference interval of 0.15 ng/mL. Cardiac
21
22 245 troponin I level was significantly higher in DCM-C dogs than those in other groups (P
23
24
25 246 < 0.001), while there was no significant difference in cTnI between NORM, EQUIV,
26
27 247 and DCM-P dogs (Table 1; Fig.1A). Median cTnI levels were also significantly higher
28
29
30 248 in dogs with VA (0.50 ng/mL [25th–75th percentiles: 0.27–0.80 ng/mL]) than those who
31
32 249 had no VA (0.19 ng/mL [0.09–0.30 ng/mL]; $P < 0.001$; Fig. 1B).
33
34
35

36 250 Receiver operator characteristic curve analysis showed that in general, cTnI performed
37
38 251 with good accuracy to differentiate different ECHO groups, performing best to
39
40
41 252 differentiate NORM from DCM-P (Area under the curve [AUC] 0.78; 95% confidence
42
43 253 interval [CI]: 0.68–0.89; $P < 0.001$) and NORM from DCM-C dogs (AUC 0.85; 95% CI:
44
45 254 0.73–0.97; $P < 0.001$). When the groups were combined, cTnI was able to differentiate
46
47
48 255 between all GD with DCM (DCM-C + DCM-P) from all GD without DCM (NORM +
49
50 256 EQUIV) with good accuracy (AUC 0.80; 95% CI: 0.72–0.88; $P < 0.001$). A cTnI
51
52
53 257 concentration of 0.199 ng/mL yielded the highest Youden index (146.1) at a sensitivity
54
55 258 of 88.2% and a specificity of 58.9% (Fig. 2A).
56
57
58

59 259 Cardiac troponin I also differentiated dogs with VA on ECG from those without
60
61 260 documented VA with good accuracy (AUC 0.82; 95% CI: 0.74–0.91; $P < 0.001$). A cTnI
62
63
64
65

261 concentration of 0.34 ng/mL yielded the highest Youden index (151.78) at a sensitivity
1 262 of 69% and a specificity of 83% (Fig. 2B).

2
3
4 263 At the time of writing, eight GD were lost to follow up, seven GD were alive and 109
5
6 264 GD had died (Table 1). Thirty-eight GD (30.6%) were determined to have died due to
7
8
9 265 cardiac disease (SCD, CD, and EU-CD). No GD with DCM were alive at the time of
10
11
12 266 analysis. Sudden cardiac death was common, with 53% of cardiac deaths being SCD.
13
14 267 Nineteen GD that had been classified as NORM or EQUIV on exam ultimately suffered
15
16 268 a cardiac death, with 12 (63%) of these being SCD.

17
18
19
20 269 Great Danes which died or were euthanised as a consequence of cardiac disease had
21
22 270 significantly higher cTnI concentration (0.44 ng/mL [25th–75th percentiles: 0.19–0.69
23
24
25 271 ng/mL]; $n = 38$) than those dogs that died from or were euthanised due to non-cardiac
26
27 272 causes (0.20 ng/mL [25th–75th percentiles: 0.14–0.35 ng/mL]; $n = 71$; $P < 0.001$; Table
28
29
30 273 1). In addition, cTnI was also significantly higher in all GD suffering SCD (0.51 ng/mL
31
32 274 [25th–75th percentiles: 0.23–0.72 ng/mL]; $n = 20$) than those dying from or euthanised
33
34
35 275 for non-cardiac causes ($P < 0.001$; Table 1; Fig.3A). Considering only NORM/EQUIV
36
37 276 dogs, median cTnI concentration of GD suffering SCD (0.33 ng/mL [25th–75th
38
39 277 percentiles: 0.20–0.52 ng/mL]; $n = 12$) was significantly higher ($P = 0.026$) than that of
40
41
42 278 GDs dying of non-cardiac causes (0.20 ng/mL [25th–75th percentiles: 0.10–0.31
43
44 279 ng/mL]; $n = 57$; Fig. 3B).

45
46
47
48 280 Median survival times for dogs in this study are summarised in table 2. Median T_{cTnI}
49
50 281 was significantly different among ECHO groups ($P < 0.001$). Kaplan-Meier survival
51
52
53 282 analysis showed that GD with cTnI level lower than 0.199 ng/mL had significantly
54
55 283 longer T_{cTnI} (3.15 years [95% CI: 2.26–3.29 years]; $n = 57$; $P = 0.001$) than GD with
56
57
58 284 cTnI level higher than this cutoff (1.25 years [95% CI: 1.37–2.23 years]; $n = 67$; Fig.
59
60 285 4A). In addition, GD without VA had significantly longer T_{cTnI} (2.44 years; 25th–75th

286 percentiles: 0.76–3.94 years; $n = 78$; $P = 0.002$) than those GD exhibiting VA (0.97
1 287 years [25th–75th percentiles: 0.36–3.06 years]; $n = 36$; Fig. 4B).

288 Cox proportional hazards analysis showed a significant effect of cTnI concentration on
289 survival time, with each 0.1 ng/mL increase in cTnI, the hazard rate increased by 14%
290 (95% CI: 9.7–18.6%; Wald $\chi^2_{(1)} = 43.07$, $P < 0.001$). No association was identified
291 between presence of VA and SCD (Logistic regression: $\chi^2_{(1)} = 2.85$, $P = 0.091$); 95%
292 CI for the odds ratio = 0.16–1.15. There was a significant positive relationship between
293 cTnI level and probability of suffering SCD (Logistic regression: $\chi^2_{(1)} = 0.93$; $P = 0.032$;
294 Fig. 5). The following equation developed from binary logistic regression analysis can
295 be used to estimate probability of SCD at any cTnI level (within the studied range [0 to
296 3.27 ng/mL]):

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

297

298 DISCUSSION

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

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

308 This study shows that cTnI is moderately accurate in determining if a GD has
1 309 echocardiographic evidence of DCM. A cTnI concentration of 0.199 ng/mL detected
2
3 310 dogs with DCM with a sensitivity of 88.2% and a specificity of 58.9%. At the currently
4
5 311 cited reference range of <0.15 ng/mL for this Immulite assay, it showed 97% sensitivity
6
7
8 312 and 42% specificity. Great Danes with VA in our study also had higher levels of cTnI.
9
10 313 A cTnI concentration of 0.34 ng/mL detected dogs with VA with a sensitivity of 69%
11
12 314 and a specificity of 83%. At the currently cited reference range of <0.15 ng/mL for this
13
14 315 Immulite assay, it showed 97% sensitivity and 36% specificity. Similar findings were
15
16 316 reported in a study of cTnI in Dobermanns [18].
17
18
19
20

