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2 Risk indicators in cats with preclinical hypertrophic cardiomyopathy: a
3 prospective cohort study.

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21 Abstract

22 *Objectives:* To identify indicators of the risk of progression of preclinical hypertrophic
23 cardiomyopathy (HCM).

24 *Methods:* A prospective cohort study following a population of cats with preclinical hypertrophic
25 cardiomyopathy. Cats serially underwent physical examination, blood pressure measurement, blood
26 sampling and echocardiography. Development of congestive heart failure (CHF), aortic
27 thromboembolism (ATE) or sudden death (SD) were considered cardiac-related events. Associations
28 between factors recorded at baseline, and on revisit examinations, and the development of a
29 cardiac-related event were explored using ROC analysis.

30 *Results:* 47 cats were recruited to the study and followed for a median period of 1135 days. 15 cats
31 (31.9%) experienced at least one cardiac-related event; 6 CHF, 5 ATE and 5 SD. One cat experienced
32 a cardiac-related event per 10.3 years of patient follow-up. Cats with increased left atrial (LA) size
33 and higher concentrations of N-terminal B-Type Natriuretic peptide (NTproBNP) at baseline were
34 more likely to experience an event. Cats with a greater rate of enlargement of left atrial size
35 between examinations were also more likely to experience an event.

36 *Conclusions and relevance:* Factors easily measured, either once or serially, in cats with preclinical
37 HCM can help to identify those at greater risk of going on to develop clinical signs.

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39

40 Introduction.

41 Hypertrophic cardiomyopathy (HCM) is the most prevalent cardiac disease of cats ¹. It is estimated
42 to affect as many as 16% of domestic cats ¹⁻³. With the UK population of pet cats thought to number
43 approximately 11.1 million^a there could be over a million affected cats in the UK.

44 Although in some cats HCM is a progressive disease, many cats remain free from clinical signs for
45 years ^{4,5}. Some cats do develop serious clinical complications including congestive heart failure,
46 thromboembolism and sudden death ⁵⁻⁸. The challenge for veterinary surgeons is to distinguish
47 those cats at greater risk of having progressive disease from those more likely to remain stable.

48 Information of prognostic value can be gained from signalment and clinical examination. Cats
49 younger at the time of diagnosis have been shown to have a longer survival time ^{4,8}. The presence
50 of an arrhythmia and audible gallop have been associated with a worse outcome ⁹ and a detectable
51 arrhythmia has been associated with a greater risk of sudden death¹⁰.

52 Echocardiography has consistently been shown to provide information of prognostic value in cats
53 with HCM. Increased left atrial size has been associated with shorter survival time and a higher
54 likelihood of developing congestive heart failure, thromboembolism or experiencing sudden cardiac
55 death ^{4,8,10-12}. Greater left ventricular wall thickness has also been associated with a greater risk of
56 death ^{7,9}.

57 More recently, two studies have demonstrated that measurement of cardiac biomarker
58 concentrations may be of prognostic value ^{13,14}. Both studies showed that higher concentrations of
59 circulating troponin were associated with a worse outcome. The study by Borgeat and others (2014)
60 also demonstrated that an N-terminal pro B-type natriuretic peptide (NTproBNP) concentration of
61 greater than 250 pmol/L was associated with a greater risk of cardiac death; however, this did not
62 remain independently associated with a worse outcome when the presence of clinical signs and left
63 atrial size were accounted for.

64 The majority of studies of feline HCM in the literature have been retrospective studies and described
65 populations of cats seen at referral centres by specialists. There is limited information about risk
66 indicators in cats with pre-clinical HCM seen by non-specialists, outside the setting of a referral
67 hospital. The aim of the current study was prospectively to follow a population of cats diagnosed

^a 2018 PDSA Animal Wellbeing (PAW) Report
<https://www.pdsa.org.uk/media/4371/paw-2018-full-web-ready.pdf>

68 with pre-clinical HCM; repeating clinical examination, systolic blood pressure measurement, blood
69 tests (including cardiac biomarkers) and echocardiography approximately annually.

