Critical appraisal Jung et al., 2015 – Cohort questions

Introduction	
Are the aims clearly stated?	Yes • Identify prevalence of AF in medium-sized to large breed dogs with congestive heart failure (CHF) due to myxomatous mitral valve regurgitation (MMVD) • Assess impact of AF on survival • Determine effect of rate control on survival.
Methods	
Is the study design suitable for the aims?	Yes, with limitations: • Electronic medical record retrospective cohort • Survival analysis potentially flawed by excluding patients who survived or were lost to follow-up rather than by using censored data. This can bias hazard ratio estimates. • Prevalence estimates may have been biased by loss to follow-up and referral setting
What population of animals was being studied?	Medium and large breed dogs (>15 kg) with CHF secondary to MMVD recorded in the electronic medical records of the University of California (Davis) William R Pritchard Vet Med Teaching hospital from Jan 1, 2005-Dec 31, 2010 (n=64). Boxers, Doberman Pinchers, dogs with concurrent ventricular arrhythmia or congenital heart disease or infective endocarditis or systemic illness, or were alive or lost to follow-up were excluded from analysis.
Was a control (non exposed) group used?	Combined prevalence and prognostic study; no control groups but multiple comparisons: • Risk factors for AF

	• AF effect on survival • Effect of rate control on AF survival • Effect of diltiazem or diltiazem combined with digoxin on heart rate and survival.
Is the control group appropriate?	See above
How long did they follow the population for?	879 days longest (endpoints reached much sooner for most)
What exposures were measured?	AF prognostic and treatment factors: ECG, echocardiography parameters, weight, age, sex, CHF therapy (pimobendan, enalapril, furosemide), electrolytes, renal values Mortality prognostic and treatment factors (overall): AF, echocardiography parameters, weight, age, sex, CHF therapy (pimobendan, enalapril, furosemide), electrolytes, renal values Mortality prognostic and treatment factors (AF only): Heart rate control, diltiazem, digoxin-diltiazem
Is it clear what measurements were carried out in the study?	Yes
Were the correct measurements chosen? Do they that reflect (or are they strongly related to) the outcome of interest?	Yes Yes • Primary outcome was death from cardiac-related disease (defined as sudden death or euthanasia from refractory or worsening CHF). This is a clinically relevant endpoint. • Holter monitoring would have been preferred over non-ambulatory ECG (acknowledged by authors).

Were previously established validated methods used to make the measurements? (e.g. Glasgow pain score, International Units etc)	Yes
Did the exposure precede the outcome?	Yes
What outcomes were measured?	• Death attributable to cardiac disease (including euthanasia) • Atrial fibrillation • Average heart rate (>160 BPM, <160 BPM)
Are the outcomes clinically relevant?	Yes
Are the statistical methods described?	Yes
Was the statistical significance level stated?	Yes
Was the sample size justified?	No
Was ethical approval obtained?	Not stated
Overall, are the methods described in enough detail that you could repeat them?	Yes
Results	
Were the basic data adequately described?	Yes
Do the numbers add up?	Yes

Are all subjects accounted for?	Yes
Was the statistical significance (p value) stated in the results? Is this consistent with the methods? (It should be stated in the sample size or power calculation)	Yes Unknown
What were the main findings/key results?	Median heart rate lower with diltiazem-digoxin combination (144 BPM) than with diltiazem alone (180 BPM) Median survival time with diltiazem-digoxin (130 days) was improved compared with diltiazem alone (35 days, p=0.024). AF patients with adequate rate control (<160 BPM) had improved survival (<160 BPM--171 days, >160 BPM—61 days; p=0.002) AF was associated with decreased median survival time (AF--142 days, non-AF--234 days; p=0.002) 52% of dogs (33/64) were diagnosed with AF initially (n=21) or subsequent to CHF (n=12). Larger dogs were at higher risk for AF (p=0.016).
Discussion and conclusion	
What do the main findings/key results mean?	There was a high prevalence of atrial fibrillation in large breed dogs with CHF and MMVD with increased body weight a significant risk factor for development of AF. AF was associated with decreased overall survival times in dogs with MMVD and CHF. However, the use of digoxin in combination with diltiazem in AF patients was