21 317 Sixty-nine percent of GD in this study had elevated cTnI based on the reference
22
23 318 interval for the assay used (0.15 ng/mL). Elevated cTnI has been reported in dogs with
24
25 319 systemic disease [15, 28], azotaemia [15], gastric dilatation-volvulus [13], snake-bite
26
27 320 envenomation [14], Ehrlichiosis [12] and pyometra [11]. We excluded any dog
28
29 321 identified as having systemic disease at the time of examination, but we cannot
30
31 322 completely exclude that some patients were suffering from other conditions that might
32
33 323 affect their cTnI concentration. It is also possible that there is some breed variation in
34
35 324 circulating cTnI levels, as suggested in other breeds [29, 30] and indeed a higher
36
37 325 normal cTnI level has been reported for this breed in a previous study albeit using a
38
39 326 different assay [21]. Alternatively, some of these GD may have had early myocardial
40
41 327 disease that was not detectable on ECHO; this has been reported in Dobermanns,
42
43 328 where incipient DCM was identified by cTnI assays [18, 28].
44
45
46
47
48
49
50

51 329 Despite the low specificity of cTnI for detection of DCM and/or VA, an increased value
52
53 330 identifies a GD which benefits from a full veterinary health assessment. We propose
54
55 331 that cTnI has the potential for use as a first-line screening test in apparently healthy
56
57 332 GD, allowing owners to prioritise those GD for full screening.
58
59
60
61
62
63
64
65

333 Sudden cardiac death is common in dogs with DCM, particularly in Dobermanns,
1 334 where SCD is associated with increased heart size but also the presence of malignant
2
3 335 VA [31]. In GD, sudden death is also reported in populations affected by DCM [5]. Early
4
5 336 identification of affected dogs might allow anti-arrhythmic treatment to be initiated and
6
7
8 337 prevent SCD in some cases [32].
9

10
11 338 Sudden death was common in our population of GD, with 16% of all GD suffering SCD,
12
13
14 339 accounting for 53% of all cardiac deaths. Interestingly, some dogs determined as
15
16 340 NORM or EQUIV by ECHO also suffered cardiac deaths, with 63% of these being
17
18
19 341 SCD. This is in keeping with the natural history of DCM in other breeds such as
20
21 342 Dobermanns, where SCD can occur prior to any echocardiographic changes [31].
22
23

24
25 343 Importantly, GD suffering SCD had higher concentrations of cTnI in this study. Even
26
27 344 GD without DCM on ECHO (NORM+EQUIV) that suffered later SCD had higher cTnI
28
29
30 345 levels than other GD in these ECHO groups. Probability of SCD was shown to increase
31
32 346 with increasing cTnI concentration. These findings are similar to reported increases in
33
34
35 347 cTnI in Dobermanns suffering SCD [31]. Great Danes with VA identified on ECG also
36
37 348 had shorter survival times.
38
39

40 349 Sudden cardiac death in people is commonly associated with VA resulting in cardiac
41
42
43 350 arrest [33]. We speculated that there might be an association between presence of VA
44
45 351 and SCD in our population of GD, but no statistically significant association was
46
47
48 352 identified. Significant VA have been documented in dogs with high levels of cTnI
49
50 353 associated with other diseases such as envenomation [14] illustrating the importance
51
52
53 354 of excluding other potential causes. We may have incorrectly classified some GD as
54
55 355 not all dogs had Holter monitors fitted, and there is known to be day-to-day variation in
56
57
58 356 VA on Holter.
59
60
61
62
63
64
65

357 Although our study does not conclusively demonstrate that VA are the cause of SCD
1 358 in GD, this study confirms the importance of Holter screening of GD, regardless of
2
3 359 ECHO findings, to identify GD which may require antiarrhythmic medication and to
4
5
6 360 identify adverse prognostic indicators.
7
8

9 361 Atrial fibrillation is reported to be one of the most common arrhythmias in the GD
10
11 362 population [5], however, it was detected in only eight GD in this study. Interestingly,
12
13
14 363 NORM dogs with AF had normal cTnl levels, whereas the EQUIV dog with AF had
15
16 364 elevated cTnl. The influence of the presence of AF on cTnl values could not be
17
18
19 365 established due to the small numbers affected. Future studies would be useful to
20
21 366 investigate any correlation.
22
23
24

25 367 This study has demonstrated the prognostic significance of cTnl in this population of
26
27 368 GD, with higher levels of cTnl increasing the likelihood of death of any cause, and also
28
29
30 369 increasing risk of SCD. This is in keeping with other studies investigating levels of cTnl
31
32 370 in cardiac [16, 33] and non-cardiac disease [34]. Higher cTnl also showed higher
33
34
35 371 probability for fatal outcomes in our GD population with VA.
36
37

38 372 The retrospective nature of this study results in some limitations. Many of the GD in
39
40 373 this study were not examined on multiple occasions, which may have resulted in
41
42
43 374 incorrect classification of dogs with early myocardial disease. Due to low numbers of
44
45 375 repeat examinations, and a change at our center to the use of a high sensitivity cTnl
46
47
48 376 assay, only one time point was used for analysis of cTnl in these dogs, and therefore
49
50 377 the significance of changes in cTnl could not be evaluated. Further studies would be
51
52
53 378 useful in assessing the prognostic significance of changes in cTnl over time.
54
55

56 379 This study did not utilise high sensitivity troponin assays, as this was not widely
57
58
59 380 available early in the study period. High sensitivity assays have been shown to be more
60
61 381 accurate in detection of DCM with or without VA in dogs, with improved sensitivity
62
63
64
65

382 particularly in the early stages of the disease [28]. Use of a high sensitivity assay may
1 383 therefore have improved early detection of DCM-P dogs or dogs with VA. Further
2
3 384 studies into the utility of high sensitivity assays and Holter monitoring in GD are
4
5
6 385 warranted.

9 386 Classification of GD with VA was challenging. Although >100 VPC/24 h is generally
10
11
12 387 considered to be abnormal in dogs [26], the complexity of the arrhythmia is also
13
14 388 considered important [4, 35]. Our data suggests that most normal GD have <20
15
16
17 389 VPC/24 hours (unpublished data) and therefore we also considered GD with >20
18
19 390 VPC/24 hour, plus evidence of complexity, as affected by VA.

22 391 Holter monitoring was not performed in all animals, and therefore this may have led to
23
24
25 392 incorrect classification of dogs as either normal or affected by VA. For example, a
26
27 393 single VPC detected during ECHO might be associated with increased circulating
28
29
30 394 catecholamines. Nevertheless, detection of at least one VPC on a five-minute ECG
31
32 395 has been shown to have a high positive predictive value for Holter abnormalities in
33
34
35 396 Dobermanns [36].

38 397 **Conclusions**

42 398 The current data suggest that cTnI could be a useful adjunctive screening test for DCM
43
44 399 and VA in GD. Great Danes with DCM and/or VA have higher concentrations of cTnI.
45
46
47 400 Circulating levels of cTnI have prognostic significance in this population, with higher
48
49 401 levels seen in patients later suffering SCD.

