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# Arrhythmogenic right ventricular cardiomyopathy in Boxer dogs: the diagnosis as a link to the human disease

Annina S. Vischer[1, 2], David J. Connolly[3], Caroline J. Coats[1], Virginia Luis Fuentes[3], William J. McKenna[4], Silvia Castelletti[1, 5] and Antonios A. Pantazis[1, 6]

*[1] Inherited Cardiovascular Disease Unit, St Bartholomew's Hospital, London, UK; [2] Medizinische Poliklinik, Universitätsspital Basel, Switzerland; [3] Royal Veterinary College, London, UK; [4] Institute of Cardiovascular Science, University College of London, London, UK; [5] Centre for Cardiac Arrhythmia of Genetic Origin, Istituto Auxologico Italiano, Milan, Italy; [6] Cardiomyopathy Service, Royal Brompton Hospital, London, UK*

**Background. Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a myocardial disease with an increased risk for ventricular arrhythmias. The condition, which occurs in Boxer dogs, shares phenotypic features with the human disease arrhythmogenic cardiomyopathy (ACM) suggesting its potential as a natural animal model. However, there are currently no universally accepted clinical criteria to diagnose ARVC in Boxer dogs. We aimed to identify diagnostic criteria for ARVC in Boxer dogs defining a more uniform and consistent phenotype.**

**Methods and Results. Clinical records from 264 Boxer dogs from a referral veterinary hospital were retrospectively analysed. ARVC was initially diagnosed according to the number of ventricular premature complexes (VPCs) in the 24-hour-Holter-ECG in the absence of another obvious cause. Dogs diagnosed this way had more VPCs, polymorphic VPCs, couplets, triplets, VTs and R-on-T-phenomenon and syncope, decreased right ventricular function and dilatation in comparison to a control group of all other Boxer dogs seen by the Cardiology Service over the same period. Presence of couplets and R-on-T-phenomenon on a 24h-ECG were identified as independent predictors of the diagnosis. A diagnosis based on ≥100 VPCs in 24 hours, presence of couplets and R-on-T phenomenon on a 24h-ECG was able to select Boxer dogs with a phenotype most similar to human ACM.**

**Conclusion. We suggest the diagnosis of ARVC in Boxer dogs requires two out of the three following criteria: presence of ≥ 100 VPCs, presence of couplets or R-on-T-phenomenon on a 24 h-ECG. This results in a uniform phenotype similar to that described in human ACM and may result in the adoption of the term ACM for this analogous condition in Boxer dogs.**

**Key words:** arrhythmogenic right ventricular dysplasia/cardiomyopathy, ventricular tachycardia, Holter electrocardiography

## Introduction

*Humans*

Arrhythmogenic cardiomyopathy (ACM) is a genetically determined myocardial disorder defined histologically by progressive replacement of the right ventricular (RV) myocardium with adipose and fibrous tissue (1, 2). Although these abnormalities predominantly occur in the RV they may also involve the left ventricle (LV) producing a dilated phenotype (3). The fibrofatty replacement affects intercellular electrical conduction increasing the risk of life-threatening ventricular arrhythmias (4). Dilatation and regional wall motion abnormalities of the RV are frequently seen(5) and may occur at any time during the disease (6).

The clinical diagnosis is based on criteria defined by the European Society of Cardiology Task Force in 1994 (7), revised in 2010 (8). The criteria include structural changes identified by cardiac MRI, echocardiography or RV angiography, histopathology, as well as characteristic ECG-changes, documentation of arrhythmias, and family history (8).

ACM can lead to recurrent sustained or non-sustained ventricular tachyarrhythmia and/or myocardial failure and possible death. However, a significant percentage of patients experiences little or no symptoms (9). ACM in humans can imitate dilated cardiomyopathy (DCM), however it usually presents with a severity of ventricular arrhythmia disproportionate to the extent of LV dilatation (10).

*Performed at the Royal Veterinary College, London, United Kingdom*

*Address for correspondence: Dr Antonios Pantazis, Royal Brompton Hospital, Sydney Street, London SW3 6NP, United Kingdom. E-mail a.pantazis@nhs.net*

*Boxer dogs*

Arrhythmogenic right ventricular cardiomyopathy (ARVC), a disease similar to ACM in humans, was first described in Boxer dogs in 1983, which unlike cardiomyopathies in other dogs, lacked ventricular dilation and atrial fibrillation but was distinguished by extensive histological alterations including fibrofatty replacement of cardiomyocytes, ventricular premature complexes (VPCs) and frequent ventricular tachycardia (VT) (11) and can concern both RV and LV (12, 13).

Subsequent research focused on arrhythmogenic risk and the genetic causes of the disease (Supplemental Material, Table 1) and it was suggested that Boxer dogs might serve as a naturally occurring model for the human disease, based on the close clinical and pathological resemblances of the two conditions (12). However, there is no consensus on diagnostic criteria for ARVC in Boxer dogs resulting in a discrepancy of diagnostic criteria between different studies, which prevents direct comparison between them. Previously used diagnostic criteria are shown in the Supplemental Material, Table 1.

*Purpose*

Our objective was to develop diagnostic criteria for ARVC in Boxer dogs, which can be easily applied to clinical practice and do not rely solely on the number of VPCs in a single 24h-ECG, in order to standardise disease classification. We anticipate that a more uniform and consistent clinical definition of the canine phenotype will allow direct comparison with the human phenotype and enhance the utility of this naturally occurring model.

## Methods

*Data collection*

All Boxer dogs admitted to the cardiology service of The Royal Veterinary College between 2001 and 2013 were included. The reason for referral, clinical signs, and medications were obtained from clinical records. 24h-ECGs and echocardiograms were analysed retrospectively.

24h-ECGs were analysed for the number of VPCs, couplets, triplets, and episodes of VT. The morphology of VPCs, couplets, triplets and VTs were evaluated and defined as monomorphic or polymorphic. Monomorphic VPCs had the same visual vector, polymorphic VPCs had > 1 vector. The most commonly appearing morphology was used to describe the overall vector. The duration and rate of supraventricular and ventricular arrhythmias was recorded. Couplets, triplets and VTs were defined as poly-

**Table 1.** Significant clinical data characteristics between affected and control dogs, when 2 of 3 criteria were (≥100 VPCs, couplets, R-on-T in 24h-ECG) fulfilled.

| | ARVC (n=61) | Controls (n=70) | p-value |
|---|---|---|---|
| Age | 7.21 ± 3.07 | 4.63 ± 3.14 | 0.000 |
| 24h-ECG | 61 (100%) | 70 (100%) | NA |
| VPCs present | 61 (100%) | 64 (91.4%) | 0.030 |
| Number VPCs | 2240 ± 3066 | 446 ± 1858 | 0.000 |
| Polymorphic VPCs | 55 (91.7%) | 39 (60.9%) | 0.000 |
| Couplets present | 54 (88.5%) | 14 (20.0%) | 0.000 |
| Number Couplets | 565 ± 1913 | 2 ± 8 | 0.015 |
| Polymorphic couplets | 26 (49.1%) | 1 (7.7%) | 0.010 |
| Triplets present | 34 (55.7%) | 5 (7.1%) | 0.000 |
| Number Triplets | 144 ± 481 | 0 ± 0.5 | 0.013 |
| VT present | 30 (49.2%) | 7 (10.0%) | 0.000 |
| R-on-T | 46 (76.7%) | 9 (13.6%) | 0.000 |
| RA dilatation | 4 (6.6%) | 0 (0.0%) | 0.045 |
| LA diameter (mm) | 36.4 ± 9.39 | 28.2 ± 4.5 | 0.000 |
| LVEF (%) | 42.0 ± 16.5 | 53.0 ± 10.1 | 0.001 |
| LVIDd (mm) | 43.3 ± 8.7 | 37.8 ± 4.8 | 0.000 |
| LVIDs (mm) | 33.5 ± 10.2 | 26.4 ± 4.7 | 0.000 |
| FS (%) | 24 ± 11 | 30 ± 8 | 0.001 |
| AV Vmax (m/s) | 2.03 ± 0.86 | 2.55 ± 1.28 | 0.023 |

ARVC: Arrhythmogenic right ventricular cardiomyopathy, ECG: electrocardiogram, VPCs: ventricular premature complexes, VT: ventricular tachycardia, RA: right atrial, LA: left atrial, LVEF: left ventricular ejection fraction, LVIDd: enddiastolic left ventricular internal diameter, FS: fractional shortening, AV Vmax: aortic valve maximal velocity

morphic if they displayed different morphologies within their single beats. R-on-T phenomenon was defined as a VPC superimposed on the T wave from other sinus beats as postulated (14). 24h-ECGs were excluded if the printout quality was sub-optimal for analysis.

Left ventricular end-diastolic (LVIDd) and end systolic (LVIDs) diameter were measured in echocardiograms in the parasternal long-axis view using 2D images. Ejection fraction was calculated using the Simpson's method of disks (15).

DCM was defined by LVIDd > 5.18 cm, LVIDs > 3.63 cm and FS < 23% which are the 95$^{th}$ and 5$^{t}$h percentiles in normal boxers, respectively (16). The presence of aortic stenosis (AS) was determined using standard Doppler assessment of AV flow. AS was defined by a AV max velocity ≥2.25 m/s (17). Diastolic function was assessed by mitral inflow pattern using PW-Doppler (18). RVOT size was measured at end-diastole in the parasternal short axis, from the anterior RV wall to the aortic valve when echocardiographic loops of appropriate quality and the correct plane were available (15). RV function, dilatation and wall motion abnormalities were estimated visually by one investigator, blinded to the number of VPCs, and categorised as mild, moderate and severe.

