Arrhythmogenic right ventricular cardiomyopathy (ARVC)-
Impact of exercise on cardiac outcome, differential diagnoses and risk
stratification of arrhythmic events

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List of papers


Abbreviations

AEs  Arrhythmic events
ARVC Arrhythmogenic right ventricular cardiomyopathy
CMR Cardiac magnetic resonance imaging
ECG Electrocardiogram
EF Ejection fraction
ICD Implantable cardioverter defibrillator
LVGLS LV global longitudinal strain
LVMD LV mechanical dispersion
METs Metabolic equivalents
MRI Magnetic resonance imaging
PVC Premature ventricular complexes
RV Right ventricle
RVD Right ventricle basal diameter (indexed in paper 2, not indexed in paper 3)
RVFAC RV fractional area change
RVGLS RVGLS longitudinal strain
RVMD RV mechanical dispersion
RVOT Right ventricular outflow tract
ROC Receiver operating characteristics
SCD Sudden cardiac death
TAPSE Tricuspid annular plane systolic excursion
TFC 2010 ARVC Task Force Criteria
VA Ventricular arrhythmia
VT Ventricular tachycardia
Introduction

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) was first described in the modern scientific literature in 1982 in the seminal work of Frank I. Marcus et al., where Marcus and colleagues systematically described 24 ARVC patient cases (1).

Symptoms and clinical course

Arrhythmogenic right ventricular cardiomyopathy (ARVC) (2), or arrhythmogenic cardiomyopathy (AC) (3;4), is an inheritable progressive heart muscle disease with a broad phenotypic spectrum, characterized by a high incidence of life-threatening arrhythmias, sudden cardiac death (SCD) and heart failure. ARVC is one of the leading causes of SCD (5-8), and may account for approximately 5 – 20 % of SCD in young individuals and athletes in Europe. The prevalence of ARVC is estimated to be 1:1000-1:5000 (9) and SCD may be the first manifestation of disease (10).

The progression of the ARVC phenotype can be divided into four stages: 1. an early concealed phase with absent or subtle structural or electrical changes, but at risk of ventricular tachycardia (VT) and SCD; 2. overt electrical disorder, where life-threatening arrhythmias can be the first manifestation of ARVC, with only subtle regional structural changes; 3. isolated right heart failure with risk of VT and SCD and 4. biventricular heart failure, i.e. advanced ARVC with increased risk of VT and SCD (3;9) (Figure 1).
O Figure 1. The progression of the ARVC phenotype.
a. A structurally normal heart. b. The ARVC disease process of myocardial loss starts on the epicardial site, and c.
extends as a wave-front from the epicardium towards the endocardium. d. Wall thinning and biventricular aneurysm
develop at a later stage because of transmural fibro fatty tissue repair. From Basso C et al. Nat.Rev.Cardiol. 2011
Nov 29; 9 (4): 223-233, with permission.

ARVC disease progression, including life-threatening ventricular arrhythmias, heart failure and SCD
may occur during “hot phases” rather than being a continuous process (9;11). Environmental factors, such as
exercise, may promote and aggravate disease progression (Figure 2).
ARVC diagnosis

In 1994, an international Task Force proposed criteria for the clinical diagnosis of ARVC (12). At that time, clinical experience with ARVC was dominated by symptomatic index cases with overt ARVC disease, and SCD victims. Consequently, the more qualitative 1994 criteria were highly specific but lacked sensitivity for early and familial disease.

In 2010, the diagnostic ARVC Task Force Criteria (TFC) (2) were modified by introducing specific quantitative aspects to improve sensitivity while maintaining diagnostic specificity. Noteworthy, the approach of classifying structural, histological, electrocardiographic, arrhythmic, and genetic/familial features of ARVC as major and minor criteria had been maintained in the modified TFC. In this modification of TFC, quantitative criteria and abnormalities defined and proposed on the basis of comparison with normal subject data. The TFC represented a work-up framework to improve the diagnosis
and management of ARVC and facilitate clinical diagnosis in first-degree family members with incomplete expression of disease.

A definite diagnosis of ARVC is fulfilled by 2 major or 1 major and 2 minor criteria or 4 minor criteria from different categories; borderline diagnosis: 1 major and 1 minor or 3 minor criteria from different categories; possible diagnosis: 1 major or 2 minor criteria from different categories. Modified Task Force criteria are shown in Table 1.

Table 1: Modified Task Force Criteria from 2010

1. Global or regional dysfunction and structural alterations

**Major**

**2D echo criteria**
Regional RV akinesia, dyskinesia or aneurysm and one of the following measured at end diastole.
- PLAX RVOT ≥32 mm (BSA corrected ≥19mm/m²)
- PSAX RVOT ≥36 mm (BSA corrected ≥21mm/m²)
- Fractional area change ≤33%

**MRI criteria**
Regional RV akinesia or dyskinesia or dyssynchronous RV contraction and one of the following
- Ratio of RV end-diastolic volume to BSA ≥110 mL/m² (male) or ≥100 mL/m² (female)
- RV ejection fraction ≤40%

**RV angiography criteria**
Regional RV akinesia, dyskinesia or aneurysm

**Minor**

**2D echo criteria**
Regional RV akinesia or dyskinesia and one of the following measured at end diastole
- PLAX RVOT ≥29 to<32 mm (BSA corrected ≥16 to <19mm/m²)
- PSAX RVOT ≥32 to<36 (BSA corrected ≥ 18 to < 21mm/m²)
- Fractional area change >33% to ≤40%

**MRI criteria**
Regional RV akinesia or dyskinesia or dyssynchronous RV contraction and one of the following
- Ratio of RV end-diastolic volume to BSA ≥100 to <110 mL/m² (male) or ≥90 to <100 mL/m²
RV ejection fraction >40 to ≤45%

2. **Tissue characterization of wall**

**Major**
- Residual myocytes <60% by morphometric analysis (or <50% if estimated) with fibrous replacement of the RV free wall myocardium in ≥1 sample, with or without fatty replacement of tissue on endomyocardial biopsy

**Minor**
- Residual myocytes 60-75% by morphometric analysis (or 50-65% if estimated), with fibrous replacement of the RV free wall myocardium in ≥1 sample, with or without fatty replacement of tissue on endomyocardial biopsy

3. **Repolarization abnormalities**

**Major**
- Inverted T waves in right precordial leads (V1, V2, and V3) or beyond in individuals >14 years of age (in the absence of complete RBBB QRS ≥120 ms)

**Minor**
- Inverted T waves in V1 and V2 in individuals >14 years of age (in the absence of complete RBBB)
  - or in V4, V5 or V6
- Inverted T waves in leads V1, V2, V3 and V4 in individuals >14 years of age in the presence of a complete RBBB

4. **Depolarization/conduction abnormalities**

**Major**
- Epsilon wave (reproducible low-amplitude signals between end of QRS complex to onset of T wave) in the right precordial leads (V1 to V3)

**Minor**
- Late potentials by SAECG in ≥1 of three parameters in the absence of a QRSd of ≥110 ms on standard ECG
  - Filtered QRS ≥114 ms
  - Duration of terminal QRS <40 µV ≥ 38 ms
  - Root-mean-square voltage of terminal 40 ms ≤ 20 µV
  - Terminal activation duration ≥ 55 ms measured from the nadir of the S wave to the last depolarization deflection
5. Arrhythmias

Major
- Nonsustained or sustained VT of LBBB morphology with superior axis

Minor
- Nonsustained or sustained VT of RVOT configuration, LBBB morphology with inferior axis or of unknown axis
- >500 PVCs per 24 h (Holter)

6. Family history

Major
- ARVD/C in first-degree relative who meets Task Force criteria
- ARVD/C confirmed pathologically at autopsy or surgery in first-degree relative
- Identification of pathogenic mutation categorized as associated or probably associated with ARVD/C in the patient under evaluation

Minor
- History of ARVD/C in first-degree relative in whom it is not possible to determine whether the family member meets Task Force criteria.

Diagnostic criteria in ARVC, modified from Marcus et al. (2), with permission.
ARVC, arrhythmogenic right ventricular cardiomyopathy; BSA, body surface area; LBBB, left bundle branch block; PLAX RVOT, parasternal long axis right ventricular outflow tract; PSAX RVOT, parasternal short axis right ventricular outflow tract; PVC, premature ventricular complex; RBBB, right bundle branch block; RV, right ventricle; SAECG, signal averaging electrocardiography; VT, ventricular tachycardia.

ARVC inheritance and pathogenesis

The major discovery that homozygous mutations in junctional plakoglobin (JUP) were the genetic basis of the ARVC associated Naxos disease in 2000 (13), led to rapid identifications of mutations of the desmosomal genes among ARVC patients. Furthermore, modern molecular genetics techniques revealed ARVC to be mainly a desmosomal disease with an autosomal-dominant inherited trait and with variable penetrance and expression (14). Additionally, autosomal recessive pattern of inheritance was described in some forms of ARVC (Naxos and Carvajal disease) (15;16).
Desmosomes are the morphological and pathophysiological hallmark of ARVC and causative mutations in five desmosomal genes: plakophilin-2 (PKP2), plakoglobin (JUP), desmoplakin (DSP), desmoglein-2 (DSG2) and desmocollin-2 (DSC2) have been identified (17;18) (Figure3).

**Figure 3.** The cardiac desmosome and proposed roles of the desmosomes in (A) supporting structural stability through cell–cell adhesion, (B) regulating transcription of genes involved in adipogenesis and apoptosis, and maintaining proper electrical conductivity through regulation of (C) gap junctions and (D) calcium homeostasis.

Dsc2, desmocollin-2; Dsg2, desmoglein-2; Dsp, desmoplakin; Pkg, plakoglobin; Pkp2, plakophilin-2; PM, plasma membrane. From Awad MA et al. Nat Clin Pract Cardiovasc Med.2008; 5:258-267, with permission.

Desmosomes and adherent gap junctions provide structural functions by mediating cell-cell adhesion and support electrical coupling. Desmosomes are crucial for cellular mechanical strength through transmission of force from cell to cell and to the cytoskeleton. The components of the desmosomes and gap junctions work together as a protein interacting network (the connexome) that regulates excitability, cell-cell adhesion, and intercellular coupling in the heart (19).

Loss of integrity of the desmosomal components result in progressive loss of cardiac myocytes, followed by fibro-fatty replacement and altered intra- and intercellular connections with effects on ion channel remodeling. Dysfunctional intracellular signaling has been suggested to promote inflammation and fibroadiposis in ARVC (20).
Components of the connexome, such as desmosomal proteins Connexin 43 (gap junctions) and Nav1.5 (sodium channels)(19) and non-desmosomal proteins, such as ryanodine receptor 2 (RYR2), transforming growth factor β3 (TGFB3), transmembrane protein 43 (TMEM43) (9;18), Desmin (DES)(21), Phospholamban (PLN)(22), Lamin A/C (LMNA)(23) and Titin (TTN)(24) have been identified to cause ARVC phenocopies.

Up to 50-60% of probands (index patients) harbor a disease causing mutations (14). Genetic penetrance in mutation positive ARVC subjects is generally considered to be low, and phenotypic disease exhibit highly variable expressivity.

Impact of exercise on the heart in the general population and in patients and family members with ARVC

The cardiovascular benefits of regular exercise on individual health are indisputable and well established and the World Health Organization (WHO) recommends 150 min/week with moderate exercise for healthy adults (WHO 2015).

Young athletes with high intensity, frequency and duration of exercise perform way above these recommendations are considered to be the healthiest individuals of society (25). Therefore, the death of a young athlete is a tragedy for the family and the whole community. The majority of SCD in young athletes at age below 35 years are due to genetic cardiac diseases that may cause life-threatening ventricular arrhythmia (VA) during exercise (26), e.g. incidence of SCD in ARVC varies from 0.1 to 3.6% per year in young individuals (8;11).

Exercise increases wall stress by volume overload with more impact on the thin wall of the right ventricle (RV) compared to the left ventricle (LV) (27), and may therefore trigger and aggravate ARVC disease progression (28). Furthermore, athletic activity is reported to increase risk of VA in patients with ARVC (6;29), and these patients are recommended to abstain from competitive sports (30).
However, the impact of high physical activity level on myocardial function and cardiac outcome in patients and mutation positive family members with ARVC has not been fully investigated, and systematic data on exercise as a trigger of progression of ARVC in humans are lacking.

**Early-phase ARVC and the challenging differential diagnosis right ventricular outflow tract ventricular tachycardia (RVOT-VT)**

The right ventricular outflow tract (RVOT) is the most common site of origin for idiopathic frequent premature ventricular complexes (PVC), non-sustained and sustained ventricular tachycardia (VT) with a configuration of left bundle branch block and an inferior axis in patients with structurally normal hearts, known as RVOT-VT (31). RVOT-VT is usually a relatively benign condition (32) with generally well tolerated ventricular arrhythmia, caused by cAMP-mediated delayed afterdepolarizations and triggered activity (31).

However, the RVOT area may also be origin of VT in patients with ARVC (2), predisposing to ventricular arrhythmia, biventricular dysfunction and SCD, therefore far from a benign condition (3). Regardless of the underlying process, VT from the RVOT area is common in both conditions, and discrimination between idiopathic RVOT-VT and early stages of ARVC may be challenging. The treatment and prognosis differ substantially, and an incorrect diagnosis may be devastating.

Although patients with RVOT-VT commonly have no evidence of structural heart disease, frequent PVC may cause myocardial remodeling with subsequent reduced function and dilatation, further complicating the discrimination to ARVC (33).

Early stages of ARVC may have only subtle or even non-detectable electrical, functional and structural changes, despite the increased arrhythmogenic risk (34). Discrimination of ARVC from RVOT-VT patients based on findings from electrical, functional and structural alterations is important, and may improve work-up and management to prevent adverse cardiac events.
Risk stratification of arrhythmic events in early ARVC

Ventricular arrhythmia is frequent in patients with definite ARVC (2;9), however, arrhythmic events (AEs) can also be the first manifestation in the early concealed or electrical stage of ARVC, i.e. the non-definite ARVC subjects, mostly mutation positive family members without, or with only subtle myocardial changes (35). Therefore, risk stratification is still challenging, particularly in early ARVC.

The ARVC population presenting in the health care system has changed substantially over the last decade. Ten years ago, patients were most commonly diagnosed with definite or overt ARVC after surviving a life-threatening arrhythmic event. Genetic family screening now provides the opportunity to identify family members at risk of developing ARVC disease before onset of symptoms (34). Hence, risk stratification no longer includes high risk patients with obvious electrical, functional and structural myocardial pathology, but has moved to predict the transition from early, pre-symptomatic to overt electrical and myocardial ARVC disease with life-threatening cardiac events and adverse cardiac outcome (28). Identifying individuals with early stage ARVC disease highlights our medical and ethical responsibility to prevent life-threatening arrhythmic events. New tools are needed to identify patients with early ARVC at risk to improve treatment options (8).

Novel echocardiographic tools: myocardial strain and mechanical dispersion

Over the last decades, echocardiography has developed to be an established and reliable non-invasive imaging modality, and is widely available for cardiovascular investigation. Assessment of myocardial function is crucial for clinical evaluation of cardiac disease. In many cardiac diseases, patients’ prognosis is closely related to left and right ventricular function (8;36;37). The implementation of novel echocardiographic imaging modalities in clinical work-up can be helpful to detect subtle myocardial changes in cardiac disease.
Strain by 2D speckle tracking echocardiography has been demonstrated to be a more accurate and sensitive tool for quantification of myocardial function compared to LV ejection fraction (LVEF) (38;39). RV strain has been shown to assess RV function in patients with ARVC (40). Mechanical dispersion (MD) by strain is a measure of timing from 2D speckle tracking echocardiography reflecting myocardial contraction heterogeneity. A pronounced MD reflects inhomogeneous timing of myocardial contraction throughout the ventricle, while a low MD reflects a more homogeneous contraction. LV and RV mechanical dispersion have been shown to be a marker of ventricular arrhythmia in patients after myocardial infarction (38;41), in dilated cardiomyopathy (42) and also in ARVC (40).

In this thesis, myocardial strain and mechanical dispersion by speckle tracking echocardiography were used as novel imaging tools to assess myocardial function in patients with both early and advanced ARVC and in patients with RVOT-VT.

Aims of the Thesis

General

We aimed to investigate the impact of vigorous physical activity on ARVC disease progression and cardiac outcome. Secondly, we aimed to assess ARVC disease specific parameters which may help to discriminate early ARVC from the important arrhythmic differential diagnosis RVOT-VT; and thirdly, we aimed to investigate risk factors for arrhythmic events (AEs) in ARVC with emphasis on early stages of disease.

Specific

1. We aimed to investigate the impact of vigorous physical exercise on myocardial function and occurrence of life-threatening cardiac events, i.e. VT, advanced heart failure, heart transplantation and aborted cardiac arrest (ACA) in ARVC patients and mutation positive family members. We
hypothesized that vigorous physical activity may aggravate and accelerate ARVC disease progression.

2. We aimed to investigate if a multi-modality approach can help to discriminate patients with early-phase ARVC from patients with RVOT-VT. We hypothesized that a combination of electrocardiographic and sensitive novel cardiac imaging parameters, can detect electrical and subtle myocardial changes in early-phases of ARVC that may improve discrimination from RVOT-VT.

3. We aimed to investigate early markers of arrhythmic events and improve risk stratification in early ARVC. We hypothesized that a combination of electrocardiographic and novel echocardiographic parameters, including strain echocardiography, may improve arrhythmic risk stratification in early ARVC.

**Study subjects**

**Paper 1. Vigorous physical activity impairs myocardial function in patients with arrhythmogenic right ventricular cardiomyopathy and in mutation positive family members**

A total of 110 ARVC patients from Norway and Sweden were included in this cross-sectional multi-center study. Of these, 65 (59%) were consecutive ARVC index patients and 45 (41%) were mutation positive family members, 78 (71%) had definite ARVC and 32 (29%) had non-definite ARVC according to TFC. Index patients were defined as the first affected individual in a family seeking medical attention for ARVC in whom the diagnosis was confirmed. Index patients were genetically tested for ARVC-related mutations. Family members were identified by cascade genetic screening.
Paper 2. Comparison of patients with early-phase arrhythmogenic right ventricular cardiomyopathy and right ventricular outflow tract ventricular tachycardia

In this cross-sectional study, 177 patients were screened, and 165 patients with ARVC and RVOT-VT were included. Consecutive ARVC patients were recruited from Oslo University Hospital, Norway (n=105) and Lund University Hospital, Sweden (n=16). A subset of these patients (n=108) were also included in paper 1(Figure 4). The Task Force Criteria were used for diagnosis and classification of ARVC disease. Definite ARVC was diagnosed in 77 (64%) and 44 (36%) had early-phase ARVC.

Furthermore, 44 consecutive RVOT-VT patients were recruited from Oslo University Hospital, Norway. The diagnosis of RVOT-VT was based on a clinical phenotype with monomorphic VT or PVC originating from the RVOT region with documented left bundle branch block configuration and inferior electric axis. A diagnosis of RVOT-VT required excluding ischemic heart disease by negative stress test or coronary angiography and no mutations in ARVC associated genes identified by genetic testing.

Paper 3. Echocardiography Combined with ECGs Improve Identification of Arrhythmic Events in Early ARVC

In total, 162 consecutive ARVC subjects from Oslo University Hospital, Norway were included in this cross-sectional study. Of these, 86 (53%) were index patients and 76 (47%) were mutation positive family members; 89 (55%) had definite diagnosis of ARVC, and 73 (45%) had non-definite ARVC and were defined as early ARVC disease. A subset of these subjects were also included in paper 1 and 2 (n=94 and n=100, respectively) (Figure 4).
**Paper 1:**

110 ARVC subjects, both athletes and non-athletes

**Paper 2:**

108 ARVC subjects from article 1

+ 13 new consecutive ARVC subjects

+ 44 consecutive RVOT-VT subjects

**Total 165 ARVC and RVOT subjects**

**Paper 3:**

100 ARVC subjects from article 1 and article 2:

94 ARVC subjects from article 1  
(110 subjects minus 3 subjects with incomplete data, 
minus 13 Swedish subjects)

+ 6 of the new ARVC subjects from article 2

+ 62 new consecutive ARVC subjects

**Total 162 ARVC subjects**

*Figure 4. Flowchart of study subjects included in the 3 papers. ARVC, arrhythmogenic right ventricular cardiomyopathy; RVOT, right ventricular outflow tract; VT, ventricular tachycardia.*
**Methods**

**Electrocardiogram (ECG)**

Standard twelve lead ECG was obtained at the time of clinical or echocardiography examination and used in all studies. Standard twelve lead ECG’s were screened both for inverted T waves in the right precordial leads (Figure 5a and b) in the absence of complete right bundle-branch block QRS $\geq$ 120 ms, epsilon waves in the right precordial leads (2) (Figure 5a and b) and S-wave upstroke was measured as the time from the nadir of the S-wave to the isoelectric line. The configuration of PVC were analyzed to suggest their likely site of origin as described previously by Zhang et al. (43).

*Figure 5a. Precordial ECG leads from an ARVC patient at rest with typical epsilon wave in V1 and T inversions in V1 – V4 (red arrows).*
Signal-averaged ECG

Signal-averaged ECG (SAECG) was performed using a MAC® 5000-analysing system (GE Medical Systems, Milwaukee, WI, USA) and used in all studies. Time domain analysis was obtained in the band-pass filter 40 – 250 Hz. Late potentials were considered present if ≥1 of the following parameters were abnormal according to TFC (2): total filtered QRS duration ≥ 114 ms, the low amplitude (< 40 µV) late signal duration ≥ 38 ms and the last (40 ms) QRS root-mean-square voltage ≤ 20 µV(44)(Figure 6).
Figure 6. Signal average ECG (SAECG) from the same patient as in Figure 5a and b.
Clearly pathologically SAECG with filtered QRS duration 154 ms, the low amplitude late signal duration 79 ms and the last QRS root-mean-square voltage 6 μV (values in the red square box).

Holter monitoring

24-hour Holter monitoring was performed using Schiller medilog®AR4 and AR4+ to assess ventricular tachycardia and frequency of PVC in percent of total heart beats in 24 hours (%PVC) (33).