402 References

- 1
2 403 [1] Dutton E, Lopez-Alvarez J. An update on canine cardiomyopathies - is it all in
3
4 404 the genes? J Small Anim Pract 2018; 59: 455-64.
5
6
7 405 [2] Martin MW, Stafford Johnson MJ, Strehlau G, King JN. Canine dilated
8
9
10 406 cardiomyopathy: a retrospective study of prognostic findings in 367 clinical
11
12 407 cases. J Small Anim Pract 2010; 51:428-36.
13
14
15 408 [3] Vollmar C, Vollmar A, Keene BW, Fox PR, Reese S, Kohn B. Dilated
16
17
18 409 cardiomyopathy in 151 Irish Wolfhounds: characteristic clinical findings, life
19
20 410 expectancy and causes of death. Vet J 2019; 245:15-21.
21
22
23 411 [4] Wess G, Domenech O, Dukes-McEwan J, Häggström J, Gordon S. European
24
25
26 412 Society of Veterinary Cardiology screening guidelines for dilated
27
28 413 cardiomyopathy in Doberman Pinschers. J Vet Cardiol 2017;19:405-15.
29
30
31 414 [5] Stephenson HM, Fonfara S, Lopez-Alvarez J, Cripps P, Dukes-McEwan J.
32
33
34 415 Screening for dilated cardiomyopathy in Great Danes in the United Kingdom. J
35
36 416 Vet Intern Med 2012; 26:1140-7.
37
38
39 417 [6] Tarducci A, Borgarelli M, Zanatta R, Cagnasso A. Asymptomatic dilated
40
41
42 418 cardiomyopathy in Great Danes: Clinical, electrocardiographic,
43
44 419 echocardiographic and echo-Doppler features. Vet Res Commun 2003; 27:799-
45
46 420 802.
47
48
49
50 421 [7] Björnerfeldt S, Höglund K, Meadows J, Tidholm A, Dukes-McEwan J,
51
52 422 Stephenson H, Meurs K, Kresken J-G, Oyama M, Lindblad-Toh K, Andersson
53
54 423 G, Häggström H. Identifying genes predisposing Great Danes and
55
56
57 424 Newfoundland dogs to Dilated Cardiomyopathy. In Proceedings of the
58
59 425 European Society of Human Genetics Meeting 2011; Amsterdam RAI.
60
61
62
63
64
65

- 426 [8] Summerfield NJ, Boswood A, O'Grady MR, Gordon SG, Dukes-McEwan J,
1 427 Oyama MA, Smith S, Patteson M, French AT, Culshaw GJ, Braz-Ruivo L,
2
3 428 Estrada A, O'Sullivan ML, Loureiro J, Willis R, Watson P. Efficacy of
4
5 429 pimobendan in the prevention of congestive heart failure or sudden death in
6
7
8 430 Doberman Pinschers with preclinical dilated cardiomyopathy (the PROTECT
9
10
11 431 Study). *J Vet Intern Med* 2012; 26:1337-49.
- 12
13
14 432 [9] Fonfara S, Loureiro J, Swift S, James R, Cripps P, Dukes-McEwan J. Cardiac
15
16 433 troponin I as a marker for severity and prognosis of cardiac disease in dogs. *Vet*
17
18
19 434 *J* 2010; 184:334-9.
- 20
21
22 435 [10] Langhorn R, Willesen JL. Cardiac troponins in dogs and cats. *J Vet Intern Med*
23
24 436 2016; 30:36-50.
- 25
26
27 437 [11] Hagman R, Lagerstedt AS, Fransson BA, Bergström A, Häggström J. Cardiac
28
29
30 438 troponin I levels in canine pyometra. *Acta Vet Scand* 2007; 49:1-8.
- 31
32
33 439 [12] Diniz PP, De Moraes HS, Breitschwerdt EB, Schwartz DS. Serum cardiac
34
35 440 troponin I concentration in dogs with ehrlichiosis. *J Vet Intern Med* 2008;
36
37
38 441 22:1136-43.
- 39
40
41 442 [13] Schober KE, Cornand C, Kirbach B, Aupperle H, Oechtering G. Serum cardiac
42
43 443 troponin I and cardiac troponin T concentrations in dogs with gastric dilatation-
44
45
46 444 volvulus. *J Am Vet Med Assoc* 2002; 221:381-8.
- 47
48
49 445 [14] Harjen HJ, Bjelland AA, Harris J, Grøn TK, Anfinson KP, Moldal ER, Rørtveit R.
50
51 446 Ambulatory electrocardiography and serum cardiac troponin I measurement in
52
53
54 447 21 dogs envenomated by the European adder (*Vipera berus*). *J Vet Intern Med*
55
56 448 2020; 34:1369-78.
- 57
58
59
60
61
62
63
64
65

- 449 [15] Porciello F, Rishniw M, Herndon WE, Biretoni F, Antognoni MT, Simpson KW.
1 450 Cardiac troponin I is elevated in dogs and cats with azotaemia renal failure and
2
3 451 in dogs with non-cardiac systemic disease. *Aust Vet J* 2008; 86:390-4
4
5
6 452 [16] Hezzell MJ, Boswood A, Chang YM, Moonarmart W, Souttar K, Elliott J. The
7
8
9 453 combined prognostic potential of serum high-sensitivity cardiac troponin I and
10
11 454 N-terminal pro-B-type natriuretic peptide concentrations in dogs with
12
13
14 455 degenerative mitral valve disease. *J Vet Intern Med* 2012; 26:302-11.
15
16
17 456 [17] Ljungvall I, Höglund K, Tidholm A, Olsen LH, Borgarelli M, Venge P, Häggström
18
19 457 J. Cardiac troponin I is associated with severity of myxomatous mitral valve
20
21
22 458 disease, age, and C-reactive protein in dogs. *J Vet Intern Med* 2010; 24:153-9.
23
24
25 459 [18] Wess G, Simak J, Mahling M, Hartmann K. Cardiac troponin I in Doberman
26
27 460 Pinschers with cardiomyopathy. *J Vet Intern Med* 2010; 24:843-9.
28
29
30
31 461 [19] Oyama MA, Sisson DD. Cardiac troponin-I concentration in dogs with cardiac
32
33 462 disease. *J Vet Intern Med* 2004;18: 831-9.
34
35
36 463 [20] Oyama MA, Sisson DD, Solter PF. Prospective screening for occult
37
38
39 464 cardiomyopathy in dogs by measurement of plasma atrial natriuretic peptide, B-
40
41 465 type natriuretic peptide, and cardiac troponin-I concentrations. *Am J Vet Res.*
42
43
44 466 2007; 68:42-7.
45
46
47 467 [21] Rosenthal SL, Tyrrell WD, Cahill R, Clark G, Buch JS. NT-proBNP and troponin I
48
49 468 levels as screening biomarkers in Great Danes. 2014 ACVIM Forum Research
50
51
52 469 Abstracts Program. *J Vet Intern Med* 2014; 28:1006.
53
54
55 470 [22] Dukes-McEwan J, Borgarelli M, Tidholm A, Vollmar AC, Häggström J, ESVC
56
57 471 Taskforce for Canine Dilated Cardiomyopathy. Proposed guidelines for the
58
59
60 472 diagnosis of canine idiopathic dilated cardiomyopathy. *J Vet Cardiol* 2003; 5:7-
61
62 473 19.
63
64
65