*Traditional diagnosis*

The diagnosis of ARVC was made if ≥ 100 VPCs were present on the 24h-ECG(19, 20). Dogs with DCM and AS were excluded from this group and added to the control group. All other Boxer dogs were used as controls. The control group therefore consisted of normal Boxer dogs as well as dogs with AS and DCM. In order to test the diagnostic accuracy of a cut-off of 100 VPCs, we repeated all calculations after the cut-off for the diagnosis of ARVC was altered to ≥ 50 VPCs and ≥ 200 VPCs, using the same exclusion criteria. This diagnostic method is henceforth referred to as "traditional diagnosis".

*Novel diagnostic criteria*

Independent variables were identified based on the p-values obtained from Chi-Square and independent sample T-tests and then added manually in a forward fashion to the multivariable logistic regression model, starting from the lowest p-value. Independent variables were kept in the model, when Odds Ratio was significant, or removed, when Odds Ratio was non-significant, respectively.

A novel diagnostic score was developed using all significant parameters from the logistic regression model. Additionally, the criterion ≥100 VPCs was kept, as it was used in the traditional diagnosis. Each parameter was assigned with one point, therefore giving a maximal number of 3 points for each dog.

Chi-square and independent sample T-tests were repeated to compare the phenotypes when the diagnosis was based entirely on the novel diagnostic score independent of any echocardiographic exclusion. Sensitivity, specificity, positive predictive value and negative predictive value were calculated based on the traditional definitions.

*Statistical methods*

Statistical analyses were performed using SPSS Version 22 for Mac. A p-value of ≤ 0.05 was considered statistically significant. Univariable analysis consisted of Chi-square and independent sample T-tests. Receiver operating characteristic (ROC) curves and area under the curve (AUC) were utilized to find the ideal cut-off for continuous parameters. Multivariable logistic regression was used to find predictors for the disease.

## Results

Data from 264 Boxer dogs were analysed. The dogs were referred for a variety of reasons including syncope, heart failure, incidentally discovered signs such as murmurs or VPCs and for breeding examinations.

Information on presenting signs was available in 213 dogs (80.7%). 24h-ECGs were available in 131 dogs (49.6%). Echocardiograms were available in 261 dogs (98.9%).

*Traditional diagnosis*

Utilising 24h-ECGs, the dogs were diagnosed on the number of VPCs, using a cut-off of ≥50 VPCs, ≥100 VPCs and ≥200 VPCs. This resulted in an ARVC group of 66, 63 and 57 Boxer dogs, respectively. From all groups, 8 dogs were moved to the control group due to a dilated LV; 12 dogs were moved from the ≥ 50 and ≥ 100 VPCs group and 9 from the ≥ 200 VPCs group to the control group due to AS, leaving 46, 43 and 40 dogs, respectively, that fulfilled the inclusion criteria for ARVC and 218, 221 and 224 dogs, respectively, in the control group. There was no significant difference in sex between the groups, but ARVC dogs were significantly older than dogs in the control group for all cut-offs (Supplemental Material Table 2).

Syncope or pre-syncope (collapse without loss of consciousness) was a common presenting sign in ARVC dogs and significantly less so in the control group using all three cut-offs.

No 24h-ECGs needed to be excluded due to an insufficient recording time; the mean recording time was 24.4 ± 1.0 hrs. As expected, the number of VPCs was higher in all ARVC groups, as this was part of our inclusion criteria. The frequency of polymorphic VPCs, cou-

plets, triplets, VTs (Fig. 1) and R-on-T-phenomenon was significantly greater in dogs with ARVC with all cut-off criteria than in the control group. VTs in the ARVC group were also more prolonged than in control dogs (Supplemental Material Table 3).

Decreased visual RV function, visual RV dilatation and RV wall motion abnormalities, although uncommon, occurred more frequently in ARVC dogs than in the controls (Supplemental Material Table 4), however, there were no significant differences between dogs with and without ARVC regarding measured LV or RV internal diameters or LV ejection fraction (Supplemental Material Table 4).

*Novel diagnostic criteria*

ROC analysis identified the best cut-off for the number of couplets or triplets on 24-h-ECG to predict the diagnosis at 1.5 couplets or 0.5 triplets (Supplemental Material Fig. 1). These numbers were rounded to 1 couplet and 1 triplet in 24 hours for convenience and logic.

Simple binary logistic regression analysis showed that both the presence of couplets and R-on-T-phenomenon were significant predictors of the diagnosis. This remained unchanged with all the different traditional cut-off criteria (Supplemental Material Table 5).

As a result of this, the criteria to diagnose ARVC were refined to include ≥100 VPCs, presence of ≥1 couplet and R-on-T phenomenon on a 24h-ECG. Henceforth this is referred to as novel criteria.

If only one of the novel criteria was fulfilled without consideration of previously applied echocardiographic exclusion parameters, patients with RV dilatation were correctly placed in the ARVC group (Supplemental Material, Tables 6-9). Affected dogs were older, had a significantly higher prevalence of polymorphic VPCs and VTs

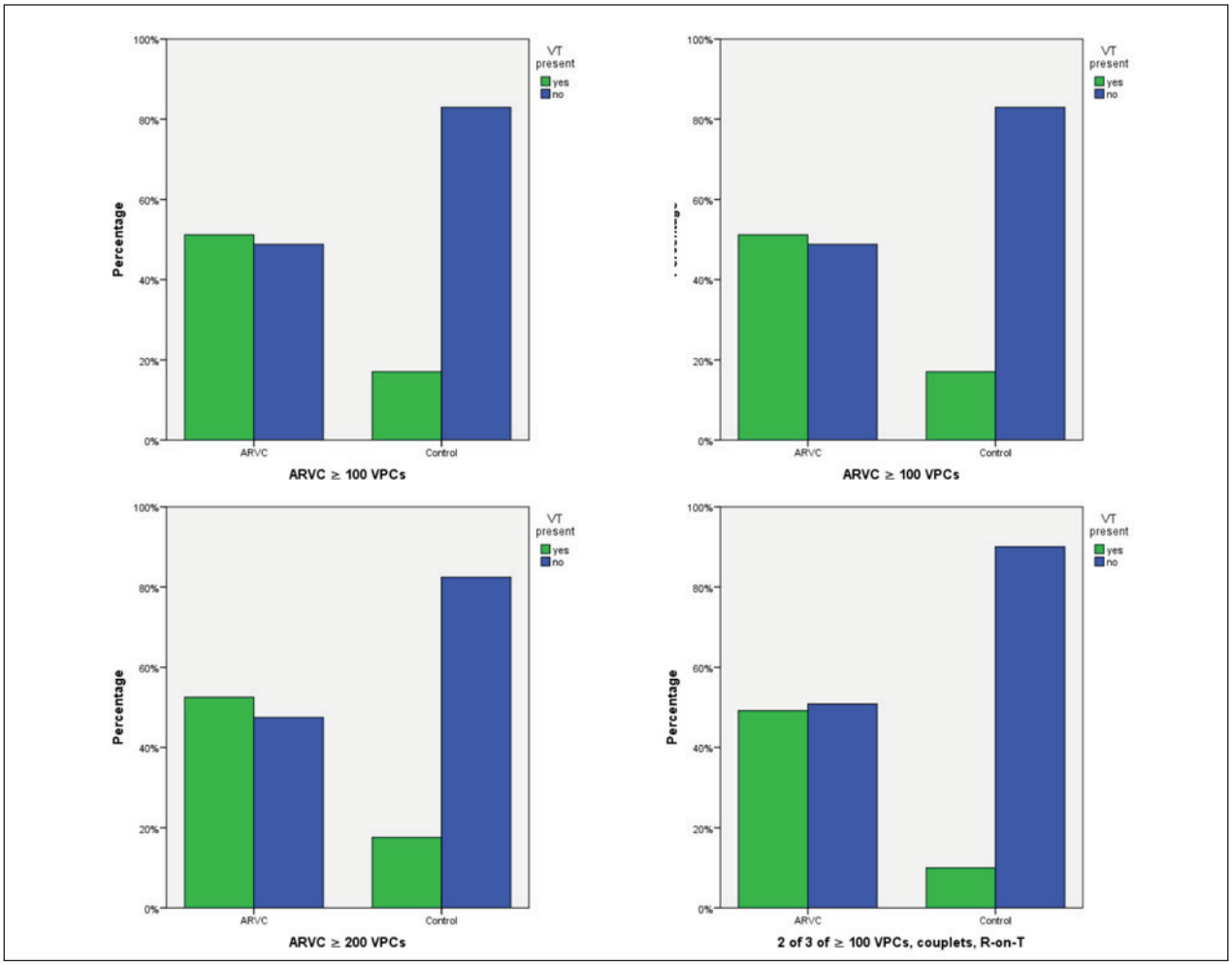


**Figure 1.** Comparison of prevalence of VT when ARVC is defined as a.) ≥100 VPCs, b.) ≥ 50 VPCs, c.) ≥ 200 VPCs, d.) 2 of 3 criteria (≥ 100 VPCs, couplets, R-on-T; only dogs with a 24h-ECG included). All remaining dogs are in Control group.

(Supplemental Material, Table 6) compared to the control group. Addition of one more novel criterion, again without application of echocardiographic exclusion parameters, further refined diagnostic efficacy (Supplemental Material, Table 6 and Fig. 1) and resulted in dogs with AS being moved to the control group (Table 1, Figure 2) and dogs with DCM being moved to the ARVC group (Table 1, Fig. 3). This effect was only marginally more pronounced when all 3 novel criteria were used.