Arrhythmia

Ventricular arrhythmias (VA) were defined as syncope with assumed arrhythmic origin, documented VT or aborted cardiac arrest (ACA) in paper 1 and 2. Arrhythmic events (AEs) were defined as VT, syncope of suspected cardiac origin, or ACA in paper 3.
Genetic analyses

Genetic testing was performed as a part of the diagnostic work-up in patients with suspected ARVC according to clinical standards and Norwegian legislation. Family members were only tested for the ARVC-related family mutation. Genomic DNA was isolated from peripheral blood. The individual exons with flanking intron sequences of the genes plakophilin-2 (PKP2), desmocollin-2 (DSC2), desmoglein-2 (DSG2), desmoplakin (DSP) and 29 of the 105 exons of the ryanodine receptor-2 (RYR2) gene were sequenced by polymerase chain reaction amplification in combination with direct sequencing. Decisions of pathogenicity was based on total evaluation including previous reports, genomic databases (45), NHLBI 6500 Exome data sets; EVS; http://evs.gs.washington.edu/EVS/, in silico programs (PolyPhen and SIFT) and family co-segregation studies. Index patients with confirmed ARVC-related desmosomal mutations in these genes were defined as ARVC mutation positive, and mutation positive family members were included by cascade genetic screening. Family members of patients with ARVC associated desmosomal variants of uncertain significance were included only if they had an ARVC phenotype and if a familial co-segregation of symptoms was present.

Exercise interview and assessment of level of physical activity

The study subjects were asked about their history of and current amount of athletic activity by direct interview or by structured telephone interviews. During the interview, we prompted the subjects to list regular and vigorous exercise since age ten. Three aspects of each regularly performed exercise were collected: intensity, frequency and duration(46) and defined as amount of physical activity. Physical activity was rated as “light”, “moderate” and “vigorous” according to the 36th Bethesda Conference, Task Force 8: Classification of Sports (47), Physical Activity and Public Health: Updated Recommendation for Adults(46) and https://sites.google.com/site/compendiumofphysicalactivities/.
Amount of physical activity was expressed in metabolic equivalents (METs) x minutes/week (METs x min/w). One MET represents an individual’s energy expenditure while sitting quietly and is approximated to 3.5 ml O₂/kg/min or 1 kcal/kg/h (48). Intensity of physical activity was graded as vigorous if ≥ 6 METs (46). Duration of regularly performed exercise was expressed in years (49). Subjects with a history of physical activity with intensity ≥ 6 METs for ≥ 4 hours a week (≥ 1440 METs x min/week) during minimum 6 years were defined as athletes. Quartiles of METS x min/week were created to explore the relationship between activity level and cardiac function and volumes.

**Echocardiography**

Patients and study subjects in all three studies underwent two-dimensional echocardiography (2D echocardiography) examination (Vivid 7 or Vivid E9, GE Vingmed, Horten, Norway). Data were digitally stored for off-line analysis and were analyzed with EchoPAC® version 112 (GE Healthcare, GE Vingmed, and Horten, Norway). Echocardiography analyses were performed blinded to clinical data, as far as possible.

**2 D Echocardiography**

By 2D echocardiography we assessed proximal RVOT diameter in the parasternal short axis view and RV basal diameter (RVOT and RVD, both not indexed (paper 1 and 3) and indexed by body surface area (paper 2), respectively (Figure 8), RV fractional area change (RVFAC) and tricuspid annular plane systolic excursion (TAPSE) from the 4-chamber view (50) and evaluated for the presence of RV wall abnormalities according to TFC. From the LV, we assessed end-diastolic diameter (LVEDD), end-diastolic volume (LVEDV) and end-systolic volume (LVESV) (not indexed values in paper 1 and 3; indexed values by body surface area in paper 2, respectively), LVEF by the modified Simpson’s biplane method and cardiac output (51;52).
Strain by speckle tracking echocardiography

Myocardial strain is based on the concept of Lagrangian strain, a measure of regional myocardial deformation of a defined cardiac segment and is defined as the change in length in dimension or length ($L_{1}-L_{0}$) normalized to the original length ($L_{0}$) of the region of interest, and is reported either as percent shortening (negative values) or percent lengthening (positive values)(53-56).

In this thesis, strain analyses of LV and RV were performed by 2D speckle tracking echocardiography from the 3 LV apical views for LV global longitudinal strain (LVGLS) and from the RV focused four chamber view for RV longitudinal strain, all with frame rates >50/s, as far as possible (57). The endocardial border was traced in each view and speckles were tracked frame by frame during the cardiac cycle. The operator manually adjusted segments that failed to track, and segments that subsequently failed to track were excluded. Peak longitudinal strain was determined in 16 LV segments (56) and averaged as a measure of LVGLS (Figure 7a).

Figure 7a. Speckle tracking longitudinal strain curves from 6 LV segments in apical 4 chamber view with peak longitudinal strain and time to peak longitudinal strain, explanation in the text. AVC, aorta valve closure.
Peak longitudinal strain from 6 segment RV model (3 septal and 3 free wall segments) was averaged to RV “global” longitudinal strain (Figure 7b) in paper 1 and 3 and peak negative longitudinal strain from 3 free wall segment RV model were averaged as RV “global” longitudinal strain (Figure 7c) in paper 2.

Figure 7b. Speckle tracking longitudinal strain curves from the apical RV-focused four-chamber view with 6 RV segments with peak longitudinal strain and time to peak longitudinal strain, explanation in the text. AVC, aortic valve closure.
Strain analyses also provide measurements of time intervals in contraction duration. Time to peak longitudinal strain for all strain curves was defined as the time from onset of Q/R on ECG to maximum myocardial segmental shortening during the cardiac cycle, and averaged from 16 LV segments for the LV (42;58) (Figure 7a shows an example for the apical LV four chamber view, white horizontal arrows indicate time to peak longitudinal strain, all papers), and from all 6 RV segments (paper 1 and 3) and from 3 RV free wall segments (paper 2) for the RV (Figure 7 b, c and 8) (40).

LV mechanical dispersion was defined as the standard deviation of time from Q/R on the ECG to peak negative longitudinal strain curves in the 16 LV segments.

RV mechanical dispersion was defined as the standard deviation of time from onset of Q/R on the ECG to peak negative longitudinal strain curves in 3 RV free wall segments or 6 RV segment (Figure 7b, c and 8). Thus, mechanical dispersion reflects contraction heterogeneity as a function of time (ms) in both ventricles.
Figure 8. Echocardiographic longitudinal strain curves from an RVOT patient, an early-phase ARVC patient and an ARVC patient with overt disease.

Upper panels: RV free wall longitudinal strain curves from the apical RV-focused four-chamber view in patients with RVOT-VT (left panel), early-phase ARVC (mid panel) and overt ARVC (right panel). Vertical white arrow indicates the amplitudes of RV strain curves, which were calculated as the average peak negative longitudinal strain in 3 RV free wall segments. Horizontal white arrows indicate time to peak strain, defined as time from onset of Q/R on the ECG to peak negative longitudinal strain. The standard deviation of time to peak strain in the same 3 RV segments was defined as RV mechanical dispersion, reflecting contraction heterogeneity. Increasing RV mechanical dispersion from left to right panels.

Lower panels: Measures of RV basal diameters in these patients. Increasing RV basal diameter from left to right panels. ARVC, arrhythmogenic right ventricular cardiomyopathy; AVC, aortic valve closure; RV, right ventricle; RVD, RV basal diameter; RVOT, right ventricular outflow tract; VT, ventricular tachycardia. From Saberniak et al. Eur Heart J Cardiovasc Imaging. 2017 Jan;18(1):62-69. Epub 2016 Feb 21, with permission.

Cardiac magnetic resonance imaging (CMR) studies

CMR was performed in a subset of patients (n=54(49%) in paper1, n=80(48%) in paper 2 and n=121(75%) in paper 3), in a 1.5 Tesla unit (Magnetom Sonata, Vision Plus or Avanto Siemens, Erlangen, Germany) using a phased array body coil. The RV free wall was imaged by axial and sagittal breath-hold T1-weighted turbo spin echo technique. Standard long and short axis breath-hold cine sequences (fast
imaging with steady-state free precession, trueFISP) were obtained to cover both ventricles. Only MRI studies including short axis images were defined as appropriate. RV and LV volumes and ejection fractions were calculated by summation of the luminal areas of the short axis images at end-diastole and end-systole, including the RV and LV outflow tracts, but excluding the papillary muscles. Post-processing analyses were performed using Qmass® MR 6.1.6 software (Medis medical imaging systems, Leiden, the Netherlands).

Reproducibility and feasibility

Paper 1: RVGLS, LVGLS and LVEF could be assessed in 95%, 92% and 99%, respectively. Only a subset of subjects (n=54, 49%) had appropriate short axis images by CMR. However, there were no differences in age, gender, heart rate or BSA between those with and without CMR. Intra and inter observer intra class correlation for LVEF by echocardiography was 0.97 (95% CI 0.96-0.98) and 0.81 (95% CI 0.73-0.88), respectively, and for RVEF by CMR 0.84 (95% CI 0.49-0.96) and 0.79 (95% CI 0.67-0.87), respectively.

Paper 2: By echocardiography, 44 (100%) RVOT-VT and 119 (98%) ARVC patients could be evaluated. RVGLS, RVMD, LVGLS, LVMD and LVEF could be assessed in 95%, 92%, 93%, 87% and 99%, respectively. A subset of 23 (52%) RVOT-VT patients and 57 (47%) ARVC patients had assessable CMR examinations. However, there were no differences in age, gender, heart rate or BSA between those with and without CMR. Intra and inter observer intra class correlation for LVEF by echocardiography was 0.96 (95% CI 0.95-0.97) and 0.80 (95% CI 0.71-0.85), respectively and for RVEF by CMR 0.96 (95% CI 0.86-0.99) and 0.92 (95% CI 0.66-0.98), respectively.

Paper 3: LVGLS analyses could be performed in 89% of patients and 88% of segments could be analyzed. RVGLS analyses could be performed in 82% of patients and 96% of segments could be analyzed. Intra- and interobserver intraclass correlation for LVGLS and LVMD by strain echocardiography, were 0.98 (95% CI 0.92-0.99) and 0.94 (95% CI 0.77-0.99) and 0.95 (95% CI 0.81-0.99) and 0.91 (95% CI
0.67-0.98), respectively. For RVGLS and RVMD values were 0.98 (95% CI 0.92-0.99) and 0.83 (95% CI 0.40-0.95) and 0.87 (95% CI 0.53-0.97) and 0.81(95% CI 0.35-0.95), respectively.

**Statistical analyses**

All papers: Continuous data were presented as mean ± standard deviation, frequency (percentages) or as median (range). Differences between groups were assessed by Chi-square test, Fisher’s exact test, Student’s t-test or one-way analysis of variance as appropriate (SPSS 20.0, Inc. Chicago, Illinois). Intra- and interobserver variability were expressed by intra class correlation values based on analyses performed by two independent investigators. C statistic was calculated by receiver operating characteristic (ROC) curves. Comparisons of C statistics were performed with the software Analyze-it®. The value closest to the upper left corner of the ROC curve was defined as giving optimal combined sensitivity and specificity. Two-sided p-values < 0.05 were considered significant.

Paper 1: Correlations between amount of physical activity and RV and LV function were assessed by bivariate correlation. Quartiles of METs x min/week were created to explore the relationship between activity level and cardiac function and volumes. Logistic regression was used to find markers of athletic status, parameters with p-values <0.05 from univariable analyses were included in multivariable analyses. Multivariable logistic regression was performed to adjust the outcome of athlete status for age and gender. Kaplan Meier curves were constructed and log-rank test was performed to assess cumulative lifetime cardiac transplantation free survival. Subgroups of index and non-index subjects were analyzed separately.

Paper 2: Univariable logistic regression was used to find predictors of early-phase ARVC. Significant parameters with p-values <0.05 from univariable analyses were included in multivariable logistic regression analyses.

Paper 3: Univariable logistic regression was used to identify predictors of ventricular arrhythmia and significant parameters (p<0.05) from univariable analyses were included in multivariable logistic regression analyses. Multivariable logistic regression analyses were used to adjust for beta blocker use, age, gender and
mutation status in the total ARVC population. Correlations between continuous parameters were assessed by linear regression. We used Likelihood ratio test to evaluate if echocardiographic parameters could improve the model for arrhythmic risk stratification compared to only electrical parameters. Overfitting was tested with Akaike information criterion (AIC), suggesting smaller values indicate better model.

**Summary of results**

**Paper 1**

We included 110 consecutive ARVC patients and mutation positive family members with determined athlete status (yes/no) in this study. The definition of athlete status was fulfilled in 37(34%) subjects compared with 73(66%) non-athletes, 35(95%) of athletes had definite ARVC diagnosis compared to 43(59%) non-athletes (p<0.001). Athletes were more frequently male, and more frequently index patients compared to non-athletes (p<0.01). Therefore, all analyses were additionally performed for index patients separately and results were adjusted for gender in addition to age.

Athletes had a history of physical activity level of 3178±886 METs x min/w and non-athletes of 669±290 METs x min/w (p<0.001). Totally, 66 (60%) of the subjects had experienced VA and these events were more frequent in athletes compared to non-athletes (p<0.01) Exercise induced VA was documented in 100% of index-athletes compared to only 31% of index-non-athletes (p<0.001) (Table 2).

Genetic testing was conducted in 100 study subjects and ARVC-related mutations/variants were identified in 75 study subjects (75%). We observed ARVC-related mutations in PKP2 (n=68, 91%) and in DSP (n=2, 3%) and ARVC associated desmosomal variants in three index patients fulfilling definite ARVC diagnosis. Two family members of one of these index patients were included because of familial co-segregation of the ARVC phenotype and symptoms.

Table 2. Clinical characteristics in 110 ARVC subjects, 73 non-athletes and 37 athletes
<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Total (n=110)</th>
<th>Non-athletes (n=73)</th>
<th>Athletes (n=37)</th>
<th>p-value</th>
<th>Adjusted Odds Ratio (95% CI)</th>
<th>Adjusted p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index (n)</td>
<td>65 (59%)</td>
<td>37 (51%)</td>
<td>28 (76%)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male gender (n)</td>
<td>64 (58%)</td>
<td>35 (48%)</td>
<td>29 (78%)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Index</td>
<td>22 (60%)</td>
<td>22 (79%)</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>13 (36%)</td>
<td>7 (78%)</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at baseline echocardiography (years)</td>
<td>42±17</td>
<td>45±18</td>
<td>36±13</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Index</td>
<td>50±16</td>
<td>37±13</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>39±18</td>
<td>32±12</td>
<td>0.29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definite ARVC by TFC</td>
<td>78 (71%)</td>
<td>43 (59%)</td>
<td>35 (95%)</td>
<td>&lt;0.001</td>
<td>22.92 (4.27 – 123.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Index (n=59)</td>
<td>32 (87%)</td>
<td>27 (96%)</td>
<td>0.22</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Non-index (n=19)</td>
<td>11 (31%)</td>
<td>8 (89%)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-definite ARVC by TFC</td>
<td>32 (29%)</td>
<td>30 (41%)</td>
<td>2 (5%)</td>
<td>&lt;0.001</td>
<td>0.04 (0.01 – 0.23)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Index (n=6)</td>
<td>5 (13%)</td>
<td>1 (4%)</td>
<td>0.22</td>
<td></td>
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<tr>
<td>Non-index (n=26)</td>
<td>25 (69%)</td>
<td>1 (11%)</td>
<td>&lt;0.01</td>
<td></td>
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<tr>
<td>Syncope (n)</td>
<td>40 (44%)</td>
<td>19 (33%)</td>
<td>21 (62%)</td>
<td>&lt;0.01</td>
<td>3.35 (1.29 – 8.71)</td>
<td>0.01</td>
</tr>
<tr>
<td>Index</td>
<td>12 (50%)</td>
<td>17 (68%)</td>
<td>0.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Index</td>
<td>7 (21%)</td>
<td>4 (44%)</td>
<td>0.20</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>VA (n)</td>
<td>66 (60%)</td>
<td>37 (51%)</td>
<td>29 (78%)</td>
<td>&lt;0.01</td>
<td>4.53 (1.59 – 12.94)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Index</td>
<td>34 (92%)</td>
<td>27 (96%)</td>
<td>0.63</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>3 (8%)</td>
<td>2 (22%)</td>
<td>0.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercised-induced VA (n)</td>
<td>41 (37%)</td>
<td>13 (18%)</td>
<td>28 (80%)</td>
<td>&lt;0.001</td>
<td>16.54 (5.59 – 49.98)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Index</td>
<td>11 (31%)</td>
<td>26 (100%)</td>
<td>&lt;0.001</td>
<td></td>
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</tr>
<tr>
<td>Non-index</td>
<td>2 (6%)</td>
<td>2 (22%)</td>
<td>0.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICD (n)</td>
<td>52 (47%)</td>
<td>29 (40%)</td>
<td>23 (62%)</td>
<td>0.03</td>
<td>2.58 (1.05 – 6.38)</td>
<td>0.04</td>
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<tr>
<td>Index</td>
<td>27 (73%)</td>
<td>21 (75%)</td>
<td>0.85</td>
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</tbody>
</table>
Values are n, n (%) or mean ± SD unless stated otherwise; p by Chi-square test or Fisher’s exact test, Student’s t-test or one-way analysis of variance as appropriate. Odds ratio (OR), confidence interval (CI) and adjusted p-values by multivariable logistical regression. ARVC, arrhythmogenic right ventricular cardiomyopathy; TFC, 2010 Task Force Criteria; VA, ventricular arrhythmia; ICD, implantable cardioverter-defibrillator; ECG, electrocardiogram. *Adjusted for age and male gender.

Athletes had increased RV diameters (RVOT and RV basal diameter) and reduced RV function by RVFAC, RVGLS by echocardiography and RVEF by CMR compared to non-athletes (all p<0.01). LV function by LVEF and LVGLS by echocardiography and LVEF by CMR were reduced in athletes compared to non-athletes (all p<0.001), confirming biventricular dysfunction in ARVC. Importantly, LVEF by echocardiography (Table 3) and by CMR (Table 4) was reduced both in subgroups of athlete index patients and in athlete family members (p<0.05), showing slightly reduced LV function both in index patients and mutation positive family members with athletic activity compared to those without.

Table 3. Echocardiographic parameters in 73 non-athletes and in 37 athletes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total</th>
<th>Non-athletes</th>
<th>Athletes</th>
<th>p- value</th>
<th>Adjusted Odds</th>
<th>Adjusted p- value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats per minute)</td>
<td>63±13</td>
<td>65±13</td>
<td>59±12</td>
<td>0.06</td>
<td>0.96 (0.92 - 1.00)</td>
<td>0.05</td>
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<tr>
<td></td>
<td>Index</td>
<td>Non-index</td>
<td>p-value</td>
<td>CI</td>
<td>p-value</td>
<td></td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>25±4</td>
<td>25±4</td>
<td>0.84</td>
<td>1.04 (0.91 - 1.18)</td>
<td>0.58</td>
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<tr>
<td>RVOT PSAX (mm)</td>
<td>36±9</td>
<td>34±7</td>
<td>&lt;0.01</td>
<td>1.08 (1.02 - 1.15)</td>
<td>0.01</td>
<td></td>
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<tr>
<td>RV basal diameter (mm)</td>
<td>44±9</td>
<td>42±8</td>
<td>&lt;0.01</td>
<td>1.10 (1.03 - 1.16)</td>
<td>&lt;0.01</td>
<td></td>
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<tr>
<td>TAPSE/displacement (mm)</td>
<td>18±5</td>
<td>19±4</td>
<td>&lt;0.01</td>
<td>0.88 (0.79 - 0.98)</td>
<td>0.02</td>
<td></td>
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<tr>
<td>RVFAC (%)</td>
<td>38±11</td>
<td>40±11</td>
<td>&lt;0.01</td>
<td>0.93 (0.88 - 0.98)</td>
<td>&lt;0.01</td>
<td></td>
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<tr>
<td>RVGLS (%)</td>
<td>-20.7±5.5</td>
<td>-22.0±4.8</td>
<td>&lt;0.01</td>
<td>1.18 (1.06 - 1.32)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>RV wall abnormality by TFC(n)</td>
<td>69 (63%)</td>
<td>38 (53%)</td>
<td>&lt;0.01</td>
<td>13.86 (4.35 - 44.09)</td>
<td>&lt;0.001</td>
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<tr>
<td>Cardiac output (l/min)</td>
<td>3.7±1.2</td>
<td>3.8±1.2</td>
<td>0.53</td>
<td>0.71 (0.46 - 1.08)</td>
<td>0.11</td>
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<tr>
<td>LVEDV (ml)</td>
<td>113±40</td>
<td>105±25</td>
<td>&lt;0.01</td>
<td>1.01 (1.00 - 1.03)</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Parameters</td>
<td>Total population (n=54)</td>
<td>Non-athletes (n=34)</td>
<td>Athletes (n=20)</td>
<td>p-value</td>
<td></td>
<td></td>
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<tr>
<td>LVEDVI (ml)</td>
<td>74±18</td>
<td>74±19</td>
<td>74±16</td>
<td>0.97</td>
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<td></td>
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<td>Index 84±14</td>
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<tr>
<td></td>
<td></td>
<td>Non-index 62±18</td>
<td>73±11</td>
<td>0.19</td>
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<tr>
<td>LVESVI (ml)</td>
<td>37±12</td>
<td>35±12</td>
<td>40±12</td>
<td>0.15</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Index 39±12</td>
<td>40±14</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-index 31±11</td>
<td>40±5</td>
<td>0.08</td>
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</tbody>
</table>

Values are n, n(%) or mean ± SD unless stated otherwise; p by Chi-square test or Fisher’s exact test, Student’s t-test or one-way analysis of variance (ANOVA) as appropriate. Odds ratio (OR), confidence interval (CI) and adjusted p-values by multivariable logistical regression. Multivariable logistical regression as described in text. ARVC, arrhythmogenic right ventricular cardiomyopathy; RVOT PSAX, right ventricular outflow tract parasternal short-axis; TAPSE, right ventricular tricuspid annular plane systolic excursion; RVFAC, right ventricular fractional area change; RVGLS, right ventricular global longitudinal strain; TFC, 2010 Task Force Criteria; LVEDD, left ventricular end-diastolic diameter; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVEF, left ventricular ejection fraction; LVGLS, left ventricular global strain. *Adjusted for age and gender
<table>
<thead>
<tr>
<th></th>
<th>Index</th>
<th>Non-index</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td>LVEF (%)</td>
<td>50±8</td>
<td>53±8</td>
<td>46±6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>54±10</td>
<td>46±7</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>RVEDVI (ml)</td>
<td>89±36</td>
<td>82±34</td>
<td>99±38</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>99±35</td>
<td>111±39</td>
<td>0.36</td>
<td></td>
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<tr>
<td>RVESVI (ml)</td>
<td>56±32</td>
<td>47±28</td>
<td>70±34</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>57±34</td>
<td>81±36</td>
<td>0.06</td>
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<tr>
<td>RVEF (%)</td>
<td>39±11</td>
<td>43±10</td>
<td>32±8</td>
<td>&lt;0.001</td>
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<td></td>
<td>45±13</td>
<td>30±9</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>41±7</td>
<td>37±5</td>
<td>0.17</td>
<td></td>
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</tbody>
</table>

Values are n or mean ± SD; p by Student’s t-test or one-way analysis of variance (ANOVA). ARVC, arrhythmogenic right ventricular cardiomyopathy; LVEDVI, left ventricular end-diastolic volume indexed; LVESVI, left ventricular end-systolic volume indexed; LVEF, left ventricular ejection fraction; RVEDVI, right ventricular end-diastolic volume indexed; RVESVI, right ventricular end-systolic volume indexed; RVEF, right ventricular ejection fraction.