- 474 [23] Scharnhorst V, Vader HL, van der Graaf F. Characteristics of the cardiac
1 475 troponin I assay on the Immulite 2000 analyzer. Clin Chem 2002; 48:1626-7.
2
3
4 476 [24] Cornell CC, Kittleson MD, Torre PD, Häggström J, Lombard CW, Pedersen HD,
5
6 477 Vollmar A, Wey A. Allometric scaling of M-mode cardiac measurements in
7
8
9 478 normal adult dogs. J Vet Intern Med 2004;18:311-21.
10
11
12 479 [25] Brown DJ, Rush JE, MacGregor J, Ross Jr JN, Brewer B, Rand WM. M-mode
13
14 480 echocardiographic ratio indices in normal dogs, cats, and horses: a novel
15
16
17 481 quantitative method. J Vet Intern Med 2003;17:653-62.
18
19
20 482 [26] Wess G. Screening for dilated cardiomyopathy in dogs. J Vet Cardiol 2022;
21
22 483 40:51-68
23
24
25
26 484 [27] Spratt DP, Mellanby RJ, Drury N, Archer J. Cardiac troponin I: evaluation of a
27
28 485 biomarker for the diagnosis of heart disease in the dog. J Small Anim Pract
29
30
31 486 2005; 46:139-45.
32
33
34 487 [28] Klüser L, Maier ET, Wess G. Evaluation of a high-sensitivity cardiac troponin I
35
36 488 assay compared to a first-generation cardiac troponin I assay in Doberman
37
38
39 489 Pinschers with and without dilated cardiomyopathy. 2019; 33:54-63.
40
41
42 490 [29]. LaVecchio D, Marin LM, Baumwart R, Iazbik MC, Westendorf N, Couto CG.
43
44 491 Serum cardiac troponin I concentration in retired racing greyhounds. J Vet Intern
45
46
47 492 Med 2009; 23:87-90.
48
49
50 493 [30] Baumwart RD, Orvalho J, Meurs KM. Evaluation of serum cardiac troponin I
51
52 494 concentration in Boxers with arrhythmogenic right ventricular cardiomyopathy.
53
54
55 495 Am J Vet Res 2007; 68:524-528.
56
57
58
59
60
61
62
63
64
65

- 496 [31] Klüser L, Holler PJ, Simak J, Tater G, Smets P, Rügamer D, Küchenhoff H,
1 497 Wess G. Predictors of sudden cardiac death in Doberman pinschers with dilated
2
3 498 cardiomyopathy. J Vet Intern Med 2016; 30:722-32.
4
5
6 499 [32] Calvert CA, Brown J. Influence of antiarrhythmia therapy on survival times of 19
7
8 500 clinically healthy Doberman pinschers with dilated cardiomyopathy that
9
10 experienced syncope, ventricular tachycardia, and sudden death (1985-1998).
11 501 J Am Anim Hosp Assoc 2004; 40:24-8.
12
13
14 502
15
16 503 [33] Borgeat K, Sherwood K, Payne JR, Luis Fuentes V, Connolly DJ. Plasma
17
18 cardiac troponin I concentration and cardiac death in cats with hypertrophic
19 504 cardiomyopathy. J Vet Intern Med 2014; 28:1731-7.
20
21
22 505
23
24 506 [34] Langhorn R, Thawley V, Oyama MA, King LG, Machen MC, Trafny DJ, Willesen
25
26 JL, Tarnow I, Kjelgaard-Hansen M. Prediction of long-term outcome by
27 507 measurement of serum concentration of cardiac troponins in critically ill dogs
28
29 508 with systemic inflammation. J Vet Intern Med 2014; 28:1492-7.
30
31
32 509
33
34 510 [35] Meurs KM, Spier AW, Wright NA, Hamlin RL. Use of ambulatory
35
36 electrocardiography for detection of ventricular premature complexes in healthy
37 511 dogs. J Am Vet Med Assoc. 2001; 218:1291-1292.
38
39
40 512
41
42 513 [36] Wess G, Schulze A, Geraghty N, Hartmann K. Ability of a 5-minute
43
44 electrocardiography (ECG) for predicting arrhythmias in Doberman Pinschers
45 514 with cardiomyopathy in comparison with a 24-hour ambulatory ECG. J Vet
46
47 515 Intern Med 2010; 24:367-71.
48
49
50 516
51
52
53 517
54
55
56 518
57
58
59 519
60
61
62
63
64
65

520

1 521

- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10
- 11
- 12
- 13
- 14
- 15
- 16
- 17
- 18
- 19
- 20
- 21
- 22
- 23
- 24
- 25
- 26
- 27
- 28
- 29
- 30
- 31
- 32
- 33
- 34
- 35
- 36
- 37
- 38
- 39
- 40
- 41
- 42
- 43
- 44
- 45
- 46
- 47
- 48
- 49
- 50
- 51
- 52
- 53
- 54
- 55
- 56
- 57
- 58
- 59
- 60
- 61
- 62
- 63
- 64
- 65

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

Status	Cause	Median (25 th –75 th percentiles) of cTnl concentration (ng/mL)					
		ALL GD	ECHO group				
			Normal	EQUIV	DCM-P	DCM-C	
Overall	Total <i>N</i>	124	53	37	21	13	
	cTnl	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	<i>n</i> (%)	8 (6.5)	6 (11.3)	1 (2.7)	0 (0.0)	1 (7.7)	
	cTnl	0.11 ^b (0.03–0.18)	0.06 (0.02–0.15)	0.15	-	0.22	
Alive	<i>n</i> (%)	7 (5.6)	6 (11.3)	1 (2.7)	0 (0.0)	0 (0.0)	
	cTnl	0.10 ^b (0.05–0.12)	0.07 (0.05–0.10)	0.12	-	-	
Dead	<i>n</i> (%)	109 (87.9)	41 (77.4)	35 (94.6)	21 (100.0)	12 (92.3)	
	cTnl	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)	
	All	<i>n</i> (%)	38 (30.6)	8 (19.5)	11 (31.4)	10 (47.6)	9 (75.0)
		cTnl	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)
Card-iac	SCD	<i>n</i> (%)	20 (16.1)	5 (12.2)	7 (20.0)	6 (28.6)	2 (16.7)
		cTnl	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	<i>n</i> (%)	5 (4.0)	0 (0.0)	1 (2.9)	1 (4.8)	3 (25.0)
cTnl		0.25 ^{ab} (0.21–0.53)	-	0.21	0.25	0.53 (0.18–2.86)	
	EU-CD	<i>n</i> (%)	13 (10.5)	3 (7.3)	3 (8.6)	3 (14.3)	4 (33.3)
cTnl		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)	

		<i>n</i> (%)	71 (57.3)	33 (80.5)	24 (68.6)	11 (52.4)	3 (25.0)
	All	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)
		<i>n</i> (%)	21 (16.9)	9 (22.0)	6 (17.1)	4 (19.0)	2 (16.7)
	Non-cardiac	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)
		<i>n</i> (%)	50 (40.3)	24 (58.5)	18 (51.4)	7 (33.3)	1 (8.3)
	EU-NC	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.