Dogs with RV dilatation, which did not fulfil any of the novel criteria, had either a severe valvular or subvalvular AS, and were therefore correctly assigned to the non-ARVC group, or did not have a 24h-ECG and were therefore not eligible for the correct diagnosis (Supplemental Material, Table 6). Syncope was prevalent in both ARVC and control dogs, however statistically significantly more so in the ARVC group.

## Discussion

ARVC in Boxer dogs has been proposed as a useful model for the human disease because of their similarity at the clinical and pathological levels (12). Furthermore the prevalence is estimated to be higher than in humans and disease progression more rapid (21). However, in order to fully exploit this model, clear diagnostic criteria are required. To date, there are no universally accepted diagnostic criteria for ARVC in Boxer dogs, with inclusion criteria in different studies varying considerably, occasionally in a contradictory manner. Diagnosis is traditionally based on the number of VPCs with or without echocardiographic changes (Supplemental Material, Table 1). However, the number of VPCs documented on 24h-ECGs recorded from the same dog may vary as much as 80% (22). We therefore aimed to identify diagnostic criteria, which would describe a more uniform pheno-

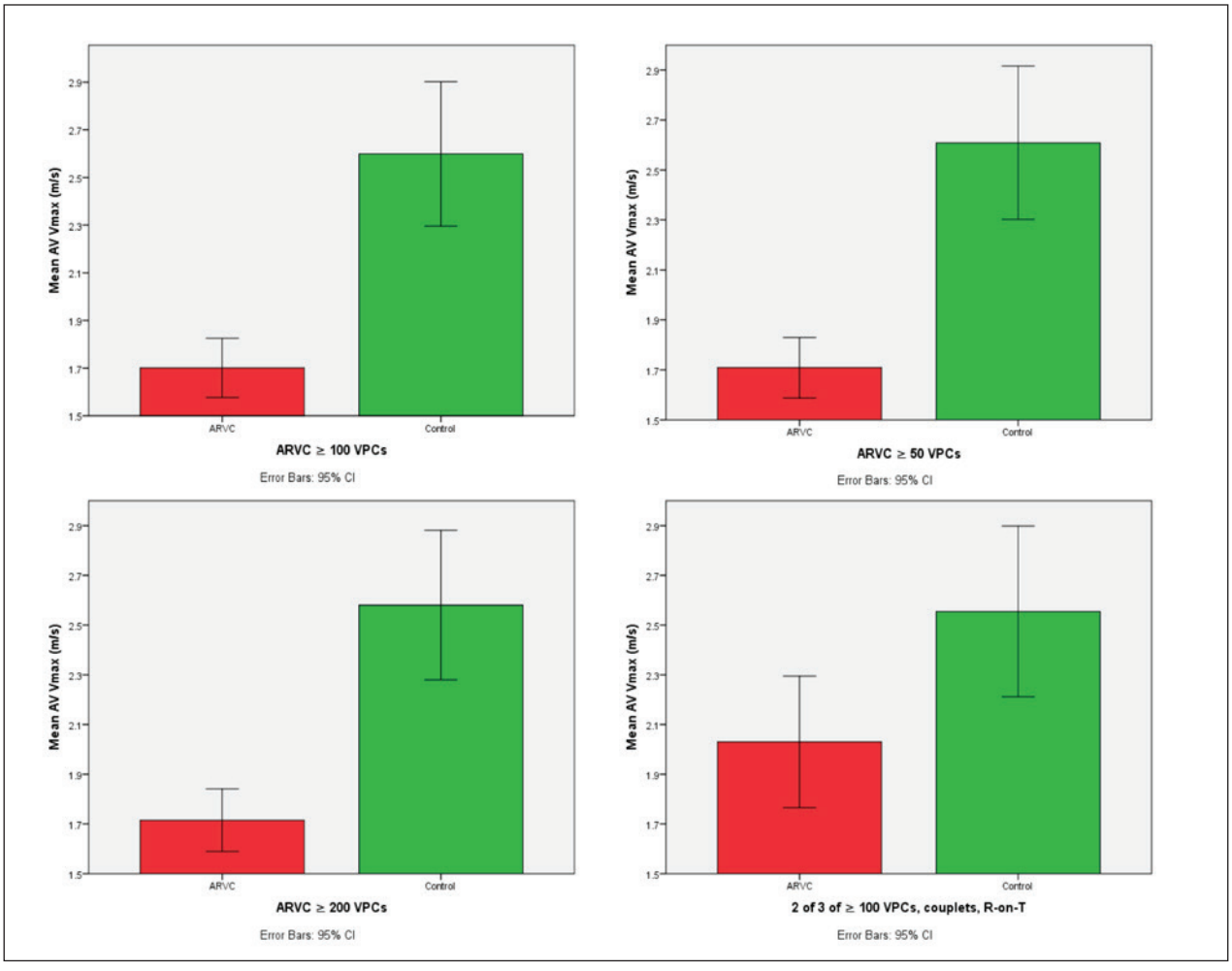


**Figure 2.** Comparison of mean AV Vmax (m/s), when ARVC is defined as a.) ≥100 VPCs, b.) ≥ 50 VPCs, c.) ≥ 200 VPCs, d.) 2 of 3 criteria (≥ 100 VPCs, couplets, R-on-T; only dogs with 24h-ECG included). All remaining dogs are in Control group. Whiskers symbolise 95% confidence intervals.

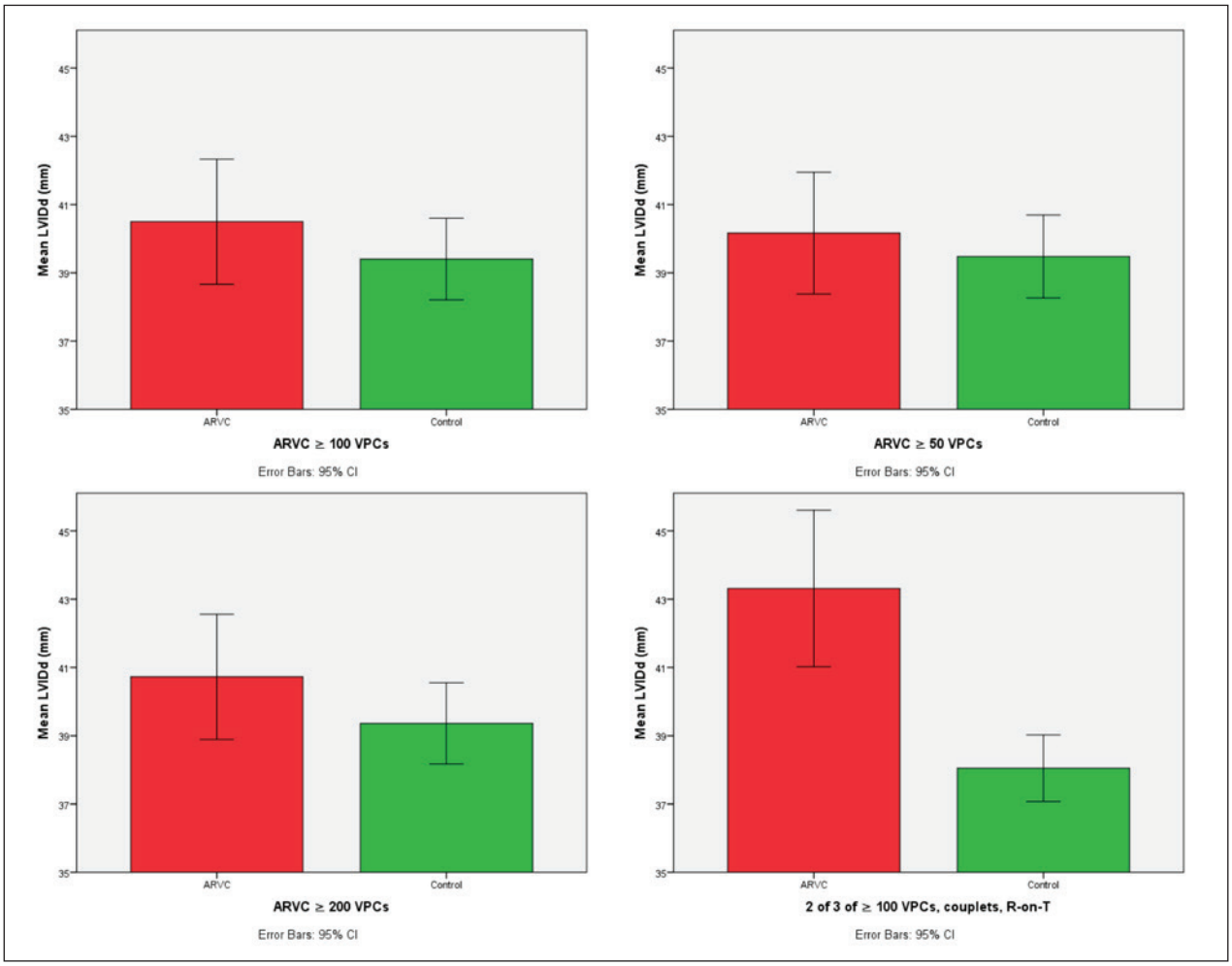


**Figure 3.** Comparison of mean left ventricular end-diastolic internal diameter (mm), when ARVC is defined as a.) ≥100 VPCs, b.) ≥ 50 VPCs, c.) ≥ 200 VPCs, d.) 2 of 3 criteria (≥ 100 VPCs, couplets, R-on-T; only dogs with a 24h-ECG included). All remaining dogs are in Control group. Whiskers symbolise 95% confidence intervals.

type, clinically applicable and not solely related on the number of VPCs on a single 24h-ECG.