Athletic activity by METs x minutes/week correlated with reduced RV and LV function, and with increased RVOT diameter and LV volumes by echocardiography and CMR (not shown) (all p<0.05) (Figure 9).
Figure 9. Bar charts displaying relationship between amounts of physical activity expressed as quartiles of METs x minutes / week during minimum 6 years and echocardiographic findings in RV and LV in 110 ARVC subjects. There was a significant correlation between increasing activity in quartiles and increased RVOT diameter, increased LV volumes and reduced function by RV strain and LVEF (all \( p < 0.05 \)). \( P \) values from bivariate correlation.

METS, metabolic equivalents; FAC, fractional area change; RVOT, right ventricular outflow tract; RV, right ventricular; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVEF, left ventricular ejection fraction. From Saberniak J et al. Eur J Heart Fail. 2014 Dec; 16(12):1337-44, with permission.

An amount of \( \geq 900 \) METs \( \times \) min/w (\( \geq 2.5 \) h vigorous exercise/week) during minimum 6 years showed a C statistics of 0.80 (95% CI 0.71-0.90) to optimally discriminate subjects with exercise induced VA (sensitivity: 76% (95% CI 0.60-0.88), specificity: 79% (95% CI 0.67-0.88)). Athletes experienced more frequently VA-episodes and more ICD implantation at younger age compared to non-athletes (all \( p < 0.01 \)).

Importantly, only athletes underwent heart transplantation (log rank \( p < 0.001 \)).
In this study, 165 consecutive patients with early-phase and advanced ARVC and RVOT-VT were included. Compared to the total ARVC population, were RVOT-VT patients more frequently female (p<0.01), had more frequently non-sustained VT (p<0.001) and had more frequent PVC during Holter monitoring (p<0.001). Aborted cardiac arrest occurred in 13 patients, all of whom had ARVC. SAECG was less pathological in RVOT-VT compared to ARVC patients (Table 5).

Table 5. Clinical characteristics in 44 RVOT-VT and 121 ARVC patients

<table>
<thead>
<tr>
<th></th>
<th>RVOT-VT (n=44)</th>
<th>Total ARVC (n=121)</th>
<th>p-value</th>
<th>Early-phase ARVC (n=44) vs. RVOT-VT</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis (years)</td>
<td>47±14</td>
<td>42±17</td>
<td>0.08</td>
<td>39±17</td>
<td>0.01</td>
</tr>
<tr>
<td>Male gender (n)</td>
<td>13(30%)</td>
<td>69(57%)</td>
<td>&lt;0.01</td>
<td>22(50%)</td>
<td>0.08</td>
</tr>
<tr>
<td>BSA (m2)</td>
<td>1.9±0.2</td>
<td>1.9±0.2</td>
<td>0.88</td>
<td>1.9±0.2</td>
<td>0.86</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>66±13</td>
<td>63±13</td>
<td>0.19</td>
<td>68±13</td>
<td>0.61</td>
</tr>
<tr>
<td>Syncope (n)</td>
<td>25(61%)</td>
<td>44(44%)</td>
<td>0.06</td>
<td>12(29%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ventricular arrhythmia (n)(TFC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sustained VT (n)</td>
<td>5(14%)</td>
<td>65(55%)</td>
<td>&lt;0.001</td>
<td>5(12%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Non-sustained VT (n)</td>
<td>24(67%)</td>
<td>9(8%)</td>
<td>&lt;0.001</td>
<td>4(9%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ACA (n)</td>
<td>0(0%)</td>
<td>13(13%)</td>
<td>0.01</td>
<td>1(2%)</td>
<td>0.48</td>
</tr>
<tr>
<td>RFA (n)</td>
<td>22(50%)</td>
<td>45(43%)</td>
<td>0.42</td>
<td>4(10%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ICD (n)</td>
<td>4(9%)</td>
<td>61(50%)</td>
<td>&lt;0.001</td>
<td>6(14%)</td>
<td>0.50</td>
</tr>
<tr>
<td>SAECG ≥1 pathological value (TFC)</td>
<td>16 (46%)</td>
<td>50 (55%)</td>
<td>0.35</td>
<td>12 (34%)</td>
<td>0.33</td>
</tr>
<tr>
<td>%PVC by Holter</td>
<td>18.6±15.3</td>
<td>1.9±6.5</td>
<td>&lt;0.001</td>
<td>1.5±7.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Beta-blocker (n)</td>
<td>42(98%)</td>
<td>67(65%)</td>
<td>&lt;0.001</td>
<td>11(26%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Amiodarone (n)</td>
<td>2(5%)</td>
<td>26(25%)</td>
<td>&lt;0.01</td>
<td>1(2%)</td>
<td>1.00</td>
</tr>
<tr>
<td>ARVC related mutation (n) TFC</td>
<td>0(0%)</td>
<td>82(75%)</td>
<td>&lt;0.001</td>
<td>37(84%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Values are n, n(%) or mean ± SD; p by Chi-square test or Fisher’s exact test, Student’s t-test or by one-way analysis of variance as appropriate. ACA, aborted cardiac arrest; ARVC, arrhythmogenic right ventricular cardiomyopathy; BSA, body surface area; ICD, implantable cardioverter defibrillator; PVC, premature ventricular complexes; RFA, radio-frequency ablation; RV, right ventricle; RVOT, right ventricular outflow tract; SAECG, signal-averaged electrocardiogram; TFC, ARVC 2010 Task Force Criteria; VT, ventricular tachycardia.

By cardiac imaging, all RV diameters were within normal range in the RVOT-VT patients, compared to the increased RV diameters in the total ARVC population (all p<0.05). Furthermore, RV function was impaired both by echocardiography and by CMR, and RVMD was pronounced in ARVC patients (all p<0.001). RV function was in the lower normal range in RVOT-VT patients. LV function was impaired by echocardiographic LVEF and LVGLS (all p<0.05), and LVMD was more pronounced in ARVC compared to RVOT-VT (p<0.01) (Table 6).

Table 6. Echocardiographic parameters in 44 RVOT-VT and 119 ARVC patients

<table>
<thead>
<tr>
<th></th>
<th>RVOT-VT (n=44)</th>
<th>Total ARVC (n=119)</th>
<th>p-value vs. RVOT-VT</th>
<th>Early-phase ARVC (n=43)</th>
<th>p-value vs. RVOT-VT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV wall motion abnormality (TFC) (n)</td>
<td>4 (9%)</td>
<td>50 (42%)</td>
<td>&lt;0.001</td>
<td>3 (7%)</td>
<td>1.00</td>
</tr>
<tr>
<td>RVOT diameter (mm/m²)(TFC)</td>
<td>17±3</td>
<td>19±4</td>
<td>&lt;0.01</td>
<td>18±3</td>
<td>0.17</td>
</tr>
<tr>
<td>RVD (mm/m²)</td>
<td>19±2</td>
<td>23±4</td>
<td>&lt;0.001</td>
<td>21±3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RVFAC (%) (TFC)</td>
<td>46±5</td>
<td>38±11</td>
<td>&lt;0.001</td>
<td>46±7</td>
<td>0.96</td>
</tr>
<tr>
<td>TAPSE (mm)</td>
<td>22±4</td>
<td>18±5</td>
<td>&lt;0.001</td>
<td>20±4</td>
<td>0.09</td>
</tr>
<tr>
<td>RVGLS (%)</td>
<td>-27.1±5.0</td>
<td>-22.7±7.3</td>
<td>&lt;0.001</td>
<td>-26.4±5.3</td>
<td>0.53</td>
</tr>
<tr>
<td>RVMD (ms)</td>
<td>15±11</td>
<td>32±29</td>
<td>&lt;0.001</td>
<td>22±15</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>LV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac output (l/min)</td>
<td>4.4±1.2</td>
<td>3.8±1.2</td>
<td>&lt;0.01</td>
<td>4.2±1.1</td>
<td>0.52</td>
</tr>
</tbody>
</table>
Patients with early-phase ARVC had also lower %PVC by Holter monitoring as electrical parameters compared to RVOT-VT patients. Lower %PVC by Holter monitoring was a strong and independent marker of early-phase ARVC (Adjusted OR 0.77 (95%CI 0.64-0.92)), (p<0.01). <2 %PVC discriminated optimally between early-phase ARVC and RVOT-VT with an AUC of 0.93 (95%CI 0.86–1.00) by C statistics.

In patients with early-phase ARVC, ECG morphology indicated, that the probable site of origin of PVC more frequently originated from the RV lateral free wall compared to patients with RVOT-VT, with PVC origin mainly in the septal part of the RVOT (both p<0.001). Only subjects with early-phase ARVC had PVC with likely origin in the LV (Table 7).

Table 7. Results from analysis of ECG pattern of PVC/VT in early-phase ARVC vs. RVOT-VT to identify the site/focus of arrhythmia according to the ECG algorithm of Zhang et al.(43)

<table>
<thead>
<tr>
<th>Site of origin of arrhythmia according to ECG pattern (43)</th>
<th>RVOT-VT (n=43)</th>
<th>Early-phase ARVC (n=9)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Values are n, n(%); p by Fisher’s exact test. ARVC, arrhythmogenic right ventricular cardiomyopathy; ECG, electrocardiogram; LV, left ventricle; PVC, premature ventricular complexes; RV, right ventricle; RVOT, right ventricular outflow tract; VT, ventricular tachycardia.

RVEF by CMR, and indexed RV basal diameter (RVD), RVMD and LVGLS by echocardiography were imaging markers of early-phase ARVC (all p<0.05 compared to RVOT-VT). By C statistics, RVEF <43%, RVD >21 mm and RVMD >22ms had optimal ability to discriminate between early-phase ARVC and RVOT-VT. Furthermore, RVEF had best ability by C statistics to discriminate between early-phase ARVC and RVOT-VT with a cut-off value of <43% RVEF by CMR: AUC of 0.83 (95% CI 0.70 – 0.97) (Figure 10).

Figure 10. Receiver operating characteristic (ROC) curves for the ability of RV parameters to discriminate between 44 patients with early-phase ARVC and 44 RVOT-VT patients. RVEF by CMR and RVD and RV mechanical dispersion by echocardiography had good ability to discriminate early-phase ARVC from RVOT-VT patients. ARVC, arrhythmogenic right ventricular cardiomyopathy; AUC, area under the curve; CMR, cardiac magnetic resonance imaging; RVD, indexed right ventricular basal diameter; RVEF, right ventricular ejection fraction; RVMD,

In multivariable analysis, RVD and RVMD were the only independent imaging predictors of early-phase ARVC (all p<0.05) (Table 8).

Table 8. Multivariable analysis of parameters to predict status of early-phase ARVC (n=44) vs. RVOT-VT (n=44)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis (years)</td>
<td>0.96</td>
<td>0.90-1.03</td>
<td>0.23</td>
</tr>
<tr>
<td>RVEF by CMR (%)</td>
<td>0.88</td>
<td>0.76-1.02</td>
<td>0.09</td>
</tr>
<tr>
<td>RVD (mm/m2)</td>
<td>2.29</td>
<td>1.10-4.78</td>
<td>0.03</td>
</tr>
<tr>
<td>RV mechanical dispersion (ms)</td>
<td>1.09</td>
<td>1.00-1.18</td>
<td>0.04</td>
</tr>
<tr>
<td>LVGLS (%)</td>
<td>1.41</td>
<td>0.84-2.34</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Values are odds ratios (OR), confidence interval (CI) and adjusted p-values by multivariable logistical regression. ARVC, arrhythmogenic right ventricular cardiomyopathy; CI, confidence interval; CMR, cardiac magnetic resonance imaging; LVGLS, left ventricular global longitudinal strain; RVD, indexed right ventricular basal diameter; RVEF, right ventricular ejection fraction.

RVEF by CMR was decreased (41±8% vs. 49±4%, p<0.001), RVD indexed was larger (21±3mm/m2 vs. 19±2mm/m2, p<0.01) and RVMD was more pronounced (22±15ms vs.15±13ms, p=0.03) in early-phase ARVC vs. RVOT-VT patients.

**Paper 3**

In total, 162 consecutive ARVC subjects were included in this study. AEs events occurred in 84(52%) patients in total, in 69(78%) with overt and in 15(21%) with early ARVC. Also, 50(31%) patients had been or were implanted with an ICD at time of study inclusion, including 4 subjects with early ARVC. Appropriate ICD therapies had been given in 15 patients, none of whom had early disease. The diagnosis of
early ARVC was mainly based on family mutations (n=58), minor depolarization abnormalities (n=22), and minor criteria for ventricular arrhythmias (n=15) (Figure 2). CMR was performed in 121(75%) ARVC subjects, including 50(68%) early ARVC subjects.

In the total ARVC population, RV basal diameter (RVD) and RVOT diameter were markers of AEs (both p<0.001). RV function by RVFAC and RVGLS were decreased in subjects with arrhythmias (both p<0.01) and RVMD was more pronounced (p=0.001). An RVD of 41 mm and RVOT diameter of 34 mm optimally detected subjects with AEs. RV and LV dimensions were larger and RV and LV function were impaired in those with AEs (all p<0.05), also when adjusted for beta blocker use, age, gender and mutation status in multivariable analyses (all p≤0.05). Furthermore, LVMD was more pronounced in those with AEs (p=0.001). All 3 parameters from SAECG, Epsilon waves and T-wave inversion were markers of AEs in the total ARVC population (all p≤0.001).

In patients with overt ARVC, 69 (78%) had experienced AEs (17 ACA, 40 sustained VT, and 11 non-sustained VT and 1 cardiac syncope). Increased RVOT diameter and decreased RVFAC were markers of AEs. Interestingly, also the new echocardiographic parameters RVD and RVGLS were markers of arrhythmia with similar ability to identify patients with AEs (C statistics 0.75(95%CI: 0.61-0.88) and 0.68(95%CI 0.54-0.82) respectively, p=0.36). Parameters from SAECG were abnormal in overt ARVC patients, but were not markers of arrhythmias.

In subjects with early ARVC, 15 (21%) had experienced AEs (5 sustained VT, 9 non sustained VT and 1 cardiac syncope without documented VT). RVD was larger in subjects with AEs (both p=0.05), and RVMD was more pronounced (p=0.003) (Table 9). By ROC analyses, RVD and RVMD had similar ability to detect AEs (C statistics 0.74(95%CI 0.59-0.90) and 0.62(95%CI 0.47-0.78) respectively, p=0.20.). Neither echocardiographic parameters of RV function (RVFAC, RVGLS) nor parameters of LV function were markers of AEs in early ARVC. However, all parameters from SAECG were arrhythmic markers (all p<0.05) (Table 9), with comparable discriminative ability (p>0.59 for all comparisons). Interestingly,
RVMD correlated with the electrical parameter filtered QRS duration from signal averaged ECG (R=0.38, p=0.009) (Figure 11).

Table 9. Comparison of 73 early ARVC subjects without (n=58) and with (n=15) arrhythmic events.

<table>
<thead>
<tr>
<th></th>
<th>No arrhythmic events (n=58)</th>
<th>Arrhythmic events (n=15)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female (n (%))</strong></td>
<td>32 (55)</td>
<td>6 (40)</td>
<td>0.29</td>
</tr>
<tr>
<td><strong>Echocardiography</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV diameter (mm)</td>
<td>37±5</td>
<td>40±4</td>
<td>0.05</td>
</tr>
<tr>
<td>RVFAC (%)</td>
<td>45±8</td>
<td>43±6</td>
<td>0.45</td>
</tr>
<tr>
<td>RVGLS (6 segm)(%)</td>
<td>-24.2±3.3</td>
<td>-23.8±2.9</td>
<td>0.69</td>
</tr>
<tr>
<td>RV mechanical dispersion (6 segm)(ms)</td>
<td>26±11</td>
<td>39±15</td>
<td>0.003</td>
</tr>
<tr>
<td>RVOTsax (mm)</td>
<td>32±5</td>
<td>35±5</td>
<td>0.09</td>
</tr>
<tr>
<td>LVEDV (ml)</td>
<td>112±28</td>
<td>115±31</td>
<td>0.75</td>
</tr>
<tr>
<td>LVESV (ml)</td>
<td>48±14</td>
<td>50±15</td>
<td>0.69</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>58±4</td>
<td>57±4</td>
<td>0.43</td>
</tr>
<tr>
<td>LVGLS (16 segm)(%)</td>
<td>-20.5±2.4</td>
<td>-20.1±1.7</td>
<td>0.57</td>
</tr>
<tr>
<td>LV mechanical dispersion (16 segm)(ms)</td>
<td>33±14</td>
<td>39±31</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Electrical parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filtered QRS (ms)</td>
<td>109±7</td>
<td>115±8</td>
<td>0.005</td>
</tr>
<tr>
<td>HFLA (ms)</td>
<td>32±7</td>
<td>37±5</td>
<td>0.02</td>
</tr>
<tr>
<td>RMS (uV)</td>
<td>38±14</td>
<td>27±13</td>
<td>0.02</td>
</tr>
<tr>
<td>Epsilon waves (n (%))</td>
<td>0(0)</td>
<td>2(13)</td>
<td>0.04</td>
</tr>
<tr>
<td>Incomplete RBBB (n(%))</td>
<td>2(3)</td>
<td>1(7)</td>
<td>0.59</td>
</tr>
<tr>
<td>QRS (ms)</td>
<td>92±13</td>
<td>94±8</td>
<td>0.43</td>
</tr>
<tr>
<td>S-wave upstroke (ms)</td>
<td>39±7</td>
<td>38±8</td>
<td>0.59</td>
</tr>
<tr>
<td>S-wave upstroke &gt;55ms (n (%))</td>
<td>1 (2)</td>
<td>0(0)</td>
<td>1.00</td>
</tr>
<tr>
<td>T-wave inversions (n (%))</td>
<td>3(5)</td>
<td>2(13)</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Values are n, n(%) or mean ± SD; p by Chi-square test or Fisher’s exact test, Student’s t-test or one-way analysis of variance (ANOVA). GLS, global longitudinal strain; HFL, high-frequency low-amplitude signals; LV, left ventricular;
LVEDV, left ventricular end diastolic volume; LVEF, left ventricular ejection fraction; LVESV: left ventricular end systolic volume; RBBB, right bundle branch block; RMS, root-mean-square voltage of terminal 40 ms.; RV diameter, right ventricular basal diameter; RVFAC, right ventricular fractional area change; RVGLS, right ventricular global longitudinal strain; RVOT, right ventricular outflow tract diameter.

Figure 11. Correlation between filtered QRS duration and RV mechanical dispersion in early ARVC

To assess arrhythmic risk in early ARVC subjects, we evaluated the presence of major and minor electrical abnormalities and echocardiographic parameters (Table 9) as given by the TFC (2). In patients with early ARVC and AEs, 67% had abnormal electrical parameters and 67% had abnormal echocardiographic findings by the early parameters (RVD ≥40 mm and/or RVMD ≥37 ms). Interestingly, 87% of those with arrhythmias had at least one of the electrical or echocardiographic abnormalities. We
tested the ability of major and minor electrical abnormalities to detect subjects with AEs in a logistic regression model (Figure 12). The model’s ability to detect AEs increased significantly when adding the two new early echocardiographic markers, RVD and RVMD, both in early ARVC and in the total ARVC population.

Figure 12. Chi Square statistics for models to identify subjects with arrhythmic events in (a) early ARVC (n=73) and (b) the total ARVC population (n=162). Electrical abnormalities were defined according to the TFC as epsilon waves in V1 to V3, pathological signal averaged ECG and T-wave inversions in V1-V2 or beyond. RVD and RVMD were included as continuous variables. Chi square statistics was used a measure of the ability to detect arrhythmic events, and showed that the model for identifying subjects with arrhythmic events was improved both in early ARVC (χ² increased from 4.7 to 10.0, p=0.05; AIC 58 and 57, respectively, indicating that overfitting is of more concern in this model) and in the total ARVC population (χ² increased from 28.1 to 48.1, p<0.001; AIC 155 and 139, respectively, indicating better fit also when adjusting for inclusion of more parameters) when adding measurements of RVD and RVMD to presence of electrical abnormalities alone. ARVC, arrhythmogenic right ventricular cardiomyopathy; RVD, right ventricular basal diameter; RVMD, right ventricular mechanical dispersion; TFC, Task Force Criteria 2010. From Leren IS, Saberniak J et al. JACC Cardiovasc Imaging. 2016 Oct 14. Doi: 0.1016/j.jcmg.2016.06.011. Epub ahead of print, with permission.
Discussion

This thesis focused on patients and family members with ARVC, with particular focus on the impact of vigorous exercise on adverse cardiac outcome in ARVC, the challenging differential diagnosis from other common arrhythmic disease and risk assessment of AEs in early stages of ARVC disease. We introduced new insight in risk factors and risk stratification of ventricular arrhythmia and cardiac outcome in ARVC patients and mutation positive family members at early and advanced stages of disease, and provided the important differential diagnosis early stage ARVC vs. RVOT-VT.