525
526
527

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

Status	Cause	Total <i>N</i>	Time-to-death (or follow-up) after last cTnl analysis (years)				
			All GDs	ECHO group			
				Normal	EQUIV	DCM-P	DCM-C
Overall			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		<i>n</i> (%)	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		<i>n</i> (%)	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		<i>n</i> (%)	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	<i>n</i> (%)	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	<i>n</i> (%)	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	<i>n</i> (%)	13 (10.5)	3 (7.3)	3 (8.6)	3 (14.3)	4 (33.3)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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

^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. ^bActual 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.

532
533
534

535 Figure captions

1 536 **Figure 1.** Box-and-whisker plot showing the concentration of cTnI in [A] GD in each of
2
3
4 537 the four echocardiographic groups and [B] in GD with/without VA (all ECHO groups).
5
6 538 Within each box plot, horizontal line indicates the median, black circle indicates the
7
8
9 539 mean, and error bars around the circle represent standard error of the mean. Different
10
11 540 superscripts indicate statistically significant differences between groups.
12
13
14 541 cTnI: cardiac troponin I; DCM-C: clinical DCM; DCM-P: preclinical DCM; ECHO:
15
16 542 echocardiography; EQUIV: equivocal; GD: Great Danes; NORM: normal; VA:
17
18 543 Ventricular arrhythmias.

22 544 **Figure 2.** Receiver operator characteristic curves demonstrating diagnostic
23
24 545 performance of cTnI to differentiate between GD both (A) with echocardiographic
25
26 546 changes associated with DCM (DCM-P+ DCM-C) and those without DCM (NORM+
27
28
29 547 EQUIV) and (B) with VA noted on ECG and those showing no VA.
30
31
32 548 AUC: area under the curve; cTnI: cardiac troponin I; DCM-C: clinical DCM; DCM-P:
33
34 549 preclinical DCM; ECG: electrocardiogram; ECHO: echocardiography; EQUIV:
35
36 550 equivocal; GD: Great Danes; NORM: normal; VA: Ventricular arrhythmias.

40 551 **Figure 3.** Box and whisker plot showing the concentration of cTnI in [A] all GD suffering
41
42 552 sudden cardiac death (SCD) versus non-cardiac deaths (NC/EU-NC) and [B] only GD
43
44 553 without DCM [NORM + EQUIV] suffering SCD versus all non-cardiac deaths (NC/EU-
45
46
47 554 NC). Within each box plot, horizontal line indicates the median, black circle indicates
48
49
50 555 the mean, and error bars around the circle represent standard error of the mean. Within
51
52 556 each figure, different superscripts indicate statistically significant differences between
53
54
55 557 groups.

57 558

559 cTnI: cardiac troponin I; ECHO: echocardiography; EQUIV: equivocal; EU-NC:
1 560 euthanasia due to non-cardiac causes; GD: Great Danes; NC: non-cardiac death;
2
3 561 NORM: normal; SCD: sudden cardiac death.
4
5

6
7 562 **Figure 4.** Kaplan Meier curve comparing the time-to-death after last cTnI analysis (in
8
9 563 years) [A] according to the receiver operating characteristic calculated cTnI cut-off
10
11 564 value of 0.199 ng/mL in all GD and [B] in GD with VA recorded on ECG versus GD
12
13
14 565 without detected VA.
15

16
17 566 cTnI: cardiac troponin I; ECG: electrocardiogram; GD: Great Danes; VA: Ventricular
18
19 567 arrhythmias.
20
21

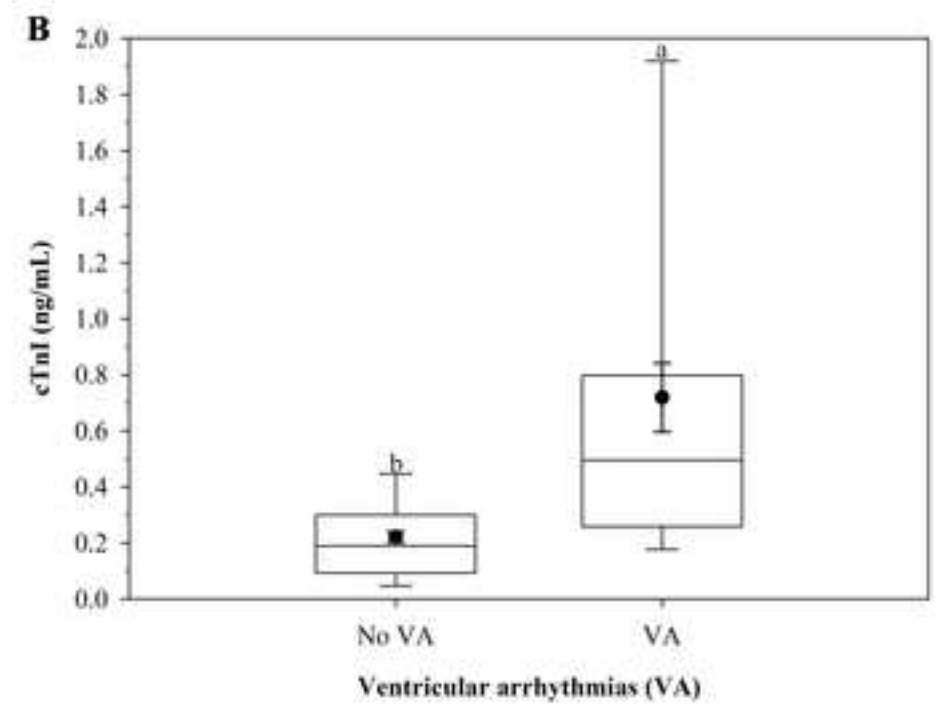
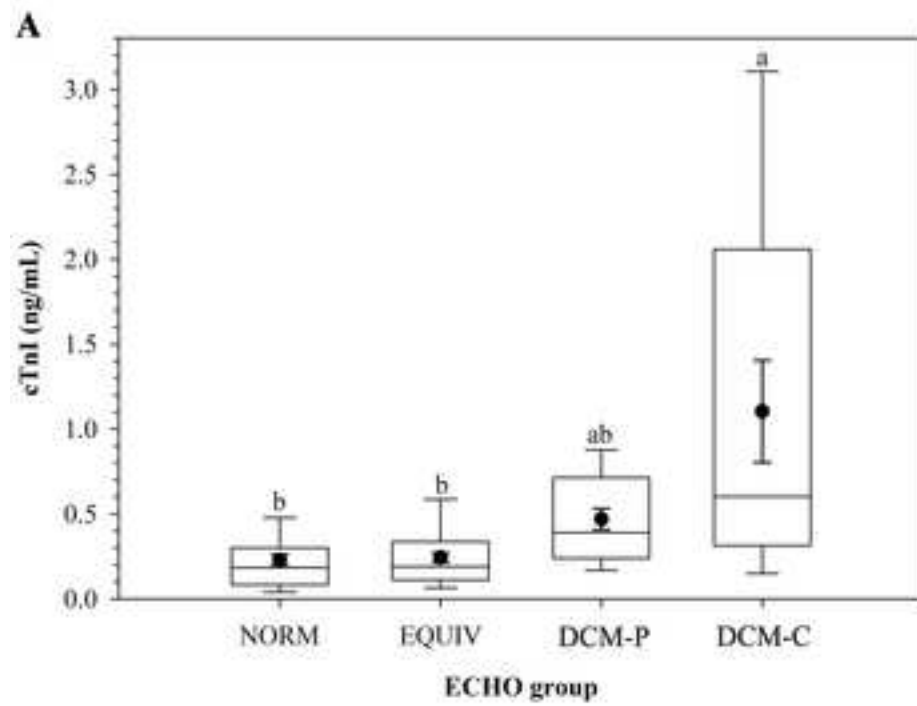
22 568 **Figure 5.** Predicted probability and 95% CI from a statistically significant ($P = 0.032$)
23
24
25 569 binary logistic regression model investigating the effect of cTnI concentration on the
26
27 570 probability of GD suffering SCD.
28