Our main findings are that there are significant differences with regard to presentation with syncope, presence of polymorphic VPCs, couplets, triplets, VTs and R-on-T phenomenon in 24h-ECGs, as well as decreased RV function and RV dilatation in echocardiograms between affected and control groups, when using the number of VPCs documented on a 24h-ECG as an inclusion criterion, and echocardiographic signs of AS or DCM as exclusion criteria. Between cut-off values of ≥ 50, ≥ 100 or ≥ 200 VPCs, a cut-off of ≥100 VPCs showed the clearest distinction between the two groups. Multivariable analysis identified couplets and R-on-T phenomenon on 24h-ECGs as clinically useful predictors for ARVC. Therefore, we suggest new criteria for the diagnosis of ARVC consisting of ≥ 100 VPCs, presence of couplets and R-on-T phenomenon on a 24h-ECG.

There is currently no gold standard against which to compare these results. However, when the three diagnostic criteria outlined above are applied without using echocardiographic exclusion criteria, with 2 out of 3 criteria needing to be satisfied for a diagnosis of ARVC, we were able to diagnose a group of dogs presenting with a phenotype closely resembling the phenotype described in human ACM with ventricular arrhythmia, dilatation of the RVOT, presentation with syncope and without signs of valvular disease. Dogs diagnosed with ARVC using only the novel criteria also had larger LV diameters and a lower velocity across the aortic valve in comparison to control dogs, and this finding excludes AS as a common reason for arrhythmia. The use of these three 24h-ECG criteria should enable a clearer description of the phenotype. However, future prospective studies will be required to test their robustness.

ARVC in Boxer dogs is characterized by ventricular tachyarrhythmia, syncope and myocardial dysfunction (12). Necropsies reveal RV chamber dilatation in about a third of cases and histopathological changes closely resembled those seen in human patients (12).

RV dilatation and dysfunction are a mainstay of the diagnosis in humans(8). We identified RV dilatation in a small percentage of Boxer dogs with ARVC, as previously described (23). In humans with ACM, electrical changes often precede structural changes (6). The low prevalence of RV dilatation in Boxer dogs may indicate that RV dilatation is characteristic of more progressive disease and that a proportion of dogs die before these structural changes develop(11). When applying the novel criteria, the mean LV diameter was larger in dogs with ARVC than in the control group. ACM in humans is also known to involve the LV which may have an appearance similar to DCM, however, is generally associated with a more arrhythmic presentation than DCM(10). The finding of biventricular or even left dominant involvement has led to the term "arrhythmogenic cardiomyopathy" (24, 25). The original description of ARVC in Boxer dogs included both an arrhythmic and a dilated phenotype (11). Further studies are required to determine whether both phenotypes are an expression of the same disease. However, based on our results and previous publications commenting on left ventricular involvement in Boxer dogs (12, 13), we recommend the use of the term arrhythmogenic cardiomyopathy in Boxer dogs in view of the condition's close similarity to the human disease.

In human patients, the diagnostic and prognostic value of couplets is unresolved (26-29). In our study, we identified a correlation between the number of couplets and the number of VPCs. Furthermore, binary logistic regression showed couplets to be an independent predictor for the diagnosis of ARVC in Boxer dogs. The occurrence of the R-on-T phenomenon in Boxer dogs with ARVC has been previously described (30) and this study confirms its diagnostic value.

Our novel criteria, based on knowledge from human ACM with respect to arrhythmia and structural changes, appear robust and therefore likely to have beneficial diagnostic utility. This further refinement of the ARVC phenotype in Boxer dogs should endorse its use as a model of human disease.

*Limitations*

This is a retrospective study on a heterogeneous and biased cohort of Boxer dogs, which needed to be referred to a tertiary centre for inclusion. Not all dogs were examined with all diagnostic modalities, although dogs without a 24h-ECG were excluded from the ARVC group. Therefore, the control group may have included dogs with ARVC, which were misdiagnosed. Follow up data in these dogs is lacking, so dogs assigned to the control group may develop ARVC later in life or show an incomplete expression. Also we have not assessed survival data in this study.

The main limitation, however, is, that there is no gold standard for the diagnosis of ARVC. Furthermore, our criteria are based on the hypothesis that ACM in humans and ARVC in Boxer dogs are the same disease (12). Our criteria need confirmation, using prospective longitudinal studies and possibly histological analysis.

## Conclusions

We suggest 3 diagnostic criteria, which include ≥ 100 VPCs in 24 hours, presence of couplets and R-on-T phenomenon in a 24h-ECG, of which 2 must be fulfilled as a more robust and straightforward way of diagnosing ARVC in Boxer dogs. ARVC in Boxer dogs progresses more rapidly compared to humans and could therefore serve as an excellent research model for the human disease. Studying the canine version of the disease allows further research on the genetic cause and its therapy, which may be translated into the human clinic. The novel findings of couplets and R-on-T phenomenon will need further evaluation in both Boxer dogs and humans. In view of the close analogy between disease in Boxer dogs and humans and in particular the presence of both right and left ventricular involvement we suggest adopting the term arrhythmogenic cardiomyopathy for the canine condition.

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# Appendix

**Supplemental Table 1. Topics of previous studies on Boxer dog arrhythmogenic right ventricular cardiomyopathy and the inclusion/exclusion criteria used.**

| Author | Topic | Inclusion criteria | Exclusion criteria |
|---|---|---|---|
| Spier 2004[1] | Spontaneous variability in the frequency of ventricular arrhythmias | ≥ 2 years old, no evidence of structural heart disease (echo), ≥ 500 VPC/24h | 24h-ECG < 20 h |
| Basso 2004[2] | A new animal model of human ARVC | Substantial ectopy or syncope, died or euthanized | NA |
| Baumwart 2005[3] | Plasma brain natriuretic peptide concentration | ≥ 1000 VPC/24h | Abnormal left ventricular shortening (<25%), increased left ventricular chamber size with respect to body size, notable valvular regurgitation |
| Meurs 2006[4] | Expression of the cardiac ryanodine receptor | > 1000 VPC/24h of RV origin, and, when present, syncope, sudden cardiac death or right heart failure | NA |
| Baumwart 2007[5] | Serum cardiac troponin I concentration | ≥ 1000 VPC/24h, echocardiographic variables within reference range limits | 24h-ECG < 20 h |
| Smith 2007[6] | Omega-3 fatty acids | ≥ 95 VPC/24h | Symptomatic heart disease, concurrent major diseases, left ventricular outflow tract velocity >3 m/s, arrhythmic medication |
| Oyama 2008[7] | Calstabin2 deficiency | > 1000 VPC/24h with LBBB morphology and when present, syncope or SCD | NA |
| Scansen 2009[8] | Temporal variability of ventricular arrhythmias | > 1 year old, ≥100 VPC/24h, normal systolic function (fractional shortening > 25%) in echo | 24h-ECG < 24h, current antiarrhythmic medication |
| Baumwart 2009[9] | Magnetic resonance imaging of right ventricular morphology and function | > 1000 VPC/24h and, when present, syncope | NA |
| Meurs 2010[10] | Deletion in the 3' untranslated region of striatin | ≥ 500 VPC/24h of right ventricular origin and, when present, syncope | Echocardiographic abnormalities suggestive of congenital heart disease or dilated cardiomyopathy |
| Palermo 2011[11] | Clinical presentation, diagnostic findings and survival | History of syncope or exercise intolerance, presence of ECG and/or 24h-ECG-abnormalities | Unavailability of one of the investigations, gross left atrial dilatation, aortic velocity > 2.4 m/s, congenital or other acquired heart diseases, myocardial failure secondary to rapid supraventricular tachycardia, systemic diseases that might affect the cardiovascular system |
| Oxford 2011[12] | Ultrastructural changes in cardiac myocytes | "We used a combination of electrocardiographic and histopathologic characteristics to confirm a phenotypic diagnosis | NA |
| Caro-Vadillo 2013[13] | Retrospective study of survival | > 100 VPC/24h, normal left ventricular chamber on echo (LVEDD/BSA < 4.35 cm/m2, LVESD <2.91 cm/m2) | NA |
| Mõtsküla 2013[14] | Prognostic value of 24-hour ambulatory ECG (Holter) monitoring | Referral for clinical signs | 24h-ECG <19 h, documented congenital heart disease, aortic blood flow velocities ≥ 2.25 m/s |
| Meurs 2013[15] | Association of dilated cardiomyopathy with the striatin mutation genotype | Adult, frequent ventricular tachyarrhythmias, normal LV systolic function, positive for striatin mutation | NA |
| Meurs 2014[16] | Natural history of arrhythmogenic right ventricular cardiomyopathy in the boxer dog: a prospective study | > 1 year old, ≥ 300 VPC/24h without an obvious cause for the arrhythmia, evaluated at least 2 years sequentially | Echocardiographic abnormalities suggestive of congenital heart disease, evidence of serious systemic disease |
| Oxford 2014[17] | Change in β-catenin localization suggests involvement of the canonical Wnt pathway | Extensive histopathological examination and striatin mutation OR evidence of cardiac dysfunction on echocardiogram OR > 10% VPC/24h. | NA |
| Cattanach 2015[18] | Pedigree-based genetic appraisal | Clinically diagnosed based on echocardiography, 24h-ECG according to the criteria specified by Meurs[19], exclusion of other causes of arrhythmia: Supposedly >100 VPC/24h, normal echo | Rescue dogs and other dogs without pedigree |