Paper 1. Vigorous physical activity impairs myocardial function in patients with arrhythmogenic right ventricular cardiomyopathy and in mutation positive family members

In paper 1, we confirmed that vigorous athletic activity increased penetrance and phenotypic expression of ARVC disease in a large European cohort. For the first time in humans, we showed a linear relationship between amount of physical activity and AVRC manifestations, with reduced biventricular myocardial function and adverse arrhythmic outcome in both ARVC patients and mutation positive family members. This apparent dose-response relationship might be considered as a continuum of duration and intensity of athletic activity and may define the important interaction between genetic and environmental risk factors. Our results were consistent by conventional and novel echocardiographic and CMR parameters from TFC in this multi-modality approach.

ARVC athletes had consistently reduced biventricular myocardial function, more structural abnormalities by echocardiographic and CMR parameters, and increased risk of VA compared to ARVC non-athletes. Athletes among the mutation positive family members had significantly reduced LV function, more structural RV abnormalities, increased RV diameter and fulfilled more often definite ARVC by TFC compared to non-athletic family members.
Our results could hypothetically be a result of physiological athletic changes. However, heart rate, cardiac output and BMI were not different. LVEF in ARVC index patients was reduced below values that could be considered as physiological in athletes (60). Reduced LV function in athletes below physiological values was further underscored by the finding of reduced LV global strain. ARVC athletes had clearly dilated RV dimensions and severely reduced RV function, which were not attributable to common physiological changes (60;61).

We found that athletic activity was related to adverse arrhythmic outcome with a higher frequency and earlier onset of VA, higher frequency and earlier ICD implantation and only athletes needed cardiac transplantation. Age of training onset correlated to the age at ICD implantation.

In our paper, we speculated that myocardial dysfunction and adverse cardiac outcome in ARVC might be related to a continuum of physical activity. Our results could be interpreted as different from reports from other groups suggesting an existing exercise threshold in desmosomally healthy individuals, causing the “acquired ARVC” (62). Our new findings might suggest no existing exercise threshold value for recommendations of physical exercise in ARVC mutation positive subjects, i.e. both in patients and in mutation positive family members, to prevent adverse effects on myocardial function and increased risk of arrhythmia.

Our paper indicated that athletic activity was an important environmental risk factor, which accelerated and aggravated ARVC disease progression and worsened cardiac outcome in both patients and in mutation positive family members. The clinical implication of our findings was emphasized in the editorial accompanying the paper (63). Our results may have impact on future sports and ARVC guidelines.

Paper 2. Comparison of patients with early-phase arrhythmogenic right ventricular cardiomyopathy and right ventricular outflow tract ventricular tachycardia

In paper 2, we showed a new diagnostic multi-modality approach by electrical and novel cardiac imaging parameters to compare early-phase ARVC from RVOT-VT, the most common arrhythmic disease
originating from the RVOT. The discrimination between ARVC and RVOT-VT may be obvious in advanced ARVC, but is clinically challenging in early stages of ARVC.

As expected, our total ARVC population had more severe disease, indicated by more frequent ACA and obvious pathological structural and functional cardiac changes compared to RVOT-VT patients. Severe arrhythmias were more prevalent already in early-phase ARVC, highlighting the different prognosis of these conditions. Myocardial function and structure were affected also in early-phase ARVC disease, and these subtle changes might help to discriminate early-phase ARVC from RVOT-VT.

The three novel imaging parameters: RVMD, RVD and LVGLS, none of them included in the TFC, and one parameter from TFC, RVEF by CMR, were sensitive multi-modality imaging markers of early-phase ARVC compared to RVOT-VT. Our results confirmed subtle biventricular changes also in early stages of ARVC (64), and RVD and RVMD were independent markers of early-phase ARVC disease versus RVOT-VT. The novel imaging parameter RVD was easy to obtain as an echocardiographic parameter and currently not included in the TFC (2), however, the RV dimensions are load dependent, and RV dilatation is noted in athletes (62). Therefore, the clinical value of RV dimension may be limited. Segmental strain analyses provide time measurements of regional myocardial contraction duration heterogeneity in both the LV and RV, and MD has previously been reported as an important novel marker of arrhythmias in ARVC and several other cardiac diseases (40;58;65). Mechanical dispersion may be a link between electrical properties and dispersed myocardial function. This link may be essential in both in early-phase and advanced ARVC (66).

In this paper, RVEF by CMR was the best cardiac imaging parameter to discriminate between early-phase ARVC and RVOT-VT. However, RVEF is included in the TFC and was used as a primary parameter to differentiate between RVOT-VT and ARVC patients, and the impact of RVEF may therefore be overestimated. Furthermore, evaluation of a patient’s diagnosis should not rely on only one single parameter. We propose the combined use of CMR and echocardiography parameters to discriminate early-
phase ARVC from RVOT-VT. The novel echocardiographic parameters may be of help to select patients for further CMR studies and in centers where CMR is not widely available.

%PVC was a strong and independent electrical marker of early-phase ARVC, i.e. lower %PVC indicated more likely early-phase ARVC compared to RVOT-VT. Work-up of patients with suspected RVOT-VT and moderate frequency of PVC (in our study <2%PVC/24 h) should be evaluated by the TFC to avoid overlooking the more severe ARVC diagnosis.

Frequent PVC may cause adverse myocardial remodeling with subsequent reduced function and dilatation, and thus further complicate the discrimination to early-phase ARVC (33). The moderate number of PVC in early-phase ARVC found in our paper, and the frequent origin of PVC in the RV lateral free wall in ARVC, have also been reported by others (31;43). This supports that early-phase ARVC is more likely in patients with moderate frequency of PVC originating from the lateral free wall of the RVOT, compared to RVOT-VT with very frequent PVC originating from the septal part of the RVOT.

Our paper supported the diagnostic value of electrical parameters and novel and conventional cardiac imaging parameters in the important discrimination between early-phase ARVC and RVOT-VT. This broader diagnostic approach, including electrical and new imaging parameters might help to correctly diagnose early-phase ARVC and RVOT-VT, and aid treatment decisions.

**Paper 3. Echocardiography combined with ECGs improve identification of arrhythmic events in early ARVC**

Risk stratification of arrhythmias and SCD in ARVC subjects is difficult and challenging in early stages of ARVC. ARVC mutation positive family members with no apparent or only early disease constitute a substantial proportion of current ARVC populations seen in cardiomyopathy clinics. Risk stratification of AEs in these individuals has not been sufficiently addressed. In our study, 21% of ARVC subjects with early disease had experienced AEs, underlining the importance of identifying early markers of arrhythmias.
In the total ARVC population, RV function was decreased and RVMD was pronounced, as shown previously (40), and interestingly, RV dimensions were larger in subjects with previous AEs, reflecting the well known RV affection in ARVC. The novel echocardiographic parameter RVD was a marker of AEs, a parameter easily to obtain and not included in the current TFC (2). The TFC is a diagnostic score, and was primarily not intended for identifying subjects with AEs. However, our results showed, that RVD may have a potential in both ARVC diagnostics, staging and risk assessment (67).

RVOT diameter, a criterion from TFC, was also a marker of AEs. The optimal RVOT diameter to detect patients with AEs was 34mm, even lower than the TFC diagnostic value (36mm).

Importantly, the novel imaging parameters RVD and RVMD were arrhythmic markers, also in early ARVC. Our findings indicated that subtle changes in RV dimension and function might be the first RV changes in progression to a higher level of arrhythmic risk, although the retrospective study design may limit interpretation of cause and effect. Mechanical dispersion, reflecting inhomogeneous contraction, has previously been shown to be a marker of arrhythmias in ARVC, and other cardiac diseases (40;41;58). Subtle structural and functional changes and fibrosis in particular may reflect substrates for pronounced MD and hence increased arrhythmic risk. As shown previously, asymptomatic ARVC mutation positive family members had more pronounced RVMD than healthy controls (40), indicating a possible continuum of risk.

The frequent involvement of the LV in ARVC is now commonly recognized (9). In the total ARVC population, LV function and timing parameters were markers of previous AEs, indicating biventricular disease in advanced ARVC. However, in early ARVC, we found no affection of LV parameters. Our results may imply a later onset of LV dysfunction compared to RV dysfunction in our population.

Previous studies have indicated that electrical dysfunction precede structural findings in ARVC (34). Our study supports these findings, with one third of patients with early ARVC having pathological SAECG. However, the value of SAECG in risk stratification of arrhythmias is less clear. Some studies have reported signal averaged ECG parameters to be associated with arrhythmic risk and to relate to the extent of myocardial fibrosis (68;69). In our study, all three electrical parameters from SAECG were markers of AEs
in early ARVC, supporting the importance of this assessment in early ARVC disease, when risk
stratification for arrhythmias is most challenging.

Current risk stratification of SCD, and hence ICD indications, in ARVC are based on the presence of
previous syncope, VT or ACA, and RV/LV dysfunction, proband status, accumulated athletic activity and a
definite ARVC diagnosis by TFC (8). However, these markers are most useful in advanced ARVC. Risk
stratification of AEs, including SCD in ARVC subjects is still difficult to predict, and most challenging in
eye stages of ARVC (3;19), when AEs may occur prior to major structural changes.

The addition of novel imaging parameters RVD and RVMD to electrical parameters improved the
ability to identify patients with AEs compared to the electrical changes given by the TFC 2010 alone (Figure
12), both in early ARVC and in the total ARVC population. RVMD correlated with electrical parameters in
eye ARVC (Figure 11), indicating that pronounced MD, as a marker of arrhythmia may reflect early
electromechanical interactions important for arrhythmogenicity.

Our paper showed, that novel sensitive tools from echocardiography and electrical parameters by
signal averaged ECGs were markers of AEs in ARVC subjects at risk. The addition of early structural and
functional changes by echocardiography improved the prediction of AEs in both in early and advanced
ARVC compared to electrical parameters alone. The clinical implication of our findings was emphasized in
the editorial accompanying the paper (70). We suggest including measurements of RV diameter and RV
mechanical dispersion in a broader imaging approach when evaluating risk stratification of arrhythmias in
ARVC subjects.
Moving from multi-modality diagnostic tests towards multi-modality risk stratification in ARVC

In this thesis, a broader multi-modality approach with conventional diagnostic criteria by TFC and novel, echocardiographic parameters, not included in TFC, was used to improve risk stratification of cardiac outcome in early stage and advanced ARVC.

Potential life-threatening arrhythmias remain a major concern for ARVC patients and the clinical challenge in work-up and clinical management of the individual ARVC patient primarily lies in the early ARVC stages without or with only subtle phenotypic expression. The combination of novel cardiac imaging tools and electrical parameters identifying subtle structural, functional and electrical pathology may have a potential to be applied not only in diagnostic testing but also as markers of arrhythmic risk and adverse cardiac outcome. E.g. we could show that the electrical diagnostic TFC parameters of SAECG (filtered QRS, HFLA and RMS) had a potential when used as a marker of arrhythmic risk in early ARVC. The novel cardiac imaging parameters RVMD and RVD had potential when used as markers for adverse cardiac outcome, in diagnostics and in risk assessment of AEs in early and advanced ARVC.

Our findings may indicate that more pathological values from SAECG, RV dilatation and pronounced MD without overt affection of myocardial function may be the first changes in ARVC disease progression and may imply a transition to a level of higher risk of arrhythmic events.

Furthermore, our results may provide a step moving from multi-modality diagnostic tests towards multi-modality risk stratification in ARVC to improve cardiac outcome in ARVC.
Methodology: Strengths and limitations of cardiac myocardial imaging by strain echocardiography

2D strain speckle tracking by echocardiography were used in all papers as a sensitive tool for detection of subtle myocardial alterations by assessment of segmental myocardial function and by time intervals of regional myocardial contraction duration (54;58). Strain echocardiography is supposed to be attractive, fast and robust in use and has the ability to identify myocardial dysfunction on a regional level (57). Furthermore, strain echocardiography may be a more direct measure of global myocardial function compared the volume-based ejection fraction.

There is a growing body of evidence showing that the assessment of myocardial function by strain echocardiography by speckle tracking techniques provides incremental new information in the clinical setting (71) and strain echocardiography has been recently implemented in several guidelines (72-74). Strain echocardiography has the possibility to take a closer look at biventricular myocardial function and, hence cardiac outcome.

In general, all kinds of ultrasound noise reduce the tracking ability and image quality. High temporal and spatial resolution is essential to provide accuracy and robustness of the measurements and the analyses performed. Furthermore, the acquisition of good image quality is essential for reducing inter- and intra observer variability of tracking data. In this thesis, reproducibility and feasibility studies of strain measurements were satisfying.

A number of software algorithms are available on the marked and the use of these techniques has revealed existing variability among vendors in the definition and modality of measuring parameters and hence, the results output (56). In our papers, all echocardiographic examinations were performed on GE Vingmed echo machines and analyzed by EchoPAC software 112. However, we used both Vivid 7 and Vivid E9 GE echo machines, which might have a minor impact on our results.

Speckle tracking strain echocardiography has the advantage of relatively angle independency, but is sensitive to reverberations (motionless artifacts forming acoustic shadowing) and out of plane motion (speckles move not only in one plane, but in all three dimensions).
Load dependency of the LV and RV is a known limitation for all echocardiographic methods, including speckle tracking strain echocardiography. Changes in preload and/or afterload conditions have impact on strain/deformation values (57). However, in our papers, all subjects were in hemodynamically stable conditions.

Low frame rate, i.e. low temporal resolution will lead to a lack of time of information and may result in reduced strain values and inaccurate timing intervals. In our papers we used satisfying frame rates. However, due to method dependency, we may have missed peak strain values and hence, correct values of global longitudinal strain mechanical dispersion.

**Limitations**

**General limitations:**

In this thesis, all papers had a cross-sectional design and a relatively limited number of study subjects, thereby reducing the strength of our conclusions. Prospective follow-up multi-center studies should be performed to increase numbers of patients and thereby provide better estimates of clinical variation and better strengths of results.

The echocardiographic limitations are listed in the chapter above.

**Paper specific limitations:**

Paper 1 had cross-sectional design and data on athletic activity were collected by direct interview or by telephone calls with possible information bias. Future studies should attempt to quantify exercise intensity, frequency and duration directly with devices which can accurately track amount of physical activity.

Our paper was not designed to fully answer if ARVC mutation positive family members should be restricted in physical activity. However, our paper underlined current guidelines which recommend patients
with definite ARVC to refrain from competitive sports. Future prospective follow-up studies may be needed, including larger numbers of athletes with both definite and non-definite ARVC for comprehensive work-up and to further advice the family members.

Only CMR studies including short axis images were defined as appropriate (59). Therefore, only a subset of subjects (49%) had an appropriate CMR study which might have influenced our results. However, this subset did not differ from those without available CMR regarding age, gender, index status or athlete status.

Paper 2 had a cross-sectional design including a relatively limited number of RVOT-VT patients, and hence, may not allow for evaluation of sufficient predictive ability. This might limit the interpretation of markers of discrimination between early-phase ARVC and RVOT VT. The study populations were predefined by their clinical diagnosis when recruited from our cardiological department. Future prospective follow up studies should include and investigate unselected actual patients to validate our results. Furthermore, future prospective studies should follow up both early-phase ARVC and RVOT-VT patients for a longer period of time, to explore any overlap between these diagnoses. Although we excluded all patients with definite ARVC criteria from our RVOT-VT cohort, we cannot exclude that patients diagnosed with RVOT-VT will develop criteria for ARVC in the future.

Only a subset of patients (48%) had an appropriate CMR study (59), which may have influenced our results. However, this subset did not differ from patients without available CMR regarding age, gender and BSA, neither in the early-phase nor in the advanced ARVC population. Furthermore, RVEF by CMR from TFC was used as a primary parameter to differentiate between RVOT-VT and ARVC patients and the impact of RVEF by CMR may therefore be overestimated.

Paper 3 had a cross-sectional, single center design with inherent limitations. The limited number of with early ARVC subjects with AEs makes the model for identifying these subjects vulnerable to overfitting. Our analyses may be confounded by inclusion of mutation positive family members who will remain ARVC non-penetrant, i.e. asymptomatic ARVC subjects.
AEs were obtained retrospectively at inclusion which might limit the overall applicability and interpretation of markers of AEs. The precise role of CMR findings, performed in most subjects, might need more evaluation and might add important information to arrhythmic risk assessment. Future studies should prospectively follow subjects with early ARVC disease to confirm our results. All echocardiographic analyses were performed in a blinded fashion; however the presence of an ICD lead might have demasked arrhythmic status in few cases.

Main conclusions

General conclusions:

We used novel echocardiography parameters, currently not included in TFC, and electrical parameters from TFC in a comprehensive approach to improve work-up and management of ARVC patients. These novel tools added important information in assessment of risk factors of cardiac outcome in ARVC, to discriminate early-phase ARVC from RVOT-VT and to predict AEs in early ARVC. In addition, they showed potential in research, diagnostics and risk stratification. Our results might be a step moving from multi-modality diagnostics towards multi-modality risk stratification in ARVC patients and family members, to improve cardiac outcome in this patient group.

Specific conclusions:

Paper 1

Athletic activity was a risk factor which aggravated LV and RV myocardial dysfunction, increased risk of arrhythmic events and accelerated the onset of life-threatening ARVC manifestations both in ARVC patients and in mutation positive family members. Our results may have impact on future sports guidelines and ARVC guidelines.
**Paper 2**

In early-phase ARVC, the electrical parameter %PVC was the most powerful marker to discriminate early-phase ARVC from RVOT-VT. By conventional and novel cardiac imaging parameters, early-phase ARVC patients had slightly lower RV function by RVEF by CMR, more pronounced RV mechanical dispersion and larger RV basal diameter compared to RVOT-VT patients. This novel diagnostic approach may add help to correctly diagnose early-phase ARVC and RVOT-VT patients and aid treatment decisions.

**Paper 3**

In patients with early ARVC disease, electrical parameters from SAECG and the two new RV echocardiographic parameters, RV diameter and RV mechanical dispersion, were markers of arrhythmic events. The addition of early structural changes increased the ability to predict arrhythmic events compared to electrical parameters alone. We suggested including measurements of RV diameter and RV mechanical dispersion in risk stratification in ARVC.
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Paper I

Saberniak J, Hasselberg NE, Borgquist R, Platonov PG, Sarvari SI, Smith HJ, Ribe M, Holst AG, Edvardsen T, Haugaa KH

Vigorous physical activity impairs myocardial function in patients with arrhythmogenic right ventricular cardiomyopathy and in mutation positive family members

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Vigorous physical activity impairs myocardial function in patients with arrhythmogenic right ventricular cardiomyopathy and in mutation positive family members

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Aims Exercise increases risk of ventricular arrhythmia in subjects with arrhythmogenic right ventricular cardiomyopathy (ARVC). We aimed to investigate the impact of exercise on myocardial function in ARVC subjects.

Methods and Results We included 110 subjects (age 42 ± 17 years), 65 ARVC patients and 45 mutation-positive family members. Athletes were defined as subjects with ≥4 h vigorous exercise/week (≥1440 metabolic equivalents (METs x minutes/week)) during a minimum of 6 years. Athlete definition was fulfilled in 37/110 (34%) subjects. We assessed right ventricular (RV) and left ventricular (LV) myocardial function by echocardiography, and by magnetic resonance imaging (MRI). The RV function by RV fractional area change (FAC), RV global longitudinal strain (GLS) by echocardiography, and RV ejection fraction (EF) by MRI was reduced in athletes compared with non-athletes (FAC 34 ± 9% vs. 40 ± 11%, RVGLS – 18.3 ± 6.1% vs. – 22.0 ± 4.8%, RVEF 32 ± 8% vs. 43 ± 10%, all P < 0.01). LV function by LVEF and LVGLS was reduced in athletes compared with non-athletes (LVEF by echocardiography 50 ± 10% vs. 57 ± 5%, LVEF by MRI 46 ± 6% vs. 53 ± 8%, and LVGLS – 16.7 ± 4.2% vs. – 19.4 ± 2.9%, all P < 0.01). The METs x minutes/week correlated with reduced RV and LV function by echocardiography and MRI (all P < 0.01). The LVEF by MRI was also reduced in subgroups of athlete index patients (46 ± 7% vs. 54 ± 10%, P = 0.02) and in athlete family members (47 ± 3% vs. 52 ± 6%, P < 0.05).

Conclusion Athletes showed reduced biventricular function compared with non-athletes in ARVC patients and in mutation-positive family members. The amount and intensity of exercise activity was associated with impaired LV and RV function. Exercise may aggravate and accelerate myocardial dysfunction in ARVC.

Keywords Arrhythmogenic cardiomyopathy • Heart failure • Exercise • Myocardial function • Ventricular arrhythmia

Introduction Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited cardiac cardiomyopathy predisposing to ventricular arrhythmia (VA) and sudden cardiac death in apparently previously healthy young individuals. Molecular genetic studies have revealed that pathomechanisms involve dysfunction in cardiac desmosomes. Desmosomal dysfunction ultimately leads to apoptosis and fibro-fatty replacement of the myocardium. The cardiac phenotype presents as a dilated right ventricle with wall thinning and
aneurysms, and the occurrence of life-threatening VA. Pathology is not restricted to the right ventricle (RV) and left ventricular (LV) involvement is common.1

Although the benefit of physical activity on individual health is indisputable1,2, exercise may have adverse effects in patients with underlying desmosomal dysfunction.4 A high level of physical activity is reported to increase risk of VA in patients with ARVC4,5 and these patients are recommended to refrain from competitive sports.6 However, the impact of a high level of physical activity on myocardial function in patients with ARVC has not been fully investigated. Exercise increases wall stress by volume overload and sympathetic stimulation, which may trigger the disease,7 and case reports indicate a high prevalence of ARVC in competitive athletes.8 Furthermore, experimental studies support that vigorous exercise accelerates RV dysfunction in mice with ARVC-related mutations.9 However, in humans, systematic data on exercise as a trigger of biventricular cardiomyopathy and data on how exercise affects mutation positive family members are sparse. Information about the effect of physical activity on a genetically altered myocardium is of utmost importance for patients with ARVC, and in particular for their mutation-positive family members in whom overt myocardial dysfunction has not yet been detected.

We aimed to investigate the impact of vigorous exercise on myocardial function in patients with ARVC and in their mutation positive family members. We hypothesized that myocardial function is more reduced in ARVC subjects with a history of athletic activity compared to those without athletic activity.