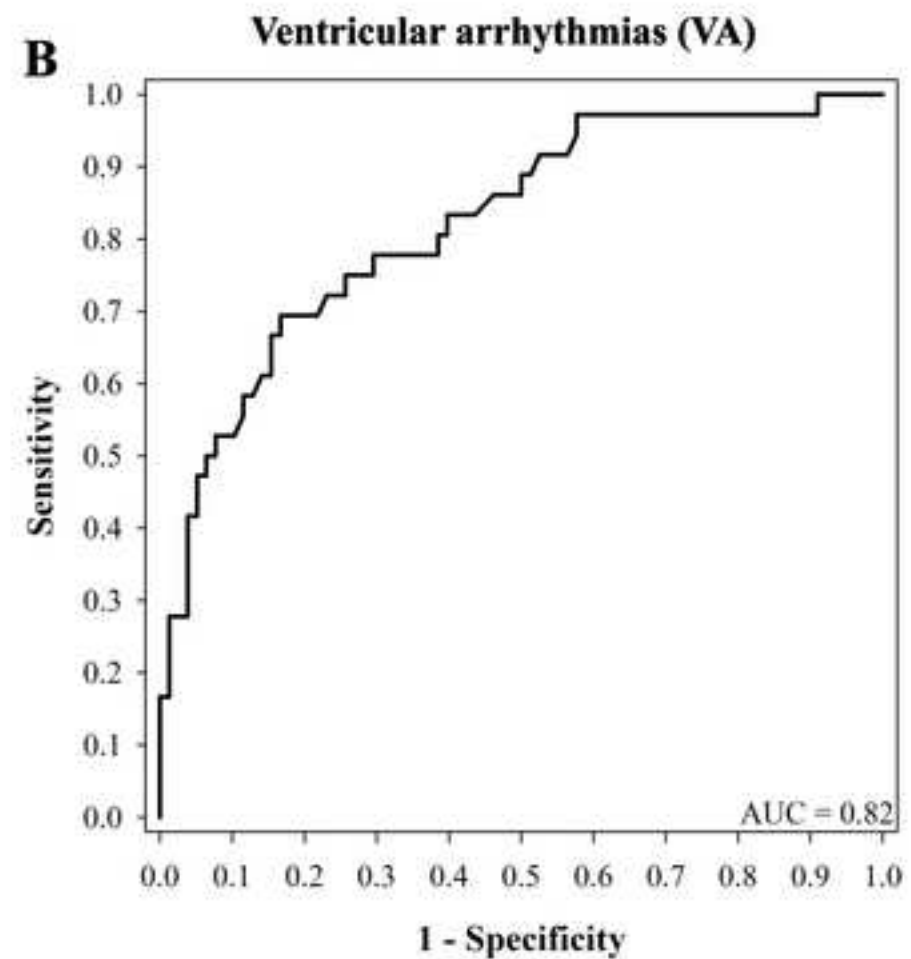
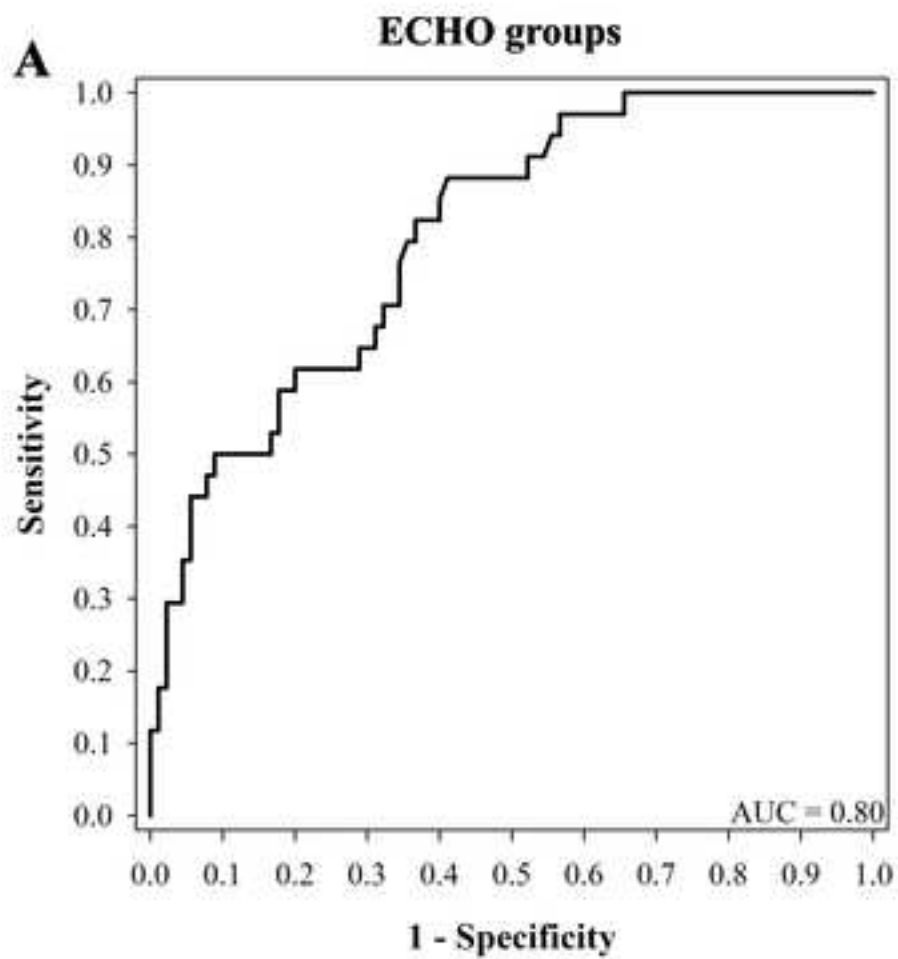
29
30 571 CI: confidence intervals; cTnI: cardiac troponin I; GD: Great Danes; SCD: sudden
31
32 572 cardiac death.
33
34