	associated with improved rate control and survival times versus use of diltiazem alone.
Was a casual relationship between the outcome and exposure suggested? Is it justified?	Multiple associations were found in this study and some were thought to be causal: 1. AF was significantly associated with larger body size but this finding was not covered in the discussion. 2. AF was significantly associated with decreased cardiac-related survival. Causal relationship is implied by the authors in their discussion of tachycardia-induced myocardial failure. 3. Rate control in AF was significantly associated with improved cardiac-related survival. This conclusion is justified by the data and by what is known about management of human atrial fibrillation in the authors' discussion. 4. Diltiazem-digoxin therapy was superior to diltiazem monotherapy in reducing cardiac-related mortality in dogs with AF. This conclusion is justified by the data presented, subject to the limitations of an observational study at high risk of unmeasured confounding and informative censoring. 5. Diltiazem-digoxin therapy is superior to diltiazem monotherapy in achieving adequate rate control (<160 BPM) with AF. This conclusion is supported by the finding that median HR was less on combination than on monotherapy but no statistical analysis was performed.
Are the negative findings discussed? How are the negative findings interpreted?	Limitations are discussed (low power, selection and clinician bias, different CHF treatments, lack of ambulatory ECG monitoring) with suggestion that a prospective randomized clinical trial is needed.

Does the discussion reflect the results?	Yes with the exception of omitting discussion of risk factors for AF and potential effects of referral hospital selection bias for prevalence of AF
Interpretation	
What are the clinical implications of this study?	In dogs >15 kg with CHF secondary to MMVD, presence of atrial fibrillation appears to be a risk factor for earlier cardiac-related death. In dogs with rapid AF (HR>160), treatment with digoxin in combination with diltiazem improved rate control and cardiac-related survival times versus treatment with diltiazem monotherapy. There are caveats related to flaws in the study design and analysis: 1. High prevalence of AF in this study may reflect selection bias of referral hospital. We think it likely that dogs with AF may be more likely to be referred than dogs without AF. 2. Observational study in which interventions were not randomly assigned poses high risk for unmeasured confounding (clinician, patient). 3. Use of cardiac-related death as both inclusion criteria and endpoint for cohort may heavily bias survival analysis. Failure to include right censored data does not allow assessment of informative censoring. 4. Study period straddled introduction of pimobendan (50% of patients did not receive pimobendan). Since pimobendan has been shown to significantly increase survival times in dogs with CHF secondary to MMVD, stratified analysis adjusting for pimobendan would have improved confidence in analysis. 5. The authors appeared to use overly stringent significance criteria ($p<0.05$) for consideration of

Are the subjects in the study similar to those in the BET/your own?	additional candidate variables in their Cox proportional hazards analysis, rejecting multivariate analysis since only AF reached this threshold in univariate analysis. Candidate covariates are typically included in model building if $p<0.2$ in univariate analysis (or if of likely clinical relevance); use of more stringent significance criteria can omit important covariates[1], a situation we think likely given that pimobendan was not considered a significant factor in the Cox model under stringent selection. We are concerned too that the authors report using Wilcoxson signed-rank testing to compare continuous variables between the AF and No-AF groups. This test is used to compare variables in matched or paired samples and is inappropriate for two independent groups. Yes
General	
Who funded this study?	Not stated

Comments: Median diltiazem dose was 1 mg/kg TID (standard release formulation) aside from two dogs who received extended release diltiazem at 3 mg/kg BID (Dilacor XR). Median digoxin dose was 0.004 mg/kg BID.

[1]Bursac, Z., Gauss, C. H., Williams, D. K., & Hosmer, D. W. (2008). Purposeful selection of variables in logistic regression. *Sourcecode for Biology and Medicine*, *3*(1), 17.