VPC: ventricular premature beat; LBBB: left bundle branch block; SCD: sudden cardiac death; echo: echocardiogram; LVEDD: left ventricular end-diastolic diameter; BSA: body surface area; LVESD: left ventricular end-systolic diameter

Supplemental Table 2. Baseline characteristics of arrhythmogenic right ventricular cardiomyopathy (ARVC) and cont
off at ≥ 50, ≥ 100 and ≥ 200 ventricular premature complexes (VPC), respectively.

| Parameter | ARVC: Cut-off ≥ 50 VPC | | | ARVC: Cut-off ≥ 100 VPC | | | ARVC: Cu |
|---|---|---|---|---|---|---|---|
| | ARVC (n = 46) | Controls (n = 218) | p-value | ARVC (n = 43) | Controls (n = 221) | p-value | ARVC (n = 40) |
| Male Sex | 22 (47.8%) | 114 (55.0%) | 0.417 | 20 (46.5%) | 122 (55.2) | 0.319 | 18 (45.0%) |
| Age | 7.22 ± 3.39 | 4.89 ± 3.20 | **0.000** | 7.31 ± 3.36 | 4.91 ± 3.21 | **0.000** | 7.18 ± 3.35 |
| Echocardiogram | 45 (97.8%) | 216 (99.0%) | 0.438 | 42 (97.7%) | 219 (99.1%) | 0.415 | 39 (97.5%) |
| 24h-ECG | 46 (100%) | 85 (39.0%) | **0.000** | 43 (100%) | 88 (39.8%) | **0.000** | 40 (100%) |
| (Pre-) Syncope | 35 (83.3%) (n = 42) | 85 (49.7%) (n = 171) | **0.000** | 33 (84.6%) (n = 39) | 87 (50.0%) (n = 174) | **0.000** | 31 (86.1%) (n = 36) |
| (Pre-) Syncope at exertion | 14 (33.3%) | 40 (23.4%) | 0.234 | 13 (33.3%) | 41 (23.6%) | 0.224 | 12 (33.3%) |
| BNP | 2022 ± 789 (n = 7) | 1673 ± 1184 (n=33) | 0.462 | 2022 ± 789 (n = 7) | 1673 ± 1184 (n = 33) | 0.462 | 2022 ± 789 (n = 7) |
| Troponin | 1.71 ± 3.51 (n = 16) | 0.72 ± 2.32 (n=63) | 0.177 | 1.80 ± 3.61 (n = 15) | 0.72 ± 2.30 (n = 64) | 0.148 | 1.80 ± 3.61 (n = 15) |

BNP: brain natriuretic peptide.

Supplemental Table 3. 24h-ECG results of arrhythmogenic right ventricular cardiomyopathy (ARVC) and control group, with a cut-off of ≥ 50, ≥ 100 and ≥ 200 ventricular premature complexes (VPC), respectively.

| Parameter | ARVC: Cut-off ≥ 50 VPC | | | ARVC: Cut-off ≥ 100 VPC | | | ARVC: Cut-off ≥200 VPC | | |
|---|---|---|---|---|---|---|---|---|---|
| | ARVC (n = 46) | Controls (n = 85) | p-value | ARVC (n = 43) | Controls (n = 88) | p-value | ARVC (n = 40) | Controls (n = 91) | p-value |
| Time 24h-ECG (h) | 24.4 ± 1.0 | 24.4 ± 1.1 | 0.821 | 24.3 ± 1.0 | 24.4 ± 1.0 | 0.473 | 24.3 ± 1.0 | 24.4 ± 1.0 | 0.583 |
| Mean HR (bpm) | 92 ± 17 | 92 ± 24 | 0.902 | 93 ± 18 | 91 ± 24 | 0.708 | 93 ± 18 | 91 ± 24 | 0.717 |
| VPC present | 46 (100%) | 79 (92.9) | 0.090 | 43 (100%) | 82 (93.2%) | 0.177 | 40 (100%) | 85 (93.4%) | 0.177 |
| Number VPC | 2339 ± 3129 | 624 ± 2051 | **0.000** | 2498 ± 3177 | 605 ± 2018 | **0.000** | 2673 ± 3228 | 590 ± 1986 | **0.000** |
| Polymorphic VPC | 41 (91.1) | 53 (67.1%) | **0.002** | 38 (90.5%) | 56 (68.3%) | **0.007** | 35 (89.7%) | 59 (69.4%) | **0.014** |
| VPC negative lead I | 20 (45.5%) | 27 (34.2%) | 0.248 | 20 (48.8%) | 27 (32.9%) | 0.115 | 19 (50.0%) | 28 (32.9%) | 0.107 |
| VPC positive lead II | 18 (40.9%) | 34 (43.0%) | 0.851 | 18 (43.9%) | 34 (41.5%) | 0.848 | 18 (47.4%) | 34 (40.0%) | 0.554 |
| VPC negative lead III | 18 (50.0%) | 29 (43.9%) | 0.678 | 17 (51.5%) | 30 (43.5%) | 0.526 | 14 (46.7%) | 33 (45.8%) | 1.000 |
| Couplets present | 38 (82.6%) | 30 (35.3%) | **0.000** | 37 (86.0%) | 31 (35.2%) | **0.000** | 34 (85.0%) | 34 (37.4%) | **0.000** |
| Number Couplets | 680 ± 2183 | 39 ± 197 | **0.008** | 727 ± 2252 | 38 ± 194 | **0.005** | 781 ± 2327 | 36 ± 191 | **0.003** |
| Polymorphic couplets | 15 (40.5%) | 12 (41.4%) | 1.000 | 15 (41.7%) | 12 (40.0%) | 1.000 | 15 (45.5%) | 12 (36.4%) | 0.617 |
| Triplets present | 24 (52.2%) | 15 (17.6%) | **0.000** | 24 (55.8%) | 15 (17.0%) | **0.000** | 23 (57.5%) | 16 (17.6%) | **0.000** |
| Number Triplets | 172 ± 547 | 10 ± 63 | **0.008** | 184 ± 565 | 10 ± 62 | **0.005** | 198 ± 583 | 10 ± 61.0 | **0.003** |
| Polymorphic triplets | 5 (20.0%) | 5 (33.3%) | 0.457 | 5 (20.0%) | 5 (33.3%) | 0.457 | 5 (21.7%) | 5 (29.4%) | 0.717 |
| VT present | 22 (47.8%) | 15 (17.6%) | **0.000** | 22 (51.2%) | 15 (17.0%) | **0.000** | 21 (52.5%) | 16 (17.6%) | **0.000** |
| Number VT | 183 ± 1064 | 2 ± 8 | 0.118 | 195 ± 1100 | 2 ± 8 | 0.099 | 210 ± 1140 | 2 ± 8 | 0.082 |
| Duration longest VT (beats) | 28 ± 36 | 14 ± 17 | 0.181 | 28 ± 36 | 14 ±17 | 0.181 | 29 ± 36 | 14 ± 17 | 0.134 |
| HR fastest VT (bpm) | 268 ± 61 | 247 ± 53 | 0.294 | 268 ± 61 | 247 ± 53 | 0.294 | 270 ± 62 | 245 ± 51 | 0.197 |
| Polymorphic VT | 3 (13.6%) | 2 (13.3%) | 1.000 | 3 (13.6%) | 2 (13.3%) | 1.000 | 3 (14.3%) | 2 (12.5%) | 1.000 |
| R-on-T | 33 (73.3%) | 22 (27.5%) | **0.000** | 32 (76.2%) | 23 (27.7%) | **0.000** | 31 (79.5%) | 24 (27.9%) | **0.000** |
| Number of beats R-on-T | 689 ± 2926 | 17 ± 23 | 0.288 | 708 ± 2971 | 19 ± 26 | 0.273 | 731 ± 3018 | 19 ± 26 | 0.254 |
| R-on-T % of all beats | 26.6 ± 75.7 | 36.4 ± 40.1 | 0.581 | 25.0 ± 76.3 | 38.3 ± 40.2 | 0.447 | 25.7 ± 77.4 | 36.8 ± 40.1 | 0.526 |

HR: heart rate; VT: ventricular tachycardia.