70 We hypothesized that in a population of cats with preclinical HCM certain of these measurements
71 would be of prognostic value and serial measurements would give additional valuable information
72 regarding outcome.

73 **Materials and methods**

74 **Setting**

75 Cats with HCM were identified from among cats with heart murmurs that were referred for further
76 investigation from first opinion veterinarians in northern England to one of two investigators both
77 RCVS cardiology certificate holders (VI and PT) between July 2010 and November 2015. Cats
78 underwent examination either in the practice in which they would normally be examined or in
79 another primary care practice near to their usual practice. Owners gave informed consent for their
80 cats to be enrolled in the study. All the procedures undertaken as part of the study were standard
81 diagnostic and monitoring procedures appropriate for patients with preclinical hypertrophic
82 cardiomyopathy and therefore the protocol did not undergo ethical review. The study underwent
83 review and was funded by Petsavers.

84 Cats underwent a full clinical examination, systolic blood pressure measurement, blood tests and
85 echocardiography.

86 **Physical Exam**

87 A complete physical examination was performed at each point of contact with each patient. Body
88 weight, condition score and pulse quality were recorded. Murmur intensity, the presence or
89 absence of gallop sounds, the presence or absence of arrhythmias, heart rate and lung sounds were
90 noted after thoracic auscultation.

91 **Blood pressure measurement**

92 Systolic blood pressure was measured non-invasively by Doppler sphygmomanometry (Ultrasonic
93 Doppler flow detector, Model 811-B, Parks Medical Electronics Inc, Aloha, OR, USA) prior to
94 collection of blood. Cuffs were placed on the right or left forelimb, and a mean of three consecutive
95 readings was recorded.

96 **Blood tests**

97 Blood was collected by jugular venepuncture into plain, serum gel and EDTA tubes. After clotting,
98 plain tube samples were centrifuged and the serum transferred to a clean plain tube. The serum gel

99 and an EDTA tube were centrifuged within 5 minutes of collection. EDTA plasma was then separated
100 into a plain tube. A separate EDTA tube was submitted to the laboratory with a freshly prepared
101 blood film. Samples were refrigerated and then sent via courier to a commercial laboratory (Idexx
102 Laboratories, Wetherby) for the following tests: urea, creatinine, glucose, alanine aminotransferase
103 (ALT), sodium, potassium, total thyroxine, cardiac troponin I^b (cTnI), NTproBNP^c, and a complete
104 blood count.

105 **Echocardiography**

106 For each echocardiographic examination cats were clipped and placed in right and then left lateral
107 recumbency on an ultrasound examination table. An ultrasound unit equipped with a 3.5-8 MHz
108 probe and ECG monitoring (Vivid-I, 7S-RS probe, GE Medical Systems, Milwaukee, WI, USA) was used
109 for all examinations. Each cat had all echocardiographic examinations performed by the same
110 observer.

111 A standard echocardiographic examination was performed¹⁵ using the using the 7S-RS probe at an
112 appropriate frequency setting to optimise image quality. Simultaneous ECG monitoring was achieved
113 for all cats except those intolerant of the ECG leads. Images were digitally stored and measurements
114 were obtained from still or looped images. All reported linear measurements were obtained from
115 two-dimensional images. Echocardiographic variables obtained were the average of at least 3
116 measurements from discrete cardiac cycles. The following parameters were measured at each
117 echocardiographic examination. The long axis left atrial measurement i.e. the maximal dimension
118 parallel to the plane of the mitral annulus measured at end-ventricular systole was measured in the
119 frame just before the mitral valve opening¹⁶. The left atrial and aortic dimensions were obtained
120 from short-axis images. They were measured on the first diastolic frame obtained after closure of
121 the aortic valve. The aorta was measured parallel to the commissure of the non- and right-coronary
122 aortic valve cusps. The left atrial dimension was measured parallel to the commissure of the left- and
123 noncoronary aortic valve cusps. The ratio of left atrial size to aortic root was then calculated¹⁷.