Methods

Study subjects

In this cross-sectional study, subjects were recruited from two university hospitals (Oslo University Hospital, Norway and Lund University Hospital, Sweden). We used the 2010 Task Force Criteria (2010 TFC) in the diagnostics of ARVC.10 Index patients were genetically tested for ARVC-related mutations. Family members positive for a truncation, frameshift or splice site mutation, or with an ARVC-related missense mutation that co-segregated with ARVC phenotype in the family were included.

The age of the ARVC subjects at echocardiographic examination, at implantable cardioverter defibrillator (ICD) implantation, and at cardiac transplantation was recorded. Ventricular arrhythmias were defined as syncope with assumed arrhythmic origin, documented sustained ventricular tachycardias (VTs) and aborted cardiac arrests. Exercise induced VA were defined as VA occurring during physical activity. Any ARVC related medication received at time of echocardiographic examination was recorded.

Written informed consent was given by all study participants. The study complied with the Declaration of Helsinki and was approved by the Regional Committees for Medical Research Ethics in Sweden and Norway.

Athlete definition

The study participants were asked about their history of and current amount of athletic activity by direct interview or by telephone calls. Amount of physical activity was expressed in metabolic equivalents (METs) x min/week. One MET represents an individual's energy expenditure while sitting quietly and is approximated to 3.5 ml/kg.min or 1 kcal/kg.h.11 Intensity of physical activity was graded as vigorous if ≥ 6 METs.12 The duration of regularly performed exercise was expressed in years. Subjects with a history of physical activity with intensity ≥ 6 METs for ≥ 4 hours/week (≥ 1440 METs x min/week) during minimum 6 years were defined as athletes. Quartiles of METs x min/week were created to explore the relationship between activity level and cardiac function and volumes.

Echocardiographic study

All subjects underwent an echocardiographic study (Vivid 7 and Vivid E9, GE Vingmed, Horten, Norway) and were analyzed off-line (EchoPac®, GE Vingmed). We assessed the following RV parameters: diameter of RV outflow tract (RVOT) from parasternal short axis view (PSAX), RV basal diameter (RVBD), RV fractional area change (FAC), tricuspid annular plane systolic excursion (TAPSE),13,14 and evaluated for the presence of RV wall abnormalities by 2010 TFC.10 From the LV we assessed end-diastolic diameter (LVEDD), end-diastolic (LVEDV) and end-systolic (LVESV) volume, ejection fraction (LVEF) and cardiac output.15 Values were considered abnormal according to current guidelines.16,17 Strain analyses were performed by two-dimensional (2D) speckle tracking echocardiography. The LV strain was derived from 2D images from the three apical views,18 and RV strain was derived from the four-chamber view with focus on the right ventricle.19 Peak systolic longitudinal strain was determined in 16 LV segments averaged to LV global longitudinal strain (LVGLS). Peak systolic longitudinal strain from six RV segments was averaged to RVGLS. The LVIF was assessed by two independent observers blinded to all clinical information and athlete status.

Magnetic resonance imaging

Cardiac magnetic resonance imaging (MRI) was performed in a subset of patients using a 1.5 Tesla scanner (Magnetom Vision Plus o’ Magnetom Sonata; Siemens, Erlangen, Germany) and a phased array body coil. The RV and LV were covered by axial and sagittal breath-hold 11-TT turbo spin echo (TSE) images, and by multiple short axis cine images using a breath-hold segmented balanced gradient echo sequence (fast imaging with steady-state free precession, trueFISP). Two-chamber and four-chamber cine acquisitions were obtained. Calculation of RV and LV volumes were accomplished by summing the luminal areas on the short axis images at end-diastole and end-systole, including the RV and LV outflow tracts.20 Only MRI studies including short axis images were defined as appropriate. Post-processing analyses were performed using Qmass® MR 6.1.6 software (Medis Medical Imaging Systems, Leiden, the Netherlands). Interobserver analyses for RV and LV volumes were performed in 10 patients.

Genetic analyses

Genomic DNA was isolated from peripheral blood. The individual exons with flanking intron sequences of the plakophilin-2 (PKP2), desmocollin-2 (DSC2), desmoglein-2 (DSG2), desmopakin (DSP) genes and 29 of the 105 exons of the ryothidine receptor-2 (RYR2) gene were sequenced by polymerase chain reaction amplification in combination with direct sequencing. Genetic screening was performed in family members of patients with pathogenic mutations. Family members of patients with variants of uncertain significance (VUS)
Table 1: Clinical characteristics in 110 arrhythmogenic right ventricular cardiomyopathy (ARVC) subjects, 73 non-athletes and 37 athletes

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Total (n = 110)</th>
<th>Non-athletes (n = 73)</th>
<th>Athletes (n = 37)</th>
<th>P-value</th>
<th>Adjusted odds ratio (95% CI)</th>
<th>Adjusted P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index (n)</td>
<td>65 (59%)</td>
<td>37 (51%)</td>
<td>28 (78%)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male gender (n)</td>
<td>64 (58%)</td>
<td>35 (48%)</td>
<td>29 (78%)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>22 (60%)</td>
<td>22 (79%)</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at baseline echocardiography (years)</td>
<td>42 ± 17</td>
<td>45 ± 18</td>
<td>36 ± 13</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Index</td>
<td>50 ± 16</td>
<td>37 ± 13</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definite ARVC by TFC</td>
<td>78 (71%)</td>
<td>43 (59%)</td>
<td>35 (95%)</td>
<td>&lt;0.001</td>
<td>22.92 (4.27-123.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Index (n = 59)</td>
<td>32 (67%)</td>
<td>27 (90%)</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index (n = 19)</td>
<td>13 (76%)</td>
<td>8 (89%)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-definite ARVC by TFC</td>
<td>22 (29%)</td>
<td>2 (5%)</td>
<td>&lt;0.001</td>
<td>0.04 (0.01-0.22)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Index (n = 6)</td>
<td>5 (13%)</td>
<td>1 (4%)</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index (n = 26)</td>
<td>25 (69%)</td>
<td>1 (11%)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syncope (n)</td>
<td>40 (44%)</td>
<td>19 (33%)</td>
<td>21 (62%)</td>
<td>&lt;0.01</td>
<td>3.35 (1.29-8.71)</td>
<td>0.01</td>
</tr>
<tr>
<td>Index</td>
<td>12 (50%)</td>
<td>17 (68%)</td>
<td>0.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>7 (21%)</td>
<td>4 (44%)</td>
<td>0.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VA (n)</td>
<td>66 (60%)</td>
<td>37 (51%)</td>
<td>29 (78%)</td>
<td>&lt;0.01</td>
<td>4.53 (1.59-12.94)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Index</td>
<td>34 (92%)</td>
<td>27 (96%)</td>
<td>0.63</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>3 (8%)</td>
<td>2 (22%)</td>
<td>0.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercised-induced VA (n)</td>
<td>41 (37%)</td>
<td>13 (18%)</td>
<td>28 (80%)</td>
<td>&lt;0.001</td>
<td>16.54 (5.99-49.98)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Index</td>
<td>11 (31%)</td>
<td>26 (100%)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>2 (6%)</td>
<td>2 (22%)</td>
<td>0.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICD (n)</td>
<td>52 (47%)</td>
<td>29 (40%)</td>
<td>23 (62%)</td>
<td>0.03</td>
<td>2.58 (1.05-6.38)</td>
<td>0.04</td>
</tr>
<tr>
<td>Index</td>
<td>27 (73%)</td>
<td>21 (75%)</td>
<td>0.85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>2 (6%)</td>
<td>2 (22%)</td>
<td>0.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at ICD implantation (years)</td>
<td>39 ± 16</td>
<td>45 ± 16</td>
<td>33 ± 13</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Index</td>
<td>46 ± 16</td>
<td>34 ± 13</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>29 ± 11</td>
<td>28 ± 16</td>
<td>0.95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart transplantation (n)</td>
<td>5 (5%)</td>
<td>0 (0%)</td>
<td>5 (14%)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARVC mutation positive (n)</td>
<td>72 (68%)</td>
<td>53 (79%)</td>
<td>22 (67%)</td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-blocker (n)</td>
<td>62 (56%)</td>
<td>33 (56%)</td>
<td>29 (78%)</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amiodarone (n)</td>
<td>23 (21%)</td>
<td>8 (14%)</td>
<td>15 (41%)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ARVC: arrhythmogenic right ventricular cardiomyopathy; TFC: 2010 Task Force Criteria; VA: ventricular arrhythmia; ICD, implantable cardioverter–defibrillator; ECG, electrocardiogram.

*Adjusted for age and male gender.

were included only if they had an ARVC phenotype and if a familial co-segregation of symptoms was present.

Statistical analyses

Data were presented as mean±standard deviation or as median (range). Differences between groups were assessed by chi-square test and Fisher's exact test and Student's t-test (SPSS 20.0; SPSS Inc., Chicago, IL, USA). The subgroups of index and non-index subjects were analysed separately. Correlations between the amount of physical activity and RV and LV function were assessed by Pearson bivariate correlation. Kaplan–Meier curves were constructed and log-rank test was performed to assess cumulative lifetime cardiac transplantation-free survival. The C-statistic was calculated by receiver operating characteristic (ROC) curves for amount of physical activity that optimally detected those with arrhythmic events and those with RV/LV. The value closest to the upper left corner of the ROC curve was defined as giving optimal sensitivity and specificity. Multivariate logistic regression was performed to adjust the outcome of athlete status for age and gender. Intra-observer and inter-observer variability was expressed as intraclass correlation coefficient. Two-sided P-values <0.05 were considered significant.

Results

Patients characteristics and athlete status

We included 110 subjects of which athlete status could be determined. Of the 110, 65 (59%) were consecutive ARVC index patients and 45 (41%) were mutation-positive family members (Table 1). The definition of athlete status was fulfilled in 37 (34%), while 73 (66%) were non-athletes (Table 1). Athletes had a history
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total</th>
<th>Non-athletes (n = 73)</th>
<th>Athletes (n = 37)</th>
<th>P-value</th>
<th>Adjusted odds ratio (95% CI)</th>
<th>Adjusted P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats per min)</td>
<td>63 ± 13</td>
<td>65 ± 13</td>
<td>59 ± 12</td>
<td>0.06</td>
<td>0.96 (0.92–1.00)</td>
<td>0.05</td>
</tr>
<tr>
<td>Index</td>
<td>61 ± 11</td>
<td>58 ± 11</td>
<td></td>
<td>0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>68 ± 14</td>
<td>65 ± 13</td>
<td></td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25 ± 4</td>
<td>25 ± 4</td>
<td>25 ± 4</td>
<td>0.84</td>
<td>1.04 (0.91–1.18)</td>
<td>0.58</td>
</tr>
<tr>
<td>Index</td>
<td>26 ± 4</td>
<td>25 ± 4</td>
<td></td>
<td>0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>24 ± 3</td>
<td>24 ± 3</td>
<td></td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right ventricular parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVOT PSAX (mm)</td>
<td>36 ± 9</td>
<td>34 ± 7</td>
<td>39 ± 10</td>
<td>&lt;0.01</td>
<td>1.08 (1.02–1.15)</td>
<td>0.01</td>
</tr>
<tr>
<td>Index</td>
<td>36 ± 8</td>
<td>40 ± 11</td>
<td></td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>32 ± 5</td>
<td>35 ± 6</td>
<td></td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right ventricular basal diameter (mm)</td>
<td>44 ± 9</td>
<td>42 ± 8</td>
<td>47 ± 9</td>
<td>&lt;0.01</td>
<td>1.10 (1.03–1.16)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Index</td>
<td>46 ± 8</td>
<td>49 ± 9</td>
<td></td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>37 ± 4</td>
<td>41 ± 5</td>
<td></td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVOTAPSE/displacement (mm)</td>
<td>18 ± 5</td>
<td>19 ± 4</td>
<td>16 ± 5</td>
<td>&lt;0.01</td>
<td>0.88 (0.79–0.98)</td>
<td>0.02</td>
</tr>
<tr>
<td>Index</td>
<td>19 ± 5</td>
<td>17 ± 5</td>
<td></td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>19 ± 4</td>
<td>19 ± 3</td>
<td></td>
<td>0.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVFAC (%)</td>
<td>38 ± 11</td>
<td>40 ± 11</td>
<td>34 ± 9</td>
<td>&lt;0.01</td>
<td>0.93 (0.88–0.98)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Index</td>
<td>36 ± 10</td>
<td>32 ± 9</td>
<td></td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>45 ± 9</td>
<td>40 ± 8</td>
<td></td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVGLS (%)</td>
<td>20.7 ± 5.5</td>
<td>−22.0 ± 4.8</td>
<td>−18.3 ± 6.1</td>
<td>&lt;0.01</td>
<td>1.18 (1.06–1.32)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Index</td>
<td>−20.6 ± 5.4</td>
<td>−17.4 ± 6.5</td>
<td></td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>−23.4 ± 3.8</td>
<td>−21.4 ± 3.1</td>
<td></td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV wall abnormalities by 2010 TFC</td>
<td>69 (63%)</td>
<td>38 (53%)</td>
<td>31 (84%)</td>
<td>&lt;0.01</td>
<td>13.86 (4.35–44.09)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Index</td>
<td>28 (76%)</td>
<td>25 (89%)</td>
<td></td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>10 (29%)</td>
<td>6 (67%)</td>
<td></td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricular parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>3.7 ± 1.2</td>
<td>3.8 ± 1.2</td>
<td>3.6 ± 1.3</td>
<td>0.53</td>
<td>0.71 (0.46–1.08)</td>
<td>0.11</td>
</tr>
<tr>
<td>Index</td>
<td>3.6 ± 1.1</td>
<td>3.3 ± 1.3</td>
<td></td>
<td>0.42</td>
<td></td>
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<tr>
<td>Non-index</td>
<td>4.0 ± 1.2</td>
<td>4.6 ± 1.0</td>
<td></td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVESV (mL)</td>
<td>113 ± 49</td>
<td>105 ± 25</td>
<td>128 ± 56</td>
<td>&lt;0.01</td>
<td>1.01 (1.00–1.03)</td>
<td>0.11</td>
</tr>
<tr>
<td>Index</td>
<td>108 ± 27</td>
<td>129 ± 63</td>
<td></td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>103 ± 23</td>
<td>125 ± 21</td>
<td></td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVESV (mL)</td>
<td>54 ± 31</td>
<td>47 ± 15</td>
<td>67 ± 47</td>
<td>&lt;0.01</td>
<td>1.03 (1.00–1.06)</td>
<td>0.03</td>
</tr>
<tr>
<td>Index</td>
<td>49 ± 17</td>
<td>70 ± 53</td>
<td></td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>44 ± 11</td>
<td>55 ± 12</td>
<td></td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVF (%)</td>
<td>54 ± 8</td>
<td>57 ± 5</td>
<td>50 ± 10</td>
<td>&lt;0.001</td>
<td>0.87 (0.80–0.95)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Index</td>
<td>55 ± 6</td>
<td>48 ± 11</td>
<td></td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>58 ± 3</td>
<td>56 ± 3</td>
<td></td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVGLS (%)</td>
<td>18.5 ± 3.7</td>
<td>−19.4 ± 2.9</td>
<td>−16.7 ± 4.2</td>
<td>&lt;0.001</td>
<td>1.25 (1.06–1.46)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Index</td>
<td>−18.2 ± 2.8</td>
<td>−16.0 ± 4.4</td>
<td></td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-index</td>
<td>−20.4 ± 2.7</td>
<td>−19.0 ± 2.5</td>
<td></td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ARVC, arrhythmogenic right ventricular cardiomyopathy; RVOT PSAX, right ventricular outflow tract parasternal short-axis; RVOTAPSE, right ventricular tricuspid annular plane systolic excursion; RVFAC, right ventricular fractional area change; RVGLS, right ventricular global longitudinal strain; 2010 TFC, 2010 Task Force Criteria; LVEDD, left ventricular end-diastolic diameter; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVEF, left ventricular ejection fraction; LVGLS, left ventricular global strain.

*Adjusted for age and gender.

The activity level of 3178 ± 886 METs·min/week and non-athletes of 669 ± 290 METs·min/week, (P < 0.001). In athletes, age at start of activity was 11 ± 3 years and the duration of activity ≥ 1440 METs·min/week was 11 ± 4 years. According to the definition employed, all athletes had a history of athletic activity ≥ 6 years. Athletes were more frequently male, had more frequently definite ARVC by 2010 TFC and were more frequently index patients compared with non-athletes (all P < 0.01) (Table 1). Therefore, all analyses were also performed for index patients separately and results were adjusted for gender in addition to age.
Echocardiographic and MRI findings

By echocardiography, the ARVC population had reduced RV function by RVFAC (38 ± 11%) and increased RV (44 ± 9 mm) and RVOT (36 ± 9 mm) diameters compared with established normal values in healthy subjects (Table 2). Values for LV function and volumes by echocardiography were normal (Table 2).

Athletes were younger at the time of baseline echocardiography (P < 0.01), which in the majority of cases coincided with the time of diagnosis (Table 1). In addition, by separate analysis of index patients, athletes were younger at first echocardiographic examination. Resting heart rate was not lower in athletes vs. non-athletes (P = 0.06), or in index patients (P = 0.24), or in mutation positive family members (P = 0.62) (Table 2). Cardiac MRI was performed in 54 subjects, 20 athletes and 34 non-athletes.

RV function and diameters

Athletes had increased RV diameters compared with non-athletes and reduced RV function by RVFAC, TAPSE, and RV GLS (all P < 0.01) (Table 2) and reduced RVEF by MRI (P < 0.001) (Table 3). Athletes had a higher frequency of wall abnormalities by 2010 TFC (Table 2). Increased amount of physical activity in quartiles correlated with increased RVOT diameter (R = 0.24, P = 0.02) and with reduced RV strain (R = 0.21, P = 0.03) by echocardiography (Figure 1). Increasing amount of physical activity expressed as METs × min/week correlated with reduced RVEF by MRI (R = -0.51, P < 0.001) (Figure 2). By ROC analysis, 840 METs × min/week (≈2.3 h vigorous exercise/week) during a minimum of 6 years optimally detected those with RVFAC < 35% with a sensitivity of 69% [95% confidence interval (CI) 0.53–0.82], and specificity of 49% [95% CI 0.36–0.62].

LV function and volumes

The LV EF by echocardiography and MRI was lower in athletes compared with non-athletes in the total population (both P < 0.01), in the subgroups of index patients (P < 0.01 and P = 0.02, respectively) and in mutation-positive family members (both P < 0.05; Tables 2 and 3). Reduced LVEF and LV GLS by echocardiography correlated with increasing physical activity expressed as METs × min/week during a minimum of 6 years (R = -0.41, P < 0.001 and R = 0.32, P < 0.01, respectively; Figure 1).

Arrhythmias and outcome

In total, 66 (60%) of 110 subjects had experienced VA, and these events were more frequent in athletes compared with non-athletes (P < 0.01; Table 1) and occurred at younger age in athletes (36 ± 13 years vs. 50 ± 16 years, P < 0.01). Patients with VA had a history of a higher amount of METs × min/week during a minimum of 6 years compared with those without (1739 ± 1434 METs × min/week vs. 1177 ± 1056 METs × min/week, P = 0.03).

Table 3: Magnetic resonance imaging parameters in 54 arrhythmogenic right ventricular cardiomyopathy (ARVC) study participants

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total population (n = 34)</th>
<th>Non-athletes (n = 20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDVI (mL)</td>
<td>74 ± 18</td>
<td>74 ± 19</td>
<td>74 ± 16</td>
</tr>
<tr>
<td>Index</td>
<td>84 ± 14</td>
<td>75 ± 18</td>
<td>78 ± 18</td>
</tr>
<tr>
<td>Non-index</td>
<td>62 ± 18</td>
<td>73 ± 11</td>
<td>71 ± 11</td>
</tr>
<tr>
<td>LVEV6I (mL)</td>
<td>37 ± 12</td>
<td>35 ± 12</td>
<td>40 ± 12</td>
</tr>
<tr>
<td>Index</td>
<td>39 ± 12</td>
<td>40 ± 14</td>
<td>40 ± 14</td>
</tr>
<tr>
<td>Non-index</td>
<td>31 ± 11</td>
<td>40 ± 5</td>
<td>40 ± 5</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>50 ± 8</td>
<td>53 ± 8</td>
<td>46 ± 6</td>
</tr>
<tr>
<td>Index</td>
<td>54 ± 10</td>
<td>46 ± 7</td>
<td>47 ± 7</td>
</tr>
<tr>
<td>Non-index</td>
<td>52 ± 6</td>
<td>47 ± 3</td>
<td>48 ± 3</td>
</tr>
<tr>
<td>RVEDVI (mL)</td>
<td>89 ± 36</td>
<td>82 ± 34</td>
<td>99 ± 38</td>
</tr>
<tr>
<td>Index</td>
<td>99 ± 35</td>
<td>111 ± 30</td>
<td>105 ± 30</td>
</tr>
<tr>
<td>Non-index</td>
<td>63 ± 19</td>
<td>73 ± 18</td>
<td>73 ± 18</td>
</tr>
<tr>
<td>RVEV6I (mL)</td>
<td>56 ± 32</td>
<td>47 ± 28</td>
<td>70 ± 34</td>
</tr>
<tr>
<td>Index</td>
<td>57 ± 34</td>
<td>81 ± 36</td>
<td>79 ± 36</td>
</tr>
<tr>
<td>Non-index</td>
<td>36 ± 11</td>
<td>46 ± 12</td>
<td>46 ± 12</td>
</tr>
<tr>
<td>RVEF (%)</td>
<td>39 ± 11</td>
<td>43 ± 10</td>
<td>32 ± 8</td>
</tr>
<tr>
<td>Index</td>
<td>45 ± 13</td>
<td>30 ± 9</td>
<td>30 ± 9</td>
</tr>
<tr>
<td>Non-index</td>
<td>41 ± 7</td>
<td>37 ± 5</td>
<td>37 ± 5</td>
</tr>
</tbody>
</table>

Athletes were younger at time of ICD implantation (P < 0.01; Table 1). The age of onset of training correlated to the age ICD implantation (R = 0.48, P = 0.02).

Exercise-induced VA occurred in 41 (37%) subjects and occurred more frequently in athletes (P < 0.001). Amount of METs × min/week was higher in those with exercise-induced arrhythmias compared with those without [2373 ± 1369 METs × min/week vs. 929 ± 864 METs × min/week, P < 0.001]. An amount of 900 METs × min/week (≥2.5 h vigorous exercise/week) during a minimum of 6 years showed a C-statistics of 0.80 (95% CI 0.71–0.90) to discriminate subjects with exercise-induced VA [sensitivity 76% (95% CI 0.60–0.88), specificity 79% (95% CI 0.67–0.88)]

Five patients, all athletes, underwent cardiac transplantation, while no non-athletes were transplanted (log rank P < 0.001; Figure 2). Of the five transplanted, all had biventricular end-stage heart failure and four had additional repetitive untreatable VA.