Supplemental Table 4. Echocardiographic results of arrhythmogenic right ventricular cardiomyopathy (ARVC) and control group, with a cut-off of ≥ 50, ≥ 100 and ≥ 200 ventricular premature complexes (VPC), respectively.

| Parameter | ARVC: Cut-off ≥ 50 VPC | | | ARVC: Cut-off ≥ 100 VPC | | | ARVC: Cut-off ≥ 200 VPC | | |
|---|---|---|---|---|---|---|---|---|---|
| | ARVC (n = 45) | Controls (n = 216) | p-value | ARVC (n = 42) | Controls (n = 219) | p-value | ARVC (n = 47) | Controls (n = 214) | p-value |
| Decreased RV function | 3 (6.7%) | 2 (1.0%) | **0.045** | 3 (7.1%) | 2 (1.0%) | **0.037** | 3 (7.7%) | 2 (1.0%) | **0.030** |
| RV dilatation | 5 (11.1%) | 4 (2.0%) | **0.012** | 5 (11.9%) | 4 (2.0%) | **0.009** | 4 (10.3%) | 5 (2.4%) | **0.039** |
| RV wall motion abnormality | 4 (8.9%) | 3 (1.5%) | **0.023** | 4 (9.5%) | 3 (1.5%) | **0.018** | 4 (10.3%) | 3 (1.5%) | **0.014** |
| RA dilatation | 3 (6.7%) | 6 (3.0%) | 0.217 | 3 (7.1%) | 6 (3.0%) | 0.186 | 2 (5.1%) | 7 (3.4%) | 0.638 |
| Aortic diameter (mm) | 19.8 ± 2.2 | 19.2 ± 3.3 | 0.313 | 19.8 ± 2.2 | 19.2 ± 3.3 | 0.285 | 19.8 ± 2.2 | 19.2 ± 3.3 | 0.345 |
| LA diameter (mm) | 33.1 ± 8.8 | 30.9 ± 7.9 | 0.146 | 33.6 ± 9.0 | 30.9 ± 7.8 | 0.066 | 33.1 ± 8.0 | 31.0 ± 8.1 | 0.181 |
| LVEF (%) | 47.8 ± 13.2 | 50.0 ± 16.5 | 0.532 | 47.5 ± 13.4 | 50.0 ± 16.5 | 0.467 | 47.0 ± 13.3 | 50.2 ± 16.4 | 0.365 |
| IVSd (mm) | 10.6 ± 1.7 | 10.3 ± 2.0 | 0.382 | 10.7 ± 1.7 | 10.3 ± 2.0 | 0.286 | 10.6 ± 1.7 | 10.4 ± 2.0 | 0.469 |
| LVIDd (mm) | 40.2 ± 5.8 | 39.5 ± 7.5 | 0.582 | 40.5 ± 5.7 | 39.4 ± 7.5 | 0.390 | 40.7 ± 5.6 | 39.4 ± 7.5 | 0.294 |
| LVPWd (mm) | 10.6 ± 1.9 | 10.8 ± 1.8 | 0.497 | 10.5 ± 1.9 | 10.9 ± 1.8 | 0.265 | 10.4 ± 1.7 | 10.9 ± 1.8 | 0.138 |
| LVIDs (mm) | 30.3 ± 7.2 | 28.5 ± 8.6 | 0.215 | 30.8 ± 7.0 | 28.4 ± 8.5 | 0.105 | 31.0 ± 7.0 | 28.4 ± 8.5 | 0.086 |
| FS (%) | 25.7 ± 0.5 | 28.4 ± 10.3 | 0.126 | 25.0 ± 9.3 | 28.5 ± 10.3 | 0.052 | 25.0 ± 9.5 | 28.5 ± 10.3 | 0.059 |
| TAPSE (mm) | 15.3 ± 4.5 | 15.5 ± 2.8 | 0.925 | 15.3 ± 4.5 | 15.5 ± 2.8 | 0.925 | 15.9 ± 4.5 | 15.2 ± 2.9 | 0.576 |
| MV E Velocity (m/s) | 0.80 ± 0.23 | 0.82 ± 0.21 | 0.625 | 0.81 ± 0.23 | 0.82 ± 0.21 | 0.830 | 0.81 ± 0.23 | 0.82 ± 0.21 | 0.830 |
| MV Deceleration time (ms) | 122 ± 29 | 127 ± 40 | 0.658 | 124 ± 29 | 126 ± 40 | 0.830 | 124 ± 29 | 126 ± 40 | 0.830 |
| MV Deceleration slope (m/s2) | 7.2 ± 3.1 | 6.4 ± 2.3 | 0.311 | 7.2 ± 3.1 | 6.4 ± 2.3 | 0.329 | 7.20 ± 3.12 | 6.44 ± 2.31 | 0.830 |
| MV A Velocity (m/s) | 0.54 ± 0.15 | 0.55 ± 0.15 | 0.678 | 0.53 ± 0.14 | 0.56 ± 0.15 | 0.474 | 0.53 ± 0.14 | 0.55 ± 0.15 | 0.329 |
| MV E/A | 1.58 ± 0.79 | 1.56 ± 0.53 | 0.880 | 1.62 ± 0.81 | 1.55 ± 0.53 | 0.584 | 1.62 ± 0.81 | 1.55 ± 0.53 | 0.584 |
| AV Vmax (m/s) | 1.71 ± 0.33 | 2.56 ± 1.27 | **0.000** | 1.70 ± 0.33 | 2.55 ± 1.27 | **0.000** | 1.71 ± 0.33 | 2.54 ± 1.27 | **0.001** |
| AV max PG (mmHg) | 12 ± 5 | 32 ± 33 | **0.001** | 12 ± 5 | 31 ± 33 | **0.001** | 12 ± 5 | 31 ± 33 | **0.002** |
| RVOT PSAX (mm) | 25.0 ± 7.3 | 24.4 ± 5.5 | 0.681 | 24.7 ± 7.5 | 24.5 ± 5.5 | 0.866 | 24.7 ± 7.6 | 24.5 ± 5.4 | 0.916 |
| RVIT 4CH (mm) | 21.8 ± 10.5 | 22.7 ± 4.1 | 0.692 | 21.8 ± 10.9 | 22.7 ± 4.0 | 0.698 | 21.8 ± 10.9 | 22.7 ± 4.0 | 0.698 |
| LVIT 4CH (mm) | 33.1 ± 11.6 | 36.0 ± 5.7 | 0.269 | 32.7 ± 12.0 | 36.1 ± 5.6 | 0.215 | 32.7 ± 12.0 | 36.1 ± 5.6 | 0.215 |
| RVIT/LVIT | 0.71 ± 0.38 | 0.63 ± 0.09 | 0.281 | 0.72 ± 0.40 | 0.63 ± 0.09 | 0.225 | 0.72 ± 0.40 | 0.63 ± 0.09 | 0.225 |

RV: right ventricular; RA: right atrium; LA: left atrium; LVEF: left ventricular ejection fraction; IVSd: intraventricular septum measured in diastole; LVIDd: left ventricular internal diameter in diastole; LVPWd: left ventricular posterior wall measured in diastole; LVIDs: left ventricular internal diameter in systole; FS: fractional shortening; TAPSE: tricuspid annular plane systolic excursion; MV: mitral valve; AV: aortic valve; Vmax: maximal velocity; max PG: maximal pressure gradient; RVOT: right ventricular outflow tract; PLAX: parasternal long axis view, PSAX: parasternal short axis view; RVIT: right ventricular inflow tract; 4CH: four-chamber-view; LVIT: left ventricular inflow tract.

Supplemental Table 5 a-c. Simple binary logistic regression predicting likelihood of arrhythmogenic right ventricular cardiomyopathy (ARVC) , cut-off a.) ≥ 50, b.) ≥1 00, c.) ≥ 200 VPC for diagnosis ARVC.

| a.) | B | SE | Wald | df | P | Odds Ratio | 95% CI | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Couplets | 1.765 | 0.481 | 13.468 | 1 | 0.000 | 5.843 | 2.276 | 15.000 |
| R on T | 1.711 | 0.448 | 14.581 | 1 | 0.000 | 5.535 | 2.300 | 13.320 |
| Constant | -2.529 | 0.467 | 29.283 | 1 | 0.000 | 0.080 | | |

| b.) | B | SE | Wald | df | P | Odds Ratio | 95% CI | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Couplets | 2.051 | 0.526 | 15.193 | 1 | 0.000 | 7.779 | 2.773 | 21.825 |
| R on T | 1.863 | 0.472 | 15.551 | 1 | 0.000 | 6.441 | 2.552 | 16.257 |
| Constant | -2.975 | 0.534 | 31.048 | 1 | 0.000 | 0.051 | | |

| c.) | B | SE | Wald | df | P | Odds Ratio | 95% CI | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Couplets | 1.830 | 0.532 | 11.845 | 1 | 0.001 | 6.236 | 2.199 | 17.685 |
| R on T | 2.052 | 0.490 | 17.564 | 1 | 0.000 | 7.783 | 2.981 | 20.320 |
| Constant | -3.093 | 0.552 | 31.370 | 1 | 0.000 | 0.045 | | |

B: B coefficient; SE: standard error; Wald: Wald test; df: degrees of freedom; P: p value; CI: confidence interval.

Supplemental Table 6. Baseline characteristics of arrhythmogenic right ventricular cardiomyopathy (ARVC) and control group, 1/3, 2/3, 3/3 novel criteria fulfilled necessary for diagnosis, respectively. All dogs included.

| Parameter | ARVC: 1 of 3 novel criteria | | | ARVC: 2 of 3 novel criteria | | | ARVC: 3 of 3 novel criteria | | |
|---|---|---|---|---|---|---|---|---|---|
| | ARVC (n = 89) | Controls (n = 175) | p-value | ARVC (n = 61) | Controls (n = 203) | p-value | ARVC (n = 36) | Controls (n = 228) | p-value |
| Male Sex | 40 (44.9%) | 102 (58.3%) | **0.050** | 30 (49.2%) | 112 (55.2%) | 0.465 | 20 (55.6%) | 122 (53.5%) | 0.859 |
| Age | 6.55 ± 3.34 | 4.59 ± 3.16 | **0.000** | 7.21 ± 3.07 | 4.69 ± 3.22 | **0.000** | 7.29 ± 2.94 | 5.00 ± 3.32 | **0.000** |
| Echocardiogram | 88 (98.9%) | 173 (98.9%) | 1.000 | 60 (98.4%) | 201 (99.0%) | 0.547 | 35 (97.2%) | 226 (99.1%) | 0.357 |
| 24h-ECG | 89 (100%) | 42 (24.0%) | **0.000** | 61 (100%) | 70 (34.5%) | **0.000** | 36 (100%) | 95 (41.7%) | **0.000** |
| (Pre-) Syncope | 63 (79.7%) (n = 79) | 57 (42.5%) (n = 134) | **0.000** | 44 (80.0%) (n = 55) | 76 (48.1%) (n = 158) | **0.000** | 26 (78.8%) (n = 33) | 94 (52.2%) (n = 180) | **0.007** |
| (Pre-) Syncope at exertion | 31 (39.2%) | 23 (17.2%) | **0.001** | 19 (34.5%) | 35 (22.2%) | 0.075 | 11 (33.3%) | 43 (23.9%) | 0.278 |
| BNP | 2043 ± 1061 (n = 22) | 1356 ± 1112 (n = 18) | 0.054 | 2183 ± 925 (n = 16) | 1434 ± 1163 (n = 24) | **0.037** | 2339 ± 574 (n = 7) | 1606 ± 1176 (n = 33) | 0.118 |
| Troponin | 1.68 ± 3.89 (n = 33) | 0.38 ± 0.53 (n = 46) | **0.028** | 1.80 ± 4.21 (n = 26) | 0.49 ± 1.04 (n = 53) | **0.035** | 1.92 ± 3.85 (n = 13) | 0.73 ± 2.28 (n = 66) | 0.133 |