124 The thickness of the left ventricular free wall and interventricular septum were both measured in
125 diastole using the leading edge method¹⁸. Focal or generalized hypertrophy was characterized by a
126 thickness of ≥ 6 mm⁷. The left ventricular outflow tract maximal velocity was recorded from the left
127 parasternal long axis view. In addition, the presence or absence of the following were noted:

^b Beckman Coulter high sensitivity TnI.

^c First-generation Cardiopet proBNP assay until August 2013, second-generation Cardiopet proBNP assay thereafter.

128 systolic anterior motion of the mitral valve (SAM); chordal anterior motion (CAM); and whether a
129 dynamic left ventricular outflow velocity profile was observed ¹⁹.

130 If sedation was needed in order to complete the examinations 2.5 mg/kg ketamine (Anaestamine;
131 Animalcare) and 0.25 mg/kg midazolam (Hypnovel; Roche) were given intravenously via an IV
132 cannula.

133 ***Enrolment criteria***

134 Cats were recruited to the study during the period from July 2010 to November 2015.

135 Cats were considered eligible for inclusion in the study if diagnosed with preclinical HCM. HCM was
136 diagnosed if evidence was found of left ventricular segmental or diffuse hypertrophy of unknown
137 origin (interventricular septum (IVS) and/or left ventricular free wall (LVFW) thickness in diastole was
138 $\geq 6\text{mm}$) ⁷.

139 Cats were excluded if they were found to have a cardiac disease other than HCM, clinical signs
140 associated with HCM, or other clinically relevant disease including hypertension, hyperthyroidism,
141 diabetes mellitus, anaemia (a red blood cell count below the reference interval of the laboratory)
142 and azotaemia.

143 After the initial diagnosis, owners of cats with an aortic velocity of $\geq 4\text{m/s}$ were offered the option of
144 using atenolol at a dose rate of 6.25 mg SID or BID. No other cardiac medication was offered at this
145 stage.

146 ***Follow up***

147 Re-examinations were scheduled at approximately yearly intervals for two years after the baseline
148 visit. At re-examination cats underwent the same tests as were performed at baseline. Examinations
149 performed on individual cats were always repeated by the same investigator.

150 If follow up echocardiography showed left atrial enlargement, clopidogrel for prevention of
151 thrombo-embolism was discussed with the owners. If used, the dose was 18.75 mg SID. Atenolol
152 treatment was stopped if atrial dilation was noted.

153 Cats were followed until they experienced a cardiac-related event, were lost to follow up, died (of
154 any cause), or the study was concluded. A cat was considered to have experienced a cardiac-related
155 event if any of the following occurred; the cat experienced a thromboembolic event, developed
156 signs consistent with congestive heart failure (CHF) that required treatment or experienced sudden
157 death, assumed to be cardiac in origin.

158 Diagnosis of arterial thromboembolism (ATE) was made on the basis of characteristic clinical signs of
159 the occlusion of arterial blood supply to at least one limb. A diagnosis of CHF was made on the basis
160 of a cat developing clinical signs of tachypnoea and dyspnoea in the absence of another cause. The
161 presence of the following were considered to corroborate a clinical diagnosis of heart failure;
162 audible pulmonary crackles, a response to diuretic therapy, pulmonary infiltrates on a thoracic
163 radiograph and/or a pleural effusion on thoracic ultrasound. A cat was considered to have
164 experienced sudden death as its first cardiac related event if it was found dead by the owner having
165 been known to be normal less than 24 hours prior to being found dead in the absence of an
166 alternative explanation for the death.

167 The study was concluded in March 2018.

168 The primary outcome of interest was whether or not a cat experienced a cardiac-related event
169 during the period of follow up.

170 The following variables were recorded at baseline sex (male/female), age (years) and breed
171 (pedigree/not). The following variables were recorded at baseline and at each re-examination; body
172 weight (Kg), heart rate (bpm), murmur intensity (/6), systolic blood pressure (mmHg), BUN (mg/dL),
173 NTproBNP (pmol/L), cTn I (ng/mL), maximum left ventricular wall thickness in diastole (mm), LA:Ao
174 ratio, left atrial diameter in long axis, maximum aortic velocity (m/s), treated with atenolol (yes/no)
175 and the presence of an arrhythmia (y/n).