Genetic findings

Genetic testing for ARVC-related mutations was performed in 100 of the study subjects. In these, 76 ARVC-related mutations/variants were identified in 75 (75%) subjects (one patient had two mutations). We observed eight different pathogenic mutations in PKP2 (n = 68) and in DSP (n = 2). Variants of uncertain significance were
Figure 1 Bar charts displaying relationship between the amount of physical activity expressed as quartiles of metabolic equivalents (METs) x minutes/week during a minimum of 6 years and echocardiographic findings in the right ventricle and left ventricle in 110 arrhythmogenic right ventricular cardiomyopathy (ARVC) subjects. There was a significant correlation between increasing activity in quartiles and increased right ventricular outflow tract (RVOT) diameter, increased left ventricular (LV) volumes and reduced function by right ventricular (RV) strain and left ventricular ejection fraction (LVEF) (all P < 0.05). The P-values are from Pearson’s bivariate correlation. FAC, fractional area change; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume.

Figure 2 Scatter plot and regression line of 54 arrhythmogenic right ventricular cardiomyopathy (ARVC) subjects examined with cardiac magnetic resonance imaging (MRI). The amount of physical activity in metabolic equivalents (METs) x min/week during a minimum of 6 years correlated with reduced right ventricular ejection fraction (RVEF) by MRI.

Figure 3 Kaplan Meier analysis of 110 arrhythmogenic right ventricular cardiomyopathy (ARVC) patients and their mutation-positive family members. Athletes had significantly worse outcome regarding cardiac transplantation (n=5) compared with non-athletes (Log rank, P < 0.001).

found in three index patients (PKP2, n=1; DSC2, n=1; DSP, n=1) fulfilling definite ARVC diagnosis. In 25 index patients, no pathogenic mutation was found and no genetic testing was performed in 10 index patients. All these 35 fulfilled 2010 TFC for definite ARVC diagnosis. Family members of mutation negative or not-tested index patients were not included. Two family members of the index patients with a VUS in DSP were included because of familial co-segregation of the ARVC phenotype and their ARVC symptoms.

Discussion
This study showed that ARVC patients with a history of athletic activity had reduced RV and LV function compared with ARVC
non-athletes. Athletes among the asymptomatic mutation-positive family members had lower LV function and more RV abnormalities compared with non-athletic family members. The results were consistent by several echocardiographic and MRI parameters. Athletic activity was related to worse outcome, with a higher frequency and earlier onset of VA and only athletes needed cardiac transplantation. These findings suggest that athletic activity accelerates and aggravates ARVC progression and outcome.

Impact of athletic activity on myocardial function in ARVC

Experimental studies have indicated physical activity in ARVC mice to be associated with worse outcome.6 Case reports and a recent study have shown that athletic activity was associated with worse arrhythmic outcome in ARVC patients.4,8 Our study adds to the current knowledge showing a relationship between amount and intensity of athletic activity and reduced biventricular function by echocardiography and MRI in a comprehensive study of ARVC patients and in mutation-positive family members. As expected, RV function was reduced in ARVC patients.13 Importantly, ARVC athletes had a consistently reduced RV function by several parameters, and a more dilated RV compared with ARVC non-athletes; this was also found in separate analyses of index patients only. The LV function was more reduced in ARVC athletes compared with non-athletes, as shown by echocardiographic and MRI studies. Interestingly, LV function was also slightly but significantly reduced in family members with a history of athletic activity compared with non-athlete family members.

Physiological cardiac changes caused by athletic activity include hypertrophy and dilatation of the LV, leading to larger LV volumes, larger stroke volume, and lower resting heart rate. Healthy athletes may therefore have LVEF in the lower normal range19 and our results could hypothetically be a result of physiological athletic changes. Indeed, cardiac volumes were increased in athletes compared with non-athletes. However, heart rate, cardiac output and BMI were not different. Furthermore, LVEF in ARVC index patients was reduced below values that could be considered as physiological in athletes. Reduced LV function in athletes below physiological values was further underscored by the finding of reduced LV global strain. LVEF and LV GLS is not expected to be lower in athletes,15–21 and even better LVEF and LV GLS has been reported in healthy athletes compared with healthy non-athletes.22

A slightly reduced RV function and increased RV diameters have been reported in healthy athletes,23–27 while others have reported normal RV function.19,28 In the present study, ARVC athletes had clearly dilated RV dimensions and severely reduced RV function.19,29 Combined, our findings showed reduced LV and RV function in ARVC athletes compared with ARVC non-athletes.

It has been previously proposed that extreme exercise can promote an ARVC phenotype in genotype negative individuals.25–27 Our ARVC mutation-positive subjects seemed to have more severe myocardial damage, despite lower levels of exercise compared with the mutation-negative athletes from the previous studies. These findings support the theory that higher levels of physical activity are necessary to impair myocardial function in athletes with normal or non-severely altered desmosomes. We speculate that while there may be a threshold value for exercise in desmosomal healthy individuals, causing the 'acquired ARVC', no threshold value may exist in desmosomal mutation carriers. We demonstrate a linear relationship between amount of physical activity and reduced LV and RV function in our study, indicating that myocardial damage relates to a continuum of physical activity. Therefore, there may be no threshold value for recommendations of physical activity in ARVC mutation-positive subjects to prevent negative effects on myocardial function.

Clinical implications

Athletes had reduced RV and LV function and only athletes had heart transplantation, indicating that athletic activity aggravates biventricular myocardial dysfunction. Furthermore, athletes were younger at the time of ARVC diagnosis and ICD implantation indicating that athletic activity accelerated the onset of life-threatening symptoms in ARVC. The present study underlines current guidelines that recommend patients with definite ARVC to refrain from competitive sports. However, current guidelines do not sufficiently address mutation-positive family members and sparse evidence has been available to justify restrictions in physical activity in this subjects. The benefit of physical activity on individual health is indisputable and unnecessary restrictions must be avoided. This study was not designed to fully answer whether ARVC mutation positive family members should be restricted in physical activity. Prospective follow-up studies are needed to further advise the family members.

Limitations

This study had a cross-sectional design; however, data on athletic activity was collected retrospectively. Future studies should follow ARVC patients with athletic activity prospectively over an appropriate period of time.

Athletes were more frequently male and more frequently index patients than non-athletes. We expected index patients to have more severe disease and therefore, every result was analysed separately in index patients only. Furthermore, all results were adjusted for gender in addition to age. The majority of RV and LV parameters remained decreased in index athletes only and results could therefore not be attributed to the higher proportion of index patients in athletes. Adjustment for gender and age did not change our results. Only a subset of subjects (n = 54) had an appropriate MRI study that may have influenced our results.

Conclusions

The ARVC subjects with a history of athletic activity showed reduced RV and LV function compared with non-athletes by echocardiography and by MRI. The LVEF was reduced in athletes, both in index patients and in asymptomatic mutation-positive family members. Higher levels of physical activity correlated with reduced LV and RV function, and only athletes had cardiac transplantation. Diagnosis of ARVC and onset of VA occurred at younger age in

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athletes compared with non-athletes. These findings indicate that athletic activity aggravates LV and RV myocardial function in ARVC patients and in mutation-positive family members and accelerate the onset of life-threatening symptoms.

Acknowledgements

We thank all patients and family members who participated in this study.

Funding

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Conflict of interest: A.G.H. is currently an employee at Novo Nordisk, Denmark. The other authors have no conflicts of interest to report.

References

Paper II

Saberniak J, Leren IS, Haland TF, Beitnes JO, Hopp E, Borgquist R, Edvardsen T, Haugaa KH

Comparison of patients with early-phase arrhythmogenic right ventricular cardiomyopathy and right ventricular outflow tract ventricular tachycardia

Comparison of patients with early-phase arrhythmogenic right ventricular cardiomyopathy and right ventricular outflow tract ventricular tachycardia

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Aims

Differentiation between early-phase arrhythmogenic right ventricular cardiomyopathy (ARVC) and right ventricular outflow tract (RVOT)-ventricular tachycardia (VT) can be challenging, and correct diagnosis is important. We compared electrocardiogram (ECG) parameters and morphological right ventricular (RV) abnormalities and investigated if ECG and cardiac imaging can help to discriminate early-phase ARVC from RVOT-VT patients.

Methods and results

We included 44 consecutive RVOT-VT (67 ± 14 years) and 121 ARVC patients (42 ± 17 years). Of the ARVC patients, 77 had definite ARVC and 44 had early-phase ARVC disease. All underwent clinical examination, ECG, and Holter monitoring. Frequency of premature ventricular complexes (PVC) was expressed as percent per total beats/24 h (%PVC), and PVC configuration was recorded. By echocardiography, we assessed indexed RV basal diameter (RVD), indexed RVOT diameter, and RV and left ventricular (LV) function. RV mechanical dispersion (RVMD), reflecting RV contraction heterogeneity, was assessed by speckle-tracking strain echocardiography. RV ejection fraction (RVEF) was assessed by cardiac magnetic resonance imaging (CMR). Patients with early-phase ARVC had lower %PVC by Holter and PVC more frequently originated from the RV lateral free wall (both P < 0.001). RVD was larger (21 ± 3 vs. 19 ± 2 mm, P < 0.01), RVMD was more pronounced (22 ± 15 vs. 15 ± 13 ms, P = 0.03), and RVEF by CMR was decreased (41 ± 8 vs. 49 ± 4%, P < 0.001) in early-phase ARVC vs. RVOT-VT patients.

Conclusion

Patients with early-phase ARVC had structural abnormalities with lower RVEF, increased RVD, and pronounced RVMD in addition to lower %PVC by Holter compared with RVOT-VT patients. These parameters can help correct diagnosis in patients with unclear phenotypes.

Keywords

ARVC • RVOT-VT • cardiac imaging • ventricular arrhythmias

Introduction

The right ventricular outflow tract (RVOT) is the most common site of origin for idiopathic ventricular tachycardias (VT) and frequent premature ventricular complexes (PVC) in patients with structurally normal hearts, known as RVOT-VT. RVOT-VT is supposed to be a relatively benign condition,1 with generally well-tolerated ventricular arrhythmias. However, the RVOT area may also be origin of VT in patients with arrhythmogenic right ventricular cardiomyopathy (ARVC),2 an inherited cardiomyopathy predisposing to ventricular
arrhythmias, biventricular dysfunction, and sudden cardiac death and therefore a far from benign condition. Regardless of the underlying process, VT from the RVOT area with a left bundle branch block and inferior axis is common in both conditions, and to distinguish between idiopathic RVOT-VT and early stages of ARVC is challenging. The treatment and prognosis, however, differ substantially, and an incorrect diagnosis may be devastating. Although patients with RVOT-VT commonly have structurally normal ventricles, frequent PVC may cause myocardial remodelling with subsequent reduced function and dilation. These secondary and often reversible structural changes in patients with RVOT-VT further complicate the discrimination to early-phase ARVC, but a comprehensive comparison between these entities has not been previously performed. Furthermore, patients in the early stages of ARVC often have only subtle or even non-detectable structural changes, despite the risk of arrhythmias. Risk of arrhythmias in overt ARVC is reported to originate from fibro-fatty replacement of myocardium forming the substrate for VT. In early phases of ARVC, other mechanisms may be involved including disruptive electrical conduction in addition to assumed early fibrosis. In contrast, monomorphic origin of PVC and VT in RVOT-VT is thought to be a result of triggered activity.

Diagnostic accuracy in ARVC was improved by the ARVC 2010 Task Force Criteria (TFC), classifying ARVC diagnosis in possible, borderline, and definite ARVC and including the echocardiographic parameters right ventricular (RV) morphology and function, RVOT diameter, and RV fractional area change. The discrimination of RVOT from RVOT-VT is particularly challenging in the early phases of ARVC patients. We aimed to compare findings from echocardiogram (ECG) and morphological RV abnormalities including new echocardiographic strain parameters, to distinguish patients with early-phase ARVC from RVOT-VT. We hypothesized that sensitive imaging parameters can detect subtle myocardial changes in early phases of ARVC that are relevant to distinguish early-phase ARVC from RVOT-VT.

Methods

Study patients

In this cross-sectional study, consecutive RVOT-VT patients were recruited from Oslo University Hospital, Norway. The diagnosis of RVOT-VT was based on a clinical phenotype with monomorphic VT or monomorphic PVC originating from the RVOT region with documented left bundle branch block and inferior axis. Furthermore, a diagnosis of RVOT-VT required maximal exercise test negative for ischemia or coronary angiography without significant stenosis and no mutations in ARVC associated genes identified by genetic testing.

Consecutive ARVC patients were recruited from Oslo University Hospital, Norway (n = 105) and Lund University Hospital, Sweden (n = 16). A subset of these patients (n = 108) have been previously reported. The 2010 TFC were used for diagnosis and classification of ARVC disease.

All patients underwent clinical examination, and we recorded medical history, use of antiarrhythmic medications, and occurrence of ventricular arrhythmias. Ventricular arrhythmias were defined as syncpe with assumed arrhythmic origin, documented non-sustained/sustained VT, and aborted cardiac arrests. Treatments such as radio frequency ablation, implantable cardioverter defibrillator implantation, and cardiac transplantation were recorded.

Written informed consent was given by all study participants. The study complied with the Declaration of Helsinki and was approved by the Regional Committees for Medical Research Ethics in Sweden and Norway.

Twelve-lead electrocardiogram, signal-averaged ECG, and 24-h Holter monitoring

Twelve-lead ECG was obtained, and the configuration of PVC was analyzed according to their likely site of origin as described previously by Zhang et al. Signal-averaged ECG was performed using a MAC® 5000-analysing system (GE Medical Systems, Milwaukee, WI, USA). Time domain analysis was obtained in the band-pass filter 40–250 Hz. Low potentials were considered present if ≥1 of the following parameters were abnormal: total filtered QRS duration ≥114 ms, the low amplitude (<40 μV) late signal duration ≥38 ms, and the last (40 ms) QRS root-mean-square voltage <0.2 mV. Twenty-four-hour Holter monitoring was performed using Schiller medilog®AR4 and AR4+ to assess VT and frequency of PVC in percent of total heart beats in 24 h (%PVC).

Echocardiographic studies

Patients underwent echocardiographic examination (Vivid 7 or Vivid E9, GE Vingmed, Horten, Norway). Data were digitally stored for off-line analysis (EchoPac®, GE Vingmed). Echocardiographic analyses were performed blinded to clinical data. We assessed RVOT diameter from the parasternal short axis and RV basal diameter (RVD) (both indexed by body surface area), RV fractional area change (RV FAC), and tricuspid annular plane systolic excursion (TAPSE) from the four-chamber view. Strain analyses were performed by 2D speckle-tracking echocardiography. RV strain by speckle-tracking technique was traced from the four-chamber view focusing on the right ventricle. Peak negative longitudinal strains from the RV free wall segments were averaged as RV strain (Figure 1). Time-to-peak longitudinal strain was defined as the time from onset of Q/R on ECG to peak negative longitudinal RV strain. RV mechanical dispersion was defined as the standard deviation of time-to-peak strain from RV free wall segments. From the left ventricle (LV), we assessed end-diastolic diameter (LVEDD), end-diastolic volume and end-systolic volume (all indexed by body surface area), ejection fraction (LVEF) by the modified Simpson’s biplane method, and cardiac output. LV strain was traced from the three apical views. LV global longitudinal strain (LVGLS) and LV mechanical dispersion were calculated similarly to RV from 16 LV segments. Echocardiographic parameters were considered abnormal according to current guidelines.

Cardiac magnetic resonance imaging

Cardiac magnetic resonance imaging (CMR) was performed in a subset of patients (n = 80), in a 1.5 Tesla unit (Magnetom Sonata, Vision Plus or Avanto Siemens, Erlangen, Germany) using a phased array body coil. The RV free wall was imaged by axial and sagittal breath-hold T1-weighted turbo spin echo technique. Standard long- and short-axis breath-hold cine sequences (fast imaging with steady-state free precession, trueFISP) were obtained to cover both ventricles. RV and LV volumes and ejection fractions were calculated by summation of the luminal areas of the short-axis images at end-diastole and end-systole, including the RV and LV outflow tracts, but excluding the papillary muscles. Post-processing analyses were performed using Qmass® MR 6.1.6 software (Medis Medical Imaging Systems, Leiden, the
Figure 1. Echocardiographic longitudinal strain curves from an RVOT-VT patient, an early-phase ARVC patient, and an ARVC patient with overt disease. Upper panels: RV free wall longitudinal strain curves from the apical RV-focused four-chamber view in patients with RVOT-VT (left panel), early-phase ARVC (mid-panel), and overt ARVC (right panel). Vertical white arrow indicates the amplitudes of RV strain curves, which were calculated as the average peak negative longitudinal strain in three RV free wall segments. Horizontal white arrows indicate time-to-peak strain, defined as time from onset of Q/R on the ECG to peak negative longitudinal strain. The standard deviation of time-to-peak strain in the same three RV segments was defined as RV mechanical dispersion, reflecting contraction inhomogeneity. Patients with early-phase ARVC (mid-panel) showed more pronounced RV mechanical dispersion compared with RVOT-VT patients (left panel). Lower panels: Measures of RV basal diameters in these patients. Patients with early-phase ARVC (mid-panel) had larger diameters compared with patients with RVOT-VT (left panel). ARVC, arrhythmogenic right ventricular cardiomyopathy; AVC, aortic valve closure; RV, right ventricle; RVD, RV basal diameter; RVOT, right ventricular outflow tract; VT, ventricular tachycardia.

Netherlands). Inter- and intraobserver analyses for RVEF were performed in 10 patients.

Genetic analyses
Genomic DNA was isolated from peripheral blood from a subset of RVOT-VT patients and from ARVC index patients and their family members. The individual exons with flanking intron sequences of the genes plakophilin-2 (PKP2), desmocollin-2 (DSP2), desmoglein-2 (DSG2), desmoplakin (DSP), and 29 of the 105 exons of the ryanodine receptor-2 (RYR2) gene were sequenced by polymerase chain reaction amplification in combination with direct sequencing. Index patients with confirmed pathogenic mutations in these genes were defined as ARVC mutation positive, and mutation-positive family members were included by cascade genetic screening.

Statistical analyses
Data were presented as mean ± standard deviation. Differences between groups were assessed by χ² test and Fisher’s exact test for categorical variables and unpaired Student’s t-test for continuous variables (SPSS 20.0, Inc., Chicago, IL). Logistic regression was used to find predictors of early-phase ARVC. Parameters with p-values < 0.05 from univariable analyses were included in multivariable analyses. C-statistics were calculated by receiver operating characteristic (ROC) curves to assess the parameters’ ability to differentiate between early-phase ARVC and RVOT-VT patients. The value closest to the upper left corner of the ROC curve was defined as giving optimal sensitivity and specificity. Two-sided p-values < 0.05 were considered significant.

Results

Clinical characteristics
In all, 177 patients were screened for participation in the study, 56 patients with suspected RVOT-VT and 121 ARVC subjects. Patients with suspected RVOT-VT were referred to our hospital for clinical workup and eventual radio frequency catheter ablation. Clinical workup showed that 12 (21%) of these patients had a different etiology of ventricular arrhythmias, 7 had definite ARVC by 2010 TFC, 3 had LVOT-VT, 1 had ischaemic heart disease, and 1 had dilated
cardiomyopathy, and they were excluded from the RVOT-VT group. The RVOT-VT population therefore included 44 patients (Table 1). Of these, 26 (59%) were genetically tested for ARVC-related mutations with negative results.

We included 121 ARVC patients, 73 (60%) were index patients, and 48 (40%) were mutation-positive family members. Definite ARVC was diagnosed in 77 (64%), and 44 (36%) had early-phase ARVC.

Comparison between RVOT-VT patients and the total ARVC population

RVOT-VT patients were more frequently female compared with the total ARVC population ($P < 0.001$) (Table 1). Ventricular arrhythmias occurred in 29 (69%) RVOT-VT and in 74 (62%) ARVC patients ($P = 0.43$) (Table 1). Aborted cardiac arrest occurred in 13 patients, all of whom had ARVC. RVOT-VT patients had less frequently sustained VT ($P < 0.001$), but more frequently non-sustained VT ($P < 0.001$) and more frequent PVC during Holter monitoring ($P < 0.001$) compared with ARVC patients (Table 1). Parameters obtained by signal-averaged ECG were less pathological in RVOT-VT patients compared to ARVC (all $P < 0.05$).

Imaging results

By echocardiography, 44 (100%) RVOT-VT and 119 (98%) ARVC patients could be evaluated. A subset of 23 (52%) RVOT-VT patients and 57 (47%) ARVC patients had assessable CMR examinations ($P = 0.39$).

All RV diameters were within normal range in the RVOT-VT patients compared with increased RV diameters in the total ARVC population (all $P < 0.05$) (Table 2). Furthermore, RV function was decreased both by echocardiography and by CMR, and RV mechanical dispersion was pronounced in ARVC patients (all $P < 0.001$), whereas RV function was in the lower normal range in RVOT-VT patients (Tables 2 and 3). LV function was reduced by echocardiographic LVEF and LVGLS, and LV mechanical dispersion was more pronounced in ARVC compared with RVOT-VT (Table 2).

Intra- and interobserver intraclass correlation for RVEF by CMR was 0.96 (95% CI 0.86–0.99) and 0.92 (95% CI 0.66–0.98), respectively.

Comparison between patients with RVOT-VT and with early-phase ARVC

Of the 44 early-phase ARVC patients, 35 (80%) were mutation-positive family members and 2 (4%) were mutation-positive index patients. The remaining 7 (16%) were index patients with borderline (n = 6) and possible (n = 1) ARVC. These 7 patients had experienced ventricular arrhythmias suggestive of ARVC, in addition to having pathologic signal-averaged ECG (n = 5), RV dysfunction or fatty infiltration by CMR (n = 4), and dilated RVD (n = 4); hence, their phenotypes were considered clinically suggestive of early-phase ARVC.