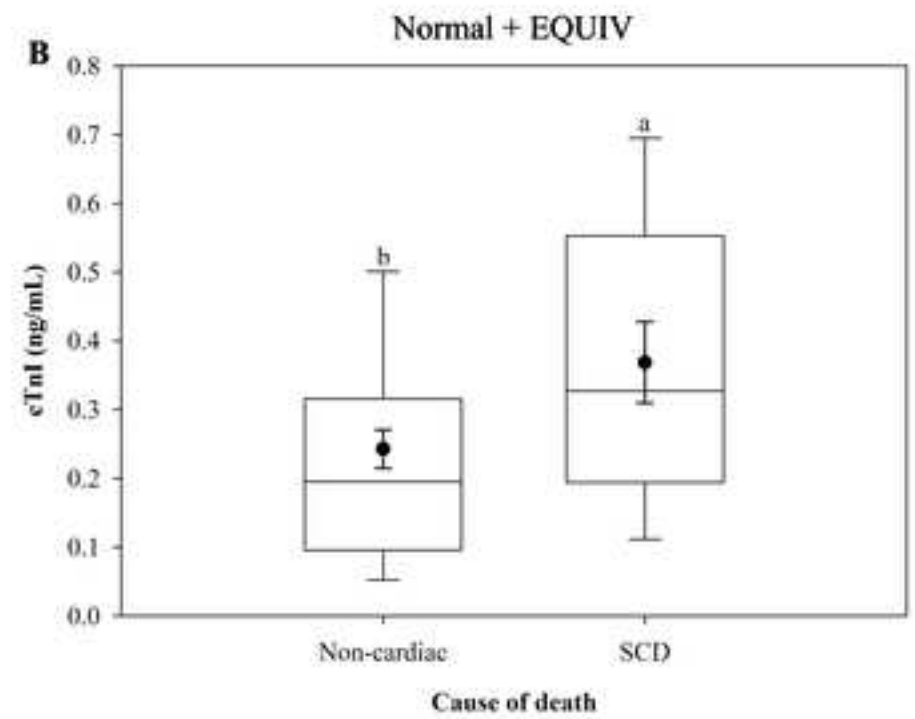
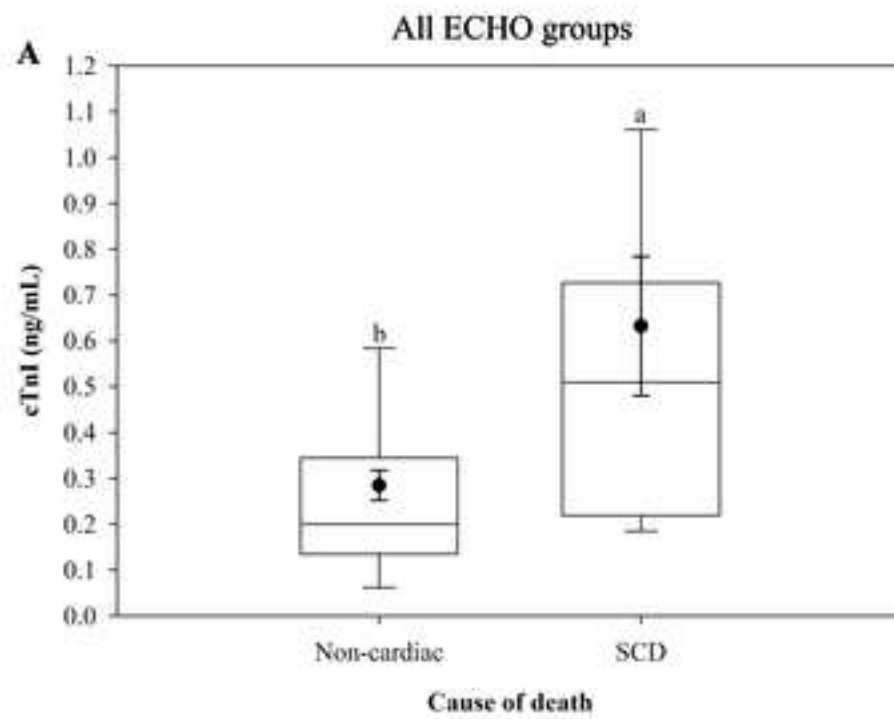
35
36 573
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

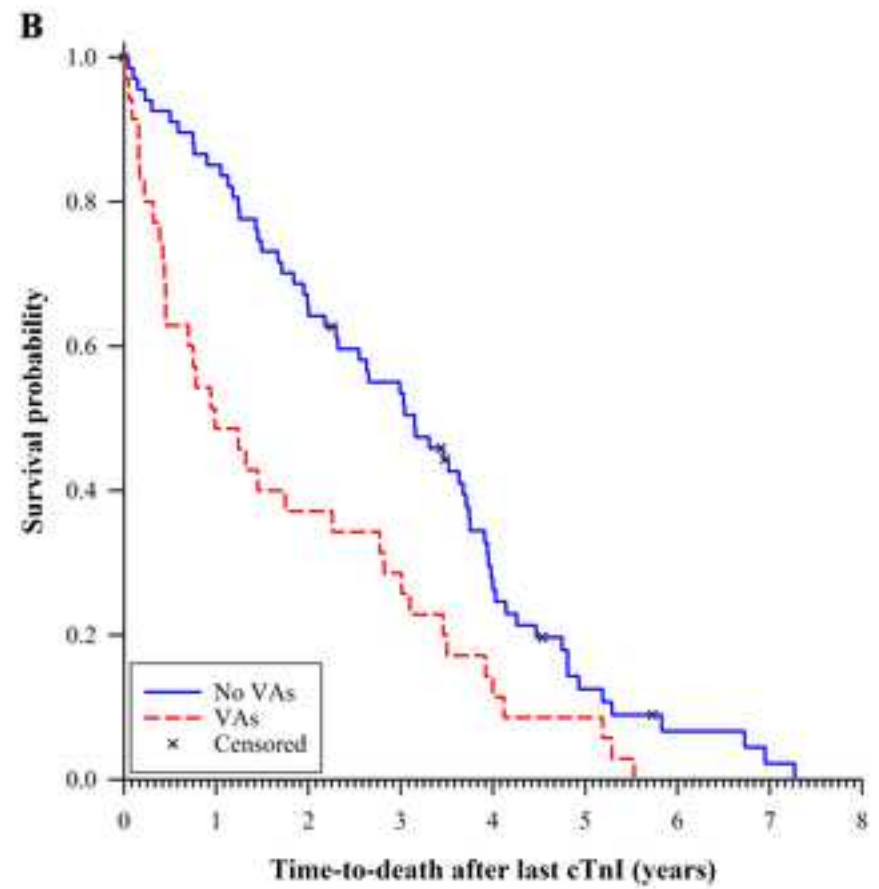
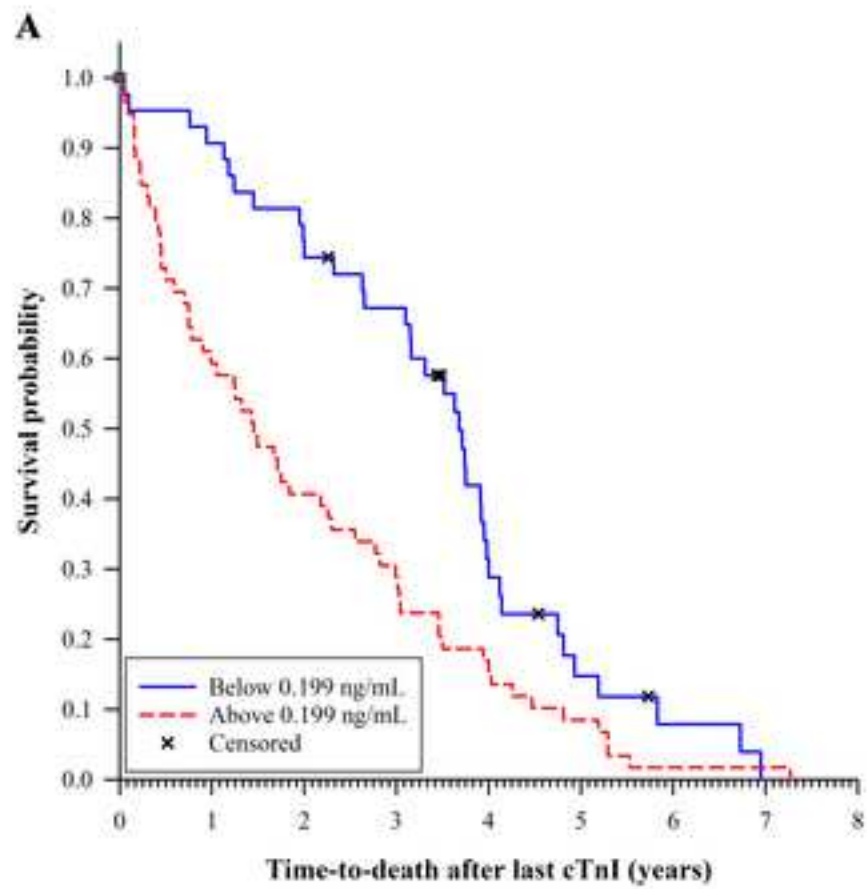
574 Footnotes