176 The upper limit of detection of the NTproBNP assay was 1500 pmol/L, cats with values above the
177 upper limit were ascribed a concentration of 1500 pmol/L.

178 An a priori power analysis was not conducted but the study planned to recruit fifty cats.

179 ***Statistical analysis***

180 Descriptive statistics for continuous variables are reported as median values and range; for
181 categorical and ordinal variables, they are reported as frequency and proportions.

182 Variables at baseline were compared between cats that went on to experience an event and those
183 that did not. Continuous variables were compared using an independent samples Mann-Whitney U
184 test. Categorical variables were compared with a Chi-square or Fisher's exact as appropriate.

185 Those variables where the distribution differed significantly between cats that experienced an event
186 and those that did not were evaluated further for their ability to discriminate between the two
187 groups using ROC analysis. A cut-off value was calculated with the best discriminatory ability on the
188 basis of the co-ordinate points of the ROC curve and commonly used cut-off values.

189 For the two variables shown by the ROC analysis to best discriminate between cats experiencing an
190 event and those that did not, the predictive ability of combining these variables was examined. Cats
191 were classified as having none, one or both values of these variables above the cut-offs determined
192 by the ROC analysis and the proportions of cats in each group were compared for likelihood of
193 experiencing an event.

194 Time to event analyses were undertaken comparing cats with neither, one or both values of
195 variables above the chosen cut-offs using Kaplan-Meier and Log-rank analysis. Cats that were lost to
196 follow up, died of a non-cardiac cause or survived until the end of the study were right-censored in
197 the analysis at the point of their last known contact with investigators or the time of death.

198 Finally, a graph was plotted with values of the two variables on the axes illustrating those cats that
199 did and did not experience an event.

200 To determine whether cats at risk of an event could be identified by repeated measurement of
201 characteristics identified to be associated with the likelihood of an event at baseline, the following
202 analyses were undertaken. For those variables that differed significantly between cats that
203 subsequently experienced an event and those that did not at the baseline visit, values for the
204 absolute change in the variable $((\text{measurement at visit } n+1) - (\text{measurement at visit } n))$ and the
205 percentage change in the variable between visits were calculated $(100 * ((\text{measurement at visit } n+1) - (\text{measurement at visit } n)) / (\text{measurement at visit } n))$. The absolute and percentage change of each
206 variable from the previous visit were compared between cats that subsequently experienced an
207 event and those that did not. For those variables that demonstrated a significant difference between
208 groups, an ROC curve was plotted using the absolute or percentage change in the variable as the
209 discriminator and subsequent event yes/no as the outcome.
210

211 Results

212 47 cats were diagnosed with preclinical HCM and recruited to the study. Baseline characteristics of
213 the cats are summarised in Table 1.

214 Of the 47 cats, fifteen experienced at least one cardiac-related event (32%); six developed signs
215 consistent with CHF (13%), five experienced sudden death (11%) and five experienced ATE (9%). One
216 cat experienced CHF and ATE concurrently. Four cats experienced death due to non-cardiac causes
217 (9%). Twenty eight cats (60%) were alive and known not to have experienced a cardiac-related event
218 at the time of last contact with the investigators. Figure 1 is a flow chart illustrating the outcome for
219 all 47 cats recruited to the study.

220 The median time of follow up for all cats in the study was 1135 days (range 215 – 2456 days) i.e.
221 greater than 3 years. The median time in study for those cats that experienced an event was 1016
222 days (range 215 – 1811 days). The median time in study for those cats that did not experience an
223 event was 1210.5 days (range 264 – 2456 days). In total there were 56,444 days (154.6 years) of
224 patient follow up meaning that there was one event per 10.3 years of patient follow up giving an
225 incidence rate of 9.7% (95% CI 5.4 – 16%) per year.

226 Eight cats required sedation in order to perform at least one of their echocardiographic
227 examinations. Seven cats required sedation at the first examination of which five were subsequently
228 examined (once $n = 2$ or twice $n = 3$) without the need for sedation. One cat sedated at the initial
229 examination required sedation at both subsequent examinations and one cat required sedation at
230 the second re-examination only. One cat that did not require sedation at the baseline visit and first
231 re-examination required sedation for the second re-examination.