Early-phase ARVC patients were younger than the RVOT-VT patients at diagnosis ($P = 0.01$) and had less frequently ventricular arrhythmias and PVC (Table 1). Lower %PVC by Holter was a strong and independent marker of early-phase ARVC (Table 1) [adjusted odds ratio (OR) 0.77 (95% CI 0.64–0.92) P < 0.01]. A %PVC of <2 discriminated optimally between early-phase ARVC and RVOT-VT with an AUC of 0.93 (95% CI 0.86–1.00).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical characteristics in 44 RVOT-VT and 121 ARVC patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>RVOT-VT</td>
<td>Total ARVC</td>
</tr>
<tr>
<td>(n = 44)</td>
<td>(n = 121)</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>47 ± 14</td>
</tr>
<tr>
<td>Male gender (n)</td>
<td>13 (30%)</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>66 ± 13</td>
</tr>
<tr>
<td>Syncope (n)</td>
<td>25 (61%)</td>
</tr>
<tr>
<td>Ventricular arrhythmia (n) (TFC)</td>
<td>29 (69%)</td>
</tr>
<tr>
<td>Sustained VT (n)</td>
<td>5 (14%)</td>
</tr>
<tr>
<td>Non-sustained VT (n)</td>
<td>24 (67%)</td>
</tr>
<tr>
<td>ACA (n)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>RFA (n)</td>
<td>22 (50%)</td>
</tr>
<tr>
<td>ICD (n)</td>
<td>4 (9%)</td>
</tr>
<tr>
<td>SAECG ≥1 pathological value (TFC)</td>
<td>16 (46%)</td>
</tr>
<tr>
<td>%PVC by Holter</td>
<td>186 ± 153</td>
</tr>
<tr>
<td>Beta-blocker (n)</td>
<td>42 (98%)</td>
</tr>
<tr>
<td>Amiodarone (n)</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>ARVC-related mutation (n) (TFC)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Mean ± SD. P by Student’s t-test.

ACA, aborted cardiac arrest; ARVC, arrhythmogenic right ventricular cardiomyopathy; BSA, body surface area; ICD, implantable cardioverter defibrillator; PVC, premature ventricular complexes; RFA, radio frequency ablation; RV, right ventricle; RVOT, right ventricular outflow tract; SAECG, signal-averaged electrocardiogram; TFC, ARVC 2010 Task Force Criteria; VT, ventricular tachycardia.
Comparison of early-phase ARVC and RVOT-VT

Table 2  Echocardiographic parameters in 44 RVOT-VT and 119 ARVC patients

<table>
<thead>
<tr>
<th></th>
<th>RVOT-VT (n = 44)</th>
<th>Total ARVC (n = 119)</th>
<th>P-value vs. RVOT-VT</th>
<th>Early-phase ARVC (n = 43)</th>
<th>P-value vs. RVOT-VT</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV wall motion abnormality (TFC)</td>
<td>4 (9%)</td>
<td>50 (42%)</td>
<td>&lt;0.001</td>
<td>3 (7%)</td>
<td>1.00</td>
</tr>
<tr>
<td>RVOT diameter (mm/m²) (TFC)</td>
<td>17 ± 3</td>
<td>19 ± 4</td>
<td>&lt;0.01</td>
<td>18 ± 3</td>
<td>0.17</td>
</tr>
<tr>
<td>RVD (mm/m²)</td>
<td>19 ± 2</td>
<td>22 ± 4</td>
<td>&lt;0.001</td>
<td>21 ± 3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RV FAC (%) (TFC)</td>
<td>46 ± 5</td>
<td>38 ± 11</td>
<td>&lt;0.001</td>
<td>46 ± 7</td>
<td>0.96</td>
</tr>
<tr>
<td>TAPSE (nm)</td>
<td>22 ± 4</td>
<td>18 ± 5</td>
<td>&lt;0.001</td>
<td>20 ± 4</td>
<td>0.09</td>
</tr>
<tr>
<td>RV strain (%)</td>
<td>-27.1 ± 5.0</td>
<td>-22.7 ± 7.3</td>
<td>&lt;0.001</td>
<td>-26.4 ± 5.3</td>
<td>0.53</td>
</tr>
<tr>
<td>RVMD (ms)</td>
<td>15 ± 11</td>
<td>32 ± 29</td>
<td>&lt;0.001</td>
<td>22 ± 15</td>
<td>0.03</td>
</tr>
<tr>
<td>LV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>4.4 ± 12</td>
<td>3.8 ± 12</td>
<td>&lt;0.01</td>
<td>4.2 ± 1.4</td>
<td>0.52</td>
</tr>
<tr>
<td>LVEDD (mm/m²)</td>
<td>27 ± 3</td>
<td>27 ± 3</td>
<td>0.44</td>
<td>27 ± 4</td>
<td>0.97</td>
</tr>
<tr>
<td>LVEDV (mL/m²)</td>
<td>61 ± 11</td>
<td>58 ± 17</td>
<td>0.29</td>
<td>58 ± 17</td>
<td>0.10</td>
</tr>
<tr>
<td>LVESV (mL/m²)</td>
<td>28 ± 7</td>
<td>27 ± 14</td>
<td>0.61</td>
<td>25 ± 6</td>
<td>0.53</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>57 ± 5</td>
<td>54 ± 8</td>
<td>0.03</td>
<td>58 ± 4</td>
<td>0.85</td>
</tr>
<tr>
<td>LVGDS (%)</td>
<td>-21.3 ± 2.7</td>
<td>-18.8 ± 3.7</td>
<td>&lt;0.001</td>
<td>-19.9 ± 2.5</td>
<td>0.02</td>
</tr>
<tr>
<td>LVMD (ms)</td>
<td>39 ± 12</td>
<td>54 ± 27</td>
<td>&lt;0.01</td>
<td>40 ± 11</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Mean ± SD, P by Student’s t-test.
ARVC, arrhythmogenic right ventricular cardiomyopathy; LV, left ventricle; LVEDD, indexed left ventricular end-diastolic diameter; LVEDV, indexed left ventricular end-diastolic volume; LVGDS, left ventricular global longitudinal strain; LVESV, indexed left ventricular end-systolic volume; LVMD, left ventricular mechanical dispersion; RV, right ventricle; RVD, indexed right ventricular basal diameter; RVFAC, right ventricular fractional area change; RVMD, RV mechanical dispersion; RVOT, indexed right ventricular outflow tract; TAPSE, tricuspid annular plane systolic excursion; TFC, ARVC 2010 Task Force Criteria.

Table 3  CMR parameters in 23 RVOT-VT and 57 ARVC patients

<table>
<thead>
<tr>
<th>Clinical categories</th>
<th>RVOT-VT (n = 23)</th>
<th>ARVC total (n = 57)</th>
<th>P-value vs. RVOT-VT</th>
<th>Early-phase ARVC (n = 19)</th>
<th>P-value vs. RVOT-VT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender (n)</td>
<td>8 (35%)</td>
<td>32 (56%)</td>
<td>0.08</td>
<td>10 (53%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>11 ± 11</td>
<td>12 ± 16</td>
<td>0.76</td>
<td>36 ± 17</td>
<td>0.11</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>0.65</td>
<td>1.9 ± 0.2</td>
<td>0.94</td>
</tr>
<tr>
<td>RVEDV (mL/m²) (TFC)</td>
<td>75 ± 17</td>
<td>88 ± 36</td>
<td>0.09</td>
<td>69 ± 19</td>
<td>0.36</td>
</tr>
<tr>
<td>RVESV (mL/m²)</td>
<td>38 ± 9</td>
<td>56 ± 33</td>
<td>&lt;0.001</td>
<td>40 ± 12</td>
<td>0.43</td>
</tr>
<tr>
<td>RVEF (%)</td>
<td>49 ± 4</td>
<td>39 ± 12</td>
<td>&lt;0.001</td>
<td>41 ± 8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVEDV (mL/m²)</td>
<td>74 ± 21</td>
<td>74 ± 18</td>
<td>0.90</td>
<td>69 ± 17</td>
<td>0.51</td>
</tr>
<tr>
<td>LVESV (mL/m²)</td>
<td>35 ± 14</td>
<td>37 ± 13</td>
<td>0.59</td>
<td>34 ± 10</td>
<td>0.78</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>53 ± 6</td>
<td>51 ± 8</td>
<td>0.21</td>
<td>52 ± 7</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Mean ± SD, P by Student’s t-test.
ARVC, arrhythmogenic right ventricular cardiomyopathy; BSA, body surface area; CMR, cardiac magnetic resonance imaging; LVEDV, indexed left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; RVESV, indexed left ventricular end-systolic volume; RVEF, right ventricular ejection fraction; RVEDV, indexed right ventricular end-diastolic volume; RVOT, right ventricular outflow tract; TFC, ARVC 2010 Task Force Criteria.

by C-statistics. ECG morphologies of the PVC were available in 43 RVOT-VT patients and in 9 patients with early-phase ARVC (Table 4). In patients with RVOT-VT, site of origin of PVC was mainly the septal part of the RVOT (98%), while only 22% of patients with early-phase ARVC had this origin (P < 0.001). In early-phase ARVC, 56% had origin of PVC in the RV free wall and 22% had PVC origin in the LV.

RVD, RV mechanical dispersion, LVGDS, and RVEF by CMR were markers of early-phase ARVC (all P < 0.05 compared with RVOT-VT, Tables 2 and 3). In multivariable analysis, RVD and RV mechanical dispersion were the only independent imaging predictors of early-phase ARVC (Table 5). RVEF < 43%, RVD > 21 mm,, and RV mechanical dispersion > 22 ms had optimal ability to discriminate between RVOT-VT and ARVC (Figure 2).
Discussion

This study presents a new approach to compare early-phase ARVC from RVOT-VT patients. Early-phase ARVC patients had less PVC, slightly enlarged RV diameter, more dispersed RV contraction, and reduced RV function by CMR compared with RVOT-VT patients. These findings indicate that myocardial function is affected also in early-phase ARVC disease and that analyses of both structural and functional changes can help to discriminate early-phase ARVC from RVOT-VT. In patients with an unclear phenotype, the number of PVC and a few imaging parameters may help discrimination between early-phase ARVC and RVOT-VT patients.

Clinical characteristics and ventricular arrhythmias

Current workup in ARVC and RVOT-VT patients includes clinical, electrocardiographic (ECG), echocardiographic, and CMR studies, and genetic analyses may be indicated in suspected ARVC cases. The differentiation between ARVC and RVOT-VT is obvious when the ARVC phenotype is fully expressed. As expected, our total ARVC population had more severe disease, indicated by more frequent aborted cardiac arrest and clearly pathological structural and functional cardiac changes. However, differentiation between RVOT-VT and early-phase ARVC remains challenging. Interestingly, one-fifth of the referred RVOT-VT patients screened for participation in our study were excluded due to other etiology of their arrhythmias, and more than half of these fulfilled the 2010 TFC for definite ARVC.

Early-phase ARVC patients were younger than the RVOT-VT patients, a result of the early detection of ARVC mutation positive by family screening. Furthermore, early-phase ARVC patients had less frequent PVC and arrhythmias, with <2% PVC in early-phase ARVC compared with 18% PVC in RVOT-VT patients and less frequent nVT. The relatively low number of PVC in early-phase ARVC and the frequent origin of PVC in the lateral free wall have also been reported by others and emphasize that RVOT-VT is most likely in patients with very frequent monomorphic PVC originating from the septal part of the RVOT. Importantly, severe arrhythmias were more prevalent in ARVC compared with RVOT-VT and all aborted cardiac arrests occurred only in ARVC patients, highlighting the different prognosis of these conditions.

Structural and functional findings

All RV imaging parameters used in the ARVC 2010 TFC were pathological in ARVC patients compared with low normal values in RVOT-VT patients. However, only RV diameter and RV mechanical dispersion were independent markers of early-phase ARVC disease. RV mechanical dispersion has previously been reported as a marker of arrhythmias in ARVC, and this study showed that mechanical dispersion can also distinguish between early-phase ARVC and RVOT-VT. Mechanical dispersion may have the ability to detect subtle changes in contraction and might be explained by diffuse cell necrosis causing electrical and myocardial remodeling in ARVC in contrast to RVOT-VT.

LV function by global longitudinal strain was reduced in early-phase ARVC compared with RVOT-VT patients, indicating subtle biventricular affection also in early stages of ARVC.

In this study, RVFE by CMR was the best imaging parameter to discriminate between early-phase ARVC and RVOT-VT. However, RVFE is included in the ARVC 2010 TFC and was used as a primary parameter to differentiate between RVOT-VT and ARVC patients, and the impact of RVFE may therefore be underestimated. Some previous studies have suggested that the majority of RVOT-VT patients have structurally normal hearts by CMR, while others found subtle RV abnormalities by CMR which were associated with worse outcome. However, evaluation of a patient’s diagnosis should not rely on one single parameter, and we propose the combined use of CMR and echocardiographic parameters to discriminate early-phase ARVC from RVOT-VT. The echocardiographic parameters can be of help to select patients for further CMR studies and in centers where CMR is not widely available.

Clinical implications

One-fifth of referred RVOT-VT patients were re-diagnosed, and more than half of these had definite ARVC reflecting that discrimination between these diseases is challenging. Our findings indicate that patients with no structural abnormalities and very frequent
PVC with appropriate morphology were more likely to have RVOT-VT than early-phase ARVC. Patients with a moderate number of PVC (in our study <2%/PVC/24 h) and suspected RVOT-VT should be evaluated by the ARVC 2010 TFC to detect those fulfilling definite ARVC criteria to avoid overlooking the more severe ARVC disease. The majority of our patients with early-phase ARVC were genetic positive, and therefore we had a genetic gold standard for our diagnosis. However, 50% of ARVC patients in general are reported to be genetic negative or with variants of uncertain significance. In these patients and in patients with unclear phenotypes, the number of PVC and a few CMR and echocardiographic parameters may help the discrimination between early-phase ARVC and RVOT-VT patients.

**Limitations**
This study had a cross-sectional design, and future studies should follow both ARVC and RVOT-VT patients for a longer period of time to explore overlap between the diagnoses and deterioration in cardiac function. Although we excluded all patients with definite ARVC criteria from our RVOT-VT population, we cannot exclude that patients diagnosed with RVOT-VT in our study will develop criteria for ARVC in future. Only a subset of patients (n = 80, 48%) had an appropriate CMR study, which may have influenced our results. However, equal proportions of early-phase ARVC and RVOT-VT patients had CMR studies with no significant differences in characteristics (Table 3).

**Conclusions**
As expected, the total ARVC population had more severe structural pathology compared with RVOT-VT patients. In early-phase ARVC, %PVC was the most powerful marker to distinguish ARVC from RVOT-VT, and >2%/PVC per 24 h was suggestive of RVOT-VT diagnosis. By imaging, early-phase ARVC patients had slightly lower

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**Figure 2** ROC curves for the ability of parameters to discriminate between 44 patients with early-phase ARVC and 44 RVOT-VT patients. RVEF by CMR and RVD and RV mechanical dispersion by echocardiography had good ability to discriminate early-phase ARVC from RVOT-VT patients. ARVC, arrhythmogenic right ventricular cardiomyopathy; AUC, area under the curve; CI, confidence interval; CMR, cardiac magnetic resonance imaging; RVD, indexed right ventricular basal diameter; RVEF, right ventricular ejection fraction; RVMD, right ventricular mechanical dispersion.
RV function, more pronounced RV mechanical dispersion, and larger RV diameter compared with RVOT-VT patients. Careful cardiac imaging may help to correctly diagnose early-phase ARVC and RVOT-VT patients and aid treatment decisions.

Funding

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Conflict of interest: none declared.

References

Paper III

Leren IS, Saberniak J, Haland TF, Edvardsen T, Haugaa KH

Echocardiography Combined with ECGs Improve Identification of Arrhythmic Events in Early ARVC

Combination of ECG and Echocardiography for Identification of Arrhythmic Events in Early ARVC

Ida S. Leren, MD, PhD, Jørg Sabornisk, MD, PhD, Trine F. Haland, MD, PhD, Thor Edvardsen, MD, PhD, Kristina H. Haugaa, MD, PhD

Abstract

OBJECTIVES The aim of this study was to investigate early markers of arrhythmic events (AEs) and improve risk stratification in early arrhythmogenic right ventricular cardiomyopathy (ARVC).

BACKGROUND AEs are frequent in patients with ARVC, but risk stratification in subjects with early ARVC is challenging.

METHODS Early ARVC disease was defined as possible or borderline ARVC diagnosis according to the ARVC Task Force Criteria 2010. We performed resting and signal averaged electrocardiogram (ECG). Using echocardiography, we assessed right ventricular (RV) outflow tract diameter and right ventricular basal diameter (RV diameter). Global longitudinal strain and mechanical dispersion (MD) from strain echocardiography were assessed in both the right and left ventricle. AEs were defined as documented ventricular tachycardia, cardiac syncope, or aborted cardiac arrest.

RESULTS Of 162 included subjects with ARVC (41 ± 16 years of age, 47% female), 73 had early ARVC, including mutation positive family members not fulfilling definite ARVC diagnosis. AEs occurred in 15 (21%) subjects with early ARVC. Those with AEs in early disease had larger RV diameter (40 ± 4 mm vs. 37 ± 5 mm), more pronounced RVMD (39 ± 15 ms vs. 26 ± 11 ms), and more pathological signal averaged ECGs compared with those without AEs (all p < 0.05). Adding measurements of RV diameter and RVMD to electrical parameters improved identification of subjects with AEs compared with electrical parameters alone (p = 0.05).

CONCLUSIONS ECG parameters, RV diameter, and RVMD were markers of previous arrhythmic events in patients with early ARVC. A combination of electrical and echocardiographic parameters improved identification of subjects with AEs in early ARVC disease. (J Am Coll Cardiol Img 2016;■:■:■) © 2016 by the American College of Cardiology Foundation. Published by Elsevier. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Arrhythogenic right ventricular cardiomyopathy (ARVC) is an inheritable cardiomyopathy that predisposes to life-threatening arrhythmias and heart failure, and is one of the leading causes of sudden cardiac death (SCD) in young individuals (1). Ventricular arrhythmias are frequent in patients with ARVC and, importantly, arrhythmias may occur also in early stages of disease, making risk stratification challenging (2). Furthermore, the patients with ARVC seen in cardiological clinics have changed over the past decade. Fifteen years ago, patients were typically diagnosed with overt ARVC after they had survived a life-threatening arrhythmic event. Genetic family screening now identifies individuals at risk of developing ARVC before onset of symptoms of disease. Hence risk

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stratification has moved from assessing obvious myocardial pathology to predicting the transition from asymptomatic, mutation positive ARVC to electrical disease with potential life-threatening ventricular arrhythmias (3). Of importance, outcome is favorable in patients detected early and treated according to guidelines (4). Tools are needed to detect early disease and to optimize medication and timing of implantation of a cardioverter defibrillator (ICD) to prevent SCD becoming the first symptom of ARVC.

The Task Force Criteria revised in 2010 (TFC 2010) improved sensitivity and specificity of ARVC diagnosis (5). Newer echocardiographic techniques, such as strain echocardiography, have shown promising results in ARVC risk stratification in previous studies (6,7). We aimed to explore early markers of ARVC disease and their association with previous ventricular arrhythmias. We hypothesized that a combination of electrocardiographic parameters and imaging parameters, including strain echocardiography, may improve risk stratification of arrhythmic events in subjects with ARVC.

METHODS

STUDY POPULATION. In this cross-sectional study, consecutive patients referred to the Department of Cardiology, Oslo University Hospital, Rikshospitalet, between 2008 and 2014 with suspected ARVC were evaluated for inclusion. Probands were genetically screened and family members of probands with disease-causing mutations were tested and included if mutation-positive. All patients fulfilling borderline, possible, or definite ARVC diagnosis by the TFC 2010 (5) at their visit were invited to participate. No patient refused consent to participate. Exclusion criteria were significant ischemic heart disease or coexisting heart disease of other origin. All participants underwent clinical examination. Arrhythmic events were reviewed retrospectively at inclusion and defined as documented nonsustained or sustained ventricular tachycardia (VT) by Holter monitoring, exercise test or ICD recordings, syncope of suspected cardiac origin, or aborted cardiac arrest. Medication use, ICD presence, and eventual prior ICD therapies at the time of the echocardiographic examination were recorded.

ELECTROCARDIOGRAPHY. Twelve-lead electrocardiogram (ECG) was obtained at the time of the echocardiographic examination. Signal-averaged ECGs were performed (8) and patients with complete bundle branch block were excluded from signal-averaged ECGs analyses (5). S-wave upstroke was measured as the time from the nadir of the S-wave to the isoelectric line (9).

ECHOCARDIOGRAPHIC STUDIES. Two-dimensional echocardiographic studies were performed on a Vivid 7 or 9 (GE Healthcare, Horten, Norway) and data were analyzed with Echopac version 112 (GE Healthcare). Data analyses were performed blinded to patient clinical data.

From 2-dimensional echocardiography, we assessed proximal right ventricular outflow tract (RVOT) diameter in the parasternal short-axis view. We assessed right ventricular basal diameter (RV diameter) and right ventricular fractional area change (RV FAC) from the 4-chamber view (10). Left ventricular (LV) end-diastolic volume, LV end-systolic volume, and left ventricular ejection fraction (LVEF) were calculated by the modified Simpson biplane method.

We assessed longitudinal strains by speckle tracking technique from the 3 apical views for LV global longitudinal strain (11) and from the 4-chamber view for right ventricular (RV) global longitudinal strain, all at frame rates >50/s. The endocardial border was traced in each view. The operator manually adjusted segments that failed to track; segments that subsequently failed to track were excluded. LV global longitudinal strain was defined as the average of peak negative longitudinal strain from a 16-segment LV model (11). LV mechanical dispersion was defined as the standard deviation of time from Q/R on the ECG to peak negative longitudinal strain in the same 16 segments. Peak negative longitudinal strain from 6 RV segments was averaged as a measure of RV function (RV global longitudinal strain) (6). RV mechanical dispersion was calculated as the SD of time from Q/R on ECG to peak negative longitudinal strain from 6 RV segments (Figure 1).

CARDIAC MAGNETIC RESONANCE. Cardiac magnetic resonance was performed as previously described (12) and parameters for TFC 2010 were analyzed (5).