VPC: ventricular premature complexes; BNP: brain natriuretic peptide

Supplemental Table 7. 24h-ECG results of arrhythmogenic right ventricular cardiomyopathy (ARVC) and control group, 1/3, 2/3, 3/3 novel criteria fulfilled necessary for diagnosis, respectively. All dogs included.

| Parameter | ARVC: 1 of 3 novel criteria | | | ARVC: 2 of 3 novel criteria | | | ARVC: 3 of 3 novel criteria | | |
|---|---|---|---|---|---|---|---|---|---|
| | ARVC (n = 89) | Controls (n = 42) | p-value | ARVC (n = 61) | Controls (n = 70) | p-value | ARVC (n = 36) | Controls (n = 95) | p-value |
| Time 24h-ECG (h) | 24.4 ± 1.0 | 24.5 ± 1.1 | 0.484 | 24.4 ± 0.9 | 24.4 ± 1.1 | 0.868 | 24.3 ± 0.8 | 24.4 ± 1.1 | 0.429 |
| Mean HR (bpm) | 95 ± 24 | 85 ± 16 | **0.009** | 100 ± 26 | 85 ± 15 | **0.000** | 101 ± 29 | 88 ± 18 | **0.004** |
| VPC present | 88 (98.9%) | 37 (88.1%) | **0.013** | 61 (100%) | 64 (91.4%) | **0.030** | 36 (100%) | 89 (93.7%) | 0.123 |
| Number VPC | 1802 ± 2996 | 7 ± 11 | **0.000** | 2582 ± 3342 | 45 ± 167 | **0.000** | 2920 ± 3495 | 585 ± 1821 | **0.000** |
| Polymorphic VPC | 73 (83.9%) | 21 (56.8%) | **0.002** | 55 (91.7%) | 39 (60.9%) | **0.000** | 35 (97.2%) | 59 (67.0%) | **0.000** |
| VPC negative lead I | 36 (41.9%) | 11 (29.7%) | 0.230 | 28 (47.5%) | 19 (29.7%) | 0.063 | 20 (55.6%) | 27 (31.0%) | **0.014** |
| VPC positive lead II | 39 (45.3%) | 13 (35.1%) | 0.325 | 24 (40.7%) | 28 (43.8%) | 0.855 | 14 (38.9%) | 38 (43.7%) | 0.691 |
| VPC negative lead III | 33 (46.5%) | 14 (45.2%) | 1.000 | 27 (54.0%) | 20 (38.5%) | 0.164 | 17 (56.7%) | 30 (41.7%) | 0.195 |
| Couplets present | 68 (76.4%) | 0 (0.0%) | **0.000** | 54 (88.5%) | 14 (20.0%) | **0.000** | 36 (100%) | 32 (33.7%) | **0.000** |
| Number Couplets | 388 ± 1601 | 0 ± 0 | **0.005** | 565 ± 1913 | 2 ± 8 | **0.015** | 895 ± 2438 | 25 ± 150 | **0.001** |
| Polymorphic couplets | 27 (40.9%) | NA | NA | 26 (49.1%) | 1 (7.7%) | **0.006** | 18 (50.0%) | 9 (30.0%) | 0.133 |
| Triplets present | 38 (42.7%) | 1 (2.4%) | **0.000** | 34 (55.7%) | 5 (7.1%) | **0.000** | 26 (72.2%) | 13 (13.7%) | **0.000** |
| Number Triplets | 99 ± 403 | 0 ± 0 | **0.003** | 144 ± 481 | 0 ± 0 | **0.013** | 235 ± 613 | 4 ± 27 | **0.000** |
| Polymorphic triplets | 10 (25.6%) | 0 (0.0%) | 1.000 | 10 (28.6%) | 0 (0.0%) | 0.306 | 6 (23.1%) | 4 (28.6%) | 0.718 |
| VT present | 33 (37.1%) | 4 (9.5%) | **0.001** | 30 (49.2%) | 7 (10.0%) | **0.000** | 21 (58.3%) | 16 (16.8%) | **0.000** |
| Number VT | 96 ± 766 | 0 ± 1 | 0.421 | 140 ± 924 | 0 ± 1 | 0.209 | 235 ± 1201 | 1 ± 4 | 0.058 |
| Duration longest VT (beats) | 24 ± 31 | 7 ± 4 | 0.282 | 26 ± 33 | 9 ± 6 | 0.207 | 32 ± 37 | 9 ± 5 | **0.019** |
| HR fastest VT (bpm) | 259 ± 60 | 261 ± 52 | 0.967 | 266 ± 59 | 234 ± 52 | 0.193 | 271 ± 56 | 244 ± 59 | 0.159 |
| Polymorphic VT | 5 (15.2%) | 0 (0.0%) | 1.000 | 5 (16.7%) | 0 (0.0%) | 0.245 | 4 (19.0%) | 1 (6.3%) | 0.259 |
| R-on-T | 55 (62.5%) | 0 (0.0%) | **0.000** | 46 (76.7%) | 9 (13.8%) | **0.000** | 36 (100%) | 19 (21.3%) | **0.000** |
| Number of beats R-on-T | NA | NA | NA | 450 ± 2487 | 13 ± 25 | 0.563 | 631 ± 2805 | 21 ± 31 | 0.350 |
| R-on-T % of all beats | NA | NA | NA | 26.4 ± 68.1 | 51.9 ± 24.6 | 0.275 | 24.0 ± 72.2 | 43.1 ± 41.7 | 0.293 |

VPC: ventricular premature complexes; HR: heart rate; VT: ventricular tachycardia.

Supplemental Table 8. Echocardiographic results of arrhythmogenic right ventricular cardiomyopathy (ARVC) and control group, 1/3, 2/3, 3/3 novel criteria fulfilled necessary for diagnosis, respectively. All dogs included.