232 Baseline variables that differed significantly between cats going on to experience an event and those
233 that did not were as follows; LA:Ao ratio ($p < 0.001$), NTproBNP concentration ($p = 0.001$) and LA
234 long axis measurement ($p = 0.025$). For all three variables, values were higher in the group of cats
235 that went on to experience an event.

236 Results of the ROC analysis testing the ability of these three variables to discriminate between those
237 cats that went on to experience an event and those cats that did not are illustrated in table 2. Cut-off
238 values are derived from the co-ordinate points of the ROC curves for the two most promising
239 discriminators and the sensitivity, specificity and positive and negative likelihood ratios calculated on
240 the basis of these cut-offs.

241 The numbers of cats experiencing an event (and not experiencing an event) according to whether
242 they had none, one or both risk indicators above the proposed cut offs are reported in tables 3 and
243 4. A Kaplan Meier graph illustrating the proportion of cats remaining free of an event against time
244 for the three groups of cats (neither risk indicator elevated, one risk indicator elevated, and both risk
245 indicators elevated) is shown in Figure 2. Cats without either risk factor were significantly less likely to
246 experience an event compared to those with one factor ($P = 0.018$) and cats with both risk factors (P
247 < 0.001). The median time to event was not reached in the group with neither risk factor. The
248 median time to event was 1693 days (95% CI 665 - 2720 days) for cats with one risk factor and 1016
249 days (95% CI 647 – 1384 days) for cats with both risk factors however the difference between these
250 groups was not significant ($P = 0.124$).

251 A graph illustrating the concentrations of NTproBNP and left atrial to aortic ratios of individual cats
252 measured at baseline is illustrated in Figure 3. Those cats that experienced sudden cardiac death

253 appear as red dots, those that experienced CHF appear as blue dots and those that experienced ATE
254 appear as yellow dots. Those that did not experience an event appear as black dots.

255 A significantly greater proportion of cats that experienced an event received atenolol at some point
256 during their follow-up (P = 0.046).

257 At the first revisit examination the absolute and percentage change in LA:Ao, LA Long and NTproBNP
258 concentration did not differ between cats that went on to experience an event and those that did
259 not (Table 5a). At the second revisit the absolute and percentage change in LA:Ao from the previous
260 visit were greater in cats that went on to experience an event (Table 5b). The absolute change in the
261 NTproBNP concentration was lower in those cats that went on to experience an event compared to
262 those that did not (Table 5b). The absolute and percentage change in LA:Ao were significantly
263 associated with the likelihood of an event in the ROC analysis (Table 6). As can be seen from figure 1,
264 ten events occurred after the second revisit examination and data were only available for nine of
265 those cats representing only 60% of all cats that experienced an event.

266 Discussion

267 This is the first study to prospectively follow a cohort of cats with preclinical hypertrophic
268 cardiomyopathy managed in a primary care setting by non-specialists. It provides additional
269 information about the natural history of this common disease, confirms the value of known
270 echocardiographic risk indicators and demonstrates the value of measurement of circulating
271 biomarkers in identifying cats at greater risk of going on to experience a cardiac-related event. It also
272 provides information regarding the value of serial evaluation of risk indicators.

273 The findings in the described population of cats confirm that many cats with pre-clinical
274 hypertrophic cardiomyopathy can live for long periods without experiencing a cardiac-related event.
275 It has previously been reported that for many cats with HCM, particularly those that are free of
276 clinical signs at the time of diagnosis, the disease can be a relatively benign and slowly progressive or
277 non-progressive^{4,5,11}. In the current study, during more than 150 years of patient follow up only 15
278 cats experienced cardiac-related events, occurring at a rate of one event per 10.3 years. The three
279 individual events that were considered cardiac-related events; the onset of CHF, aortic
280 thromboembolism and sudden or unexpected death, occurred with similar frequency. In the
281 population as a whole, fewer than one third of the affected cats experienced an event in a period of
282 follow up of, on average, three years.