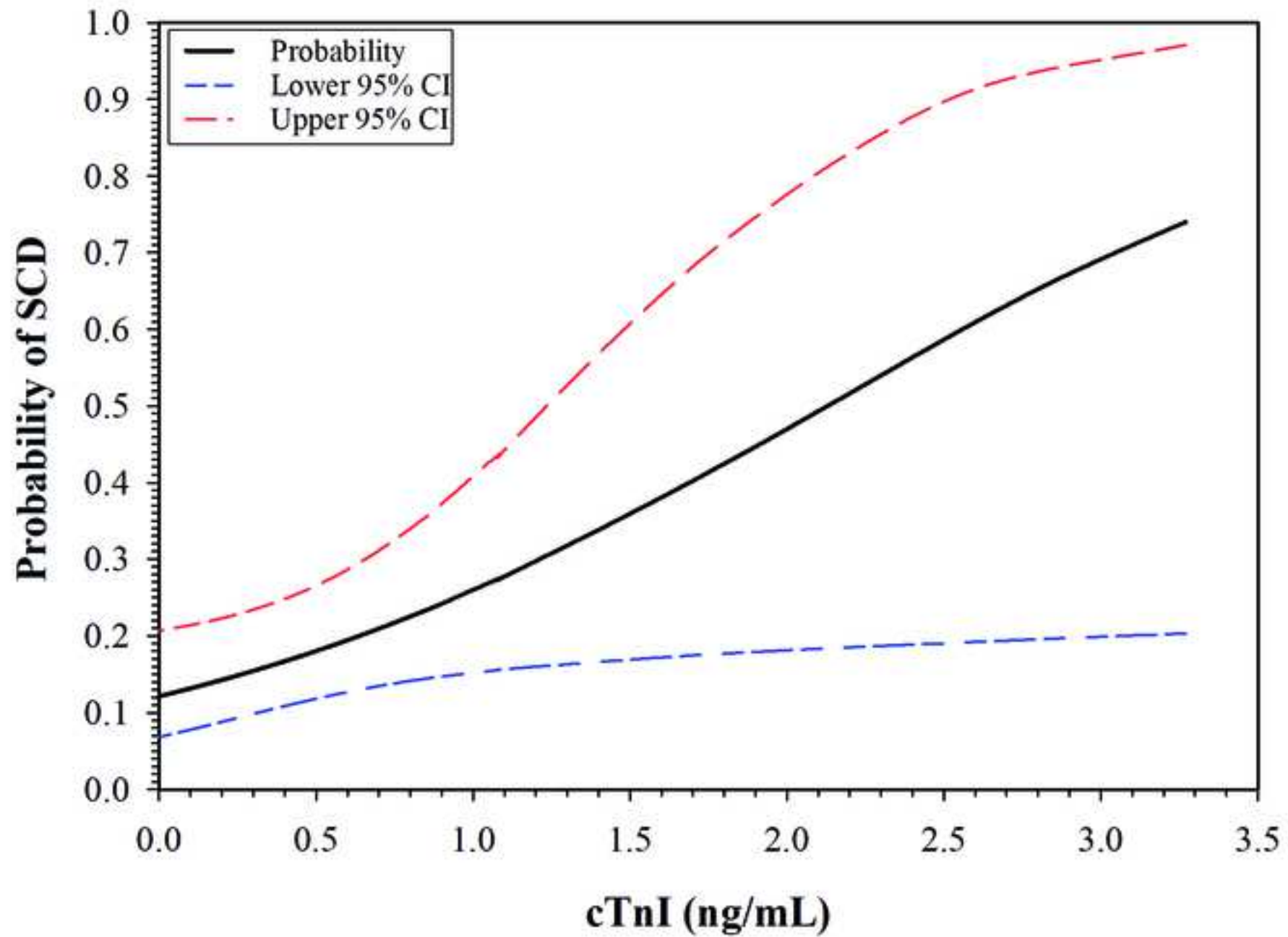
- 1
2 575 ^e Immulite 2000 cTnI assay (Siemens; Surrey).
3
4 576 ^f Vivid 7 Echocardiography machine; GE, Buckinghamshire, UK with an M4S
5
6
7 577 multifrequency phased array sector transducer.
8
9
10 578 ^g Echopac; GE, Buckinghamshire, UK.
11
12
13 579 ^h SAS. (2013). The SAS system for Windows, release 9.4. In Cary, NC, USA: SAS
14
15 580 Institute Inc.
16
17
18 581 ⁱ IBM SPSS (2017). IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM
19
20
21 582 Corp
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65











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/m²)	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/m²)	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 \pm SE (minimum– maximum) while non-normally distributed data are presented as median (25th -75th 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.

Journal of Veterinary Cardiology

The following information is required for submission:

Author contribution

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:

A handwritten signature in black ink, appearing to read "J. Wepheuser". The signature is written in a cursive style with a large, looping initial "J".

Date: ...28th March 2022....