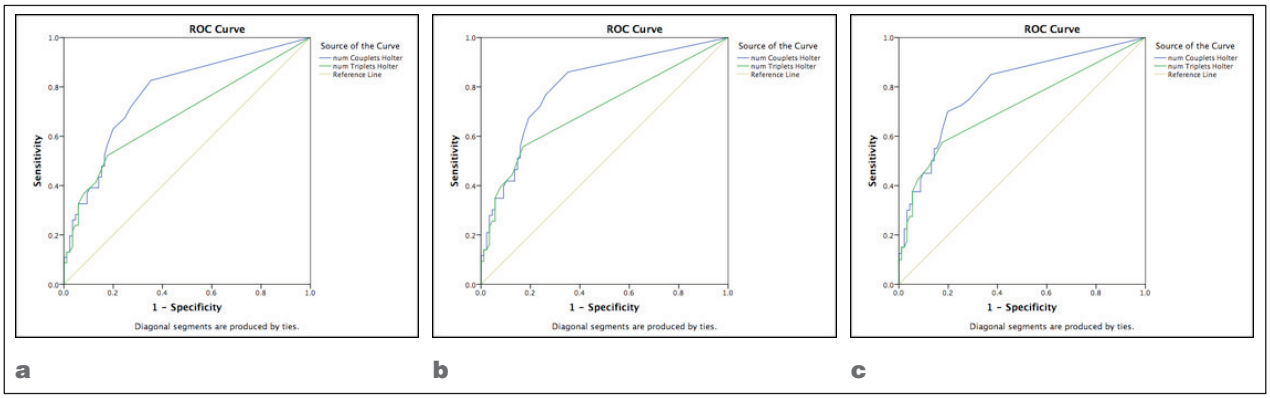
| Parameter | ARVC: 1 of 3 novel criteria | | | ARVC: 2 of 3 novel criteria | | | ARVC: 3 of 3 novel criteria | | |
|---|---|---|---|---|---|---|---|---|---|
| | ARVC (n=88) | Controls (n=173) | p-value | ARVC (n=60) | Controls (n=201) | p-value | ARVC (n=35) | Controls (n=226) | p-value |
| Decreased RV function | 4 (4.7%) | 1 (0.6%) | **0.032** | 4 (6.9%) | 1 (0.5%) | **0.003** | 4 (11.4%) | 1 (0.5%) | **0.000** |
| RV dilatation | 5 (5.9%) | 4 (2.5%) | 0.184 | 5 (8.6%) | 4 (2.2%) | **0.022** | 4 (11.4%) | 5 (2.4%) | **0.009** |
| RV wall motion abnormality | 5 (5.9%) | 2 (1.3%) | **0.039** | 5 (8.6%) | 2 (1.1%) | **0.003** | 5 (14.3%) | 2 (1.0%) | **0.000** |
| RA dilatation | 4 (4.7%) | 5 (3.1%) | 0.550 | 4 (6.8%) | 5 (2.7%) | 0.145 | 4 (11.4%) | 5 (2.4%) | **0.008** |
| Aortic diameter (mm) | 19.4 ± 3.3 | 19.3 ± 3.0 | 0.857 | 19.5 ± 2.3 | 19.3 ± 3.4 | 0.643 | 19.5 ± 2.3 | 19.3 ± 3.2 | 0.707 |
| LA diameter (mm) | 33.2 ± 9.3 | 30.1 ± 6.8 | **0.010** | 36.4 ± 0.4 | 29.3 ± 6.5 | **0.000** | 37.5 ± 10.4 | 30.1 ± 6.9 | **0.000** |
| LVEF (%) | 45.4 ± 15.7 | 53.1 ± 15.5 | **0.010** | 42.0 ± 16.5 | 53.1 ± 14.0 | **0.000** | 40.6 ± 15.9 | 51.4 ± 15.0 | **0.005** |
| IVSd (mm) | 10.5 ± 1.9 | 10.3 ± 1.9 | 0.545 | 10.6 ± 1.9 | 10.3 ± 1.9 | 0.436 | 10.7 ± 1.9 | 10.4 ± 1.9 | 0.412 |
| LVIDd (mm) | 41.1 ± 8.3 | 38.4 ± 5.9 | **0.009** | 43.3 ± 8.7 | 38.0 ± 5.7 | **0.000** | 43.7 ± 8.2 | 38.8 ± 6.6 | **0.000** |
| LVPWd (mm) | 10.8 ± 1.8 | 10.7 ± 1.8 | 0.766 | 10.7 ± 1.9 | 10.8 ± 1.8 | 0.770 | 10.5 ± 1.7 | 10.8 ± 1.8 | 0.398 |
| LVIDs (mm) | 30.9 ± 9.5 | 27.4 ± 6.9 | **0.004** | 33.5 ± 10.2 | 27.0 ± 6.4 | **0.000** | 34.7 ± 10.4 | 27.7 ± 7.3 | 0.000 |
| FS (%) | 26.5 ± 10.5 | 28.8 ± 9.9 | 0.107 | 24.1 ± 11.0 | 29.4 ± 9.4 | **0.001** | 22.0 ± 10.7 | 29.0 ± 9.7 | **0.000** |
| TAPSE (mm) | 15.2 ± 3.8 | 16.0 ± 2.9 | 0.601 | 15.2 ± 3.9 | 15.8 ± 3.1 | 0.630 | 15.5 ± 4.9 | 15.4 ± 2.9 | 0.934 |
| MV E Velocity (m/s) | 0.84 ± 0.24 | 0.80 ± 0.18 | 0.364 | 0.84 ± 0.26 | 0.81 ± 0.19 | 0.482 | 0.79 ± 0.25 | 0.82 ± 0.20 | 0.586 |
| MV Deceleration time (ms) | 115 ± 35 | 134 ± 38 | **0.035** | 118 ± 30 | 128 ± 40 | 0.348 | 123 ± 38 | 126 ± 38 | 0.798 |
| MV Deceleration slope (m/s2) | 7.0 ± 2.6 | 6.3 ± 2.4 | 0.231 | 6.7 ± 2.5 | 6.6 ± 2.5 | 0.872 | 6.01 ± 2.66 | 6.67 ± 2.48 | 0.512 |
| MV A Velocity (m/s) | 0.55 ± 0.14 | 0.55 ± 0.15 | 0.767 | 0.52 ± 0.14 | 0.56 ± 0.15 | 0.218 | 0.49 ± 0.13 | 0.56 ± 0.15 | 0.057 |
| MV E/A | 1.60 ± 0.75 | 1.54 ± 0.43 | 0.589 | 1.67 ± 0.85 | 1.51 ± 0.44 | 0.156 | 1.74 ± 1.00 | 1.53 ± 0.50 | 0.199 |
| AV Vmax (m/s) | 2.29 ± 1.09 | 2.47 ± 1.27 | 0.342 | 2.03 ± 0.86 | 2.52 ± 1.28 | **0.019** | 1.76 ± 0.54 | 2.50 ± 1.25 | **0.007** |
| AV max PG (mmHg) | 24 ± 29 | 30 ± 32 | 0.246 | 19 ± 20 | 31 ± 33 | **0.032** | 13 ± 10 | 30 ± 33 | **0.015** |
| RVOT PSAX (mm) | 25.0 ± 6.8 | 23.8 ± 4.4 | 0.421 | 25.8 ± 7.0 | 23.6 ± 5.2 | 0.097 | 27.1 ± 6.8 | 23.6 ± 5.6 | **0.016** |
| RVIT 4CH (mm) | 22.8 ± 8.1 | 22.0 ± 3.6 | 0.694 | 23.5 ± 7.7 | 21.5 ± 5.4 | 0.316 | 25.4 ± 9.7 | 21.6 ± 5.1 | 0.108 |
| LVIT 4CH (mm) | 35.1 ± 9.6 | 35.1 ± 5.0 | 0.972 | 36.0 ± 7.2 | 34.4 ± 8.4 | 0.520 | 38.2 ± 6.8 | 34.2 ± 8.0 | 0.158 |
| RVIT/LVIT | 0.67 ± 0.28 | 0.63 ± 0.10 | 0.515 | 0.68 ± 0.31 | 0.63 ± 0.09 | 0.442 | 0.72 ± 0.44 | 0.64 ± 0.09 | 0.282 |

RV: right ventricular; RA: right atrium; LA: left atrium; LVEF: left ventricular ejection fraction; IVSd: intraventricular septum measured in diastole; LVIDd: left ventricular internal diameter in diastole; LVPWd: left ventricular posterior wall measured in diastole; LVIDs: left ventricular internal diameter in systole; FS: fractional shortening; TAPSE: tricuspid annular plane systolic excursion; MV: mitral valve; AV: aortic valve; Vmax: maximal velocity; max PG: maximal pressure gradient; RVOT: right ventricular outflow tract; PLAX: parasternal long axis view, PSAX: parasternal short axis view; RVIT: right ventricular inflow tract; 4CH: four-chamber-view; LVIT: left ventricular inflow tract.

Supplemental Table 9. Cases with right ventricular (RV) dilatation: fulfilled criteria, clinical context.

| Patient Nr | 1 criterion fulfilled | 2 criteria fulfilled | 3 criteria fulfilled | Clinical context |
|---|---|---|---|---|
| 11 | No | No | No | No 24h-ECG, RV dilatation due to fluid overload |
| 25 | Yes | Yes | Yes | Progressive abdominal distention, ascites, dilatation of all 4 chambers, systolic dysfunction, moderate TR |
| 35 | Yes | Yes | Yes | Recurrent collapses, high arrhythmic burden, mild LV dilatation |
| 56 | Yes | Yes | Yes | Syncope during exercise, generalised cardiomegaly, sustained VT during follow up |
| 101 | Yes | Yes | No | SVT during routine examination, later presyncope, cough, moderate MR |
| 102 | No | No | No | Lethargy, dilatation of all 4 chambers, no 24h-ECG |
| 136 | Yes | Yes | Yes | Abdominal distention, ascites, chaotic arrhythmia incl. AF, rapid ventricular runs, severe TR, clinically interpreted as ARVC |
| 155 | No | No | No | Murmur at routine examination, syncope during excitement, severe AS, VT in 24h-ECG |
| 222 | No | No | No | Murmur detected at first vaccination, severe PS, TR, right heart failure |

TR: tricuspid valve regurgitation; LV: left ventricular; VT: ventricular tachycardia; MR: mitral valve regurgitation; AF: atrial fibrillation; ARVC: arrhythmogenic right ventricular cardiomyopathy; AS: aortic stenosis; PS: pulmonic valve stenosis




**Supplemental Figure 1.** Receiver operated characteristic (ROC) curves for number of couplets and triplets.
**a.)** ROC curve for number of couplets and triplets, diagnosis based on ≥ 50 ventricular premature complexes (VPC). 1.5 couplets are the best cut-off for diagnosis of arrhythmogenic right ventricular cardiomyopathy (ARVC) with a sensitivity of 71.7% and a specificity of 72.9%, area under the curve (AUC) 0.774 (95% confidence interval (CI) 0.689-0.859). 0.5 triplets are the best cut-off for the diagnosis. Sensitivity 52.2%, specificity 82.4%, AUC 0.685 (95% CI 0.584-0.786)
**b.)** ROC Curve for number of couplets and triplets, diagnosis based on ≥ 100 VPC. 1.5 couplets are the best cut-off for diagnosis of ARVC with a sensitivity of 76.7% and a specificity of 73.9%, AUC 0.800 (95% CI 0.718-0.882). 0.5 triplets are the best cut-off for the diagnosis. Sensitivity 55.8%, specificity 83%, AUC 0.707 (95% CI 0.605-0.808)
**c.)** ROC Curve for number of couplets and triplets, diagnosis based on ≥ 200 VPC. 1.5 couplets are the best cut-off for diagnosis of ARVC with a sensitivity of 72.5% and a specificity of 71.4%, AUC 0.796 (95% CI 0.711-0.882). 0.5 triplets are the best cut-off for the diagnosis. Sensitivity 57.5%, specificity 82.4%, AUC 0.717 (95% CI 0.613-0.821).

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