283 Many previous studies have demonstrated the value of left atrial to aortic ratio as an indicator of
284 cats at greater risk of an adverse outcome^{4,8,10-12}. In the current study this result was confirmed

285 with cats having a LA:Ao ≥ 1.5 approximately 4 times more likely to experience a cardiac-related
286 event. The current study also demonstrated that NTproBNP concentrations, when considered in
287 isolation, were of similar predictive value to LA:Ao. Cats with a concentration ≥ 700 pmol/L were also
288 approximately four times more likely to experience a cardiac-related event. These markers appeared
289 to be complementary in their ability to identify cats at higher risk. Cats with values of both indicators
290 above the cut-off were the most likely to experience an event and did so more quickly.

291 In contrast to previous studies^{13,14} cTnI did not prove to be a useful indicator of the risk of a cardiac-
292 related event in this population. One possible reason for this is that the cats recruited to this study
293 were all at the preclinical stage of their disease. If the release of troponin from myocardium is a late
294 event in the course of HCM then it may only be a good indicator of risk in populations including
295 those in the later stages of their disease i.e. those not at the preclinical stage of the disease. Another
296 possible reason for the lack of demonstrated association is that the population described in the
297 current study is relatively small – however this cohort is larger and was followed for longer than
298 both of those previously described^{13,14}.

299 Both of the identified risk indicators, LA:Ao and NTproBNP, were evaluated serially in this
300 population. Cats that subsequently experienced a cardiac-related event appeared to have a greater
301 absolute change and percentage change in LA:Ao in the time interval prior to their experiencing the
302 event. This suggests there is value in serial monitoring of LA:Ao. However it is worth noting that
303 fewer than two thirds of the cats (30 in total) contributed data to this analysis. Some cats had
304 already experienced the event before they were re-examined or their owners chose not to return for
305 subsequent examinations. Clearly serial measurements can only be of value in those patients in
306 which they can be obtained. Methods of prognostication for cats that are only seen on a single
307 occasion must also be used because cats may experience an event before they are re-examined and
308 owners may not be willing to wait for a second examination before an opinion on their cat's
309 likelihood of experiencing an event is given.

310 Unexpectedly those cats that experienced an event had a lower absolute change in NTproBNP
311 concentration between their first and second revisit. This may be a consequence of there being an
312 upper limit for the highest concentration of NTproBNP that can be registered by the assay involved.
313 Cats with concentrations above 1500 pmol/L which had an increase in concentration would not be
314 correctly identified by this method of measurement. This would mean the analysis would only
315 correctly recognise elevations in cats that initially had lower concentrations, but not in those that
316 initially had high concentrations. It makes it doubtful, using the current assay, that there will be

317 value in serial measurement of NTproBNP in cats despite the concentrations measured at the first
318 visit being good indicators of risk.

319 It is interesting to note that the cut-off value in this study proposed to distinguish cats at greater risk
320 was 700 pmol/L. This is considerably higher than cut-offs that were previously proposed to
321 distinguish cats in heart failure from those with respiratory distress due to other causes, and higher
322 than cut-offs proposed to distinguish cats with preclinical cardiomyopathy from cats without
323 cardiomyopathy²⁰. There may be several reasons for this, firstly the feline NTproBNP assay has been
324 through several iterations and it may be that absolute values obtained from earlier versions of the
325 assay are not directly comparable to those from more recent iterations. Secondly every cat in the
326 current study was known to have heart disease and the differentiation being made is between those
327 with “worse” heart disease and milder heart disease. This may mean that a higher cut-off is required
328 to distinguish those two groups compared to a cut-off being used to distinguish cats without heart
329 disease from those with heart disease.

330 Treatment with atenolol was offered to cats in which an elevated left ventricular outflow tract
331 velocity was found because at the time our study was designed it was believed that beta-blockade
332 may improve outcome in cats with preclinical hypertrophic obstructive cardiomyopathy and such
333 treatment was widely recommended by cardiologists²¹. Systematically withholding such treatment
334 to cats in the study was considered unethical. However, as our study progressed a trial was
335 published which failed to show a benefit of atenolol administration in cats with hypertrophic
336 obstructive cardiomyopathy¹¹. Treatment was not consistently administered in all cases in which it
337 was prescribed. One clinical trial had suggested that once in heart failure, cats receiving atenolol did
338 less well than those not receiving atenolol²² and for that reason treatment was withdrawn in cats
339 where evidence of disease progression was found.