STAGING OF ARVC DISEASE. To assess the stage of ARVC disease (asymmetric or early), we used the TFC 2010 criteria (5). Patients who fulfilled definite ARVC diagnosis (>2 major or 4 minor criteria, or 1 major and 2 minor criteria) were defined as overt ARVC. Subjects fulfilling borderline ARVC (1 major + 1 minor or 3 minor criteria) and possible ARVC (1 major or 2 minor criteria) were defined as early ARVC.

GENETIC ANALYSES. Genetic testing was performed as previously described (12) as part of the diagnostic workup in subjects with suspected ARVC. Cascade genetic screening was performed in family members.
of mutation-positive index patients. Only family members of ARVC patients with confirmed pathogenic mutations were included.

**Statistical Analyses.** Comparisons of proportions were performed by the chi-square test or Fisher exact test when appropriate. Continuous data were presented as mean ± SD and compared by unpaired Student t test (SPSS version 21.0, SPSS Inc., Chicago, Illinois). Multivariable logistic regression analyses were used to adjust for beta-blocker use, age, sex, and mutation status in the total ARVC population. Overfitting was tested with Akaike information criterion (AIC).

Correlations between continuous parameters were assessed by linear regression. The C-statistic was calculated by receiver operating characteristic (ROC) curves. The value closest to the upper left corner of the ROC curve was defined as giving optimal sensitivity and specificity. Comparisons of C-statistics were performed with the software Analyze-it (Analyse-it Software, Ltd., Leeds, United Kingdom). We used likelihood ratios test to evaluate if echocardiographic parameters could improve the model for identifying subjects with arrhythmic events compared with electrical parameters only. Two sided p values ≤0.05 were considered statistically significant. Intraobserver and interobserver variability analyses were performed in 10 random study patients and expressed by intraclass correlation values.

All participants gave written informed consent. The study complied with the Declaration of Helsinki and was approved by the Regional Committees for Medical Research Ethics.

**RESULTS**

We included 162 subjects with ARVC, including 86 (53%) index patients and 76 (47%) mutation-positive family members (41 ± 16 years of age, 45% female) (Table 1). A definite diagnosis of ARVC was present in 80 (50%) subjects, defined as overt ARVC, while 31 (19%) had borderline and 42 (26%) had possible ARVC diagnosis (S), defined as early ARVC disease (n = 73, 45%). Of subjects with early ARVC, 58 (79%) were mutation-positive family members and 15 (21%) had clinically suspected ARVC in addition to fulfilling possible or borderline ARVC diagnostic criteria.

Arrhythmic events occurred in 84 (52%) subjects in total; 69 (78%) with overt and 15 (21%) with early ARVC. Median time from first arrhythmic event to echocardiography was 3.2 months (interquartile range: 0.1 months, 5.9 years). At time of echocardiography, 57 (35%) patients were treated with beta blockers, 10 (6%) with amiodarone, and 1 (1%) with flecainide. Also, 50 (37%) patients had or were implanted with an ICD at the time of study inclusion, including 4 subjects with early ARVC. Appropriate
TABLE 1 Clinical Characteristics in 162 Subjects With ARVC

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Subjects With ARVC (n = 162)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>41 ± 16</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.5 ± 4.5</td>
</tr>
<tr>
<td>Cardiac arrest</td>
<td>17 (10)</td>
</tr>
<tr>
<td>Definite/borderline/possible</td>
<td>89/31/42</td>
</tr>
<tr>
<td>Female</td>
<td>76 (47)</td>
</tr>
<tr>
<td>Resting heart rate, beats/min</td>
<td>63 ± 13</td>
</tr>
<tr>
<td>Index patients</td>
<td>86 (53)</td>
</tr>
<tr>
<td>Syncope</td>
<td>47 (29)</td>
</tr>
<tr>
<td>Ventricular arrhythmias</td>
<td>84 (52)</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n (%), or n.

ARVC = arrhythmogenic right ventricular cardiomyopathy; BMI = body mass index.

ICD therapies had been given in 15 patients, none of whom had early disease.

Overt ARVC subjects had changes in all TFC 2010 (5) diagnostic categories, with the exception of tissue characterization by endomyocardial biopsy, which was missing in the majority of subjects (Figure 2). The diagnosis of early ARVC was mainly based on family mutations (n = 58), minor depolarization abnormalities (n = 22), and minor criteria for ventricular arrhythmias (n = 15) (Figure 2). Cardiac magnetic resonance was performed in 121 (75%) subjects with ARVC, including 50 (68%) with early ARVC.

TOTAL ARVC POPULATION. In the total ARVC population, RV and RVOT diameters were markers of arrhythmic events (both p < 0.001) (Table 2). RV function by RV FAC and RV global longitudinal strain were decreased in subjects with arrhythmic events (both p < 0.01) (Table 2) and RV mechanical dispersion was more pronounced (p = 0.001). By ROC analyses, RV diameter had a marginally higher C-statistic to detect subjects with arrhythmic events compared with RVOT diameter (0.78 [95% confidence interval (CI): 0.71 to 0.85] vs. 0.70 [95% CI: 0.62 to 0.78]).

In patients with overt ARVC, diagnosis was based on findings from all categories from the Task Force Criteria revised in 2010, with the exception of tissue characterization (missing data). Diagnosis in patients with early ARVC was mainly based on genetic mutations, minor depolarization abnormalities, and minor arrhythmias. Depolarization: Epsilon waves (major) or abnormal signal-averaged ECG (minor). Repolarization: ECG T-wave inversions (V1-V2 [minor], V1-V3 or beyond [major]). ARVC = arrhythmogenic right ventricular cardiomyopathy; other abbreviations as in Figure 1.
p = 0.06). An RV diameter of 41 mm and an RVOT diameter of 34 mm optimally detected subjects with arrhythmic events. RV and LV dimensions were larger and RV function and LVEF were lower in those with arrhythmic events (all p ≤ 0.05) (Table 2), also when adjusted for beta-blocker use, age, sex, and mutation status in multivariable analyses (all p ≤ 0.05).

QRS duration did not differ between ARVC with and without arrhythmic events, whereas T-wave inversions were more prevalent in subjects with arrhythmic events (p < 0.001) (Table 2). All 3 parameters from signal-averaged ECGs were markers of arrhythmic events (all p < 0.001) (Table 2).

**PATIENTS WITH OVERT ARVC.** In patients with overt ARVC, 69 (78%) had experienced arrhythmic events (17 aborted cardiac arrests, 40 sustained VTs, 11 nonsustained VTs, and 1 cardiac syncope without documented ventricular arrhythmia). Of the remaining 20 (22%) with overt ARVC and no arrhythmic events, 18 (90%) were ARVC-mutation positive. As expected, increased RVOT diameter and decreased RV FAC were markers of arrhythmic events. Interestingly, also the new parameters RV diameter and RV global longitudinal strain were arrhythmic markers, with a similar ability to identify patients with arrhythmic events (C-statistic, 0.75 [95% CI: 0.61 to 0.88] and 0.68 [95% CI: 0.54 to 0.82], respectively; p = 0.36). Parameters from signal-averaged ECGs were abnormal in overt patients with ARVC, but were not markers of arrhythmias (Online Table 1).

**SUBJECTS WITH EARLY ARVC.** In subjects with early ARVC, 15 (21%) had experienced arrhythmic events (5 sustained VT, 9 nonsustained VT, and 1 cardiac syncope without documented VT). RV diameter was larger in subjects with arrhythmic events (p = 0.05). Table 2 and RV mechanical dispersion was more pronounced (p = 0.003). By ROC analyses, RV mechanical dispersion and RV diameter had similar ability to detect arrhythmic events (C-statistic, 0.74 [95% CI: 0.59 to 0.90] and 0.62 [95% CI: 0.47 to 0.78], respectively; p = 0.20; optimal cutoffs were 2.78 ms and ≥40 mm, respectively). Neither standard parameters of RV function (RV FAC, RV global longitudinal strain) nor parameters of LV function were markers of arrhythmic events in early ARVC (Table 3), whereas all parameters from signal-averaged ECG were arrhythmic markers (all p < 0.05) (Table 3) with comparable discriminative ability (p > 0.59 for all comparisons). Optimal cutoff values were 2.78 ms for filtered QRS, ≥37 ms for high-frequency low-amplitude signal and ≤32 µV for RMS (sensitivity, specificity, and C-statistics, Online Table 2). Interestingly, mechanical dispersion correlated with the electrical parameter filtered QRS duration from signal-averaged ECG (R = 0.38, p = 0.009) (Figure 3). There were no significant differences in QRS duration or presence of T-wave inversions (p = 0.27) between subjects with and without arrhythmic events (Table 2). Only 1 subject had S-wave upstroke >55 ms (Table 3).

**EARLY ARVC ARRHYTHMIC RISK ASSESSMENT.** To assess arrhythmic risk in subjects with early ARVC, we evaluated the presence of major and minor electrical abnormalities as given by the TFC 2010 (5) (depolarization: epsilon waves in V1 to V3, pathological signal-averaged ECG; repolarization: T-wave inversions). In patients with early ARVC and arrhythmic events, 67% had abnormal electrical parameters (8 [54%] depolarization and 21 [15%] repolarization abnormalities by TFC 2010 [5]) and 67% had abnormal echocardiographic findings by the early parameters (RV diameter >40 mm and/or RV mechanical dispersion >37 ms). Interestingly, 87% of those with arrhythmias had at least 1 of the electrical or echocardiographic abnormalities. We tested the ability of major and minor electrical abnormalities to detect subjects with arrhythmic events in a logistic regression model (Figure 4). The model’s ability to detect
TABLE 2 Comparison of 73 Subjects With Early ARVC Without and With Arrhythmic Events

<table>
<thead>
<tr>
<th></th>
<th>No Arrhythmic Events (n = 58)</th>
<th>Arrhythmic Events (n = 15)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female</strong></td>
<td>32 (55)</td>
<td>6 (40)</td>
<td>0.29</td>
</tr>
<tr>
<td><strong>Echocardiography</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV diameter, mm</td>
<td>37 ± 6</td>
<td>40 ± 4</td>
<td>0.05</td>
</tr>
<tr>
<td>RV FAC, %</td>
<td>45 ± 8</td>
<td>43 ± 6</td>
<td>0.45</td>
</tr>
<tr>
<td>RV GLS, % (5 segments)</td>
<td>-24.2 ± 3.3</td>
<td>-23.8 ± 2.9</td>
<td>0.69</td>
</tr>
<tr>
<td>RV mechanical dispersion, mm (5 segments)</td>
<td>26 ± 11</td>
<td>39 ± 15</td>
<td>0.003</td>
</tr>
<tr>
<td>RVOT sa, mm</td>
<td>32 ± 5</td>
<td>35 ± 5</td>
<td>0.09</td>
</tr>
<tr>
<td>LVEDV, ml</td>
<td>112 ± 28</td>
<td>115 ± 31</td>
<td>0.75</td>
</tr>
<tr>
<td>LVESV, ml</td>
<td>46 ± 14</td>
<td>50 ± 15</td>
<td>0.69</td>
</tr>
<tr>
<td>LVF, %</td>
<td>58 ± 4</td>
<td>57 ± 4</td>
<td>0.43</td>
</tr>
<tr>
<td>LV GLS, % (16 segments)</td>
<td>-20.5 ± 2.4</td>
<td>-20.1 ± 1.7</td>
<td>0.57</td>
</tr>
<tr>
<td>LV mechanical dispersion, mm (16 segments)</td>
<td>33 ± 14</td>
<td>39 ± 31</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Electrical parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filtered QRS, ms</td>
<td>109 ± 7</td>
<td>115 ± 8</td>
<td>0.005</td>
</tr>
<tr>
<td>HFLA, ms</td>
<td>32 ± 7</td>
<td>37 ± 5</td>
<td>0.02</td>
</tr>
<tr>
<td>RMS, µV</td>
<td>38 ± 14</td>
<td>27 ± 13</td>
<td>0.02</td>
</tr>
<tr>
<td>Epsilon waves</td>
<td>0 (0)</td>
<td>2 (13)</td>
<td>0.04</td>
</tr>
<tr>
<td>Incomplete RBBB</td>
<td>2 (3)</td>
<td>1 (7)</td>
<td>0.59</td>
</tr>
<tr>
<td>QRS, ms</td>
<td>92 ± 13</td>
<td>94 ± 8</td>
<td>0.43</td>
</tr>
<tr>
<td>S-wave upstroke, ms</td>
<td>39 ± 7</td>
<td>38 ± 8</td>
<td>0.59</td>
</tr>
<tr>
<td>S-wave upstroke &gt;0.5 ms</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>1.00</td>
</tr>
<tr>
<td>T-wave inversions</td>
<td>3 (5)</td>
<td>2 (13)</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Values are n (%) or mean ± SD. p by independent Student t test or chi-square test.

RBBB = right bundle branch block; other abbreviations as in Table 2.

Arrhythmic events increased significantly when adding the 2 early echocardiographic markers of arrhythmic events, RV diameter and RV mechanical dispersion, both in early ARVC (chi-square increased from 4.7 to 10.0, p = 0.05 [Figure 4A]; AIC 58 and 57, respectively) and in the entire ARVC population (chi-square increased from 28.1 to 48.1, p < 0.001 [Figure 4B]; AIC 155 and 139, respectively).

FEASIBILITY AND VARIABILITY ANALYSES. LV and RV strain analyses could be performed in 89% and 82% of patients and 88% and 96% of segments could be analyzed, respectively. Intraobserver and interobserver intraclass correlation for LV global longitudinal strain and LV mechanical dispersion, were 0.98 (95% CI: 0.92 to 0.99) and 0.94 (95% CI: 0.77 to 0.99) and 0.95 (95% CI: 0.81 to 0.99) and 0.91 (95% CI: 0.67 to 0.98), respectively, and for RV global longitudinal strain and RV mechanical dispersion, values were 0.98 (95% CI: 0.92 to 0.99) and 0.83 (95% CI: 0.40 to 0.95), respectively, and 0.87 (95% CI: 0.33 to 0.97) and 0.81 (95% CI: 0.35 to 0.95), respectively.

DISCUSSION

Risk stratification of arrhythmias and SCD in subjects with ARVC is difficult and most challenging in the early stages of ARVC. In this study, 21% of ARVC subjects with early disease had experienced arrhythmic events, underlining the importance of identifying early markers of arrhythmias. This study showed that both electrical parameters by signal-averaged ECGs and parameters from echocardiography were markers of arrhythmic events in early ARVC disease. Of importance, the addition of a few echocardiographic parameters to electrical measures improved identification of subjects with a history of arrhythmic events.

RV STRUCTURAL AND FUNCTIONAL CHANGES. In the total ARVC population, RV function was worse and RV dimensions were larger in those with a history of arrhythmic events, reflecting the well-known RV affection in ARVC and its relation to ventricular arrhythmias. Interestingly, RV diameter was a good marker of arrhythmic events, a parameter easily obtained and not included in the current TFC 2010 (5). However, the TFC 2010 is a diagnostic score and was not intended for identifying subjects with arrhythmic events. Our study may imply that RV diameter >41 mm might be associated with increased arrhythmic risk. RVOT diameter, which is included in the TFC 2010, was also a marker of arrhythmic events. Interestingly, the optimal RV diameter to detect patients with arrhythmic events was 34 mm and therefore even lower than the TFC 2010 diagnostic value (36 mm) (5).

Importantly, echocardiographic parameters were also arrhythmic markers in early ARVC with larger RV diameter and more pronounced RV mechanical dispersion in subjects with a history of arrhythmic events. These findings may indicate that dilation without visible affection of ventricular function may be the first RV changes and may imply a patient’s transition to a level of higher risk of ventricular arrhythmia, although the retrospective study design inhibits interpretation of cause and effect. Mechanical dispersion, reflecting inhomogeneous contraction, has previously been shown to be a marker of arrhythmias in ARVC and other cardiac diseases (7,12–15). In ARVC, structural changes, and fibrosis in particular, are likely substrates for the increased mechanical dispersion. RV mechanical dispersion in ARVC detects segments with subtle dyskinesia in early disease and may reflect the start of fibrosis and arrhythmic risk. We have previously shown that asymptomatic ARVC mutation-positive family members also had more pronounced RV mechanical dispersion than healthy controls, indicating a continuum of risk (7). The current study included a large cohort and focused on arrhythmias in early disease.
and showed the additive effect of imaging to electrical parameters in detecting high risk individuals.

**LV Structural and Functional Changes.** LV affection in ARVC is now commonly recognized (16). In the total ARVC population, parameters of LV function and timing, including LVEF, were markers of previous arrhythmic events. These findings support that overt ARVC disease commonly include the left ventricle and that LV dysfunction is an important risk factor of cardiac death (17). The relatively small difference in LVEF between those with and without arrhythmic events may be explained by the high proportion of patients with early disease, with no affection of LV parameters. Our results may indicate that onset of LV dysfunction occurred later compared with RV dysfunction in our population. This study cannot answer if the relatively preserved LV function was due to a future RV-predominant ARVC type in our population. Future studies should investigate if LV dysfunction precedes early RV dysfunction in patients who develop LV-predominant ARVC disease. However, in early ARVC, the future predominant ventricle of disease remains unknown and both ventricles should therefore be assessed carefully.

**Electrical Parameters.** Pathological signal-averaged ECGs are common in early ARVC (18). Our study supports this finding, with one-third of patients with early ARVC showing pathological signal-averaged ECGs. However, the value of signal-averaged ECG in risk stratification of arrhythmias is less clear. Some studies have reported signal-averaged ECG parameters to be associated with arrhythmic risk and to relate to the extent of myocardial fibrosis (19,20). In our study, these parameters were markers of arrhythmic events in early ARVC, emphasizing the importance of this assessment in early disease. In patients with overt ARVC, signal-averaged ECGs were clearly pathological, but were not markers of arrhythmic events, indicating a threshold value for arrhythmic risk. In the total ARVC population, also the presence of T-wave inversions and epsilon waves were markers of arrhythmic events.

**Early ARVC Arrhythmic Risk Assessment.** Current risk stratification of SCD and hence ICD indications are based on the presence of previous syncope, VT or aborted cardiac arrest, and RV/LV dysfunction, proband status, accumulated athletic activity, and a definite ARVC diagnosis by TFC 2010 (6,21-24). However, these markers are most useful in overt ARVC. We suggest including both electrical and echocardiographic parameters to assess arrhythmic risk in early ARVC. The addition of RV diameter and RV mechanical dispersion improved the ability to identify patients with arrhythmic events compared with the electrical changes given by the TFC 2010 alone (Figure 4), both in early ARVC and in the total ARVC population. Interestingly, mechanical dispersion was related to electrical parameters, indicating that pronounced mechanical dispersion may reflect early electromechanical interactions important for arrhythmogenicity.

In a recent paper, time from onset of QRS to start of regional shortening in the sub-tricuspid area was a marker of ventricular arrhythmias in asymptomatic ARVC mutation carriers (25), emphasizing the role of echocardiography in risk stratification. Alterations in s’ have also been described in ARVC, including early ARVC (26,27).

In our population, only 1 subject with early ARVC had an S-wave upstroke >55 ms, which has previously been described as a common and early finding in ARVC (9,29) and a predictor of arrhythmic events (25).
This discrepancy may be explained by the different definitions of S-wave upstroke (9,25).

CLINICAL IMPLICATIONS. ARVC mutation-positive family members with no apparent or only early disease constitute a substantial proportion of current ARVC populations seen in cardiomyopathy clinics. Risk stratification of SCD in these individuals has not been sufficiently addressed and they are currently followed every 1 to 2 years from age 15 with Holter monitoring, signal-averaged ECG, and echocardiography (28). The retrospective nature of arrhythmic events in relation to the echocardiography did not allow for causal interpretation, but there seemed to be an association between arrhythmias and echocardiographic alterations. We suggest that in subjects with pathological signal-averaged ECG, the presence of RV diameter ≥40 mm and mechanical dispersion ≥37 ms might incline the clinician to more frequent arrhythmia monitoring to detect episodes of arrhythmias and support the evaluation of ICD implantation.

STUDY LIMITATIONS. This study was cross-sectional in design with the inherent limitations. The limited number of patients with early ARVC makes the model for identifying subjects with arrhythmic events vulnerable to overfitting. Our analyses may be confounded by inclusion of mutation-positive family members who will remain ARVC nonpenetrant. Future and larger studies should prospectively follow subjects with early ARVC disease to confirm our results.

All echocardiographic analyses were performed in a blinded fashion; however, the presence of an ICD lead might have demasked arrhythmic status in a few cases.

Cardiac magnetic resonance imaging scans were lacking in 32% of early ARVC subjects, hence the TFC 2010 score may have been underestimated in a few subjects.

CONCLUSIONS

Subjects with ARVC and arrhythmic events had increased RV diameters and decreased RV and LV function by echocardiography. In patients with early ARVC disease, electrical parameters from signal-averaged ECGs and the 2 RV echocardiographic parameters RV diameter and RV mechanical dispersion, were markers of arrhythmic events. The addition of early structural changes increased the ability to detect subjects with arrhythmic events compared with electrical parameters alone. We suggest including measurements of RV diameter and LV mechanical dispersion when evaluating arrhythmic risk in subjects with ARVC.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: ARVC is characterized by structural and functional changes. Electrical changes are shown to occur prior to structural changes included in the 2010 TFC. However, this study showed that 2 novel RV parameters by echocardiography, RV mechanical dispersion and RV diameter, were also markers of arrhythmic events in early ARVC. These measures, not included in the 2010 TFC, may be important for risk stratification of arrhythmias in early disease.

TRANSLATIONAL OUTLOOK: Risk stratification of ventricular arrhythmias in ARVC is challenging, particularly in early disease. Genetic family screening has resulted in a substantial proportion of mutation-positive family members in ARVC clinics with no apparent or early ARVC disease in whom risk stratification for ventricular arrhythmias is not fully addressed. In this study, we showed that including novel RV measures by echocardiography was superior to electrical parameters alone in identifying subjects with a history of arrhythmic events, both in the total ARVC population and in patients with early ARVC disease. This might imply that in asymptomatic mutation-positive family members with pathological signal-averaged ECGs, the findings of RV diameter =40 mm and mechanical dispersion =37 ms should lead the clinician to more frequent arrhythmia monitoring to detect episodes of ventricular arrhythmias.

REFERENCES


KEY WORDS arrhythmic risk, arrhythmogenic right ventricular cardiomyopathy, echocardiography, signal averaged ECG, ventricular arrhythmias

APPENDIX For supplemental tables, please see the online version of this article.