340 A significantly greater proportion of cats that experienced an event received atenolol at some point
341 in the duration of the study, but it should not be concluded that this represents a detrimental effect
342 of the treatment. Treatment was not randomly allocated nor were investigators blinded to
343 treatment allocation. It is possible that there was some degree of allocation bias, with cats
344 administered treatment being somehow different to those to which treatment was not
345 administered.

346 The current study has several limitations.

347 The number of cats followed in the study is relatively small, however there are very few large
348 prospective studies of cats with HCM in the literature and none conducted in a non-specialist

349 setting. The low number of cats and the low event rate mean that the total number of cats
350 experiencing events contributing to the analyses is only 15. The low number of events means that
351 sub-analyses of the three separate cardiac-related events would not be worthwhile. It also means
352 that multivariable analysis cannot be undertaken. The complementary value of measurement
353 NTproBNP and LA:Ao is however suggested by analyses including the Kaplan Meier analysis and
354 examination of the proportions of cats with none, one and two elevated risk indicators going on to
355 experience an event. To conclusively demonstrate an independent and complementary value of the
356 two tests a larger study with a greater number of events would be required.

357 The diagnosis of HCM was made using published guidelines by cardiologists with an advanced post-
358 graduate qualification in cardiology, but was not confirmed by a specialist or by post-mortem
359 examination. Two investigators made the diagnoses and carried out the follow up examinations on
360 the cases described, however the agreement between the two investigators and the reproducibility
361 of their findings was not evaluated.

362 The study was conducted over a long period of time, during which the assay for the measurement of
363 Feline NTproBNP was changed. This may have introduced a confounding factor particularly in the
364 evaluation of serial concentrations. The duration of the period over which the study was conducted
365 also resulted in the recommendations for treatment of preclinical HCM and prevention of ATE
366 changing during the period of the study. Treatment was therefore variable over the period of the
367 study and conclusions regarding the efficacy of treatment cannot be made.

368 The diagnosis of ATE was made on the basis of clinical signs in the majority of cases and post-
369 mortem examination or advanced imaging were not performed. A diagnosis of sudden death was
370 made on the basis of the owner's description and presumed to be cardiac in origin. The diagnosis of
371 CHF was made on the basis of clinical presentation and response to therapy and was not confirmed
372 by diagnostic imaging in every case. Confirmation of a diagnosis of CHF can be challenging in cats
373 and the performance of diagnostic imaging is not possible in every case, especially at the time of an
374 emergency presentation for breathlessness.

375 Conclusions

376 In conclusion this study has demonstrated that a larger left atrium and/or higher concentrations of
377 NTproBNP on initial examination of cats with preclinical HCM indicates cats at higher risk of
378 experiencing CHF, ATE or sudden cardiac death. In cats that underwent serial measurement of LA:Ao
379 those with increasing left atrial size had a greater risk of experiencing the same events compared to
380 those in which left atrial size was static or reduced. Although the measurement of LA:Ao requires

381 ultrasound equipment and expertise, the measurement of NTproBNP is widely available (through a
382 diagnostic laboratory) and may help to identify patients with preclinical HCM at greater risk when
383 echocardiography is not available.

384

385 Conflict of interest

386 The authors declare no conflict of interest relating to this manuscript.

387

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389 This study was funded by BSAVA PETSАVERS Charity.

390

391 Ethical approval

392 This work involved the use of non-experimental animals only (including owned or unowned animals
393 and data from prospective or retrospective studies).

394 Established internationally recognised high standards ('best practice') of individual veterinary clinical
395 patient care were followed. Ethical approval from a committee was therefore not necessarily
396 required.

397

398 Informed consent

399 Written informed consent was obtained from the owner or legal custodian of all animals described
400 in this work for the procedures undertaken.

401 No animals or humans are identifiable within this publication, and therefore additional informed
402 consent for publication was not required.

403

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